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* 9,379 volunteer abstracts, 18 symposia abstracts, 17 history of neuroscience abstracts and 46 teaching of neuroscience abstracts.

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34.	Sensory development: somatosensory	Poster	mAM				
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Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
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626.	Neuroglia and myelin V	Poster					fAM
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42.	Acetylcholine: neuronal nicotinic receptors I	Poster	mAM				
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206.	Neural-immune interactions: other interleukins and cytokines	Poster		tuAM			
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345.	Neuroendocrine regulation: other	Poster			wAM		
54.	Neuroendocrine regulation: prolactin	Poster	mAM				
538.	Regulation of autonomic function	Slide				thPM	
207.	Regulation of autonomic function: gastrointestinal and visceral afferents	Poster		tuAM			
59.	Regulation of autonomic function: genitourinary	Poster	mAM				
347.	Regulation of autonomic function: other	Poster			wAM		
58.	Respiratory regulation: central networks/patterns	Poster	mAM				
348.	Respiratory regulation: chemoreception	Poster			wAM		
208.	Respiratory regulation: transmitters/receptors	Poster		tuAM			
209.	Temperature regulation and fever	Poster		tuAM			
205.	Water and osmotic regulation	Poster		tuAM			
THEME F: SENSORY SYSTEMS							
435.	Auditory system: anatomy I	Poster			wPM		
500.	Auditory system: anatomy II	Poster				thAM	
67.	Auditory system: central physiology I	Poster	mAM				
166.	Auditory system: central physiology II	Slide		tuAM			
353.	Auditory system: central physiology III	Poster			wAM		
499.	Auditory system: cochlea	Poster				thAM	
434.	Auditory system: neurochemistry	Poster			wPM		
588.	Auditory, vestibular and lateral-line hair cells	Poster				thPM	

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
502.	Chemical senses: central pathways—olfaction	Poster				thAM	
436.	Chemical senses: pathways—gustation	Poster			wPM		
258.	Chemical senses: peripheral mechanisms	Slide		tuPM			
501.	Chemical senses: peripheral mechanisms—olfaction, carotid body	Poster				thAM	
354.	Chemical senses: peripheral mechanisms—taste	Poster			wAM		
135.	Invertebrate sensory systems	Poster	mPM				
528.	**Molecular Biology of Olfaction**	**SYMP**				**thPM**	
430.	Pain modulation: behavior	Poster			wPM		
289.	Pain modulation: brainstem and thalamus	Poster		tuPM			
212.	Pain modulation: cognitive, autonomic and endocrine	Poster		tuAM			
290.	Pain modulation: descending	Poster		tuPM			
130.	Pain modulation: dorsal horn	Poster	mPM				
62.	Pain modulation: hyperalgesia	Poster	mAM				
292.	Pain modulation: peripheral	Poster		tuPM			
129.	Pain modulation: peripheral neuropathy	Poster	mPM				
291.	Pain modulation: pharmacology and endocrinology	Poster		tuPM			
428.	Pain modulation: spinal I	Poster			wPM		
429.	Pain modulation: spinal II	Poster			wPM		
351.	Pain modulation: supraspinal	Poster			wAM		
211.	Pain pathways: dorsal horn	Poster		tuAM			
128.	Pain pathways: hyperalgesia	Poster	mPM				
350.	Pain pathways: supraspinal	Poster			wAM		
94.	Pain: pharmacology	Slide	mPM				
167.	Pain: physiology and behavior	Slide		tuAM			
63.	Retina and photoreceptors I	Poster	mAM				
171.	Retina and photoreceptors II	Slide		tuAM			
431.	Retina and photoreceptors: amacrine, ganglion and glial cells	Poster			wPM		
352.	Retina and photoreceptors: photoreceptors, horizontal and bipolar cells	Poster			wAM		
60.	Somatic and visceral afferents I	Poster	mAM				
210.	Somatic and visceral afferents II	Poster		tuAM			
349.	Somatic and visceral afferents: touch	Poster			wAM		
582.	Somatosensory cortex and thalamocortical relationships: anatomy	Poster				thPM	
644.	Somatosensory cortex and thalamocortical relationships: development and plasticity	Poster					fAM
583.	Somatosensory cortex and thalamocortical relationships: physiology	Poster				thPM	
645.	Somatosensory cortex and thalamocortical relationships: psychophysics and networks	Poster					fAM
584.	Somatosensory cortex and thalamocortical relationships: thalamus	Poster				thPM	
316.	Somatosensory system	Slide			wAM		
61.	Spinal cord	Poster	mAM				
427.	Subcortical somatosensory pathways: lemniscal and spinothalamic	Poster			wPM		
498.	Subcortical somatosensory pathways: trigeminal	Poster				thAM	
101.	Subcortical visual pathways	Slide	mPM				
64.	Subcortical visual pathways: LGN	Poster	mAM				
432.	Subcortical visual pathways: cortico-tectal, Accessory Optic System, centrifugals	Poster			wPM		
65.	Subcortical visual pathways: retina and colliculus	Poster	mAM				
454.	**Thalamic Mechanisms of Nociception**	**SYMP**				**thAM**	
586.	Visual behavior: clinical studies	Poster				thPM	
587.	Visual behavior: psychophysics and eye movements	Poster				thPM	
169.	Visual cortex: anatomical studies	Slide		tuAM			
132.	Visual cortex: anatomy of extrastriate cortex	Poster	mPM				
134.	Visual cortex: anatomy of striate cortex	Poster	mPM				
537.	Visual cortex: behavior and behavioral correlates	Slide				thPM	
585.	Visual cortex: brain imaging techniques	Poster				thPM	

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
11.	Visual cortex: functional circuits and oscillations I	Slide	mAM				
131.	Visual cortex: functional circuits and oscillations II	Poster	mPM				
99.	Visual cortex: functional organization of striate cortex	Slide	mPM				
433.	Visual cortex: motion processing I	Poster			wPM		
464.	Visual cortex: motion processing II	Slide				thAM	
256.	Visual cortex: neuronal response properties	Slide		tuPM			
66.	Visual cortex: physiology of extrastriate cortex	Poster	mAM				
133.	Visual cortex: physiology of striate cortex	Poster	mPM				
313.	Visual cortex: theoretical studies	Slide			wAM		
	THEME G: MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION						
136.	Basal ganglia and thalamus I	Poster	mPM				
137.	Basal ganglia and thalamus II	Poster	mPM				
293.	Basal ganglia and thalamus III	Poster		tuPM			
294.	Basal ganglia and thalamus IV	Poster		tuPM			
356.	Basal ganglia and thalamus V	Poster			wAM		
437.	Basal ganglia and thalamus VI	Poster			wPM		
503.	Basal ganglia and thalamus VII	Poster				thAM	
178.	Cerebellum I	Slide		tuAM			
357.	Cerebellum II	Poster			wAM		
438.	Cerebellum III	Poster			wPM		
504.	Cerebellum IV	Poster				thAM	
140.	Circuitry and pattern generation I	Poster	mPM				
442.	Circuitry and pattern generation II	Poster			wPM		
539.	Circuitry and pattern generation III	Slide				thPM	
18.	Control of posture and movement I	Slide	mAM				
441.	Control of posture and movement II	Poster			wPM		
590.	Control of posture and movement III	Poster				thPM	
591.	Control of posture and movement IV	Poster				thPM	
218.	Control of posture and movement: arm movement I	Poster		tuAM			
647.	Control of posture and movement: arm movement II	Poster					fAM
360.	Control of posture and movement: clinically related studies	Poster			wAM		
648.	Control of posture and movement: locomotion	Poster					fAM
592.	Invertebrate motor function	Poster				thPM	
103.	Motor systems and sensorimotor integration: cortex I	Slide	mPM				
213.	Motor systems and sensorimotor integration: cortex II	Poster		tuAM			
214.	Motor systems and sensorimotor integration: cortex III	Poster		tuAM			
355.	Motor systems and sensorimotor integration: cortex IV	Poster			wAM		
397.	Motor systems and sensorimotor integration: cortex V	Slide			wPM		
649.	Muscle I	Poster					fAM
650.	Muscle II	Poster					fAM
19.	Oculomotor I	Slide	mAM				
102.	Oculomotor II	Slide	mPM				
296.	Oculomotor III	Poster		tuPM			
358.	Oculomotor IV	Poster			wAM		
295.	Oculomotor system: saccades	Poster		tuPM			
589.	Reflex function I	Poster				thPM	
646.	Reflex function II	Poster					fAM
138.	Spinal cord and brainstem I	Poster	mPM				
139.	Spinal cord and brainstem II	Poster	mPM				
217.	Spinal cord and brainstem III	Poster		tuAM			
359.	Spinal cord and brainstem IV	Poster			wAM		
440.	Spinal cord and brainstem V	Poster			wPM		

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
244.	**The Role of Sensory Information in the Guidance of Voluntary Movement**	**SYMP**		**tuPM**			
439.	Vestibular system: behavioral responses	Poster			wPM		
216.	Vestibular system: morphology and physiology	Poster		tuAM			
215.	Vestibular system: neurochemistry	Poster		tuAM			
	THEME H: OTHER SYSTEMS OF THE CNS						
594.	Association cortex and thalamocortical relations	Poster				thPM	
68.	Brain metabolism and blood flow I	Poster	mAM				
177.	Brain metabolism and blood flow II	Slide		tuAM			
361.	Brain metabolism and blood flow III	Poster			wAM		
532.	Brain metabolism and blood flow IV	Slide				thPM	
143.	Comparative neuroanatomy	Poster	mPM				
2.	**Emerging Principles of Organization Within the Midbrain Periaqueductal Gray Matter**	**SYMP**	**mAM**				
141.	Limbic system I	Poster	mPM				
142.	Limbic system II	Poster	mPM				
219.	Limbic system III	Poster		tuAM			
386.	Limbic system IV	Slide			wPM		
593.	Other systems of the CNS: hypothalamus	Poster				thPM	
	THEME I: NEURAL BASIS OF BEHAVIOR						
376.	Aging and behavior I	Poster			wAM		
600.	Aging and behavior II	Poster				thPM	
6.	Biological rhythms and sleep I	Slide	mAM				
92.	Biological rhythms and sleep II	Slide	mPM				
365.	Biological rhythms and sleep III	Poster			wAM		
366.	Biological rhythms and sleep IV	Poster			wAM		
510.	Biological rhythms and sleep V	Poster				thAM	
511.	Biological rhythms and sleep VI	Poster				thAM	
512.	Biological rhythms and sleep VII	Poster				thAM	
382.	**Cognitive and Neurobiological Consequences of Normal Aging: From Rats to Primates**	**SYMP**			**wPM**		
227.	Drugs of abuse: alcohol, barbiturates and benzodiazepines I	Poster		tuAM			
322.	Drugs of abuse: alcohol, barbiturates and benzodiazepines II	Slide			wAM		
448.	Drugs of abuse: alcohol, barbiturates and benzodiazepines III	Poster			wPM		
154.	Drugs of abuse: amphetamine and other stimulants	Poster	mPM				
656.	Drugs of abuse: behavioral effects of cocaine	Poster					fAM
153.	Drugs of abuse: benzodiazepines and barbiturates	Poster	mPM				
157.	Drugs of abuse: cannabinoids and opioids	Poster	mPM				
599.	Drugs of abuse: cocaine and biochemistry	Poster				thPM	
155.	Drugs of abuse: cocaine and development	Poster	mPM				
450.	Drugs of abuse: cocaine and dopamine neuronal systems	Poster			wPM		
612.	Drugs of abuse: cocaine and other stimulants	Slide					fAM
516.	Drugs of abuse: cocaine's interaction with non-dopamine systems	Poster				thAM	
228.	Drugs of abuse: cocaine—other studies	Poster		tuAM			
515.	Drugs of abuse: ethanol and GABA	Poster				thAM	
598.	Drugs of abuse: ethanol and monoamines	Poster				thPM	
375.	Drugs of abuse: interaction of cocaine, DA-altering drugs and the VTA	Poster			wAM		
229.	Drugs of abuse: nicotine, cocaine, et al.	Poster		tuAM			
156.	Drugs of abuse: opioids	Poster	mPM				
302.	Drugs of abuse: pharmacology of cocaine	Poster		tuPM			
449.	Drugs of abuse: stimulants I	Poster			wPM		

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
657.	Drugs of abuse: stimulants II	Poster					fAM
369.	Hormonal control of reproductive behavior I	Poster			wAM		
370.	Hormonal control of reproductive behavior II	Poster			wAM		
371.	Hormonal control of reproductive behavior III	Poster			wAM		
372.	Hormonal control of reproductive behavior IV	Poster			wAM		
144.	Human cognition I	Poster	mPM				
505.	Human cognition II	Poster				thAM	
394.	Human cognition: blood flow/metabolism	Slide			wPM		
145.	Human cognition: electrophysiology I	Poster	mPM				
252.	Human cognition: electrophysiology II	Slide		tuPM			
315.	Human cognition: neuropsychology	Slide			wAM		
396.	Ingestive behavior: NPY, galanin and insulin	Slide			wPM		
514.	Ingestive behavior: nutrients, serotonin and insulin	Poster				thAM	
597.	Ingestive behavior: peptides	Poster				thPM	
314.	Ingestive behavior: salt appetite and food intake	Slide			wAM		
446.	Ingestive behavior: taste aversion and neural mechanisms	Poster			wPM		
513.	Ingestive behavior: water and salt intake	Poster				thAM	
13.	Invertebrate learning and behavior I	Slide	mAM				
224.	Invertebrate learning and behavior II	Poster		tuAM			
299.	Invertebrate learning and behavior III	Poster		tuPM			
398.	Invertebrate learning and behavior IV	Slide			wPM		
147.	Learning and memory: pharmacology—acetylcholine	Poster	mPM				
654.	Learning and memory: pharmacology—benzodiazepines	Poster					fAM
509.	Learning and memory: pharmacology—excitatory amino acids	Poster				thAM	
222.	Learning and memory: pharmacology—monoamines	Poster		tuAM			
221.	Learning and memory: pharmacology—opioids	Poster		tuAM			
362.	Learning and memory: pharmacology--other I	Poster			wAM		
655.	Learning and memory: pharmacology—other II	Poster					fAM
146.	Learning and memory: physiology I	Poster	mPM				
220.	Learning and memory: physiology II	Poster		tuAM			
297.	Learning and memory: physiology III	Poster		tuPM			
445.	Learning and memory: physiology IV	Poster			wPM		
508.	Learning and memory: physiology V	Poster				thAM	
651.	Learning and memory: systems and functions—conditioning I	Poster					fAM
652.	Learning and memory: systems and functions—conditioning II	Poster					fAM
653.	Learning and memory: systems and functions—conditioning III	Poster					fAM
443.	Learning and memory: systems and functions—misc. I	Poster			wPM		
444.	Learning and memory: systems and functions—misc. II	Poster			wPM		
506.	Learning and memory: systems and functions—models	Poster				thAM	
168.	Learning and memory: systems and functions—neuropsychology I	Slide		tuAM			
507.	Learning and memory: systems and functions—neuropsychology II	Poster				thAM	
595.	Learning and memory: systems and functions—spatial I	Poster				thPM	
596.	Learning and memory: systems and functions—spatial II	Poster				thPM	
226.	Monoamines and behavior: 8-OH-DPAT and noradrenaline	Poster		tuAM			
301.	Monoamines and behavior: accumbens, striatum and frontal cortex	Poster		tuPM			
447.	Monoamines and behavior: behavioral effects of dopamine	Poster			wPM		
7.	Monoamines and behavior: human disease and animal models	Slide	mAM				
151.	Monoamines and behavior: serotonin	Poster	mPM				
368.	Monoamines and behavior: striatum and DA receptors	Poster			wAM		
298.	Motivation and emotion I	Poster		tuPM			
364.	Motivation and emotion II	Poster			wAM		
148.	Neural plasticity I	Poster	mPM				
257.	Neural plasticity II	Slide		tuPM			
363.	Neural plasticity III	Poster			wAM		
606.	**Neurobehavioral Mechanism of Salt Intake Behavior**	**SYMP**					**fAM**
223.	Neuroethology: avian vocalization and audition	Poster		tuAM			

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
149.	Neuroethology: invertebrates, electric fish	Poster	mPM				
367.	Neuroethology: tetrapods—bat echolocation	Poster			wAM		
152.	Neuropeptides and behavior I	Poster	mPM				
373.	Neuropeptides and behavior II	Poster			wAM		
374.	Neuropeptides and behavior III	Poster			wAM		
95.	Psychotherapeutic drugs	Slide	mPM				
158.	Psychotherapeutic drugs: clozapine and dopamine antagonists	Poster	mPM				
303.	Psychotherapeutic drugs: lithium, benzodiazepines and antidepressants	Poster		tuPM			
230.	Psychotherapeutic drugs: sigma receptors and antipsychotics	Poster		tuAM			
96.	Stress	Slide	mPM				
225.	Stress and neuronal systems	Poster		tuAM			
150.	Stress: chronic	Poster	mPM				
300.	Stress: general	Poster		tuPM			

THEME J: DISORDERS OF THE NERVOUS SYSTEM

669.	Affective disorders	Poster					fAM
670.	Affective disorders: serotonin	Poster					fAM
603.	Degenerative disease: Parkinson's IV	Poster				thPM	
15.	Degenerative disease: Alzheimer's—ß-amyloid I	Slide	mAM				
72.	Degenerative disease: Alzheimer's—ß-amyloid II	Poster	mAM				
305.	Degenerative disease: Alzheimer's—ß-amyloid III	Poster		tuPM			
325.	Degenerative disease: Alzheimer's—ß-amyloid IV	Slide			wAM		
601.	Degenerative disease: Alzheimer's—ß-amyloid V	Poster				thPM	
602.	Degenerative disease: Alzheimer's—ß-amyloid VI	Poster				thPM	
616.	Degenerative disease: Alzheimer's—ß-amyloid VII	Slide					fAM
236.	Degenerative disease: Alzheimer's—CSF	Poster		tuAM			
235.	Degenerative disease: Alzheimer's—animal models	Poster		tuAM			
239.	Degenerative disease: Alzheimer's—clinical observations	Poster		tuAM			
306.	Degenerative disease: Alzheimer's—cognition	Poster		tuPM			
240.	Degenerative disease: Alzheimer's—etiologic toxins	Poster		tuAM			
234.	Degenerative disease: Alzheimer's—growth factors	Poster		tuAM			
307.	Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters	Poster		tuPM			
97.	Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters	Slide	mPM				
518.	Degenerative disease: Alzheimer's—neuropharmacology and neurotransmitters	Poster				thAM	
93.	Degenerative disease: Alzheimer's—other	Slide	mPM				
238.	Degenerative disease: Alzheimer's—plaques and tangles	Poster		tuAM			
233.	Degenerative disease: Alzheimer's—postmortem analysis	Poster		tuAM			
237.	Degenerative disease: Alzheimer's—tau	Poster		tuAM			
73.	Degenerative disease: Huntington's disease	Poster	mAM				
395.	Degenerative disease: Parkinson's I	Slide			wPM		
451.	Degenerative disease: Parkinson's II	Poster			wPM		
519.	Degenerative disease: Parkinson's III	Poster				thAM	
658.	Degenerative disease: Parkinson's V	Poster					fAM
520.	Degenerative disease: other	Poster				thAM	
604.	Developmental disorders	Poster				thPM	
159.	Epilepsy: anticonvulsant drugs	Poster	mPM				
71.	Epilepsy: basic mechanisms I	Poster	mAM				
232.	Epilepsy: basic mechanisms II	Poster		tuAM			
378.	Epilepsy: basic mechanisms III	Poster			wAM		
517.	Epilepsy: basic mechanisms IV	Poster				thAM	
70.	Epilepsy: human studies and animal models I	Poster	mAM				

Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
231.	Epilepsy: human studies and animal models II	Poster		tuAM			
377.	Epilepsy: human studies and animal models III	Poster			wAM		
69.	Genetic models I	Poster	mAM				
304.	Genetic models II	Poster		tuPM			
667.	Infectious diseases	Poster					fAM
533.	Ischemia I	Slide				thPM	
610.	Ischemia II	Slide					fAM
659.	Ischemia: acidosis	Poster					fAM
661.	Ischemia: animal models	Poster					fAM
665.	Ischemia: calcium	Poster					fAM
662.	Ischemia: cellular mechanisms	Poster					fAM
666.	Ischemia: clinical studies	Poster					fAM
521.	Ischemia: drug treatment I	Poster				thAM	
522.	Ischemia: drug treatment II	Poster				thAM	
525.	Ischemia: free radicals	Poster				thAM	
524.	Ischemia: gene or protein induction	Poster				thAM	
663.	Ischemia: glia	Poster					fAM
523.	Ischemia: glucose	Poster				thAM	
527.	Ischemia: imaging	Poster				thAM	
660.	Ischemia: neurophysiology	Poster					fAM
241.	Ischemia: neurotransmitters	Poster		tuAM			
664.	Ischemia: temperature effect	Poster					fAM
526.	Ischemia: vasculature	Poster				thAM	
381.	**Mechanisms of HIV-related Injury in the Central Nervous System**	**SYMP**			**wPM**		
452.	Neuromuscular diseases I	Poster			wPM		
668.	Neuromuscular diseases II	Poster					fAM
465.	Neurotoxicity	Slide				thAM	
380.	Neurotoxicity: amphetamine	Poster			wAM		
671.	Neurotoxicity: biological	Poster					fAM
672.	Neurotoxicity: excitotoxins	Poster					fAM
674.	Neurotoxicity: metals	Poster					fAM
675.	Neurotoxicity: metals—aluminum	Poster					fAM
673.	Neurotoxicity: miscellaneous toxins	Poster					fAM
242.	Schizophrenia	Poster		tuAM			
379.	Schizophrenia: neurochemistry	Poster			wAM		
310.	**Strategies for the Study of Alzheimer Amyloidosis Using Animal Models**	**SYMP**			**wAM**		
457.	Trauma	Slide				thAM	
74.	Trauma: behavioral studies	Poster	mAM				
77.	Trauma: cellular reaction	Poster	mAM				
79.	Trauma: drug treatment	Poster	mAM				
80.	Trauma: glia/blood-brain barrier	Poster	mAM				
81.	Trauma: hypothermia	Poster	mAM				
75.	Trauma: mechanisms of injury and healing	Poster	mAM				
78.	Trauma: metabolic changes	Poster	mAM				
76.	Trauma: transmitters	Poster	mAM				
OTHER:							
82.	History of neuroscience	Poster	mAM, PM	tuAM, PM	wAM, PM	thAM, PM	fAM
84.	Teaching of neuroscience: computer-assisted instruction	Poster	mAM, PM	tuAM, PM	wAM, PM	thAM, PM	fAM
85.	Teaching of neuroscience: elementary and secondary grades	Poster	mAM, PM	tuAM, PM	wAM, PM	thAM, PM	fAM
83.	Teaching of neuroscience: undergraduate and graduate education	Poster	mAM, PM	tuAM, PM	wAM, PM	thAM, PM	fAM

2

SYMPOSIUM: EMERGING PRINCIPLES OF ORGANIZATION WITHIN THE MIDBRAIN PERIAQUEDUCTAL GRAY MATTER (PAG). M.T. Shipley, Univ. Cincinnati & R. Bandler, Univ. Sidney (Co-Chairpersons); A. Beitz, Univ. Minnesota; T. Lovick, Univ. of Birmingham; H. Fields, Univ. of San Francisco, (Discussant).

Research during the past two decades has led to the recognition that the midbrain periaqueductal gray region (PAG) plays a pivotal executive role in an organism's responses to threatening stimuli. Such responses include rapid and coordinated skeletal and autonomic adjustments, associated with significant alterations in "pain" thresholds. Roles for PAG in the regulation of pain and aversion, defensive and sexual behavior, and cardiovascular and respiratory functions have been firmly established. The use of sensitive anatomical and neurochemical techniques has also yielded much new information about the afferent and efferent connections and the neurotransmitters of the PAG. The aim of this symposium is to provide a much-needed venue for reassessing our current understanding of the neural organization of this important brain region. Consideration will be given to the hypothesis that organization within the PAG can be largely subsumed under a single fundamental principle. Namely, that *anatomical and functional specificity is expressed in the form of discrete longitudinal neuronal and afferent columns extending along the rostrocaudal axis of the PAG.* Individual presentations will focus on different aspects of PAG function, anatomy and neurochemistry, in order to examine the different manifestations of longitudinal columnar organization within the PAG, e.g., the extent to which afferent input columns co-distribute with functionally and anatomically defined output columns; somatotopic and viscerotopic organization of longitudinal columns; intrinsic and extrinsic linkages between longitudinal columns. The goal of the symposium, thus, is to provide a precise and up-to-date understanding of the organization within the PAG that underlies the integrated expression of its vital functions.

3

SYMPOSIUM. THE COMPUTATIONAL NEURON. T.J. Sejnowski, Howard Hughes Medical Institute and The Salk Institute, La Jolla, CA (Chairperson); C. Koch, Caltech, Pasadena, CA; R. J. Douglas, Oxford University, Oxford, England; W. W. Lytton, Neurology Department, University of Wisconsin, Madison, WI; A. Bell, Free University of Brussels, Belgium.

Nonlinear properties of neurons due to voltage-sensitive ionic currents and synaptic conductances have important consequences for how neurons compute. Detailed computer models of single neurons based on anatomically-reconstructed morphologies, known synaptic strengths, and measured channel kinetics are presented that illustrate new computational principles. In the introduction, Sejnowski will motivate the need for modeling single neurons and outline the computational techniques that are available. Koch will show that synaptic conductances can dynamically modulate the properties of dendrites in cortical pyramidal neurons; Douglas will demonstrate models of neurons implemented in analog VLSI silicon circuits that vastly enhancing the speed of these models; Lytton will present simulations of synaptically-driven entrainment of thalamocortical neurons and cortical pyramidal neurons. Bell will examine the question of why dendrites have active currents, making a provocative prediction for their self-organization during development. From these modeling studies a new view of computational processing is emerging in which the intrinsic dynamical properties of neurons interact with network properties to produce large-scale coherent activity in neural populations.

BIOLOGICAL RHYTHMS AND SLEEP I

6.1

MEASUREMENTS OF ELECTRICAL COUPLING BETWEEN CIRCADIAN PACEMAKER CELLS OF THE *BULLA* EYE. Michael E. Geusz* and Gene D. Block. NSF Center for Biological Timing, University of Virginia, Gilmer Hall, Charlottesville, VA 22901.

A pacemaker in the eye of the mollusk *Bulla gouldiana* generates a circadian rhythm in the frequency of compound action potentials (CAPs) in the optic nerve. The CAPs are produced from the synchronous firing of a population of about 100 cells known as the basal retinal neurons (BRNs). The BRNs are believed to be electrically coupled because CAPs persist in media with low Ca^{2+} concentrations and dye coupling between BRNs has been shown.

To assess the strength of the electrical coupling between the BRNs, simultaneous intracellular recordings were made from pairs of BRNs while -0.5 nanoamp current pulses were injected into either cell. Coupling coefficients, measured using discontinuous current clamp, averaged 0.66 $\pm$0.15 S.D. (n=12 pairs), indicating that strong coupling occurs. There was no obvious rectification when current was passed in either direction. Strong coupling was found during both the subjective day, 0.68 $\pm$0.17 S.D. (n=7), and the subjective night, 0.64 $\pm$0.14 S.D. (n=5).

Pair-wise recordings between BRNs and the H-type cells of the retina, which hyperpolarize transiently in response to light, did not show evidence of electrical coupling (n=5). Similarly, pair-wise recordings between H-type cells did not reveal electrical coupling.

Treatments that block gap junctional conductances in other systems did not block electrical coupling between BRNs. CAPs and coupling persisted in 1 mM 1-octanol, or when extracellular pH was below 5.0. Coupling persisted in 1-heptanol at 1 mM, although CAPs were blocked. Heptanol at 10 mM caused irreversible loss of CAPs. Both alcohols were administered in 0.5% by volume dimethyl sulfoxide. *Supported by NS08806 to MEG and NS15264 to GDB.*

6.2

DISSOCIATED PACEMAKER CELLS OF *BULLA* EXPRESS CIRCADIAN RHYTHM IN MEMBRANE CONDUCTANCE

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The eyes of the marine snail *Bulla gouldiana* express a circadian rhythm of compound action potentials (CAP) in vitro driven by endogenous changes in membrane potential of basal retinal neurons (BRN), which are believed to be the pacemaker cells. Using single electrode current clamp in a semi-intact eye, it has been shown that BRN membrane conductance decreases at subjective dawn and increases at subjective dusk. These changes appear to be mediated by a modulation of a potassium current.

We now report that the circadian control of membrane conductance is retained in dissociated BRNs. The cells were obtained from surgically reduced eyes. The basal part of the *Bulla* retina was dispersed in culture dishes containing modified L-15 medium and kept in constant conditions. We used morphological criteria previously confirmed with Lucifer Yellow injections to identify BRNs in dispersals. Due to the low cell density most cells did not connect with each other, although neurite outgrowth was observed after 24 h. Intracellular recordings from these unconnected neurons were performed at ZT 19 and ZT 3 on the first (n=5) and second (n=8) circadian cycles using the same methods as in the semi-intact retina. Current-voltage relations were obtained after applying current pulses with the discontinuous current clamp technique. Predawn (ZT 19) and postdawn (ZT 3) I/V curves were significantly different in slope ($p<0.01$) during both of the first two cycles in culture. The decrease in conductance near subjective dawn was consistent with results found in the semi-intact preparation. These results support the hypothesis that an individual neuron can function as a circadian pacemaker. *Supported by DFG Mi 328/1-1 to SM, NS08806 to MEG and NS15264 and NSF DIR8920162 to GDB.*

6.3

TRYPTOPHAN HYDROXYLASE MESSENGER RNA LEVELS EXHIBIT CIRCADIAN RHYTHMICITY IN *XENOPUS LAEVIS* RETINA. C. B. Green and J. C. Besharse*. Dept. of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS 66160.

Tryptophan hydroxylase is the rate-limiting enzyme in melatonin synthesis in *Xenopus laevis* retina (Cahill and Besharse, *J. Neurochem.* **54**:716-719). Melatonin is synthesized rhythmically in photoreceptors with elevated levels during the night (Cahill and Besharse, *Visual Neuroscience* **8**:487-490, 1992). This pattern of synthesis is controlled by light and a retinal circadian clock. In order to determine whether tryptophan hydroxylase is expressed in photoreceptors and whether this expression is rhythmic, we analyzed tryptophan hydroxylase mRNA by Northern blot analysis and *in situ* hybridization.

A *Xenopus* retinal cDNA library was constructed and screened, under conditions of low stringency, using rabbit tryptophan hydroxylase cDNA (Grenett *et al., Proc. Natl. Acad. Sci. USA* **84**:5530-5534, 1987)as a probe. A cDNA clone coding for *Xenopus* tryptophan hydroxylase was isolated and characterized. This clone was used as a probe for analysis of retinal tryptophan hydroxylase mRNA. Retinas were dissected throughout the normal 12 hour light: 12 hour dark cycle or throughout a 24 hour period of constant darkness. Northern blot analysis of total retinal RNA showed that the tryptophan hydroxylase message levels are low in the day and higher during the night. This expression of tryptophan hydroxylase mRNA appears to be under circadian control since rhythmic changes are also seen in constant darkness, with elevated levels during the subjective night. Preliminary results from *in situ* hybridization suggest that the tryptophan hydroxylase mRNA in the retina is located in photoreceptors. Our observations suggest that rhythmic expression of tryptophan hydroxylase may be important in the generation of the melatonin rhythms.

***Indicates signing member**

6.4

CULTURED NEURONS AND PHOTORECEPTORS OF THE APLYSIA CIRCADIAN PACEMAKER. J.W. Jacklet. Dept. Biol. Sci., Neurobiology Research Center, SUNY Albany, Albany, NY 12222.

An antiserum against a 48kD eye specific protein stains certain cells and fibers of the Aplysia retina, which contains a circadian pacemaker. It also stains the optic nerve, the optic tract of the cerebral ganglion and other central ganglia. Myomodulin antiserum stains other specific fibers, neuropil and cells of the retina including several large neurons, whose axons project in the optic nerve to the optic tract of the cerebral ganglion. The lateral terminus of the optic tract stains with both antisera and is a putative synaptic exchange area. In order to identify cells and fibers containing the antigens and preparatory to other types of analysis, the retinas were dissociated, using conventional enzymatic digestion, and cells were plated in cell culture using defined medium. Many cell types survived, grew neurites and interacted, including monopolar and bipolar neurons; large microvillous, large tufted and small fusiform photoreceptors. The monopolar and bipolar neurons are putative pacemaker neurons, which produce the CAP activity that exhibits a circadian rhythm. They have cell bodies 12-18μm in diameter and survived for more than a week in culture. Under certain conditions they extended neurites for hundreds of micrometers and interacted with other cell types by neurite association and putative synapse formation. Preliminary electrical recordings revealed several voltage sensitive outward and slow inward currents.
Supported in part by NSF BNS-8819773; University Calgary Visiting Scientist and Alberta Heritage Foundation Funds; thanks to University Calgary Neuroscience Faculty.

6.5

AN EFFECT OF THE *PERIOD* GENE ON A CIRCADIAN RHYTHM IN VISUAL SENSITIVITY IN *DROSOPHILA*.

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The *period (per)* gene, which controls the period of circadian rhythms in *Drosophila melanogaster*, is highly expressed in the fly nervous system. *Drosophila* head sections stained with anti-*per* antibody revealed high levels of *per* protein in the compound eyes and the optic lobes of wild-type flies, but no detectable *per* protein in *per* ^{01} mutants. The effects of *per* on visual physiology were investigated with electroretinogram (ERG) recordings from wild-type and *per* ^{01} flies. Responses to 1-sec flashes of light (peak wavelength 565 nm) revealed no effects of the *per* ^{01} mutation on the ERG waveform. A daily rhythm in visual sensitivity was detected in chronic recordings (12 to 33 hours) from wild-type flies, however, with the photoreceptor response to 0.5-sec flashes of light peaking in the middle of the subjective night. For these experiments, flies (1-10 days old) were reared in a 12h : 12h light-dark cycle, then kept in darkness for 12-27 hours prior to recording. Conditions of constant darkness were maintained for the duration of the experiment, except for 0.5-sec light pulses every 30 minutes. No rhythm was detected in chronic recordings from *per* ^{01} flies, suggesting that the *per* gene regulates this physiological circadian rhythm in the visual system. This result raises the possibility of a link between physiological rhythms and rhythmic *per* expression within the photoreceptor cells.

Supported by NSF (grants #BNS-9057703 and #BNS-9010691) and a Merck Summer Research Fellowship.

6.6

ANTIBODY TO THE *DROSOPHILA PERIOD* PROTEIN IDENTIFIES AN ANTIGEN THAT EXHIBITS A DIURNAL RHYTHM IN THE SUPRACHIASMATIC NUCLEUS (SCN) OF THE RAT.

K.L. Rosewell[1], K.K. Siwicki[2], J.N. Masters[3] and P.M. Wise[1].
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The *period (per)* gene of *Drosophila melanogaster* is considered an important biological clock gene because it regulates multiple behavioral rhythms. An antibody to the *per* protein, which revealed a circadian rhythm in the *per* protein of the fly nervous system, also recognizes antigens in the SCN of rats. The purpose of this study was to determine whether the rat *per*-like antigens exhibit diurnal rhythms. Rats were ovariectomized (day 0), treated with estradiol (days 7-9) and killed at 12 times of day 9. Brains were frozen, sliced and microdissected. Levels of *per*-like antigens were quantified in several brain regions including the SCN, a critical neural pacemaker, by Western analysis. The antibody to *per* protein identifies 3 proteins of approximately 160-170kD and 115kD in all the rat brain regions examined. The largest of these proteins exhibits a diurnal rhythm in the SCN which peaks in the middle of the dark and attains its nadir around lights off; levels during the light are intermediate with a tendency toward a second drop around lights on. The data demonstrate that an antigen similar to *per* exhibits a diurnal rhythm in a critical neural pacemaker region of the rat brain. They suggest that *per*-like proteins may be an important component of the mammalian clock.

6.7

MELATONIN IS DEGRADED WITHIN THE VERTEBRATE PINEAL GLAND AND BRAIN.

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Melatonin is deacetylated within the eyes of several species, and melatonin deacetylase activity occurs at sites of melatonin action within the African clawed frog *Xenopus laevis* (Grace, M.S. et al., 1991, Brain Res. 559:56-63). The vertebrate pineal gland synthesizes melatonin, and contains all of the non-melatonin methoxyindoles generated by the retinal melatonin deacetylase. We therefore set out to determine whether melatonin deacetylase activity exists within the pineal gland and elsewhere within the central nervous system. We now report the metabolism of exogenous melatonin to 5-methoxytryptamine (5MT) and 5-methoxyindoleacetic acid (5MIAA) by cultured pineal glands and brains of the lizards *Anolis carolinensis* and *Sceloporus jarrovi*. After incubation of pineal glands or whole brains with 100nM [^{3}H-methoxy]-melatonin (specific activity 83.3Ci/mmol), ^{3}H-5MT and ^{3}H-5MIAA were detected and quantified by HPLC with fluorescence detection and flow scintillation spectrometry. The production of ^{3}H-5MT and ^{3}H-5MIAA accounted for all ^{3}H-melatonin loss. Melatonin breakdown in all cases was inhibited (80-95%) by the retinal melatonin deacetylase inhibitor eserine at 100μM. Subsequent 5-methoxytryptamine deamination was inhibited by the monoamine oxidase inhibitor pargyline at 100μM. Melatonin catabolism in the pineal is rapid; the *Sceloporus* pineal gland deacetylated 37.7pmol of melatonin/mg protein/hr, while the eyecup and brain deacetylated 2.1 and 1.1pmol melatonin/mg protein/hr, respectively. These results directly demonstrate melatonin breakdown within the pineal gland and brain, and suggest that degradation may help regulate production and release of pineal melatonin. Melatonin deacetylation within the central nervous system may generate functional methoxyindoles, and may regulate levels of melatonin at its sites of action. Support: NIH Grant EY02414 (JCB); Sigma Xi Grant-in-Aid (MSG).

6.8

ELECTROPHYSIOLOGICAL EFFECTS OF BOMBESIN-LIKE PEPTIDES ON HAMSTER SUPRACHIASMATIC NUCLEUS (SCN) NEURONS IN VITRO.

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The rodent SCN functions as a light-entrainable circadian pacemaker. Gastrin releasing peptide (GRP), the mammalian analog of bombesin (BN), and its receptors are found in the SCN region receiving photic input. We investigated how micro-ejection of BN-like peptides onto hamster SCN cells in a hypothalamic slice affected extracellularly recorded firing rates. BN, GRP, Neuromedin B (NmB) and Neuromedin C (NmC) in saline/BSA vehicle were micropressure ejected onto SCN cells. In one study, an ejecting electrode containing BN (10^{6}-10^{-4}M) was positioned 20-60μm away from a recording electrode. Of 72 cells tested with BN, 50 (69.4%) showed significant increases in firing rate, while 3 of 18 cells (16.7%) were activated by pressure-ejected vehicle. In a second study, recordings and pressure ejections were made through a single electrode. Of 52 cells tested, BN (10^{-8}-10^{-4}M) activated 30 (57.7%) and suppressed firing in 5 (9.6%). Of 213 cells tested with GRP (10^{-9}-10^{-4}M), 107 (50%) were activated and 3 (1.4%) suppressed. NmC (10^{-5}-10^{-4}M) activated 12 of 20 cells tested (60%), while NmB (10^{-4}M) activated only 2 of 30 cells tested (6.7%) and vehicle ejections activated 1 of 26 cells (3.8%).

BN-like peptides activate ~50% of hamster SCN cells, apparently via the GRP receptor, and may play a role in photic entrainment.

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6.9

INTRACEREBRAL INFUSION OF VIP ANTISENSE OLIGONUCLEOTIDES INTO THE SUPRACHIASMATIC NUCLEI AFFECTS THE CIRCADIAN CORTICOSTERONE PEAK.

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Antisense oligonucleotides (oligos), presumably acting through hybridization arrest, have been used successfully *in vivo* to inhibit mating behavior in rats (McCarthy *et al.* 1991 Neurosci Abst 197.5). We employed a mixture of two 20mer antisense oligo sequences complementary to separate regions of rat VIP mRNA in an attempt to disrupt neurotransmission of a single neuropeptide that may be important in circadian aspects of suprachiasmatic nucleus (SCN) function. To detect altered SCN function we measured serum corticosterone, a hormone whose circadian pattern of secretion is regulated by the SCN. Female rats were implanted with double guide cannulae (23 g) directed stereotaxically at the SCN. Rats were divided into 3 treatments: antisense oligos (0.5 μg/side), control oligos having the same ATGC content but a scrambled sequence, or vehicle alone. Treatments were infused slowly on Day 0 with rats under ether anesthesia. Beginning at 1800 h (time of lights off) rats were maintained in constant dim red illumination until 1800 h on Day 2 when rats were decapitated within 30 sec of handling. Trunk blood was collected and sera assayed for corticosterone. Brains were sectioned to verify cannulae placement and integrity of the SCN. Preliminary results indicate that antisense treatment of the SCN was able to significantly suppress ($p<0.02$) peak corticosterone levels in intact female rats (11.3 ± 2.1μg/dl), compared to control oligos (24.0 ± 4.5μg/dl), and oil treatment (30.9 ± 3.1μg/dl). Treatments did not adversely affect the hypothalamic-pituitary-adrenal axis because rats could release high levels of corticosterone in response to stress. Additional experiments to characterize more fully the 24 h corticosterone profile are being performed. NIH AG-02224 & HD-15955.

6.10

INDUCTION OF *C*-FOS DURING SPONTANEOUS WAKEFULNESS IN THE FOREBRAIN AND DIENCEPHALON: AN IMMUNOCYTOCHEMICAL AND IN SITU HYBRIDIZATION STUDY IN THE RAT.

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The distribution of *c*-Fos was investigated with immunocytochemistry in rats sacrificed at different times of the sleep-wakefulness cycle monitored with EEG recording. The number of *c*-Fos-immunoreactive neurons was strikingly increased in the cases with EEG corresponding to prevailing wakefulness up to 4 hrs before sacrifice in respect to those in which a sleep EEG pattern had prevailed at the same interval before sacrifice. This increase was proportional to the percent of duration of wakefulness and was especially pronounced in the cerebral cortex (frontal, cingulate, entorhinal), basal forebrain, hypothalamus, midline and intralaminar thalamus. Preliminary findings based on *in situ* hybridization indicate a similar distribution of *c*-fos transcripts in these cases. A double immunocytochemical procedure aimed at visualizing *c*-Fos and the low affinity nerve growth factor receptor (NGFr) revealed that in the basal forebrain *c*-Fos was contained in NGFr-immunonegative cell bodies. Since NGFr is expressed by cholinergic neurons in the basal forebrain, these findings suggest that the spontaneous induction of *c*-Fos during wakefulness may not involve this cholinergic system. Altogether the present data indicate that subsets of forebrain and diencephalic neurons are involved in the genomic expression during the sleep-wakefulness cycle.

6.11

D1-DOPAMINE RECEPTOR-MEDIATED ACTIVATION OF C-FOS GENE EXPRESSION IN THE FETAL RAT SUPRACHIASMATIC NUCLEUS. D.R. Weaver*, S.A. Rivkees, & S.M. Reppert. Lab. Devel. Chronobiology, Mass. General Hospital, Boston MA, 02114.

The suprachiasmatic nuclei (SCN) function as a biological clock. Studies of neurotransmitter systems in the fetal SCN may reveal mechanisms for the entrainment of the fetal biological clock. We report that an activatable D1-dopamine receptor system is present in the fetal SCN.

In situ hybridization with 35S-labeled cRNA probes revealed that D1-dopamine receptor mRNA is highly expressed within the fetal SCN on gestational days (GD) 18 and 20. Cocaine (30 mg/kg) injected into pregnant dams or directly into cesarean-delivered fetuses on GD 20 selectively increased c-fos mRNA expression in the fetal SCN. Cocaine did not induce c-fos expression elsewhere in the fetal brain or in the maternal SCN. Cocaine induced c-fos expression in the fetal SCN when administered either during the day or at night. Cocaine-induced c-fos expression in the fetal SCN was mediated in part by D1-dopamine receptors, as the D1 antagonist SCH 23390 reduced the cocaine-induced induction of c-fos and the D1-dopamine receptor agonist SKF 38393 induced c-fos expression in the fetal SCN. The presence of an activatable D1-dopamine receptor system in the fetal SCN provides a mechanism through which maternal signals could entrain the fetal biological clock and may have implications for the development of circadian function in fetuses exposed to psychoactive drugs.

6.12

EFFECTS OF LOCAL ADMINISTRATION OF EXCITATORY AMINO ACID AGONISTS ON CIRCADIAN PHASE AND *c-fos* EXPRESSION IN THE HAMSTER SCN. M.A. Rea*, A. M. Michel, and L.M. Lutton. Circadian Neurobiology Research Group, Armstrong Laboratory, Brooks AFB, TX 78235.

To further characterize the role excitatory amino acids (EAA) in the photic entrainment of the SCN circadian clock, we investigated the effects of EAA agonists, on the phase of the free-running activity rhythm, and on *c-fos* expression in the hamster SCN. Male, Syrian hamsters were fitted with intracranial guide cannulae stereotaxically aimed at the SCN and housed in constant darkness in cages equipped with running wheels. At either circadian time (CT)9-10 or CT18-19, hamsters received a 300 nl injection of 1 mM NMDA, 0.01 mM AMPA, or vehicle into the region of the SCN. Some hamsters were returned to wheel cages under constant darkness for 10 - 14 days. Others were deeply anesthetized 2 hours after injection, perfused with 4% paraformaldehyde, and processed for *c-fos* immunocytochemistry.

NMDA induced c-fos expression in the SCN at both CT9-10 and CT18-19. Expression was limited to the area immediately adjacent to the injection site. However, when the injection entered the third ventricle, the pattern of *c-fos* expression was similar to that observed after a phase advancing light pulse. AMPA induced c-fos expression in the vicinity of the injection site excluding the SCN. NMDA caused phase delays of the activity rhythm at CT9-10 (vehicle -6+6 min; NMDA=-51+9 min). Neither drug altered phase when injected at CT18-19 (vehicle=-11±10 min; NMDA =-11±8 min; AMPA=5±8). The results show that EAA-induced *c-fos* expression in the SCN does not result in light-like phase shifts of the SCN circadian clock. Supported by 2312W6 (MAR).

MONOAMINES AND BEHAVIOR: HUMAN DISEASE AND ANIMAL MODELS

7.1

IN VIVO PET IMAGING AND QUANTIFICATION OF DOPAMINE TRANSPORTER SITES WITH ^{11}C-WIN 35,428 IN LESCH NYHAN SYNDROME. DF Wong*, J Harris, B Yung, RF Dannals, HT Ravert, G Chen, M Yaster, J Neumeyer, R Carpenter, W Nyhan, HN Wagner, Jr, MJ Kuhar, G Breese; Johns Hopkins Med Inst, Baltimore, MD 21205, and Research Biochemicals, Inc., Natick, MA.

Lesch Nyhan Syndrome (LNS) is an X-linked recessive, inborn metabolic disorder characterized by infantile onset of choreoathetosis, dystonia, and stereotypical self biting. Postmortem studies of 3 LNS cases and a transgenic biochemical mouse model indicate that dopamine (DA) and its synthesizing enzymes are substantially reduced in basal ganglia suggesting dopamine deficiency. Based on these studies, we hypothesized that there may be a presynaptic reduction in DA transporter sites in striatum in LNS which might be quantified with the DA reuptake site ligand, ^{11}C-WIN 35,428 (WIN). Utilizing high specific activity WIN to test this hypothesis, we imaged the dopamine transporter in vivo with PET in a 23 year old with LNS. The Caudate-Cerebellar (CA/CB) ratio at 90 min was 3.7 in the LNS patient and 5.5 in normals. LNS CA/CB ratio and Putamen/CB ratio versus time slope was reduced by 42% and 52%, respectively. Preliminary kinetic modeling demonstrated a several fold reduction in the bound over free ratio (k_3/k_4), consistent with substantial reduction in presynaptic DA transporter sites in LNS. Our previous studies of the DA system with PET in LNS (Wong et al. Soc. Neurosci. 17:678 1991) found evidence that suggests elevated D_2 DA postsynaptic receptor density (B_{max}). This data combined with the current findings may be consistent with postsynaptic DA supersensitivity or upregulation in LNS. Supported in part by HD23042 and HD240061.

7.2

THE RESPONSE OF MESOLIMBIC DOPAMINERGIC NEURONS IS OPPOSITE DEPENDING ON THE AFFECTIVE VALUE OF STIMULUS. A. Louilot*, C. Besson and M. Le Moal. Lab. de Psychobiologie des Comportements Adaptatifs. INSERM U. 259 - Université de Bordeaux II - Domaine de Carreire - 33077 Bordeaux Cedex - France.

Depending on the affective value of a stimulus, the response of dopaminergic (DA) neurons could be opposite. In order to test this hypothesis we investigated the reactivity of DAergic neurons to an appetitive stimulus before and after an aversive conditioning.

The DAergic reactivity was studied using the voltammetric measurements of DA and its main presynaptic metabolite, the DOPAC, in the nucleus accumbens of freely moving rats. The separation of the DA and DOPAC signals was obtained by the computed numerical analysis of the catechol signal. Procedure used is as following : animals were placed during one hour in the experimental cage; they were thus exposed during one hour to an appetitive olfactory stimulus (banana) and received consecutively either an injection of saline (NaCl 0.9%) (control group) or an injection of LiCl (0.15 M) (experimental group) and stayed then one more hour in the experimental cage; 72 h later animals were still exposed during one hour to the conditional olfactory stimulus (CS). The following results are obtained : during the 1st presentation of the CS the DA signal in the nucleus accumbens increased progressively to reach about 160% of the basal level after one hour; during the 2nd presentation of the CS a transient decrease in the DA signal of about 30% below the baseline was observed for the experimental group whereas an increase of about 100% above the baseline was obtained for the control group. No significant variations in the DOPAC signal were noticed.

Results of the present study showing an increase in the DAergic signal when the stimulus is appetitive and a decrease when the stimulus is aversive argue for a differential and discriminative involvement of the DAergic neurons reaching the nucleus accumbens in the affective perception of environmental stimulus.

7.3

EFFECTS OF ACUTE TRYPTOPHAN DEPLETION IN DRUG-REMITTED OBSESSIVE-COMPULSIVE DISORDER PATIENTS. L. C. Barr, L. H. Price, C. J. McDougle, P. L. Delgado, W. K. Goodman*. Yale University School of Medicine, New Haven, CT 06519.

The majority of depressed patients remitted on serotonin reuptake inhibitors (SRIs) demonstrate reemergence of depression following acute tryptophan depletion (ATD), which is thought to lower brain serotonin. This study examined the effects of ATD in drug-remitted obsessive compulsive disorder (OCD) patients. **Methods**: 15 OCD patients remitted on SRIs (7 female, 8 male; mean age = 36 ± 2 years; 10 with a lifetime history of major depression) participated in two test sessions: (1) ATD (24hr. 160mg/day, low tryptophan diet followed the next AM by a 16-amino acid drink without tryptophan) and (2) sham depletion (both diet and drink supplemented with L-tryptophan) in random order. Blind ratings of depression and obsessive compulsive (OC) symptoms were obtained. **Results**: ANOVA revealed significantly (p=.04) increased mean depression ratings on the ATD compared to the sham test day. Paired t-tests showed significant increases in Hamilton Depression Scale ratings at 420 minutes after the amino acid drink compared to 24 hours prior to the drink (p=.004). ATD had no significant effect on mean OC ratings: only one patient showed an exacerbation of OC symptoms with ATD. **Conclusions**: This study replicates preliminary findings from a smaller non-overlapping sample that ATD increases depressive, but not OC symptoms, in OCD patients remitted on SRIs. This suggests that maintenance of SRI-induced remission of OC symptoms, unlike remission of depressive symptoms, may not depend upon ongoing availability of serotonin.

7.4

AN ANIMAL MODEL FOR STUDYING THE ETIOLOGY OF NEURODEGENERATIVE DISEASES

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Asphyxia to male Sprague-Dawley rat pups was induced by a delayed cesarean section. An immunohistochemical and functional analysis was carried out on the pups at an age of 3 weeks and 5 months. The monoaminergic nerve cell body regions of substantia nigra, locus ceruleus and raphe region were studied. It was demonstrated that asphyxia time-dependently produced an increase in the number of tyrosine hydroxylase immunoreactive (TH-IR) nerve cell bodies of the substantia nigra. This increase was still present in the adult animal. Behavioral recordings of rearing and locomotion indicated a progressive aggravation of disturbances, demonstrating a bimodal curve of events. Mild asphyxia induced increased rearing and locomotion, while severe asphyxia resulted in a reversal of the enhanced rearing and locomotion. The observed behavioural changes may reflect an animal analogue for the attention deficit disorders and hyperkinetic syndrom in MBD-children, which may be related to oxygen deprivation during the human delivery. Since disturbed function in the meso-striatal and meso-limbic dopamine systems are of central importance for the development of schizophrenia and Parkinson's disease the use of this animal model may enlighten the mechanisms behind these neurodegenerative diseases.

7.5

DISCHARGE PATTERNS OF DORSAL RAPHE NEURONS AND THEIR COORDINATION ACROSS SEVERAL TIME SCALES. B. Kocsis* and R.P. Vertes, Center for Complex Systems, Florida Atlantic University, Boca Raton, FL.

Dorsal raphe (DR) nucleus projects throughout widespread regions of the forebrain and is capable of influencing numerous and diverse structures of the forebrain. It contains a morphologically and electrophysiologically heterogeneous population of cells with firing rates distributed over more than two orders of magnitude (from 0.5-1 Hz to 70-100 Hz). We recorded extracellularly 30 DR neurons in 8 unanesthetized freely moving rats during different sleep-waking states. The neurons were categorized according to their maximum firing rates (slow (S) - <10 Hz; fast (F) - 10-55 Hz; and very fast (FF) cells - >55 Hz) and their relationship to the hippocampal theta rhythm (maximum coherence between hippocampal EEG and DR neuron discharge at theta frequency was >0.5, 0.2-0.5, and <0.1 in three groups). The activity of 17 of 30 DR neurons was correlated with the theta rhythm in the hippocampus. As shown in the table, these included cells with a range of firing rates and various firing dynamics.

	FF-cells	F-cells	S-cells	total
theta-rhythmic	2	4	1	7
theta-modulated	4	3	3	10
no coherence	5	5	3	13
total	11	12	7	30

The origin and physiological significance of theta rhythmicity in DR neurons is yet to be determined. The medial septal nucleus (MS) (site of the pacemaking cells that directly drive the hippocampal theta rhythm) has been shown to send pronounced descending projections to the DR nucleus. Through these projections the MS may synchronously drive cells of the DR as it does those of the hippocampus and entorhinal cortex. The present findings suggest DR cells discharging synchronously with the theta rhythm may serve to coordinate the activity of several forebrain structures during theta associated states or behaviors. Supported by NIMH Grant MH45075 to RPV.

7.6

ROLE OF THE LOCUS COERULEUS-NORADRENERGIC (LC-NE) SYSTEM IN ANXIOUS BEHAVIOR IN RATS: DECREASED LC ACTIVITY PRODUCES ANXIOGENIC, WHILE INCREASED LC ACTIVITY PRODUCES ANXIOLYTIC EFFECTS. J.C. Stout*, M.A. Cierpial, K.M. Boericke, & J.M.Weiss. Depts. of Psychology and Psychiatry, Duke Univ., Durham, NC 27710.

The role of LC-NE activity in anxious behavior in rats in normal fear (NF) and shock-enhanced fear (SEF) conditions was investigated by manipulating the LC-NE system pharmacologically, and then assessing plasma corticosteroids, and anxious behavior in the "drink test" (Stout & Weiss [1989], Soc.for Neurosci. Abstracts, 15: 632). Effects of these manipulations of the LC-NE system were verified by assessing noradrenergic turnover in the LC and dorsal hippocampus (DH) by measuring levels of NE and 3-methoxy-4-hydroxyphenylglycol (MHPG) using high pressure liquid chromatography (HPLC). Results indicated that anxious behavior of NF rats was not significantly altered by pharmacologically-induced increases or decreases in LC-NE activity. However, in the SEF condition, reduced LC-NE activity produced by microinfusions of desipramine directly into the LC was associated with increased anxious behavior, while increased LC-NE activity produced by either, (a) a combination of idazoxan and substance P microinfused directly into the LC, (b) idazoxan infused into the lateral ventricle, or (c) chronic administration of idazoxan via an indwelling osmotic mini-pump, was associated with a reduction in anxious behavior. Alteration of LC-NE activity did not significantly affect plasma corticosterone. Analysis of noradrenergic turnover verified that the pharmacological manipulations were effective in producing the predicted alterations of LC-NE activity.

These experiments suggest that the relationship between the LC-NE system and anxious behavior is opposite to that which was postulated by Redmond et al. in 1976 (Brain Res., 116: 502-510) in that decreased LC activity appears to have anxiogenic effects, while increased LC activity appears to have anxiolytic effects.

7.7

MATERNAL INFLUENCES ON SWIM TEST BEHAVIOR IN A NEWLY DEVELOPED ANIMAL MODEL OF DEPRESSION. M.A. Cierpial*, C. Lorenz, J.V. Morgan, H. Amir & J.M. Weiss. Department of Psychiatry, Duke University Medical Center, Durham, NC 27710.

Low motor activity in a swim test is used as a measure of "depression-like" behavior in the rat, both in behavioral screens for antidepressant drugs and in a widely used animal model of depression. We have selectively bred Sprague-Dawley rats for high or low motor activity in the swim test. Swim high active (SwHi) rats struggle vigorously in the swim test and float very little, whereas swim low active (SwLo) rats struggle very little and are immobile for much of the test period. SwLo rats may represent a new animal model of depression in that they respond to chronic, but not acute, antidepressant drug treatment with increases in swim test activity.

To assess the role of the preweanling maternal environment on adult swim test behavior in SwHi and SwLo rats, we conducted a cross-fostering study in which SwHi and SwLo litters were either reared by their natural mothers (control litters), foster dams of the same line (in-fostered litters), or foster dams of the opposite line (x-fostered litters). Swim tests were conducted on male and female rats from the 6 groups (2 lines x 3 rearing conditions) at 90 days of age. The results indicated that adult swim test behavior in these lines is dependent on early rearing experience as opposed to genetic factors. Adult SwHi rats reared by SwLo dams displayed low motor activity (not significantly different from SwLo Control or In-Fostered rats) in the swim test, whereas adult SwLo rats reared by SwHi dams displayed high activity (not significantly different from SwHi Controls or In-Fostered rats). These data indicate that an adult phenotypic behavior thought to represent depression-like behavior in the rat is modifiable based on early environmental experience.

7.8

MATERNAL INFLUENCES ON CENTRAL CATECHOLAMINE RESPONSES TO STRESS IN SwHi AND SwLo RATS. J.M. Weiss*, M.A. Cierpial, & K.M. Boericke. Department of Psychiatry, Duke University Medical Center, Durham, NC 27710.

SwHi and SwLo rats have been selectively bred to display differential motor activity in a swim test and SwLo rats may be useful as a new animal model of depression. We have demonstrated that adult swim test behavior in these rat lines is dependent on early rearing experience rather than genetic factors (see accompanying abstract). Studies were conducted to determine whether alteration of the preweanling environment (by cross-fostering of litters) would impact on the functioning of brain catecholamine systems in adulthood. Adult male rats from 6 groups (2 lines [SwHi/SwLo] x 3 rearing conditions [Control/In-Fostered/Cross-Fostered]) were either sacrificed out of their home cage (HC) or exposed to mild electric tailshock (30 min. session of 1.5 mA, 3 sec, VI300 sec) and then sacrificed (SHK). Trunk blood was collected for assay of plasma corticosterone (CORT) and various brain areas were dissected out for assay of NE, MHPG, DHPG, DA, and DOPAC.

Of particular interest was that in HC rats, striatal DA concentration was higher in SwHi control and SwLo X-Fostered animals compared to SwLo control and SwHi X-Fostered rats. Mild tailshock caused a significant increase in striatal DOPAC in SwHi vs SwLo rats. SwHi X-Fostered rats had DOPAC values which were equivalent to SwLo controls, while SwLo X-Fostered rats had DOPAC values which were equivalent to SwHi controls. The data indicate that functioning of the striatal dopamine system in adulthood can be modified based on early rearing experience and may have implications with regard to animal models of depression and/or schizophrenia.

GABA RECEPTORS: STRUCTURE AND FUNCTION

8.1

MODULATION OF NUCLEUS ACCUMBENS NEURONS BY THEIR LOCAL AXON COLLATERALS. W.-X. Shi* and S. Rayport. Dept. of Psychiatry, Center for Neurobiology & Behavior, Columbia University; Dept. of Neuropathology, NYS Psychiatric Institute, NY 10032.

The axons of GABAergic medium spiny neurons not only form the principal projections from the nucleus accumbens (NA), but also branch locally to form a dense network overlapping their own dendrites. To examine the synapses formed by these local axon collaterals, we made whole cell recordings in postnatal rat NA cultures. We found that 95% of medium-sized neurons formed GABA autapses (synapses between axon collaterals and their cell of origin). Their reliable presence in culture not only suggests the possibility that such autapses exist in the intact NA, but also provides a way to study individual intrinsic NA GABA synapses. We found that, although cells expressed both $GABA_A$ and $GABA_B$ receptors, autaptic potentials were blocked only by the $GABA_A$ antagonist bicuculline and not by the $GABA_B$ antagonist saclofen, suggesting that local axons collaterals selectively interact with $GABA_A$ receptors. Activation of $GABA_B$ receptors by baclofen suppressed the autaptic potential consistent with presynaptic $GABA_B$ modulation of GABA release. Flurazepam potentiated the autaptic potential, showing the presence of benzodiazepine binding sites on the $GABA_A$ receptor complex. Autapses may contribute to dynamic signal processing in the NA in several ways: (1) They selectively inhibit responses to suprathreshold but not subthreshold stimulation. (2) They act as low-pass filters suppressing the responses to high frequency input. (3) They may serve as sites of actions for transmitters or drugs producing a modulation more complicated than simple excitation or inhibition. For example, while baclofen increased spike threshold by hyperpolarizing the cell, it enhanced the suprathreshold stimulation-induced response by blocking autaptic release, thus improving the signal-to-noise ratio. Several other GABA-active drugs including bicuculline, flurazepam and nipecotic acid each modulated NA cell responses in a different way. Since GABA synapses between neighboring NA cells that receive similar inputs should be functionally equivalent to autapses, our data suggest ways that these synapses may contribute to signal processing in the NA.

8.2

THE INFLUENCE OF GABA-UPTAKE ON THE STRENGTH OF FAST AND SLOW INHIBITORY SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS. J. S. Isaacson*, J. M. Solis, and R. A. Nicoll. Depts. of Physiology and Pharmacology, University of California, San Francisco, CA 94143.

GABA-uptake inhibitors have been found to prolong the time course of inhibitory synaptic potentials in the hippocampus (Dingledine, R. & Korn S. *J. Physiol.* 366:387, 1985). However, the specific influence of transmitter uptake on fast ($GABA_A$ receptor-mediated) and slow ($GABA_B$ receptor-mediated) inhibitory synaptic transmission is unclear. In this study, we have examined the effects of SKF 89976A, a potent GABA-uptake blocker, on monosynaptic $GABA_A$ and $GABA_B$ receptor-mediated synaptic currents in the hippocampal slice.

Synaptic currents were studied from CA1 pyramidal neurons using "blind" patch clamp recording techniques in thick (500 μm) slices of adult guinea pig hippocampus. Monosynaptic inhibitory post-synaptic currents (IPSCs) were evoked in the presence of the glutamate receptor antagonists APV and DNQX. Pure fast and slow IPSCs were isolated in the presence of $GABA_B$ or $GABA_A$ receptor antagonists, respectively.

SKF 89976A increased the amplitude and prolonged the time course of $GABA_B$ receptor-mediated synaptic currents. In contrast, SKF 89976A had no effect on the amplitude of $GABA_A$ receptor-mediated IPSCS evoked with a stong stimulus, but greatly augmented the late decay of the synaptic current. This effect was not found for $GABA_A$ receptor-mediated IPSCs evoked by a weak stimulus (activating fewer synapses) or for spontaneous unitary IPSCs.

These experiments indicate that GABA-uptake limits the strength of slow, $GABA_B$ receptor-mediated inhibition in the hippocampus. The lack of effect of SKF 89976A on weakly evoked and spontaneous $GABA_A$ receptor-mediated IPSCs suggests that uptake does not determine the time course of fast synaptic inhibition at individual synapses. However, its effects on $GABA_A$ receptor-mediated IPSCs evoked with a strong stimulus indicate that uptake is important in preventing the accumulation of transmitter from neighboring, co-activated synapses. Together, these results suggest that transmitter uptake can influence the strength of both $GABA_A$ and $GABA_B$ receptor-mediated inhibitory synaptic transmission in the hippocampus.

8.3

ARE RECEPTORS "FULL-FILLED" BY QUANTAL RELEASE? D.S. Faber*, W.S. Young and H. Korn. SUNY, Buffalo, NY 14214 and Institut Pasteur, Paris, France.

It has been proposed that in the CNS, quantization of synaptic signals is determined by saturation of postsynaptic receptors (Jack et al., 1981; Edwards et al., 1990), as illustrated by modeling with differential equations (Busch and Sakmann, 1990). We used Monte Carlo simulations of glycinergic quanta having large (~1,000) and small (~20–40) numbers of channels and compared the predictions with data from the Mauthner cell. Transition probabilities were calculated for diffusion, and glycine (A) binding to receptors (Rs) according to $2A + R \leftrightarrow A + AR \leftrightarrow A_2R$ (closed) $\leftrightarrow A_2R^*$ (opened). Vesicles contained 5 to 20×10^3 molecules of glycine, which diffused rapidly in the cleft (D from 0.5 to 1.0×10^{-5} cm^2 sec^{-1}). A range of rate constants yielded "quanta" similar to recorded ones. Simulations with fixed parameters predicted a 2–5% CV for peak amplitude and increased variance during the decay phase. While binding was 100% within 50–100 μsec after release, the delayed peak response (at 0.3–0.5 msec) was only 70–80% maximal. Off-center exocytosis and up to 20% differences in the number of molecules per vesicle each added about 2–3% variation. Miniatures recorded in different preparations and having similar numbers of activated channels had comparable variance waveforms. These results are explained by the stochastic behavior of single channels which open and close repetitively during the quantal response, so that saturated binding, if any, does not necessarily imply a fixed postsynaptic response.

8.4

A SINGLE-NUCLEOTIDE MUTATION EXPLAINS THE ENHANCED BENZODIAZEPINE SENSITIVITY OF A CEREBELLAR GRANULAR LAYER $GABA_A$ RECEPTOR IN AN ALCOHOL-SENSITIVE RAT LINE. E.R. Korpi*, M. Köhler, P. Werner and P.H. Seeburg. Lab. of Molecular Neuroendocrinology, Zentrum für Molekulare Biologie, Univ. Heidelberg, 6900 Heidelberg, Germany, and Biomedical Research Center, Alko Ltd, Helsinki, Finland.

Alcohol-sensitive ANT (Alcohol Non-Tolerant) rats, produced by selective breeding for high sensitivity to the effects of a moderate dose of ethanol (2 g/kg), are also highly sensitive to motor-impairing effects of lorazepam, a benzodiazepine agonist. The only qualitative difference in the central $GABA_A$ receptors of the ANT rats is the enhanced benzodiazepine agonist sensitivity of the cerebellar granule cell-specific "diazepam-insensitive" binding sites for [^{3}H]Ro 15-4513, which are due to the presence of α6 subunit of the $GABA_A$ receptor. Poly A^+ RNA was isolated from the cerebella of the ANT rats, cDNA prepared, and two fragments, corresponding to the extracellular and transmembrane domains of the α6 subunit coding region, were amplified by PCR, and sequenced. In the ANT rats, a single-nucleotide mutation (G to A) was found, which caused an amino acid change in the extracellular domain of the subunit protein sequence. When this mutation was expressed using wildtype α6 backbone plasmid together with β2 and γ2 subunit coding plasmids in 293 cells, the resulting binding sites of [^{3}H]Ro 15-4513 became sensitive to diazepam.

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8.5

IN VIVO SPECT MEASUREMENT OF BENZODIAZEPINE RECEPTOR NUMBER AND AFFINITY IN BABOONS: A CONSTANT INFUSION PARADIGM. M. Laruelle*, A. Abi-Dargham, M.S. Al-Tikriti, S.S. Zoghbi, Y. Zea-Ponce, Z. Rattner, E. H. Sybirska, E.O. Smith, D.S. Charney, P.B. Hoffer, R.M. Baldwin, R.B. Innis, Yale Univ./VA Med. Ctr., West Haven, CT 06516

Standard PET methods for quantification of neuroreceptors after bolus injection of a radiotracer can be roughly divided into compartmental kinetic and transient equilibrium methods. We took advantage of the long half-life of ^{123}I to develop a sustained equilibrium method based on radiotracer constant i.v. infusion. This paradigm was used to quantify [^{123}I]iomazenil binding to occipital benzodiazepine receptors in baboons. A bolus injection of 3 mCi (high specific activity, 180000 Ci/mmol) was followed by constant infusion at a rate of 0.5 mCi/hr for 420 min (n=3). Parent compound reached plasma steady state at 120 min. Occipital activity reached stable levels around 180 min. Experiments were ended by injection of receptor-saturating dose of flumazenil (0.2 mg/kg) to define nondisplaceable activity. Occipital binding potential (BP = Bmax/Kd) measured as bound/free at equilibrium (251±20) was similar to BP calculated as K1*k3/k2*k4*f1 with a classical 3 compartment model (266±19). Experiments performed at low specific activity (30-15 Ci/mmol, n=3) provided data for a Scatchard plot (Kd = 0.57 nM, Bmax = 145 nM). This sustained equilibrium method gives results consistent with the kinetic approach and may be easier to implement in humans as, under plasma steady state conditions, arterial and venous radiotracer concentrations equilibrate and no arterial sampling would be needed.

8.6

CLONING OF THE GENE ENCODING THE DIAZEPAM BINDING INHIBITOR. M. Kolmer and H. Alho*, Dept. Biomed. Sci., Univ. of Tampere, P.O. Box 607, 33101 Tampere, Finland.

Diazepam binding inhibitor (DBI), an 9-kD polypeptide that is found in nearly all tissues, has been shown to be involved in the regulation of several important cellular functions: mitochondrial steroidogenesis, synthesis of fatty acids, insulin secretion, etc. DBI expression is resticted to certain, usually higly specialized cell types. However, the molecular mechanisms that regulate the cell-specific expression of DBI are poorly understood. Human rat, mouse and bovine DBI cDNAs have previously been cloned. The gemonic structure of DBI is not known.

To study the mechanisms regulating DBI expression, rat DBI genomic gene was cloned by screening the rat genomic library with DBI cDNA (Mocchetti, et al. 1986) as a probe. As revealed by nucleotide sequencing studies, rat DBI is encoded at least by three exons, the complete structure of genomic loci encoding for rat DBI will be presented. Promoter function experiments using luciferase gene as a reporter will be discussed.

8.7

EXPRESSION OF MITOCHONDRIAL BENZODIAZEPINE RECEPTOR AND ITS PUTATIVE ENDOGENOUS LIGAND DIAZEPAM BINDING INHIBITOR (DBI) IN HUMAN BRAIN TUMORS AND C-6 GLIOMAS. H. Alho, V. Varga, I. Podkletnova and P. Helen*. Dept. of Biomed. Sci., Univ. of Tampere, PL 607, 33101 Tampere, Finland.

A regocnition site for benzodiazepines structurally different from that linked to the $GABA_A$ receptor and the "central type" benzodiazepine receptor is located in the outer membranes of mitochondria and have desingnated as mitochondrial benzodiazepine receptor (MBR). A putative endogenous ligand for MBR is the peptide diazepam binding inhibitor (DBI) that inhibits mitochondrial benzodiazepine (BZ) ligand binding on mitochondrial membranes. The cellular location of DBI- and MBR-LI was studied in human brain tumors and C-6 glioma cell lines by light and electron microscopic immunohistochemistry.

In many brain tumors DBI- and MBR-LI was identified in astrocyte-like cells. In brain glioblastomas the number of DBI and MBR immunoreactive cells was high.In particular, large multinuclear anaplastic cells showed intense immunoreactivity. A co-localization of DBI and MBR-LI was demonstrated in many tumors and C-6 cells. The role of MBR and DBI in glial cells and gliomas is unknown. They may modulate cellular growth via central BD- or mitochondrial BD-receptors, either by acting directly via these receptors or indirectly via neurosteroids regulated by the MBR's.

8.8

2-ARYL-3-INDOLEACETAMIDES (FGIN-1): LIGANDS FOR THE MITOCHONDRIAL DBI RECEPTORS (MDR) THAT ACT AS INDIRECT ALLOSTERIC MODULATORS OF $GABA_A$ RECEPTORS. E. Romeo*, A. Kozikowski[1], A. Korneyev, G. Puia, J. Auta, E. Costa and A. Guidotti. Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ. Med. School, Washington, DC 20007; [1] Mayo Clinic, Jacksonville, FL 32224

The 2-aryl-3-indoleacetamides (FGIN-1) are a new potent and specific class of ligands for the glial MDR that, by regulating intramitochondrial cholesterol transfer, increase pregnenolone formation. FGIN-1-27[N,N-di-n-hexyl2-(4-fluorophenyl)indole-3-acetamide] (115 to 230 μmol/kg, p.o.) and its prodrug FGIN-1-44 (40 to 80μmol/kg p.o.) reduce fear of novelty (neophobia) in rats exposed to an elevated plus maze test. The antineophobic effect of FGIN-1-27 or FGIN-1-44 was blocked by PK11195 - isoquinoline carboxamide - a partial agonist at MDR, but not by flumazenil, a specific blocker of the antineophobic action of benzodiazepines. Because FGIN-1-27 does not have any direct action on $GABA_A$ receptors and does not block bicuculline-induced seizures, but antagonizes the convulsant action of isoniazid, it was thought that FGIN-1-27, acting at MDR, modulate $GABA_A$ receptor function indirectly by increasing neurosteroid biosynthesis.

Indeed, the neurosteroids 3α-OH-DHP (3-15μmol/kg) and THDOC (3-15μmol/kg) elicit a dose related antineophobic action which is insensitive to the administration of PK 11195, flumazenil, and bicuculline. A stereoisomer of 3β-5α-OH-DHP - a steroid that acts as a partial agonist on GABA activated Cl^- currents and antagonizes the positive modulation by 3α-OH-DHP of $GABA_A$ receptor - reduces the antineophobic action of FGIN-1-44 (40μmol/kg). Moreover, FGIN-1-27 administered to adrenalectomized and castrated rats treated with trilostane, an inhibitor of pregnenolone metabolism, increases in a dose related manner (from 50 to 230μmol/kg po), the brain content of pregnenolone (Korneyev, Neurosci Abs, 1992). These data suggest that FGIN-1-27 and FGIN-1-44, by binding to the MDR located in glial cells or neurons indirectly act on $GABA_A$ receptors,presumably by stimulating brain's neurosteroid production

8.9

ENDOGENOUS POSITIVE ALLOSTERIC MODULATORS FOR $GABA_A$ RECEPTORS (ENDOZEPINES): A CAUSE OF STUPOR AND COMA?

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Idiopathic Recurring Stupor (IRS) is a recently described syndrome of repeated stuporous episodes with no known metabolic or structural causes, and which can be rapidly reversed with the benzodiazepine antagonist flumazenil (Ann Neurol, 1992 in press). This suggested that the syndrome could be due to excess endogenous agonists acting at the allosteric regulatory extraneuronal domain of $GABA_A$ receptors. Using chloroform extractions, silica HPLC and reverse-phase HPLC, we have purified 2 endozepines (endozepine-2 and endozepine-4) from brain (bovine, rat, and human) and cerebrospinal fluid. These are endogenous substances that act as positive allosteric modulators of $GABA_A$ receptors (J Neurochem, 1992 in press). Mass spectrometry and NMR analysis of purified endozepine-4 revealed that it has a small molecular weight with a quinoline core structure. It has an apparent high affinity for benzodiazepine binding sites on cerebellar granule cells ($\sim$20 nM) and it can displace [^{3}H]PK 11195 binding to rat brain mitochondria. Endozepine-4 potentiates GABA evoked chloride influx on patch clamped kidney 293 cells transfected with cDNA for α_1 β_1 γ_2 GABA receptor subunits. Intraventricular administration of 1-2 nmoles endozepine-4 significantly delayed bicuculline seizures. Serum or CSF from stuporous patients with IRS contained an enormous concentration of these endozepines, with levels as high as 400 nM, compared to 5-10 nM in control serum or CSF. Serum or CSF in IRS patients during normal periods contained endozepine levels similar, but greater than, control CSF and serum. These results suggest that IRS may be due to excess endozepines and provide further proof for the existence and functional role of endozepine acting as an allosteric modulator of $GABA_A$ receptors. The cause for increased endozepine is unknown.

8.10

RAT BRAIN MINCES: A MODEL TO STUDY NEUROSTEROIDOGENESIS.

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Steroids have been recently indicated as endogenous modulators of GABA-A receptor function. Moreover, several lines of evidence indicate that in brain selected populations of glial cells may be endowed with the capability of synthesizing steroids (neurosteroids) (Hu et al, PNAS, 84:8215, 1987;Guarneri et al, PNAS, 89, 1992 in press).

We used brain cortical minces (300x300 um) prepared from adult intact male rats. In minces, allowed to stabilize for 1 hr at 37°C, the content (ng/g of protein) of progesterone, dehydroepiandrosterone, pregnenolone and deoxycorticosterone, determined by specific radioimmunoassay after HPLC purification, were 18.3, 1.7, 59.7 and 6.1, respectively. These values are in the same order of magnitude of those we found in brain cortex of three-day adrenalectomized/orchiectomized rats.

The exposure of cortical minces to dibutyryl-cyclic AMP (1 mM) or forskolin (10 uM) for 30 minutes increases the content of pregnenolone (the precursor of all steroids) up to 60% and 180%, respectively, over the basal values. This confirms that also in brain cyclic AMP can stimulate steroidogenesis as previously reported in adrenal cortex, ovary and testes.

These data suggest that rat brain minces are a suitable experimental model to study the physiology and pharmacology of brain steroidogenesis, excluding interference by blood-borne steroids.

8.11

A NEW CLASS OF POTENTIAL LIGANDS FOR THE STEROID BINDING SITE ON $GABA_A$ RECEPTORS

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Anesthetic steroids are potent modulators of GABA-gated Cl^- channels, acting at a site which appears to differ from the GABA, benzodiazepine, barbiturate and picrotoxin sites. To understand structure-activity relationships at this steroid site we have synthesized and evaluated a series of substituted benz[*e*]indenes (BIs). The BIs are tricyclic structures lacking an analogous steroid A-ring.

In voltage clamped postnatal rat hippocampal neurons, BIs potentiate GABA-gated Cl^- currents with EC_{50} values between 0.1-1.0μM and maximal effects at about 10μM. The best of these agents, BI-1 (10μM), produces 467 $\pm$ 38% (N=15) increase in the peak response to 1μM GABA. By comparison the neurosteroid, 5α-pregnan-3α-ol-20-one (3α-OH-DHP) enhances GABA currents by 478 $\pm$ 20% (N=5) at 10μM. BI-1 produces a leftward shift in the GABA dose-response curve with a smaller degree of potentiation at saturating GABA concentrations. BI-1 does not alter the shape of the GABA current-voltage (IV) relationship and does not affect the GABA reversal potential. A major difference between BIs and steroids is that the BIs do not directly gate GABA currents at concentrations up to 10μM whereas Cl^- currents are activated by 3α-OH-DHP at > 1μM.

These observations indicate that removal of the steroid A-ring produces compounds which potentiate GABA currents but which are significantly less effective in directly gating Cl^- channels.

CATECHOLAMINE RECEPTORS: DOPAMINE I

9.1

THE HUMAN DOPAMINE D1 RECEPTOR EXPRESSED IN A BACULOVIRUS-INSECT CELL SYSTEM IS FUNCTIONALLY IDENTICAL TO THE NATIVE NEURONAL RECEPTOR.

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The cDNA encoding the human dopamine D1 gene (DRD1) and a cDNA encoding a c-myc protein epitope in the aminoterminus of the dopamine D1 receptor (mDRD1) were subcloned into the cloning site of the baculovirus expression vector. These viral constructs were used to infect Spodoptera frugiperda cells (Sf9), which were harvested after periods of 24 and 48 hours. Membrane preparations from cells at these time points bound [3H]SCH23390 with densities of 5 pmol/mg and 40 pmol/mg respectively, with an affinity constant of 624 pM. Membranes from Sf9 cells infected with wild-type baculovirus did not exhibit specific D1 binding. The [3H]SCH23390 labelled DRD1 and mDRD1 receptor proteins displayed a rank order of agonist and antagonist inhibition potencies similar to the native brain DRD1: SKF 38393=dopamine>(-)noradrenalin>serotonin>isoproterenol, and SCH23390>(+)butaclamol >haloperidol. Dopamine displacement of SCH23390 labelled receptor protein was biphasic, with 50% detected in the high-affinity form with Kd 12.4 nM and 50% in the low-affinity form with Kd 490 nM. The high-affinity state of the receptor was converted to low by Gpp(NH)p. The receptors were coupled to adenylate cyclase as evidenced by dopamine stimulation of enzyme activity and inhibition by SCH23390. Photoaffinity labelling of the D1 receptor and the mDRD1 receptor revealed molecular weights similar to the neuronal receptor. These data confirm that, in the Sf9 cells, the DRD1 is fully functional, and pharmacologically and biochemically similar to the native brain human DRD1.

9.2

DOPAMINE REGULATES THE CELLULAR REDISTRIBUTION OF THE HUMAN D1 DOPAMINE RECEPTOR.

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The human dopamine receptor D1 (DRD1) and a c-myc-epitope tagged human dopamine receptor D1 (mDRD1), functionally expressed in Sf9 cells were phosphorylated, palmitoylated and stimulated adenyl cyclase in an agonist-dependent manner. To further evaluate DRD1 desensitization and to determine whether redistribution of surface receptors contribute to the mechanisms regulating DRD1 activity, the specific monoclonal antibody 9E10 recognizing the c-myc epitope in the extracellular aminoterminus of DRD1 was used for immunofluorescent localization of receptors in Sf9 cells following exposure to 10 uM dopamine for 5 min and 15 min. To assess relative receptor numbers and localization, cells were permeabilized to define total receptor number or left unpermeabilized to define surface receptor number. In nonpermeabilized cells in the basal state, the surface receptor labelling pattern was diffuse. Upon exposure to dopamine, the fluorescence coalesced into large aggregates, in a time-dependent manner. Permeabilization of cells desensitized at 5 and 15 minutes revealed intense cytoplasmic fluorescence. The agonist mediated redistribution of surface receptors could be blocked by SCH23390. Cells exposed to agonist for 15 minutes and washed to remove the agonist 3 hours before fixation showed a reversal to pattern of surface labelling similar to that observed in basal cells and suggests DRD1 recycling. These results show for the first time that the number of surface DRD1 is regulated following agonist activation.

9.3

DESENSITIZATION, PHOSPHORYLATION AND PALMITOYLATION OF THE HUMAN D1 DOPAMINE RECEPTOR. G.Ng, B.Mouillac, M.Caron, D.A.Haas*, S.R. George, M.Dennis, B.F.O'Dowd and M.Bouvier, Univ.of Toronto, Ont and Univ.de Montreal and Biosignal Inc., Que, CANADA M5S 1A8

Phosphorylation and more recently palmitoylation of the B2-adrenergic receptor (B2AR) have been shown to play important roles in the ability of the receptor to interact with the stimulatory guanine nucleotide binding protein (Gs), and to activate adenylyl cyclase. In fact, it has been proposed that these post-translational modifications are involved in the process of agonist-induced desensitization. Consensus sequences for phosphorylation by protein kinase A (PKA) are conserved in the dopamine receptor D1 (DRD1), and a cysteine residue present in the carboxyl tail of the DRD1 occupies a position equivalent to that which is palmitoylated in the B2AR. To evaluate whether these covalent modifications take place in the DRD1 and play a role in agonist promoted desensitization, the human DRD1 was expressed in Sf9 cells using the recombinant baculovirus system. The expressed receptor exhibited the appropriate pharmacology and promoted dopamine-dependent activation of the adenylyl cyclase. Preincubation of the cells with 10 uM dopamine for 15 minutes induced a desensitization of the receptor which resulted in a 40% decrease in the maximal agonist-stimulated adenylyl cyclase activity. This desensitization was accompanied by an increase in the level of phosphorylation of the DRD1, as shown by immunoprecipitation of the receptor. Also accompanying the desensitization was an increase in the incorporation of tritiated palmitate in the DRD1. Thus, these results show that the DRD1 undergoes rapid agonist-promoted desensitization and that this phenomenon is accompanied by an increase in the phosphorylation level and in the palmitoylation turnover of the receptor in Sf9 cells.

9.4

GT_1 GnRH CELL LINES: A MODEL FOR D_1-DOPAMINE RECEPTORS IN THE CENTRAL NERVOUS SYSTEM. G. Martínez de la Escalera, F. Gallo, A. L. H. Choi, C. Clapp* and R. I. Weiner, Reproductive Endocrinology Center, University of California San Francisco, CA 94143, USA, and Instituto de Investigaciones Biomédicas, National University of México, México City 04510, México.

Preliminary findings suggested that GnRH release from GT_1 GnRH cell lines was regulated by dopamine (DA). We report that these cells regulate GnRH release via D_1 DA receptors and express associated molecules involved in signalling in CNS neurons. Superfusion of GT_{1-1} cells with DA for 100 min increased the amplitude of spontaneous GnRH pulses (assessed by "cluster analysis"). DA also stimulated GnRH release and increased intracellular cyclic AMP in GT_{1-7} cells in static culture. These effects were mimicked by the selective D_1 agonist (±)SKF 38390 and blocked by the D_1 antagonist R(+)SCH 23390. However, the D_2 agonist bromocryptine or antagonist spiroperidol had no effect on GnRH release or intracellular cyclic AMP levels. Northern analysis of total RNA from GT_{1-1}, GT_{1-3} and GT_{1-7} cells showed the expression of DARPP-32 (DA and cyclic AMP regulated phosphoprotein, Mr 32,000), a specific substrate for protein kinase A, generally coexpressed with D_1 receptors. These findings demonstrate that GT_1 cell lines express D_1 DA receptors coupled to adenylate cyclase. Further experiments will determine the role of DARPP-32 in signalling in GT_{1-1} cells, including its role in dopaminergic regulation of GnRH secretion. (Work supported by NIH Grant HD 08924 and The Rockefeller Foundation).

9.5

CHARACTERIZATION OF D2, D3 AND D4 DOPAMINE RECEPTORS EXPRESSED IN THE SAME CELL BACKGROUND L. Tang*, J. Hickok+, S. Harmon, R.D. Todd+ and K.L. O'Malley. Dept. Anatomy & Neurobiology, +Genetics & Psychiatry. Washington Univ. Med. Sch., St. Louis, MO 63110

The rat D2(444) cDNA, a human D3 cDNA and the rat D4 gene were subcloned into the expression vector pcDNA/neo and transfected into mouse tk$^-$ fibroblast cells. Cell lines were purified by limiting dilution. As determined by radioligand binding assays, the expressed levels of D2, D3 and D4 subtypes were 2.0, 0.5 and 0.11 pmole receptor/mg membrane protein, respectively. The pharmacological characteristics of these D2 receptor subtypes were examined by competition binding experiments. The rank order potencies for antagonists are (+)-butaclamol, D2>D3>D4; clozapine, D4>D2>D3 and (-)-sulpiride, D2>D3-D4; and for agonists: dopamine, D3-D4>D2 and quinpirole, D3>D4>D2. The D3 receptor exhibits a twelve fold higher potency for the agonist 7-(OH)-DPAT than does the D2 receptor in these cells. Functional studies demonstrated that D2 inhibits forskolin-stimulated adenylate cyclase activity, but increases inositol phosphate levels in transfected cells, while D3 shows a negative response in both assays. The D4 receptor also does not couple to adenylate cyclase. These data suggest that different D2 receptors may couple to different intracellular second messengers and thus have different signal transduction properties.

9.6

PHARMACOLOGICAL CHARACTERISATION OF THE HUMAN D_3 DOPAMINE RECEPTOR. **S.B. Freedman, S. Patel, R. Marwood, F. Emms, M. Knowles, A. Fletcher, G. Seabrook* and G. McAllister. Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex, CM20 2QR, Great Britain.**

Human D_2 and D_3 binding was characterised in CHO cells using [^{125}I]-iodosulpiride. No binding selectivity was observed for the antagonists S-sulpiride, clozapine or haloperidol or the agonists apomorphine or bromocryptine. In contrast, dopamine, quinpirole and the efficacious agonists PHNO and 6,7 ADTN showed marked apparent hD_3 selectivity.

Functional coupling was investigated in a number of cell lines in which the hD_3 receptor was transiently or stably transfected. Both biochemical and electrophysiological studies were used to test for functional coupling in CHO, HEK-293, NG108-15, LTK and GH4 C1 cells. No evidence of functional coupling was found in any of these cell lines.

These results may be explained by a number of reasons. The hD_3 receptor may be locked in a high affinity state, these cells may not have appropriate G-proteins, or alternatively this receptor may couple by a mechanism, as yet, not determined.

9.7

ALLELIC VARIATION OF THE HUMAN D4 RECEPTOR. H.H.M. Van Tol*, C.M. Wu, H.-C. Guan, K. Ohara, J.R. Bunzow, O. Civelli, J. Kennedy, P. Seeman, H.B. Niznik and V. Jovanovic. Clarke Institute of Psychiatry, Toronto, Ontario, Canada M5T 1R8, Vollum Institute, Oregon Health Sciences University, Portland, Oregon 97201, USA.

The dopamine D4 receptor belongs to the group of receptors that acts through G proteins. Structurally and pharmacologically, the D4 receptor resembles most the dopamine D2 and D3 receptors. The antipsychotic clozapine, which is devoid of drug-induced tardive dyskinesia, has a ten-fold higher affinity for the D4 than the D2 and D3 receptors. This may explain clozapine's atypical properties. Screening of various human and genomic libraries resulted in the isolation and identification of various allelic forms of the D4 receptor. These receptors vary in the frequency of a 48-bp direct-repeat present in the putative third cytoplasmic loop. Expression of the cDNA of these allelic variants in COS-7 cells indicated differences in pharmacological properties with respect to spiperone and clozapine binding. This is the first example of allelic variations for catecholamine receptors. Such repeat polymorphisms are the basis for X-linked spinal and bulbar atrophy, fragile X-syndrome and myotonic dystrophy. The D4 receptor is located in a region of the genome (11-pter) that has been implicated in bipolar affective disorder and long QT syndrome. This polymorphism could have important implications in predisposition to psychiatric disorders and drug treatment.

9.8

STIMULATION OF DOPAMINE D-2 RECEPTOR IN CHO TRANSFECTED CELLS INHIBITS ADENYLATE CYCLASE ACTIVITY VIA GUANINE NUCLEOTIDE BINDING PROTEIN DISTINCT FROM THOSE EXPRESSED BY RAT PITUITARY CELLS A. VALERIO, C. TINTI, M. RIBOLA, P.F. SPANO and M. MEMO*, Division Pharmacology, Dept. Biomedical Sciences, University of Brescia, Italy.

Transcription of the gene encoding the dopamine D-2 receptor may result in two distinct splicing variants, named D-2s and D-21. In the present study we investigate the involvement of different G-proteins in the signal transduction pathway activated by the stimulation of the two different dopamine receptor isoforms. Chinese Hamster Ovary (CHO) cells, which normally lack dopamine D-2 receptor binding sites, were stably transfected with either the D-2s or the D-21 receptor isoform cDNA. Both CHO-D2s and CHO-D21 displayed a dose-dependent inhibition of adenylate cyclase activity when exposed to dopamine. Polymerase chain reaction (PCR) was used to analyzed the pattern of expression of the mRNA encoding the different G protein α subunits in CHO and pituitary cells. We found that pituitary cells contain transcripts for Gs long, Gs short, Gi2, Gi3, Gz and Go α subunits. CHO cells contain transcripts only for Gs long, Gs short and very low levels of Go α subunit. Our results demonstrate that Gi1, Gi2, Gi3 and Giz α subunits possibly expressed by CHO cells, are encoded by mRNAs structurally different from those present in rat pituitary.

9.9

VISUALIZATION OF ANTIPSYCHOTIC INTERACTIONS WITH LIVING MESOLIMBIC NEURONS REVEALS SPECIFIC D2 BINDING AND ACIDOTROPIC UPTAKE. S. Rayport*, M. Rappaport and D. Sulzer. Depts. Psychiatry, Anatomy & Cell Biology, Ctr. Neurobiology & Behavior, Columbia University; Dept. Neuropathology, NYS Psychiatric Institute, NY 10032.

We used fluorescent derivatives of spiperone (Ariano *et al.*, Brain Res **547**: 208-222, 1991) to examine the distribution of D2-type dopamine (DA) receptors on postnatal rat mesolimbic cells in culture. 10 nM rhodamine-*N*-(*p*-amino-phenethyl)spiperone (rhodamine-NAPS, Molecular Probes) produced continuous staining of the exposed somatic membrane of subsets of cells; glial background obscured possible neurite staining. Specific membrane staining was blocked by 10 μM (+)-butaclamol but not (-)-butaclamol. In ventral tegmental area cultures, where about 25% of neurons are DAergic, about the same fraction stained; histochemistry showed these neurons were mainly DAergic. In nucleus accumbens cultures, over 50% of the medium-sized (putatively GABAergic) neurons stained, while larger neurons (putatively cholinergic) did so rarely. In contrast, 10 nM bodipy-NAPS principally stained intracellular sites in a punctate pattern that was blocked by prior fixation or 10 mM NH_4Cl, suggesting that it reflected energy-dependent acidotropic uptake. To visualize acidotropic uptake of non-derivatized antipsychotics, we stained acidic sites with the weak base vital dye acridine orange; spiperone and haloperidol abolished acidic staining at low micromolar concentrations, clozapine and raclopride were less effective, while sulpiride was ineffectual at millimolar levels. For the first time, we have visualized DA receptors on living neurons, which should prove useful in identifying neurons expressing DA receptors and in examining changes in receptor distribution. Acidotropic uptake of antipsychotics may impact on their therapeutic action, side effects, and use in positron emission tomography to evaluate D2-type receptor numbers.

9.10

AUTORADIOGRAPHIC LOCALIZATION OF [^{3}H]QUINPIROLE BINDING TO PUTATIVE D_2 AND D_3 DOPAMINE RECEPTORS IN RAT BRAIN. B. Levant*, D.E. Grigoriadis, E.B. DeSouza. Central Nervous System Diseases Research, The DuPont Merck Pharmaceutical Co., Wilmington, DE 19880-0400.

The putative D_2 dopamine receptor agonist quinpirole (LY 171,555) has been extensively used in a variety of *in vivo* and *in vitro* studies of D_2 receptor mediated effects and may have even higher affinity for the recently described D_3 dopamine receptor (Sokoloff et al., Nature, 347:146-151, 1990). In the present study, conditions for autoradiographic visualization of [^{3}H]quinpirole-labeled D_2-like dopamine receptors were optimized and binding to slide-mounted sections was characterized with respect to pharmacology, guanine nucleotide sensitivity, and regional distribution. The pharmacological profile of [^{3}H]quinpirole binding in slide-mounted brain sections was: (±)6,7-ADTN ≥ quinpirole > dopamine for putative dopamine agonists; spiperone > (+)butaclamol > (-)sulpiride > SCH 23390 >> cinanserin > (-)butaclamol for antagonists. [^{3}H]Quinpirole binding was decreased in the presence of guanine nucleotides in most brain regions except in the islands of Calleja and the molecular layer of cerebellar lobules 9 and 10. The regional distribution of [^{3}H]quinpirole binding sites roughly paralleled the distribution of [^{3}H](-)sulpiride binding sites, with greatest densities present in the olfactory bulb glomerular layer, islands of Calleja, pituitary intermediate lobe, caudate/putamen, olfactory tubercles, and nucleus accumbens However, significantly greater densities of [^{3}H]quinpirole binding than [^{3}H](-)sulpiride binding were observed in the islands of Calleja, olfactory bulb glomerular layer, and the molecular layer of cerebellar lobules 9 and 10, and somewhat more [^{3}H]quinpirole binding in the olfactory tubercles, in concordance with the high levels of expression of D_3 receptor mRNA recently reported in these brain regions (Bouthenet et al., Brain Res., 564:203-219, 1991). Higher concentrations of [^{3}H]quinpirole binding were also observed in the dorsomedial caudate and pituitary intermediate lobe. These data indicate the utility of [^{3}H]quinpirole in autoradiography to label D_3 as well as D_2 dopamine receptors.

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9.11

NICOTINE REGULATES DOPAMINE D1 AND D2 RECEPTOR GENE EXPRESSION IN RAT BRAIN. J. Tsatsos, T. Hattori* and S.R. George, Dept of Pharmacology, University of Toronto, Ont, CANADA M5S 1A8

The central nervous system effects of nicotine are mediated, at least in part, by the activation of dopaminergic systems in brain, notably in the striatum and mesolimbic regions. In the present study, the effects of nicotine administration on dopamine D1 and D2 receptor gene expression were examined. Male Sprague-Dawley rats were housed in environmental rooms and injected s.c. with nicotine 1 mg/kg/day or saline vehicle for 3 days. Animals were sacrificed and the brain regions were dissected and quickly frozen and stored at -70°C. D1 and D2 receptor mRNA was detected by Northern blot analysis with hybridization of specific 32P-labelled rat cDNA or oligonucleotide probes. Specific dopamine D1 receptor binding was quantified by [3H]SCH23390 in the presence of 1 uM (+)butaclamol, and specific D2 receptor binding by [3H]spiperone in the presence of 10 uM (-)sulpiride. There was a significant decrease in D1 receptor mRNA in the striatum and olfactory tubercle of nicotine treated rats that was accompanied by a decrease in the density of D1 dopamine receptors in striatum. There also was a significant decrease in D2 receptor mRNA in the striatum of nicotine treated rats accompanied by a reducton of D2 dopamine receptor density. D2 receptor mRNA was also decreased in neurointermediate pituitary after nicotine treatment, but was unaffected in anterior pituitary. These results suggest that nicotine alters dopamine D1 and D2 receptor gene transcription with a consequent change in expression of the receptor protein.

9.12

EXPRESSION OF mRNAs ENCODING ARPP-16/19, ARPP-21 AND DARPP-32 IN HUMAN BRAIN TISSUE S. BRENÉ*, N. LINDEFORS, J. KOPP, H. HALL, M. EHRLICH; G. SEDVALL, P. GREENGARD and H.PERSSON

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In situ hybridization with cDNA probes was used to study the expression of mRNAs encoding the phosphoproteins ARPP-16/19, ARPP-21 or DARPP-32 in human *post mortem* brain tissues, using horizontal whole hemisphere sections. Eight representative levels of the brain ranging from 25 mm to 105 mm ventral to vertex were examined. All three probes showed distinct hybridization patterns in the caudate nucleus, putamen, nucleus accumbens and the amygdaloid complex. For ARPP-16/19 mRNA a hybridization signal comparable to the signal in caudate nucleus, putamen and nucleus accumbens was also detected in the neocortex. ARPP-21 and DARPP-32 mRNA, on the other hand, were present in much lower relative levels in neocortical regions. DARPP-32 mRNA was abundant in the deep layer of the cerebellar cortex at the level of the Purkinje cell layer. High levels of ARPP-16/19 and ARPP-21 mRNA were also found in the cerebellar cortex where they were confined to superficial layers. The present results demonstrate high expression of mRNAs for the three phosphoproteins in several areas of the human brain where abundant dopamine D1 receptor binding sites have previously been localized. In situ hybridization in human postmortem tissue should be useful for future detailed studies on mRNA expression for phosphoproteins as well as other proteins in neurological disorders.

CALCIUM CHANNEL TOXINS I

10.1

PROBING NEURONAL CALCIUM CHANNELS WITH BIOTINYLATED DERIVATIVES OF OMEGA-CONOTOXINS. J. A. Haack*, P. Kinser, D. Yoshikami, B. M. Olivera. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

Omega-Conotoxins are a class of homologous peptides, isolated from predatory cone snails, that are potent inhibitors of neuronal subtypes of voltage-sensitive calcium channels (VSCC). Purified and structurally characterized, biotinylated derivatives of two homologous omega-conotoxins, omega-GVIA (*Conus geographus*) and the recently characterized omega-MVIID (*Conus magus*) were used to probe the structure/function relationships of interactions between omega-conotoxins and neuronal VSCC. The apparent affinities of biotinylated omega-GVIA derivatives, in receptor binding assays, decreased by an order of magnitude per biotin attached to the peptide. This decrease was independent of the position of the amino acid biotinylated. Preformed complexes between streptavidin and biotinylated derivatives of omega-GVIA also retain their activity in receptor binding assays, suggesting that the receptor binding site is tolerant of large structural additions in the biotinylated regions of the peptide.

In contrast, the apparent affinities of mono-biotinylated derivatives of omega-MVIID vary depending on the position of the amino acid biotinylated. Results from receptor binding assays showed that like omega-GVIA, biotinylation of the N-terminus resulted in a one order of magnitude decrease in apparent affinity. However, biotinylation of the lysine at amino acid #10 of omega-MVIID resulted in an approximately 3 orders of magnitude decrease in apparent affinity. A comparison of the receptor binding characteristics of this collection of biotinylated derivatives allows us to map out regions of the omega-conotoxins which are important for peptide interactions with neuronal VSCC.

10.2

EFFECTS OF ω-CONOTOXIN GVIA AND ω-AGATOXIN IA ON Ca^{2+} CURRENTS IN RAT SYMPATHETIC NEURONS. V.P. Bindokas*, S. Foucart, M.E. Adams+ and R.J. Miller. Dept. Pharmacol. and Physiol. Sci., Univ. of Chicago, Chicago IL, 60637; +Dept. Entomol., Univ. of California, Riverside CA, 92521.

In this study, ω-conotoxin GVIA (CgTX) and ω-agatoxin IA (AgaTX) were used to characterize the subtypes of calcium channels in acutely isolated sympathetic neurons. Neurons from adult rat superior cervical ganglia were dissociated enzymatically, plated on poly-l-lysine coated coverslips and used within 8 hrs. The whole-cell patch-clamp method was used to record transmembrane Ca^{2+} currents (I_{Ca}). Cells were placed in a perfusion chamber and the I_{Ca} was isolated by using the following medium (in mM):$CaCl_2$ 2, TEACl 138, $MgCl_2$ 2, HEPES 10, glucose 10, pH 7.4. The internal solution was (mM): CsCl 124, $MgCl_2$ 1, HEPES 10, BAPTA 10, MgATP 3.6, $tris_2$creatine phosphate, CPK (50 U/ml), trisGTP 1, pH 7.4. Pressure application of CgTX (5 μM) resulted in a 53 ± 6.1% inhibition of the peak I_{Ca} (n = 9). The remaining I_{Ca} was unaffected by noradrenaline (NA, 10 μM; n = 4) or neuropeptide Y (NPY, 300 nM; n = 3) whereas prior to the toxin treatment, NA and NPY inhibited the I_{Ca} by 37% and 13% respectively. Application of AgaTX (500 nM) resulted in 38 ± 3% inhibition of I_{Ca} (n = 5). This effect did not prevent the inhibitory action of NA on I_{Ca}. 4 of 4 neurons were further inhibited 41 ± 14% after AgaTX treatment. In a few cases, there was slow, partial recovery of I_{Ca} following AgaTX washout. It is known that CgTX blocks N-type Ca^{2+} channels. Therefore, the results observed with CgTX suggest that NA and NPY inhibit I_{Ca} via action affecting an N-type channel. The results observed with AgaTX suggest that it may inhibit another type of Ca^{2+} channel distinct from the traditional N-type.

10.3

NOVEL SNAKE TOXIN BLOCKS SELECTIVELY THE L-TYPE CALCIUM CURRENT IN RAT DENTATE GRANULE NEURONS. C.E. Niesen, Q.T. Jones*, P.L. Carlen. Playfair Neuroscience Unit, Toronto Western Hospital, Toronto, Ontario, CANADA M5T 2S8

Polypeptide toxins have revolutionized the study of ion channels in neurobiology. In an effort to develop ligands for the labelling and characterization of neuronal voltage-gated calcium channels, we have isolated a polypeptide from the venom of the black mamba snake, *Dendroaspis polylepis polylepis*. The toxin was purified by a combination of ion-exchange and reverse-phase (HPLC) chromatographic steps. Whole cell voltage-gated calcium currents (4 mM external Ca^{2+}) were recorded in dentate granule neurons from rat hippocampal slices from 2-4 week old animals. Leupeptin and an ATP-regenerating solution in the patch electrode produced stable currents for > 40 min of recording.

Bath perfusion of 1-2 μM of the mamba toxin reduced the L-type current by 70-80% with little to no effect on the T- or N-type calcium currents. Completion of the block required 6 min of toxin perfusion and appeared to be voltage-dependent. Concentrations in the range of 100-200 nM produced half-maximal reduction of the L- type current. Washout of the toxin for 20 min in control solution did not reverse its effect. Nimodipine at 2 μM produced a 50-70% block of the L-type current, indicating that at near saturating concentrations, the mamba toxin appears to be more potent. A combination of nimodipine and mamba toxin decreased the L-type current to near zero levels, regardless of their order of application.

The mamba toxin is being characterized to elucidate its 1° and 2° structure and confirm its possible identity to a toxin described by de Weille et al. (PNAS 88:2437, 1991) termed calciseptine. To our knowledge, this represents the most potent polypeptide toxin of the L-type calcium current and should prove invaluable in dissecting this channel's pharmacology and cell biology.

Supported by an MRC Fellowship.

10.4

Evidence for allosterically coupled toxin binding sites for ω-conotoxin GVIA and ω-agatoxinIIIA on the purified ω-conotoxin receptor. M.W. McEnery*, A.M. Snowman, A.H. Sharp, M.E. Adams±, and S.H. Snyder. The Johns Hopkins Univ. Sch. of Medicine, Dept. of Neuroscience, and +Univ. of California, Riverside, Dept. of Entomol. and Tox.

The N-type calcium channel is the inhibitory site of action of ω-conotoxin GVIA (CTX) and can be distinguished from the neuronal DHP receptor/L-type calcium channel. N-hydroxy-succinimidyl-4-azidobenzoate-^{125}I-CTX labels with high affinity a discrete 230 kDa band in brain membranes which represents the recognition site for CTX in the purified CTX receptor. As with the L-type channel, the ligand binding subunit comprises only a portion of the holo-receptor. We have reported the purification of the CTX receptor with the isolated receptor protein retaining reversible ligand binding (Mc Enery, et al. (1991) PNAS 88, 11095). The purified rat brain CTX receptor comprises five subunits with substantial similarity to the subunit composition of the L-type calcium channel. We have examined the affinity of the purified CTX receptor for peptide toxins considered diagnostic for the intact receptor: CTX, ω-conotoxin MVIIA, and ω-AgaIIIA, a competitive inhibitor of [^{125}I]CTX binding. We demonstrate the high affinity recognition site for ω-AgaIIIA to be dependent upon the presence of exogenous phospholipids, while CTX and MVIIA is not (McEnery (1992) Meth. in Pharm. vol 7, in press). These results suggest 1) the ω-AgaIIIA binding site can be uncoupled from the CTX binding site, a novel finding for VDCC antagonists, 2) phospholipids induce a change in the CTX receptor which selectively effects ω-AgaIIIA potency, 3) the ω-AgaIIIA binding site is in close proximity to the surface of the membrane, while CTX and MVIIA target a more hydrophilic region of the receptor.

10.5

USE OF SPIDER TOXINS TO DISCRIMINATE BETWEEN NEURONAL CALCIUM CHANNELS. I.M. Mintz*, M.E. Adams, and B.P. Bean. Dept. of Neurobiology, Harvard Med. School, Boston MA 02115 and Dept. of Entomology, University of California, Riverside CA 92521.

Venom from the spider *Agelenopsis aperta* is rich in Ca channel antagonists. The 48 amino-acid peptide ω-Aga-IVA potently blocked P-type Ca channels (Kd ~2 nM) but had no effect on T-type, L-type, or N-type channels. Identifed by ω-Aga-IVA sensitivity, P-type current contributed 90% of the high-threshold current in cerebellar Purkinje cells and variable smaller fractions in spinal cord interneurons (45%), visual cortical neurons (30%), hippocampal CA1 pyramidal cells (25%), and DRG neurons (25%). Most CA3 hippocampal neurons had little P-type current. In cells with multiple channel types, P-type current identified by ω-Aga-IVA showed no overlap with nimodipine-sensitive L-type current or ω-conotoxin-sensitive N-type current. Except for Purkinje neurons, almost all CNS neurons also possessed a fraction of high-threshold current that remained unblocked with combined application of ω-Aga-IVA, ω-conotoxin, and nimodipine.

The 76 amino-acid peptide ω-Aga-IIIA targets most or all high-threshold Ca channels. ω-Aga-IIIA blocked L-type channels potently and completely (Kd~1 nM) and N-type channels potently (Kd~1 nM) but only partially (~70%). Applied to Purkinje neurons, 200 nM ω-Aga-IIIA was saturating but blocked only ~40% of P-type current elicited at -20 mV in 5 mM Ba. The partial block of P-type Ca channels was voltage-dependent, being almost absent at potentials above -10 mV. Noise analysis suggested that ω-Aga-IIIA reduces single P-channel current in a voltage-dependent manner.

10.6

TOXITYPING CALCIUM CHANNELS IN THE MAMMALIAN BRAIN WITH RADIOLABELLED ω-AGATOXINS AND ω-CONOTOXINS. M. E. Adams[1*], R. A. Myers[2], J. Imperial[2], and B.M. Olivera[2]. [1]Depts. of Entomology and Neuroscience, Univ. of California, Riverside, CA 92521 and [1]Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

The spider toxins ω-Aga-IIIA and ω-Aga-IVA have been radioiodinated and used in combination with other ω-agatoxins and ω-conotoxin GVIA to elucidate subtypes of calcium channels in the rat brain. Using Bolton-Hunter derivitization methods for preparation of [^{125}I]ω-Aga-IIIA, specific, high affinity binding to rat brain membranes was observed. Unlabelled toxin displaced the radioligand with an IC_{50} of about 1 nM. No significant inhibition of [^{125}I]ω-Aga-IIIA binding was observed by preincubation of membranes with other ω-agatoxins, except at much higher concentrations; IC_{50} values were approximately 100 nM for ω-Aga-IA and 600 nM for ω-Aga-IVA. In reciprocal binding experiments between ω-Aga-IIIA and ω-conotoxin GVIA, a marked asymmetry was observed. Preincubation of membranes with ω-Aga-IIIA blocked all specific binding of [^{125}I]ω-conotoxin GVIA, but pre-exposure to ω-conotoxin GVIA inhibited a maximum of 20% of [^{125}I]ω-Aga-IIIA binding. This suggests that ω-Aga-IIIA and ω-conotoxin GVIA overlap in their selectivities, but that ω-Aga-IIIA recognizes a larger population of Ca channels (see also Mintz et al. and McIntosh et al., this meeting).

Inhibition of [^{125}I]ω-Aga-IVA binding to solubilized rat brain membranes by unlabelled ω-Aga-IVA yielded an IC_{50} of approximately 30 nM, which is close to the concentration that inhibits 50% of potassium-induced ^{45}Ca flux into rat brain synaptosomes. [^{125}I]ω-Aga-IVA binding was not significantly inhibited by ω-conotoxin GVIA, ω-Aga-IA, or ω-Aga-IIIA. These data, in combination with electrophysiological results (Mintz et al., this meeting), are consistent with selectivity of ω-Aga-IVA for a unique class of P-type Ca channels in the brain.

Supported by NIH grant NS24472 and GM22737

10.7

THE SPIDER TOXIN ω-Aga-IVA INHIBITS AMINO ACID NEUROTRANSMITTER RELEASE FROM HIPPOCAMPAL TISSUE IN VITRO. S.P. Burke*, C.P. Taylor and M.E. Adams. Parke-Davis Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105 and Depts. Entomology and Neuroscience, Univ. Calif. Riverside, CA 92521.

The exocytotic release of transmitter is dependent on the opening of voltage-sensitive calcium channels (VSCC) on synaptic terminals. However, the involvement of specific subtypes of VSCC is not clear. We examined the overflow of amino acids from transverse slices of rat hippocampal CA1 area by brief application of 50 mM KCl, evoking glutamate release that is greater than 95% dependent upon external Ca^{2+}.

ω-Aga-IVA is a peptide present in the venom of the funnel-web spider *Agelenopsis aperta*. This toxin blocks Ca^{2+} entry in several preparations (Mintz et al. *Nature* 355:827(1992)). We tested the effect of ω-Aga-IVA upon depolarization-induced glutamate release in a double-pulse protocol. Twenty-five min after a 1-min bolus of 50 mM KCl medium (S1), normal superfusion (1 ml/min) was halted for 15 min while the tissue slices were submerged in 200 nM ω-Aga-IVA or control medium. Superfusion was then resumed with a second 1-min bolus (S2) of 50 mM KCl. HPLC analysis of amino acids in collected fractions gave an S2/S1 ratio for glutamate release of 0.37 (±0.05 SEM) for ω-Aga-IVA compared to 0.95 (±0.05 SEM) for control medium. S2/S1 Ratios for GABA and aspartate with 200 nM ω-Aga-IVA were 0.5-0.6. Preliminary data suggest that the effect of ω-Aga-IVA is largely reversible with 15 min of washing in control medium. Others have shown that VSCCs sensitive to the marine snail toxin ω-conotoxin-GVIA contribute to the release of monoamine transmitters. Our results suggest that VSCCs sensitive to ω-Aga-VIA contribute to release of amino acid transmitters.

10.8

PROPERTIES OF TOXIN BLOCK OF PRESYNAPTIC AND SOMATIC CALCIUM CHANNELS IN CULTURED HIPPOCAMPAL PYRAMIDAL NEURONS K.P. Scholz*, M.E. Adams, R.J. Miller. Dept. of Pharm. and Physiol. Univ. of Chicago, Chicago, IL; Depts of Entomology and Neuroscience, Univ. of Cal. Riverside.

Whole-cell voltage clamp recordings of somatic Ca2+ currents (I_{Ca}) and of evoked excitatory synaptic currents (EPSCs) were performed in cultured rat hippocampal pyramidal neurons to characterize the sensitivity of presynaptic Ca2+ channels to various Ca2+ channel toxins. Four Ca2+-channel specific toxins were compared. ω-conotoxin GVIA (5 uM) was previously shown to block about 25% of the somatic I_{Ca}. SNX-111 (synthetic ω-conotoxin MVIIA) inhibited 15-25% of I_{Ca} (n=2) and appeared to have similar specificity as ω-conotoxin GVIA. SNX-183 (synthetic ω-conotoxin SVIB; Miljanich et al., 1991) inhibited 25.7 + 2.3% of I_{Ca} (n=4). All three of the above toxins (at concentrations of 5 uM) inhibited evoked EPSCs completely. ω-Aga-IIIA inhibited 58.0 + 4.5% of I_{Ca} (n=9) and inhibited EPSCs by about 90%.

It has been observed that many G-protein linked receptors inhibit I_{Ca} in a voltage-dependent manner, whereby a depolarizing pre-pulse relieves the inhibition. A component of I_{Ca} in pyramidal neurons was inhibited in this manner following activation of adenosine receptors. We have found that ω-conotoxin GVIA blocked this component of current. ω-Aga-IIIA partially blocked this same component of current and, in contrast to adenosine, the block by ω-Aga-IIIA was not relieved by a depolarizing prepulse. This difference in the inhibition of I_{Ca} between adenosine and ω-Aga-IIIA was used to test the hypothesis that the voltage-dependent relief of inhibition of I_{Ca} is involved in increasing paired-pulse facilitation of transmitter release during G-protein linked inhibition of presynaptic Ca2+ channels. ω-Aga-IIIA increased paired pulse facilitation of EPSCs to a similar degree as adenosine. This result suggests that the voltage-dependence of G-protein linked inhibition of I_{Ca} is not a major factor involved in increasing paired-pulse facilitation of transmitter release.The authors thank Neurex Corp. for the gift of SNX-111 and SNX-183.

10.9

A NOVEL POLYAMINE CALCIUM CHANNEL ANTAGONIST ISOLATED FROM VENOM OF THE SPIDER, *DOLOMEDES OKEFENOKIENSIS*. K. Kobayashi, J.B. Fischer, A.G. Knapp, L. Margolin, D. Daly, N.L. Reddy, B. Roach*, K. McCormick*, J. Meinwald*, and S.M. Goldin**. Cambridge NeuroScience, Cambridge MA; *Chem. Dept., Cornell Univ., Ithaca NY; **Cambridge NeuroScience and Dept. of Biol. Chem., Harvard Medical Sch., Boston MA.

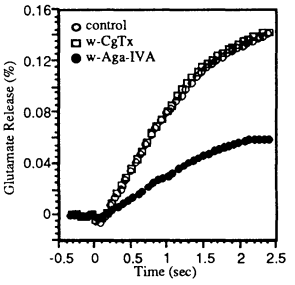
A major pathway of Ca entry into depolarized nerve cells is through voltage-activated, high-threshold Ca channels. A substantial fraction of this Ca entry is mediated through "non-N, non-L"-type Ca channels resistant to blockade by dihydropyridine Ca antagonists such as nimodipine (Regan et al. [1991] Neuron 6, 269). CNS 2103 is a polyamine Ca antagonist present in minute quantities in the venom of the domestic hunting spider *Dolomedes okefenokiensis*. It was discovered using high-throughput radioisotopic ^{45}Ca uptake assays of L-type Ca channel activity in GH4C1 clonal pituitary cells, and its ability to block L-type Ca current was confirmed using whole cell patch recordings of GH4C1 cells. CNS 2103 has been purified, its structure elucidated, and was synthesized in gram-scale quantities. Whole-cell recordings of N1E-115 neuroblastoma cells demonstrated that CNS 2103 (60 μM) reversibly blocks both L-type and dihydropyridine resistant ("R-type") high threshold channels, with no effect on T-type Ca current or voltage-acitvated Na or K channels. In whole cell recordings of primary cultures of rat brain cortical neurons, no effect of CNS 2103 was observed against membrane current generated through NMDA- and non-NMDA glutamate receptor subclasses.

We hypothesize that CNS 2103 may act at a site common to L- and "R"-type Ca channels. The broad spectrum of action of CNS 2103 compared with dihydropyridines, and its selectivity for calcium channels over other classes of ion channels, suggest that CNS 2103 may be an interesting lead towards development of drugs to treat ischemic brain injury and other CNS disorders.

10.10

Presynaptic Calcium Channels Coupled to Glutamate Release in Rat Synaptosomes Are Blocked by ω-Aga-IVA. T.J. Turner*, M.E. Adams[1], and K. Dunlap. [1]Dept. of Entomology and Neuroscience, University of California, Riverside, and Dept. of Physiology, Tufts University School of Medicine, Boston, MA 02111.

ω-Aga-IVA, a peptide toxin of the funnel web spider *Agelenopsis aperta*, selectively blocks "P-type" calcium currents in Purkinje neurons, as well as $^{45}Ca^{2+}$ uptake into rat synaptosomes, processes resistant to ω-CgTx and dihydropyridines (Mintz, et al., *Nature* **355**, 827). Since synaptosomal ^{3}H-glutamate release is also ω-CgTx resistant, we studied the effect of ω-Aga-IVA on this process using a superfusion system with a subsecond temporal resolution. ω-Aga-IVA produced a potent (IC50=30 nM) but partial block of calcium-dependent glutamate release. The toxin was more efficacious at low levels of depolarization; little effect was observed with [KCl] > 30 mM. When synaptosomes were depolarized for 1 s with 15 mM KCl, 200 nM ω-Aga-IVA blocked 56% of calcium-dependent release: ω-CgTx (10 μM) had no effect. The results indicate the ω-Aga-IVA sensitive, ω-CgTx and DHP resistant, presynaptic calcium channel coupled to glutamate release in rat brain is a "P-type" channel.



10.11

CONTRASTS BETWEEN CLONED BI AND CARDIAC L-TYPE Ca^{2+} CHANNELS EXPRESSED IN XENOPUS OOCYTES. W.A. Sather*, T. Tanabe, Y. Mori, M.E. Adams, G. Miljanich, S. Numa & R.W. Tsien. Dept. Mol. Cell. Physiol., Stanford Univ., Dept. Medical Chem., Kyoto Univ., Dept. Entomology, U.C. Riverside, Neurex Corp., Menlo Park CA 94025.

BI and cardiac L-type Ca^{2+} channels differ in their macroscopic current kinetics. In contrast to cardiac L-type channels, BI currents activate more quickly and show a more pronounced inactivation in whole cell recordings (2-40 mM Ba^{2+}).In cell-attached patches, the time course of inactivation of BI is much the same with either Ca^{2+} or Ba^{2+} as the charge carrier. BI channels displayed a slope conductance (γ) of ~15 pS and a unitary amplitude (i)~0.8 pA at 0 mV (110 mM Ba^{2+}). For L-type channels,γ~22 pS and i=1.2 pA. The pharmacology of BI and L-type channels is also markedly different. BI channels are not enhanced by the Ca^{2+} channel agonists Bay K 8644 or FPL 64176, or inhibited by nimodipine, unlike L-type channels. However, BI Ca^{2+} channels can be blocked by the Neurex conopeptides SNX-230 (MVIIC)(~50% block at 0.5 μM) and SNX-260 (near complete block at 0.5 μM), whereas L-type Ca^{2+} channels are unresponsive to SNX-260. We also examined effects of ω-Aga-IVA, a peptide toxin that potently blocks P-type channels in cerebellar Purkinje cells (IC50=5 nM in 5 mM Ba^{2+}, Mintz et al.,1992). BI channels in oocytes were only partly blocked by 200 nM ω-Aga-IVA (2 mM Ba^{2+}).

10.12

A potent peptide calcium channel blocker from *Plectreurys* spider venom contains a C terminal lipid. W. D. Branton, M. S. Rudnick*, and Yi Zhou. Department of Physiology, University of Minnesota, Minneapolis MN 55455.

We have determined the complete primary structure of PLTX-II; a potent insect calcium channel blocker found in *Plectreurys* venom. Edman microsequencing of covalently immobilized, carboxymethylated toxin yielded assignments for a stretch of 42 amino acids ending in Cys. The sequence of the first 41 residues was also confirmed by sequencing and mass spectrometry (MS) of proteolytic fragments. In addition, proteolytic digests yielded a small, but very hydrophobic fragment with a mass that accounts for the total mass of the toxin when combined with residues 1-41. This C terminal fragment contained one Cys, one Asx and one Thr residue. Microsequencing of the fragment yielded the sequence CysAsp. MS of the Pyridinethylated (PE) fragment gave a mass of 680, indicating the presence of 238 mass units of unexplained structure. Collision induced fragmentation of the ion at mass 680 was consistent with the sequence PECysAspThr-amide, with the additional 238 mass units associated with the C-terminal Threonine. Several lines of evidence are consistent with the possibility that the remaining mass is a palmitic acid ester of the C terminal Threonine. Attempted synthesis of PECys-Asp-PalmitoylThreonine-amide yielded two products with a mass of 680, and unique HPLC retention like that of the native C terminal fragment. The collision induced fragmentation pattern of one of these products was identical to the natural C-terminal peptide. The putative C terminal structure is novel, but it is closely related to recently described modifications of a number of membrane associated regulatory proteins. It is very likely that it is one of a class of novel, structurally related neurotoxins present in *Plectreurys* venom. (Supported by NIH R01 GM42829.)

VISUAL CORTEX: FUNCTIONAL CIRCUITS AND OSCILLATIONS I

11.1

SPATIOTEMPORAL RECEPTIVE FIELD STRUCTURE AND PHASE RELATIONSHIPS BETWEEN ADJACENT SIMPLE CELLS IN THE CAT'S STRIATE CORTEX. G.C. DeAngelis, G.M. Ghose, I. Ohzawa, and R.D. Freeman*, School of Optometry, Univ. of California, Berkeley, California 94720.

Nearby simple cells in the visual cortex are reported to have overlapping receptive fields which differ in spatial phase by approximately 90° (quadrature phase; Pollen & Ronner 1981) or 180° (anti-phase; Foster et al. 1983). Quadrature phase pairs are of theoretical interest because they may provide a basis set (Gabor 1946) for economical encoding of the visual image. Moreover, anti-phase pairs may be necessary to construct the antagonistic ON and OFF subregions of simple cells (eg. Ferster 1988).

We have studied phase relationships between the receptive fields of pairs of simple cells recorded from a single microelectrode positioned in the striate cortex. Responses are initially characterized using drifting sinusoidal luminance gratings. Subsequently, a reverse correlation technique (Jones & Palmer 1987) is used to obtain spatiotemporal receptive field profiles for each pair of cells. This technique allows a direct assessment of the relative spatial phase between members of a simultaneously recorded pair of neurons. In addition, the relative phase of pairs of receptive fields can be measured as a function of time over the duration of the responses (~200 ms).

Spatiotemporal receptive field profiles have been recorded for 40 pairs of nearby simple cells. Members of a pair typically have similar preferences for orientation, spatial frequency, and direction; in addition, their receptive fields are usually centered at the same spatial location. We have encountered pairs of cells that exhibit relative phase differences of approximately 90° or 180°. Other simple cell pairs have relative phases near 0° or values which are intermediate between 0° and 180°. In many cases, the relative phase between members of a pair remains constant over time. In other cases, relative phase changes dramatically with time (ie. at least one of the receptive fields is not space-time separable). These latter pairs of cells cannot be assigned to any constant-phase group. In some cases, the two members of a pair have different optimal spatial frequencies or different receptive field positions; this complicates interpretation of the phase relationship. Overall, our findings suggest that the phase relationships between adjacent simple cells are not limited to quadrature and anti-phase. (EY01175)

11.2

ANTI-HIERARCHICAL CIRCUITRY IN VISUAL CORTEX OF THE CAT AND KITTEN. G.M. Ghose, I. Ohzawa*, R.D. Freeman. Groups in Biophysics and Neurobiology, School of Optometry, Univ. of California, Berkeley, CA 94720.

Hierarchical processing of visual information specifies that receptive field properties at any stage can be accounted for by combining signals from neurons of the preceding stage. In the case of the visual cortex, it has been proposed that complex cell properties arise from the inputs of multiple simple cells (Hubel and Weisel, 1962). In order to directly investigate such hierarchical organization we have analyzed the simultaneous discharge from adjacent neurons in the cat's striate cortex. We have studied kittens at 4 weeks postnatal and adult cats to determine if this organization changes during postnatal development.

Receptive field classification of pairs of neurons was done according to the degree of response modulation to drifting sinusoidal gratings. Cross correlation analysis was performed on the responses to optimal gratings. Features in the crosscorrelograms, such as peaks or troughs, were used to group neuronal interactions into three categories: monosynaptic excitation, polysynaptic excitation, and monosynaptic inhibition.

Monosynaptic excitation of complex cells by simple cells was observed in the kitten, but not the adult cat. However, in both the kitten and the cat, excitation in the *opposite* direction (from complex to simple cells) was observed. Polysynaptic excitation in the kitten was predominantly between cells of similar cell types, whereas no such preference is apparent in the adult. Postnatal expansion of common input to nearby cortical cells can potentially explain the changes in polysynaptic excitation. Such an expansion could also create synchronous discharge which obscures the visibility of monosynaptic interactions. The existence of excitatory input to simple cells from complex cells, however, is difficult to reconcile with a strictly hierarchical model. These results suggest that the interactions between cells of different receptive field types are not completely hierarchical and change with postnatal development. (EY01175)

11.3

RESPONSE OF SIMPLE AND COMPLEX CELLS OF AREA 18 TO ORIENTATION DURING REVERSIBLE INACTIVATION OF AREA 17 IN CATS. Y. Michaud*, S. Molotchnikoff and C. Casanova. Université de Montréal, Dépt de biologie, C.P. 6128, succ. A, Montréal (Québec) CANADA H3C 3J7.

It has been hypothetized that horizontal connections within one visual cortical area link neurons (column) with similar response properties such as the prefered orientation. However the functional connections between cortical visual areas are still unclear. Thus the aim of the present investigation is to study the influence of area 17 upon the orientation tuning properties of cells in area 18. Anesthetized cats are conventionally prepared for single cell recording. Prior to injection of 300 nl of GABA in superficial layers of area 17 the dominant orientation of injected cells in determined and this orientation is correlated with the orientation of the studied unit in area 18. For simple cells (80%) the main effects during the inactivation of area 17 is a decline of the discharge to optimal orientation which results in a substantial loss of orientation selectivity. For complex cells the broadening of the orientation tuning curve is due to enhancement of responses to non-optimal orientation. Most interestingly simple cells are more frequently affected when orientation of injecting and recording sites is similar whereas complex cells are mostly affected when the two orientations are orthogonal. Thus, within the orientational domain one must consider cell types and axis of orientation.

11.4

FUNCTIONAL CONNECTIVITY BETWEEN V1 AND V2 IN THE PRIMATE. *A.W. Roe* and D.Y. Ts'o.* Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

To better understand the role of corticocortical interactions in sensory processing, we have begun to investigate the rules of functional connectivity between V1 and V2. Guided by *in vivo* optical imaging maps of the functional compartments of V1 (the blobs and interblobs) and V2 (the stripes), we targeted particular sites in V1 and V2 with a pair of electrodes. Cross correlation analysis was then used to assess the interactions and connectivity between single cells in specific V1 and V2 compartments, as well as particular rules underlying the generation of specific receptive field properties in V2.

In general, the cross-correlograms between V1 and V2 most frequently exhibited common input interactions. Often the correlogram peaks were shifted such that the firing of the V2 cell lagged the V1 cell by 5-20 ms. Peaks were usually broad (widths of 10-100ms), which may indicate a high degree of convergence of inputs from V1. Of particular interest are the interactions between color cells of the V1 blobs and the V2 color stripes. Positive (excitatory) correlations occur most often between non-oriented color cells located in V1 blobs and cells in V2 with similar color specificities, as well as between V1-V2 cell pairs that were broadband. Cells with dissimilar properties most often exhibited flat correlograms. Our results underscore the functional parcellation of corticocortical connectivity in visual cortical processing.

(Supported by grants EY06347, EY08240, ONR N00014-91-J-1865 and the Whitaker Foundation).

11.5

CORTICAL REORGANIZATION FOLLOWING BINOCULAR FOCAL RETINAL LESIONS IN THE ADULT CAT AND MONKEY. *C. Darian-Smith*, C. D. Gilbert, and T. N. Wiesel* Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

The adult neocortex is capable of significant topographic reorganization following perturbation of input. We made focal binocular retinal lesions at homologous sites to render a portion of the lateral geniculate nucleus and striate cortex (cortical 'scotoma') inactive to visual stimuli. Within two months, much of the original cortical scotoma was again physiologically responsive, and was effectively 'filled in' by surrounding retinotopic fields. Retinal lesions as large as 8° in the cat and 5° in the monkey induced cortical scotomas of up to 6 and 10mm diameter, respectively. Cortical receptive field maps made 2 to 12 months after the lesions indicate that reorganization can 'fill-in' a cortical area 6 to 7 mm in diameter in both cat and monkey. Topographic maps of the lateral geniculate nucleus (LGN) made 4 and 12 months after the retinal lesions indicated little, if any, 'recovery'. To determine the connections responsible for cortical reorganization, 4 retrograde fluorescent dyes were injected into normal cortex, the border of the original cortical scotoma, and at different positions within the reorganized cortex in each of a series of cat and monkey brains. Within the LGN, cell populations projecting to dye injections located within and close to the border of the original scotoma did not overlap spatially. These results indicate that the lateral spread of LGN afferents within the cortex (or the sprouting of these) is insufficient to account for the extent of reorganization observed. Within the cortex, labeled neurons were distributed concentrically around injection sites for a radial distance of 3-4mm. This distance is compatible with the extent of 'fill in' observed in reorganized cortex, and suggests either the strengthening of existing horizontal connections or their collateral sprouting. (Supported by NIH grants EY07968, 1EY05253, the McKnight Foundation, and the NH&MRC, Australia).

11.6

FUNCTIONAL INTERACTIONS BETWEEN MEMBERS OF ADJACENT CELL PAIRS IN THE MONKEY'S VISUAL CORTEX

Zheng Liu[1], James P. Gaska[2], Lowell D. Jacobson[2] and Daniel A. Pollen[1] [1]Division of Applied Sciences, Graduate School of Sciences and Arts, Harvard University, Cambridge, MA 02138. [2]Dept. of Neurology, Univ. of Massachusetts Medical School, Worcester, MA 01655.

Many neural network models of visual cortical function have postulated local interactions between nearby cells; however, assessment of the validity of these models has been difficult because of the paucity of supporting electrophysiological data with respect to cell connectivity. Here, we report on the connections between physically adjacent cells that were recorded simultaneously by a single electrode. Recordings were made in V1 of the anesthetized Macaque, and a Discovery workstation (BrainWave Systems, Inc.) was employed to discriminate spikes. Constraints on intercell connectivity were inferred by using cross-correlation analysis.

A total of 56 cell pairs recorded from 21 different electrode placements are reported here. Cell types include 11 simple cells, 44 complex cells and 3 cells with intermediate properties. The cell-cell combinations are 55.4% complex-complex, 25.0% simple-complex, 7.1% simple-simple, and 12.5% of pairs contain at least one cell with intermediate characteristics. For the interactions between members of complex-complex cell pairs (31), 48.4% (15) show common excitatory input, 6.4% (2) exhibit common inhibitory input, 12.9% (4) show monosynaptic excitation, and 32.2% (10) have no interaction at all. For simple-complex cell pairs (14), 21.4% (3) show common excitatory input, 42.8% (6) show no interaction, and 35.7% (5) exhibit monosynaptic excitation. Monosynaptic excitation was always directed from simple cell to complex cell. In terms of simple-simple cell pair and pairs which contain at least one "intermediate" cell, the interactions between members could be of either type. Finally, the cell's orientation and spatial frequency selectivity can be related to the interaction between cells.

Supported by NIH EY05156, AFOSR-89-0247, and DAS, Harvard University.

11.7

SYNCHRONIZATION OF NEURONAL FIRING IN AREAS V1 AND V2 OF THE MONKEY.

J. Bullier*, M.H.J. Munk and L.G. Nowak. Groupe Cerveau et Vision INSERM 94 69500 Lyon/Bron France.

Synchronization of firing between neurons located in different visual cortical areas is supposed to play a role in the binding of neuronal populations activated by the same stimulus. So far, such inter-area synchronization has only been demonstrated in the cat in which visual cortical areas receive information through many cortical and subcortical pathways. In contrast, areas V1 and V2 of the macaque monkey are thought to be organized in a serial fashion. One would expect therefore that the spikes in area V2 neurons would be displaced temporally with respect to those of neurons in V1. We have tested this possibility by computing cross-correlograms between single neurons isolated in both areas of anaesthetized and paralyzed macaque monkeys.

Several kinds of peaks could be distinguished in the cross-correlograms : broad peaks (mode 800ms), medium-sized peaks (mode 100ms) and narrow peaks (mode 30 ms). Narrow and medium-size coupling was observed between neurons with receptive field separations up to 4.5 degrees but not beyond; broad peaks were observed up to 7 degrees separation but were very rare beyond. The peaks of the cross-correlograms almost always included the origin of time and the latency most often observed was zero. In other words, many neurons in areas V1 and V2 tend to synchronize their responses to a single visual stimulus.

These results demonstrate that synchronization of firing between neurons located in different cortical areas is universal and does not depend on whether the areas are organized in parallel or serially.

11.8

SYNCHRONIZATION BETWEEN CORTICAL NEURONS DEPENDS ON ACTIVITY IN REMOTE AREAS. J. I. Nelson*, L. G. Nowak, G. Chouvet, M.H.J. Munk & J. Bullier, Groupe Cerveau et Vision, INSERM 94, 69500 Lyon/Bron, FRANCE

Synchronized neuronal firing within and between areas is mediated by cortico-cortical connections (IOVS 1992,33:1020). The connections are thought to unite cell assemblies distributed between the two recording sites (Nelson et al., Visual Neurosci.1992). Synchronized neurons in two areas are connected not only by the reciprocal projections between those areas, but also by reciprocal projections to many other areas. Do these remote areas contribute cells to the distributed assembly which synchronizes the neurons?

INTERHEMISPHERIC cross-correlograms were computed for spike trains from electrodes at the A17/18 border in each cortical hemisphere of the cat. LOCAL correlograms were also computed for neighboring neurons using spikes isolated from the same electrode. Coupling appeared with 3 different classes of correlogram peak widths: T- (1-14ms), C- (20-65ms) and H-type coupling (100-1200ms). If neurons were coupled, we reversibly inactivated PMLS in the retinotopically corresponding region with microinjections of GABA, controlled to silence the neurons around the pipette. In 9 of 15 cases, interhemispheric H-type coupling was reduced on average to 35% (3% - 69%) of original strength, typically recovering well 15 to 30 minutes later. The same effect was observed on LOCAL H-type coupling. We also blocked one A17/18 border region and saw similar decrements in local coupling around the electrode in the other hemisphere.

We conclude that temporally loose synchronization (H-coupling) of neurons within and between cortical areas depends on activity in remote areas. Since the activity from remote areas arrives over two different reciprocal projection systems (A17/18 borders interhemispherically and PMLS to A17/18 border, above) we assume all reciprocal systems participate. These projection systems radiate like a star to many areas, often skipping levels in the cortical hierarchy. We suggest that information transmission and processing in ONE area is modulated by many areas in the star-like reciprocal projection scheme tied to that area. We call this form of functional organization for information exchange in cortex "STAR TOPOLOGY."

11.9

GAMMA-OSCILLATIONS AS A VEHICLE FOR SYNCHRONIZATION. P.König*, A.K.Engel and W.Singer. Max-Planck-Institut für Hirnforschung, Deutschordenstr. 46, 6000 Frankfurt 71, F.R.G.

Increasing evidence supports the idea that cortical representations may be established by a temporal code. Based on studies of the cat visual system, we have argued that synchronization of oscillatory firing patterns may be the relevant code for the binding of distributed neurons into assemblies esthablishing cortical representations. However, these firing patterns, which can be described as recurrent bursting of neuronal groups at frequencies in the γ-band, do not seem to carry information about particular features of the visual input. Hence, their relevance for information processing has remained controversial. In this presentation, we will discuss several potential advantages of these firing patterns: (1) The observed γ-oscillations represent a population phenomenon which allows to establish local groups as intermediate building blocks of cortical representations. (2) Cells engaged in such recurrent firing patterns can be synchronized with zero phase lag despite long conduction delays. (3) Synchronization of oscillators which are not directly linked can be achieved via intermediates, even if the conduction delays show a broad distribution. (4) The broad frequency spectrum of these oscillations makes it easier to desynchronize cell groups and, thus, to segregate several coexisting representations in the temporal domain. (5) Fast oscillations are required for the segregation of several coexisting assemblies, for which a sufficient number of bursts must be accomodated within about 200 ms, the time span required for object recognition. For this purpose, oscillations in the α- and β-range would presumably be too slow. Altogether, these considerations suggest that oscillations in the γ-range be well adapted as a carrier signal, or vehicle, for response synchronization and ,hence, may offer distinct advantages for the establishment of cortical cell assemblies .

11.10

OSCILLATORY RESPONSES IN THE SUPERIOR TEMPORAL SULCUS OF ANAESTHETIZED MACAQUE MONKEYS. A.K. Engel*, A.K. Kreiter and W. Singer. Max-Planck-Institut für Hirnforschung, Frankfurt, F.R.G.

Previously, Kreiter and Singer have demonstrated oscillatory responses and their synchronization between different recording sites in the STS of an awake behaving macaque. We have now performed multi-unit recordings in the caudal STS of two anaesthetized macaque monkeys. Contrary to the results of Young et al. (Soc.Neurosci.Abstr.17,176) we have observed oscillatory responses with frequencies in the gamma range. At the majority of 70 recording sites studied quantitatively we have found that local groups of cells engaged in synchronous recurrent bursting. As in the awake monkey, the intervals between successive bursts were variable within and between trials and ranged from 15 to 35 ms. In addition, we have observed a response synchronization between spatially separate cell groups. These results corroborate the hypothesis that a temporal code may be used for assembly formation in the monkey visual system.

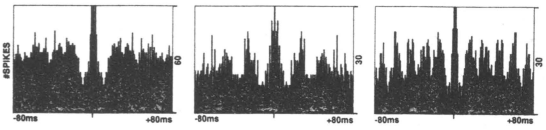
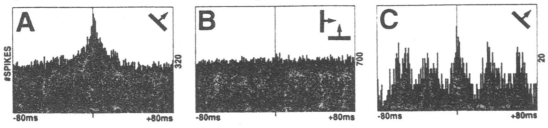


Figure: Autocorrelograms of STS responses, computed for 500ms data epochs (average over 10 trials). We frequently observed correlograms with a broad center peak flanked by troughs (left), indicating a broad band oscillation with highly variable interburst intervals. At the majority of recording sites, we also found epochs where the correlograms exhibited clear satellite peaks (middle, right), showing that the burst-and-pause sequences, which we term oscillations, had a comparatively narrow frequency band. Due to the high variability within and between trials, the correlogram modulation is often blurred if extensive averaging is performed.

11.11

STIMULUS DEPENDENT SYNCHRONIZATION IN THE CAUDAL SUPERIOR TEMPORAL SULCUS OF MACAQUE MONKEYS. A.K. Kreiter*, A.K. Engel and W. Singer. Max-Planck-Institut für Hirnforschung, Frankfurt, F.R.G.

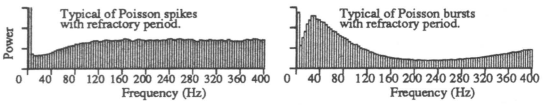
It has been inferred from experiments in cat visual cortex that synchronization of neuronal responses depends on global aspects of stimulus configuration and could serve as a mechanism to bind features of individual visual objects. To investigate whether synchronization occurs also in monkey visual cortex, we recorded with two closely spaced electrodes in the caudal STS of anaesthetized macaques. When spatially segregated cell groups with overlapping receptive fields but different preferred directions of motion were activated with a *single* light bar, moving in a direction intermediate between the respective preferences, the cells synchronized their responses (Fig. A). However, if *two* bars were moved simultaneously in the respective preferred directions, synchronization was much weaker or disappeared (Fig. B). The responding cells typically exhibited oscillatory firing patterns with frequencies in the gamma-band. Over short epochs these oscillations could have sufficiently constant frequencies to yield oscillatory cross-correlograms with multiple peaks and troughs (Fig. C, cross-correlogram of a 400ms response epoch, taken from a single trial). These results demonstrate that neurons can synchronize their activity if they are activated by a single object, whereas they desynchronize if they respond to different objects. Thus, synchronous activity might define the set of neurons whose responses represent features of the same visual object.



11.12

POWER SPECTRUM ANALYSIS OF MT NEURONS FROM AWAKE MONKEY W. Bair, C. Koch, W. Newsome[1], K. Britten[1], E. Niebur*. Computation and Neural Systems, Caltech, Pasadena, CA 91125; Dept. of Neurobio., [1]Stanford Univ. Sch. of Med., Stanford, CA 94305.

The widely held notion that nerve cells only encode information using mean firing rate has recently been questioned, in particular due to stimulus-related 30–70 Hz oscillations observed in cat V1. We investigated temporal fine structure of single cell spike trains in area MT during a demanding direction discrimination task (Newsome, Britten and Movshon, 1989) and related this to simultaneous behavioral measurements. Two characteristic power spectra are shown below. About half of 200 cells have a flat spectrum with a dip at low temporal frequencies (characteristic of a Poisson process with refractory period) while the others have a peak in the 25–50 Hz band. The peak is correlated to bursting and is explained by a cell that fires Poisson distributed bursts with a burst-related refractory period without assuming explicit oscillatory firing patterns. We find no statistically significant correlation between spectral peak and behavior.



NERVE GROWTH FACTOR I

12.1

VISUAL INPUT REGULATES THE EXPRESSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) mRNA IN RAT VISUAL CORTEX. Eero Castrén*, Hans Thoenen and Dan Lindholm. Department of Neurochemistry, Max Planck Institute for Psychiatry, Am Klopferspitz 18A, 8033 Martinsried, Germany

Neuronal activity regulates BDNF and nerve growth factor (NGF) mRNA in hippocampal neurons. We have now investigated whether physiologically relevant neuronal activity regulates the expression of these neurotrophins by studying the BDNF and NGF mRNA expression in the visual cortex, where the input can readily be manipulated. In rats that were kept in total darkness for a week, BDNF but not NGF mRNA in the visual cortex was reduced to about about 50% of that of control rats kept in normal animal house illumination with a 12-hour light-dark cycle. The exposure to normal animal house illumination after a period of total darkness for 7 days restored the BDNF mRNA to the control levels within 1 h. Darkness or light exposure had no effect on the NGF mRNA in the visual cortex. To study whether the neuronal activity of the retino-cortical pathway was responsible for the increase in BDNF mRNA, we injected tetrodotoxin (TTX) into the right eye and measured BDNF mRNA in ipsi- and contralateral visual cortex. Already six hours after the injection, the BDNF mRNA had decreased significantly in the left visual cortex compared to the right side. Intravitreal injection of saline had no effect on the BDNF mRNA in the visual cortex.

In situ hybridization showed that BDNF mRNA is expressed in individual cells in the layers II-III and VI in adult rat visual cortex. Keepting rats in darkness markedly reduced the number of BDNF mRNA positive neurons in both superficial and deep cortical layers. The neurons in layers II-III are predominantly cortico-cortical assosiation neurons, which receive input directly from the primary targets of the LGN afferents in the layer IV. Recent experiments suggest that adult sensory cortex may have more plasticity than was previously believed. Neurons, whose receptive fields have been destroyed, can aquire new receptive fields over a considerable distance and at least in visual cortex, this effect is remarkably rapid. Cortico-cortical association connections have been implicated in this process. The expression and the regulation of BDNF mRNA in the layers, where these association neurons are situated suggests that neurotrophic factors may play a role in the plasticity of adult visual cortex.

12.2

REGULATION OF NEUROTROPHIN GENE EXPRESSION IN THE RAT CNS. D. Lindholm*, E. Castren, M. Berzaghi, A. Leingärtner, F. Zafra and H. Thoenen. Dept. of Neurochemistry, Max-Planck-Institute for Psychiatry, Am Klopferspitz 18A, 8033 Planegg-Martinsried, FRG

In the CNS, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are predominantly expressed by neurons and regulated by neuronal activity. Limbic seizures and injection of kainic acid, a glutamate receptor agonist, increased mRNA levels of these factors in the hippocampus. The increase in NGF mRNA was confined to the dentate gyrus whereas BDNF mRNA increased in the hippocampal pyramidal neurons as well as in dentate granule cells. The GABA-A receptor agonist, muscimol and the NMDA receptor antagonist, MK-801 both reduced NGF and BDNF mRNA levels in the hippocampus *in vivo*. Moreover, NGF protein levels decreased in hippocampus and in the septum after muscimol and MK-801 treatments. The results demonstrate that there is a balance expression of NGF and BDNF *in vivo* in adult rats. In neonatal rats, however, hippocampal BDNF mRNA levels were not increased by kainic acid but by pilocarpine, acting on muscarinic receptors. Moreover, the relative contribution of glutamate and the cholinergic system to the up-regulation of neurotrophic gene expression seem to change with age. In contrast to NGF and BDNF, neurotrophin-3 (NT-3) is not regulated by neuronal activity. However, NT-3 mRNA levels seem to be regulated by hormones as shown in rat cerebellum during development.

12.3

NEUROTROPHIN RESPONSIVENESS OF RAT BRAIN NEURONS. B. Knüsel, D.R. Kaplan, H.R. Widmer, K. Nikolics* and F. Hefti. Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA 90089; NCI-Cancer Research and Development Center, Frederick, MD 21701; Genentech Inc., South San Francisco, CA 94080.

Expression of brain-derived neurotrophic factor (BNDF) and neurotrophin-3 (NT-3) in rat brain are regionally specific and developmentally regulated. Little is known about responsive cell populations. We show phosphorylation of *trk* type proteins after short-term (4 min) stimulation of primary cultures of different areas of embryonic rat brain with BDNF, NT-3 and NGF. Assay was by immunoprecipitation and Western blotting. NGF phosphorylation signal was detected in basal forebrain cultures only, and was much weaker than the phosphorylation signal after BDNF or NT-3 stimulation. BDNF and NT-3 evoked similar signals in cultures of basal forebrain, ventral mesencephalon, striatum and cortex and the two factors were not clearly additive when added in combination. Pretreatment of the cultures for 5 min resulted in reduced effect at later stimulation with the same factor, the reduction being proportional to the delay between pretreatment and final treatment. The relative refractory period lasted up to one day. Prolonged, 3-day pretreatment of cortical cultures with BDNF or NT-3 completely abolished any response to subsequent stimulation with the same factor, while a minor proportion of the response to the other neurotrophin remained. Our results show that neurons from different areas of embryonic rat brain are responsive to BDNF and NT-3. A major part of the response measured at the level of *trk* type proteins does not distinguish between these two related proteins. It remains to be seen whether the part of the phosphorylation signal which is selective for either factor reflects a functional response specific for each neurotrophin.

12.4

IS K-252a A NON-COMPETITIVE PARTIAL AGONIST OF HIGH AFFINITY NGF RECEPTORS? M.E. Lewis, K.V. Callison and N. Neff*, Cephalon, Inc., West Chester, PA 19380.

The protein kinase inhibitor, K-252a has been reported to inhibit a wide variety of NGF-stimulated responses in PC-12 cells, such as neurite outgrowth, ornithine decarboxylase (ODC) induction, and increased c-fos transcription. Following the identification of trk as the high-affinity NGF receptor, K-252a was reported to block the NGF-induced autophosphorylation of trk. Thus, K-252a has been suggested to act as an NGF antagonist at the level of signal transduction. Paradoxically, though, several investigators have reported that K-252a or related compounds, such as staurosporin, exhibit some NGF-like pharmacological actions. To begin to explore this paradox, we studied the effects of a wide range of concentrations of K-252a and staurosporin, in the presence or absence of NGF, on ODC activity in PC-12 cells. At low concentrations, both K-252a and staurosporin stimulated ODC activity and potentiated the ability of NGF to induce this enzyme. At higher concentrations, however, both compounds inhibited the ability of NGF to induce ODC activity, as reported before. One model to explain these findings is that the compounds act like agonists/potentiators via a high-affinity allosteric site on trk, and like antagonists via a dominant, lower affinity site on trk. In this model, therefore, K-252a could be functionally defined as a noncompetitive partial agonist at the high-affinity NGF receptor. This model generates testable predictions which are now under experimental evaluation.

12.5

INTERLEUKIN-1 BETA AND SCIATIC NERVE EFFECTS ON NEURONAL DIFFERENTIATION OF CULTURED PC-12 CELLS. E.S. Lo*, K.B. Kim[1], L.R. Ptak, J.H. Kordower, and P.M. Carvey. Dept. Neurological Sciences, Rush-Presbyterian St. Luke's Med Ctr and [1]University of Illinois School of Medicine, Chicago, IL 60612.

Previous studies have shown that Interleukin-1 Beta (IL-1) stimulates schwann cells to release nerve growth factor (NGF). NGF is known to induce the neuronal differentiation of PC-12 cells. We examined the ability of IL-1 to potentiate NGF-mediated differentiation of PC-12 cells by sciatic nerve explants. Sciatic nerve was removed from adult rats and cut into 0.5 mm segments. Between 1 to 4 sciatic nerve segments were cultured in complete media on collagen-coated plates for one week in the presence or absence of 1 to 8 pellets each containing 0.18 μg of IL-1. After 1 week, 100 μL of this "conditioned" media was collected and transferred to freshly plated, low cell density (1,750 cells/well) PC-12 cell cultures growing in PC-12 media (100 μL). After 4 days in culture, the number of cells with processes greater than 1 cell diameter was counted. Conditioned media from sciatic nerve explant cultures stimulated the differentiation of PC-12 cells into a neuronal morphology. This effect was dependent upon the number of explants in culture ($r = 0.984$). IL-1 alone also induced PC-12 differentiation in a dose-dependent fashion ($r = 0.974$). Conditioned media from cultures containing both sciatic nerve explants and IL-1 had an additive effect on PC-12 differentiation which was also dose-dependent. These results suggest that IL-1 has a direct effect on PC-12 cell differentiation and enhances NGF-mediated differentiation. Supported by NS29585 (JHK).

12.6

BRIEF DEPOLARIZING STIMULI ELICIT INCREASES IN CULTURED HIPPOCAMPAL NEURON NGF GENE EXPRESSION. R.C. Elliott*, C.F. Dreyfus, and I.B. Black, UMDNJ/Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

Previous studies in our laboratory and others have shown that addition of depolarizing concentrations of KCl or the sodium channel agonist veratridine to cultures of embryonic day 18 (E18) rat hippocampal cultures can elicit increases in NGF mRNA. To more fully define the temporal relationship between depolarization and message expression we have pursued investigations into the kinetics of this increase, starting with a more detailed time-course study of message levels over two days of exposure to KCl.

Virtually pure dissociated neuronal cultures of rat E18 hippocampi were grown in fully-defined, serum-free medium for five to six days prior to treatment with depolarizing concentrations of KCl (50 mM). Cultures were treated for 6, 24, or 48 hours, after which RNA was immediately harvested. To measure subtle changes in NGF mRNA expression, a highly sensitive and efficient solution hybridization technique (Elliott et al., Society for Neuroscience Abstracts, 1991), was utilized to quantitate message levels.

Compared to mock-treated controls, increases in NGF mRNA levels were evident as early as 6 hours after treatment began. The magnitude of the depolarization-elicited increase rose gradually as the duration of depolarization continued. After 48 hours of treatment with depolarizing agents, NGF message levels had risen by 40% over that of control groups. Our results suggest that relatively brief exposure to depolarizing stimuli increases NGF gene expression. Further experiments will determine the degree of persistence of these depolarization-induced increases in NGF mRNA levels.

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12.7

REGULATION OF TRK PROTO-ONCOGENE EXPRESSION IN HUMAN MONOCYTES. P. Ehrhard, U. Ganter, J. Bauer, A. Stalder and U. H. Otten*. Dept. of Physiology, Univ. of Basel, CH-4051 Basel, Switzerland.

Increasing evidence indicates that neurotrophins, including nerve growth factor (NGF), exert specific effects on cells of the immune system in addition to their neurotrophic actions. Recent studies have established that trk proto-oncogene, a member of the family of receptor tyrosine protein kinases, is necessary for NGF signal transduction. Using reverse transcription in combination with polymerase chain reaction the regulation of trk proto-oncogene expression in human monocytes was investigated. We found that a highly purified population of human blood monocytes expresses significant levels of trk proto-oncogene mRNA. During in vitro differentiation of human blood monocytes to macrophages, trk expression decreased suggesting maturation-dependent trk regulation. Treatment of monocytes with 1 mM dibutyryl cyclic AMP (dbcAMP) stimulated trk synthesis in a time-dependent manner. To analyse cell type-specific trk expression further we used the promonocytic cell line U 937. We found that dbcAMP (1 mM) elicited a time-dependent trk induction in phorbol-ester-differentiated U 937 cells. In contrast, no effect of dbcAMP was observed in undifferentiated cells. Our finding that treatment of human monocytes with NGF resulted in a significant increase in oxidative burst suggests that trk stimulation has functional importance. Since both NGF protein and monocytes accumulate at sites of lesion and inflammation it is attractive to postulate that NGF-trk interactions modulate nerve regeneration and immune responses.

12.8

TRANSCRIPTS FOR THE NGF RECEPTORS $gp140^{trk}$ AND $gp80^{LNGFR}$ ARE DECREASED IN DRG FOLLOWING AXOTOMY C.A. Krekoski, S. Lee, I.M. Parhad, A.W. Clark*. Depts of Pathology and Clinical Neuroscience, Univ. of Calgary, Alberta, Canada.

NGF receptor (NGFR) consists of two proteins, $gp80^{LNGFR}$ and $gp140^{trk}$ which contribute to its function. NGF induces expression of $gp80^{LNGFR}$, but its effect on $gp140^{trk}$ has not been defined. Axotomy interrupts the retrograde transport of target-derived NGF, which is severely decreased by 1 day and begins to recover by 21 days. Our hypothesis is that the decrease in supply of NGF or other target derived factors after axotomy leads to decreased expression of $gp80^{LNGFR}$ and $gp140^{trk}$. We evaluated the mRNA levels for these proteins in the DRG after axotomy. In adult rats the sciatic nerve was crushed in midthigh, or transected and ligated to prevent regeneration; the L5-L6 DRG were dissected (4, 7, 14, 30 & 60 days; 5 rats/ time point), and quantitative Northern analysis performed. $Gp80^{LNGFR}$ and $gp140^{trk}$ mRNAs were both decreased to 60% of controls at 4-14 days post axotomy (U test, $p < 0.02$) and returned to slightly above normal at 60 days, a time that coincides with reinnervation. When regeneration was blocked, $gp80^{LNGFR}$ and $gp140^{trk}$ mRNA levels were only slightly decreased (15%, 6% respectively; $p > 0.1$) at 60 days. Thus $gp80^{LNGFR}$ and $gp140^{trk}$ expression decline coordinately early after axotomy, and later recover irrespective of reinnervation. These results suggest that target derived factors are required for the maintenance of NGFR proteins early following axotomy, but that non-target derived factors can appropriate this function at later times.

12.9

REVERSAL OF AXONAL INJURY-INDUCED ABERRANT NEUROFILAMENT PHOSPHORYLATION IN SENSORY NEURONAL CELL BODIES BY NGF. B.G. Gold*†^, D.R. Austint, W.C. Mobley# and T. Storm-Dickersont. †CROET, ^Dept. Cell Biol. & Anat., Oregon Hlth Sci. Univ., Portland, OR 97201 and #Depts. Neurol. Pediat., UCSF, San Francisco, CA 94143.

We have previously proposed (Gold et al., J. Neurosci. 11:943, 1991) that the neuronal cell body response to injury (axon reaction) is initiated by an interruption of target-derived supply of NGF. In the present study, we examined the regulation by NGF on aberrant expression of phosphorylated neurofilament (pNF) epitopes (another component of the axon reaction) in dorsal root ganglion (DRG) cell bodies. Rats underwent a sciatic nerve crush or were given β,β'-iminodipropionitrile (IDPN) or 3,4-dimethyl 2,5-hexanedione (DMHD) which have been previously found to induce pNF expression in 45-50% of DRG neurons. NGF (125 ng/hr) was continuously infused into the subarachnoid space of the lumbar spinal cord via a osmotic minipump (Alzet); controls received cytochrome C. The number of immunoreactive DRG cell bodies to antibody 06-17 (directed against pNF epitopes) was reduced in animals given NGF for 14 days (to 21%, 25% and 31% in axotomized, IDPN- and DMHD-treated rats, respectively). The number of immunostained cells was not as decreased at 7 days, indicating that NGF reverses (as opposed to prevents) pNF expression. Reduction in immunostaining to the low-affinity NGF receptor (antibody 192) by these protocols was restored by NGF infusion. Since axotomy decreases high-affinity binding it is unclear how reversal of pNF expression by NGF is mediated, although loss of the low-affinity receptor (Verge et al., Soc. Neurosci. Abst. 17:1498,1991) implicates trkA. Supported by NS19611 and NS26265.

12.10

REGULATION OF NGF RECEPTOR mRNAs DURING LESION-INDUCED COLLATERAL SPROUTING IN NEURONAL POPULATIONS IDENTIFIED BY TRANSYNAPTIC NEURONAL LABELING. G.A. Kuchel[1,2,3]* and M. Blum[1], Fishberg Res. Ctr. in Neurobiology[1] and Dept. Geriatrics[2], Mt. Sinai School of Medicine, New York, NY 10029; Mtl.Gen.Hospital Res. Inst.,Montreal,Canada[3].

The rat pineal gland receives bilateral innervation from the two sympathetic superior cervical ganglia (SCG) through internal carotid nerves (ICN). We have demonstrated that levels of low-affinity NGF receptor ($p75^{NGFR}$) mRNA undergo a specific 25% increase in the SCG from which collateral neuronal sprouting takes place following a contralateral ICN cut lesion (*Exp.Neurol.*,117:3,1992).

Injection of right SCG with WGA-rhodamine resulted in labeling of some SCG neurons in the left SCG. Control experiments suggest that these cells represent a subpopulation of SCG neurons providing innervation to those target tissues which are also innervated from the opposite SCG. WGA appears to be transported anterogradely to these targets, across nerve terminals and then retrogradely to the opposite SCG. Fast Blue was injected into the left eye or left peri-aural skin and this was followed 2-3 days later by a right ICN cut or sham operation. *In-situ* hybridization using ^{35}S-riboprobes complementary for $p75^{NGFR}$ mRNA revealed significant (2-3x fold) increases in levels of this mRNA in WGA-rhodamine labeled cells, but not in cells labeled with Fast Blue, as soon as 1 day after this lesion. Preliminary studies demonstrate a similar pattern for the $p140^{prototrk}$ gene which contributes to the high-affinity NGF receptor. These changes take place before we can detect collateral sprouting and are consistent with the hypothesis that regulation of the NGF receptor genes may play a role in the sequence of events resulting in the collateral sprouting response.

12.11

DIFFERENTIAL REGULATION OF ASTROGLIAL NERVE GROWTH FACTOR mRNA CONTENT BY INTERLEUKIN-1, TPA AND STEROIDS. S.P. Pshenichkin, A.M. Szekely and B.C. Wise*. Fidia-Georgetown Institute for the Neurosciences, Georgetown University, Washington, D.C. 20007.

Nerve growth factor (NGF) expression in astroglial cells is regulated by neurotransmitters, growth factors and cytokines. Our previous studies showed that interleukin-1 (IL-1) increases NGF mRNA content and NGF secretion in primary cultures of rat cortical astrocytes. Because protein kinase C activation mediates many of the cellular effects of cytokines and because steroids are physiological antagonists of cytokine action, we studied their role in astrocyte NGF regulation by IL-1. TPA, a protein kinase C activator, increases (EC_{50}=10 nM) by 6-10 fold NGF mRNA content followed in time by increases in cell content and secretion of NGF. The induction of NGF mRNA content by TPA is transient; the maximal increase is at 3 hrs and by 18 hrs the mRNA content has returned to basal levels. IL-1 treatment (10 U/ml), on the other hand, induces a prolonged increase in NGF mRNA content. The stimulatory action of TPA on NGF expression is additive with maximal amounts of IL-1, indicating different mechanisms of action by these two agents. In contrast to the IL-1 induced stabilization of NGF mRNA, TPA fails to change NGF mRNA half-life (control, $t_{1/2}$=35 ± 5 min; TPA treated, $t_{1/2}$=45 ± 4 min). As assessed by nuclear run-on assays, TPA and IL-1 differentially affect the transcriptional rate of NGF and *c-fos* genes, the latter being implicated in the transcriptional regulation of NGF expression. The positive modulation of NGF expression by IL-1 or TPA is inhibited by dexamethasone (IC_{50}=3 nM) with maximal inhibition apparent by 2 hrs of treatment. The rank order of potency of active steroids are dexamethasone > hydrocortisone > corticosterone > aldosterone. Other ligands of the steroid/thyroid receptor superfamily fail to alter astroglial cell NGF production. Thus, there are distinct regulatory mechanisms, sensitive to steroid repression, mediating IL-1 and TPA facilitation of astroglial cell NGF expression.

12.12

RECEPTOR-MEDIATED CYTOSOLIC CA^{++} TRANSIENT IS INDEPENDENT OF RECEPTOR MEDIATED STIMULATION OF NGF SECRETION. J.B. Tuttle*, D. Layton and D.J. Creedon, Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, VA 22908

Smooth muscle cells (SMC) synthesize and secrete NGF that supports innervating neurons. Cultured SMC's increase NGF synthesis and secretion during mitotic growth, with fresh culture medium, contractile agonists (vasopressin, α-adrenergic) and muscle stretch. NGF secretion is inhibited by ß-adrenergic and adenosine (ADO) receptor activation. However, when angiotensin II (AngII) is present, AngII and ADO together increase NGF mRNA and secretion. AngII alone has no effect on NGF output. The relationship between receptor-mediated transient increases in cytosolic $[Ca^{++}]_i$ and regulation of NGF output was examined by testing for interactions between ADO and AngII during indo-1-based measurement of $[Ca^{++}]_i$. AngII (1μM) transiently elevates $[Ca^{++}]_i$, independent of the presence of ADO or the ADO receptor agonist NECA. With endogenous ADO eliminated by ADO-Desaminase (2.5U/ml), the ADO agonist NECA alone caused no change or a slight reduction in $[Ca^{++}]_i$. Thus, a negative $[Ca^{++}]_i$ transient is not required for ADO inhibition of NGF output and AngII causes a positive transient even when not activating NGF output. The data suggest changes in cytosolic Ca^{++} are not necessary and sufficient to regulate NGF synthesis and secretion in SMC's.

12.13

RAS p21: A SECOND MESSENGER FOR NEUROTROPHIC FACTORS. G.D. Borasio*[1], A. Markus[2], A. Wittinghofer[3], Y.-A. Barde[4] and R. Heumann[2]. [1]Neurologische Univ.-Klinik, Klinikum Großhadern, D-8000 München 70; [2]Lehrstuhl f. Neurobiochemie, Ruhr-Univ., D-4630 Bochum 1; [3]Abt. Biophysik, Max-Planck-Inst. f. med. Forschung, D-6900 Heidelberg; [4]Abt. Neurobiochemie, Max-Planck-Inst. f. Psychiatrie, D-8033 Martinsried, Germany

We have previously shown that cytoplasmic introduction of the oncogene product ras p21 into different types of cultured chick embryonic neurons promotes their survival and fiber outgrowth, mimicking the effects of nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF), respectively. To assess the potential signal-transducing role of the endogenous ras p21 we microinjected function-blocking anti-ras antibodies and their biologically active, affinity-purified Fab fragments into cultured chick embryonic neurons. The antibodies blocked BDNF-induced neurite outgrowth in E12 nodose ganglion neurons to below control levels, while the Fab fragments inhibited in a dose-dependent fashion the NGF-induced survival of E9 dorsal root ganglion (DRG) neurons. Both effects could be reversed by saturating the epitope-binding sites with biologically inactive ras p21 prior to microinjection. We have previously shown a strong survival-promoting activity of the protein kinase inhibitor K-252a in chick DRG neurons. This survival effect was not affected by the Fab fragments. Surprisingly, ras p21 showed no survival activity on NGF-dependent E12 chick sympathetic neurons, and the NGF-induced survival in these cells was not inhibited by the Fab-fragments. These results support the concept of an involvement of ras p21 in the intracellular signal transduction of neurotrophic factors, and raise the possibility of the existence of multiple signal transduction pathways for NGF in different neuronal populations of the chick embryo.

13.1

GABA-INDUCED INTRACELLULAR Ca^{2+} RESPONSES OF THE B PHOTORECEPTORS OF *HERMISSENDA*. K.Oka[1], E. Ito[1] C. Collin[1*], T. Yoshioka[2] and D.L. Alkon[1]. [1]Neural Systems Section, NINDS, NIH, Bethesda MD, 20892, [2] Dept. Molecular Neurobiology., Sch. Human Sci., Waseda University., Tokorozawa 359, Japan.

Previous findings implicate GABA as an inhibitory neurotransmitter released by pre-synaptic hair cells onto post-synaptic B photoreceptors in the snail *Hermissenda*. This synaptic inhibition can also be transformed into excitation after pairings of GABA stimulation and post-synaptic depolarization. Under voltage-clamp the synaptic transformation of the B cell has been shown to be caused by the modulation of $GABA_A$ and $GABA_B$ coupled I_{Cl^-} and a $I_{Ca^{2+}\_K^+}$. Because a GABA-induced $I_{Ca^{2+}}$ was ruled out, here we studied the intracellular Ca^{2+} responses to a 15 sec, 10 μM GABA pulse onto the B cell's terminals, or the Ca^{2+} responses to membrane depolarization, and their role in the synaptic transformation. We used digital ratio imaging (F340/F380) of a single fura-2 stained B cell (while it was impaled with a microelectrode). Samples were taken every .2 or 2 sec, while UV stimulation was restricted to the axon to avoid light-induced responses. 15 to 20 sec after GABA stimulation, persistent (60 sec) intracellular Ca^{2+} elevation was observed at the terminals, outlasting the GABA-post-synaptic potentials (psp). After light-GABA pairings, GABA-induced Ca^{2+} signals were prolonged by an additional 20 sec. Co^{2+} (.2mM) blocked GABA psp's, but not its Ca^{2+} signal. Membrane depolarization (with high external K^+) also induced intracellular Ca^{2+} elevation that had a much faster onset than the GABA-induced Ca^{2+} signals, that followed membrane potential, and that were blocked by Co^{2+}. These data suggest that GABA-induced Ca^{2+} elevation and synaptic transformation can play a crucial role in producing previously observed long-lasting biophysical and molecular changes following classical conditioning of the snail *Hermissenda*.

13.2

ASSOCIATIVE LEARNING PROLONGS GABA-MEDIATED Ca^{2+} RESPONSES OF THE B PHOTORECEPTORS OF *HERMISSENDA*. E.Ito, K.Oka, B.G. Schreurs, C.Collin and D.L.Alkon*. Neural Systems Section, NINDS, NIH, Bethesda MD 20892.

We studied GABA-induced intracellular Ca^{2+} responses of the post-synaptic B cells, after associative learning in *Hermissenda*, using digitized imaging (F340/F380) of fura-2 injected B cells. Associative learning resulted from 3 days of paired presentations of light and orbital rotation and not from unpaired presentations or in naive controls. While the B cell was impaled with a microelectrode, voltage-dependent Ca^{2+} signals at the terminal branches were elicited by 2sec depolarizing current pulses, followed later by a 15sec GABA perfusion (10μM) that elicited Ca^{2+} signals at the branches. The same stimulation was repeated after a series of 3 pairings of a 3sec flash of light with GABA. The sustained phase of the Ca^{2+} signal with the first GABA stimulation persisted for more than 3min in conditioned cells ($N=7$; $F340/F380=0.82\pm0.07$ (mean±SD(N-1)) at 60sec after the GABA stimulation) but control cells had only 60sec responses ($N=4$; $F340/F380=0.60\pm0.06$ at 60sec; $p<0.01$). No conditioning-specific differences were observed in the latency times or peak values of these Ca^{2+} responses. After 3 light-GABA pairings, control cells also showed 90sec Ca^{2+} responses induced by the second GABA stimulation, which was 20-30sec longer than that of the first GABA stimulation ($N=4$; $F340/F380=0.77\pm0.03$ at 90sec). Conditioned cells, however, showed higher resting Ca^{2+} levels and they did not become more prolonged after further GABA stimulation at al in the terminals ($N=4$). No significant differences were observed among groups with depolarizing-induced Ca^{2+} signals.

These data suggest that associative learning causes a GABA-induced Ca^{2+} accumulation at the terminal branches of the B cell, which plays an important role in long-term transduction of associative memory signals.

13.3

SYNAPTIC INTERACTIONS BETWEEN PAIRS OF IDENTIFIED TYPE A & B PHOTORECEPTORS IN CONDITIONED *HERMISSENDA*. R.J. Frysztak* & T. Crow. Dept Neurobiology & Anatomy, Univ Texas Med Sch, Houston, TX 77030

Classical conditioning results in intrinsic modification of the excitability of type A & B photoreceptors in *Hermissenda*. The mutually inhibitory synaptic connections between A and B photoreceptors have been well documented, however changes in the strength of connections between identified pairs of A and B photoreceptors in conditioned animals has not been previously examined. We recently reported that the lateral A photoreceptor exhibits a significant increase in the light-elicited spike discharge rate and enhanced excitability expressed by increased spike frequency to extrinsic current (Frysztak & Crow, 1991). As a first step in the analysis of the potential contribution of altered synaptic inhibition to the enhanced excitability observed in conditioned animals, we examined changes in spike elicited unitary IPSPs in type A photoreceptors. Here we report that conditioning does not significantly effect the amplitude of unitary IPSPs recorded in lateral type A photoreceptors, that are elicited by single action potentials in lateral type B photoreceptors (conditioned; $\bar{x}=0.72$mV, n=6; pseudorandom controls: $\bar{x}=0.57$mV, n=5; NS). A second assessment of synaptic inhibition consisted of an analysis of decreased spike activity in A photoreceptors produced by a current step in the lateral B photoreceptor. Consistent with the analysis of unitary IPSPs, activation of the lateral B did not significantly alter the spike frequency elicited by extrinsic current in the lateral A photoreceptor of conditioned animals ($\bar{x}_d=0.01$, n=5) as compared to controls ($\bar{x}_d=0.08$, n=5; NS). In contrast to the analysis of the pairs of lateral A and B photoreceptors, preliminary evidence suggests that unitary IPSPs recorded in the medial A, elicited by single action potentials in the medial B photoreceptor, are enhanced by conditioning. These results indicate that enhanced synaptic inhibition does not provide an important functional contribution to correlates of conditioning detected in intact lateral A photoreceptors, but potentially could contribute to altered activity in other postsynaptic targets.

13.4

CALCIUM TRANSIENTS, NOT ACCUMULATION, INDUCE BIOPHYSICAL CORRELATES OF LEARNING IN *HERMISSENDA*. L.D. Matzel*, R.F. Rogers, and D.M. Fass
Department of Psychology, Rutgers University, New Brunswick, NJ 08903

During *in vivo* and *in vitro* pairings of light and rotation, the B photoreceptors in the *Hermissenda*'s eye undergo an accumulating depolarization. This "cumulative depolarization" has been cited as a principal contributor to the initiation of Ca^{2+}-dependent processes which result in the phosphorylation of membrane proteins and a reduction of neuronal K^+ conductance indicative of memory formation. Using an *in vitro* preparation in which a 5 sec light was paired with mechanical stimulation of the vestibular organ, the statocyst, it was determined that 1, 2, or 9 pairings resulted in a progressive reduction of K^+ conductance and an increase in resistance across the soma membrane of the B photoreceptors, although a progressive depolarization was not observed following 1 pairing. The biophysical modifications were not observed when the stimulus pairing(s) was preceeded by an iontophoretic injection of the Ca^{2+} chelator EGTA into the B photoreceptor. In a subsequent experiment, the B photoreceptor was held at -60 mV (via current injection) both before and after each of 9 pairings of light and statocyst stimulation, thus eliminating any accumulation of depolarization. An increase in resistance in the B photoreceptor was observed relative to unpaired controls. Moreover, this increase was greater than that seen following pairings in which the B photoreceptor was allowed to cumulatively depolarize following each pairing. This diminution of learning by cumulative depolarization arose from a post-pairing inactivation of the voltage-dependent Ca^{2+} current, thus attenuating Ca^{2+} influx on subsequent pairings.

In total, these results indicate that cumulative depolarization is neither necessary or sufficient for the induction of learning in this system. Rather, it appears that presynaptic activity, paired with a transient postsynaptic Ca^{2+} rise is the critical determinant of memory induction.

13.5

CONDITIONED AND UNCONDITIONED RESPONSES TO ALTERED SEAWATERS IN *APLYSIA* ARE QUALITATIVELY DIFFERENT. M. Levy & A..J. Susswein*. Dept Life Sci, Bar Ilan Univ, Ramat Gan, Israel 52 900

In *Aplysia fasciata*, shocks delivered in the presence of 85%, 120% or pH 7.0 seawater leads to pairing-specific increases in the respiratory pump rate when animals are re-exposed to the same altered seawaters an hour later. Respiratory pumping is also elicited by 75%, 140% or pH 6.0 seawater, independent of previous pairing with shock. We tested the hypothesis that the learning occurs by amplifying the response to sub-threshold stimuli, so that after pairing these stimuli elicit responses similar to those elicited by supra-threshold stimuli. Both conditioned and unconditioned responses to decreased pH were affected by treatment with the neurotoxin 5,7-DHT, and by lesioning the cerebral-pleural connectives. These treatments did not affect either conditioned or unconditioned responses to changes in seawater concentration. However, significant differences were seen between conditioned and unconditioned responses with to altered seawaters. Part of the unconditioned response to altered seawaters is an increase in defensive responses, such as escape swimming and inking. Also, exposure to 75%, 140% and pH 6.0 seawaters produces sensitization of respiratory pumping upon re-exposure to these stimuli. No increase in inking was seen as part of the conditioned responses, while an increase in swimming seen after training could be attributed to sensitization as a result of shock. In addition, patterns of specificity or generalization of sensitization were different from those seen as a result of associative learning. These data indicate that associative learning does not arise from by a simple amplification of subthreshold responses, as has been suggested for classical conditioning of the gill withdrawal reflex, but rather learning leads to qualitatively different responses than those elicited by supra-threshold stimuli independent of pairing.

13.6

THE MEMORY FOR HABITUATION OF SWIMMING IN *TRITONIA* IS REGIONALIZED. G. D. Brown*, P. A. Getting, and A. O. D. Willows. Friday Harbor Laboratories, Friday Harbor, WA 98250.

Habituation is a reduced responsiveness to repetitive stimuli. When the escape swim response of the sea slug *Tritonia* is repeatedly elicited, multiple behavioral changes occur. The duration of the swim decreases (Abraham and Willows, 1971), and the strength of the swim flexion movements gets weaker (Brown et al, 1998). When the nervous system is isolated and nerve root stimulation is used to repeatedly initiate the neural program for swimming, neuronal analogs for the behavioral changes seen during habituation can be found in the flexion neurons which control the muscles used for swimming (Brown et al, 1988).

During habituation in *Triotonia*, it is likely that multiple neuronal modifications occur in the animal's nervous system. Synaptic connections made by afferents which trigger swimming decrement when repeatedly activated (Getting, 1976). The excitatory parts of swim interneuron C2 synapses also get weaker with repeated use (Snow, 1978).

It is possible to differentially stimulate parts of the swim neural circuit to determine whether the memory for habituation of specific parts of the swim behavior is distributed or regionalized. Afferents were repetitively stimulated while short circuiting the swim circuit by hyperpolarizing C2's. This produced progressively weaker bursts of action potentials in the flexion neurons without significantly changing the overall number of bursts. However, when the swim neural program for swimming was jump started by stimulation of C2's, the number of bursts decreased without a concomitant change in burst strength. These results indicate that the memory for habituation of multiple components of the swim response in *Tritonia* is regionalized.

13.7

DOES THE GIANT FIBER PATHWAY MEDIATE THE VISUALLY EVOKED ESCAPE RESPONSE OF THE FLY?

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Visual information is probably the most reliable sensory cue used by a fly to detect an approaching predator. We have recently shown that flies escape only when the visual stimulus provides darkening as well as motion (Holmqvist & Srinivasan, J Comp Physiol, 169:451-59, 1991). I then wished to know whether the visually evoked escape response is mediated by the giant fiber pathway, which has been implicated in the initiation of flight of flies.

I recorded simultaneously from the tergotrochanter muscle (TTM), which provides the main thrust of the jump, and intracellularly from several descending axons in the nerve cord of houseflies. Visual stimulation to the dorsal part of the compound eyes was provided by displaying expanding or contracting bright or dark disks on a CRT-monitor.

Unrestrained flies escaped and tethered flies occasionally showed TTM potentials in response to an expanding dark disk, but not to a bright expanding disk or a contracting disk (bright or dark). During electrical stimulation of the brain, by means of stimulating electrodes inserted into the ventral part of each compound eye, a single spike in the giant descending neuron (GDN) drives the TTM. However, when the TTM responded to visual stimulation, the GDN showed no activity. Many other descending neurons showed their highest firing rate when presented with a dark expanding disk, but so far none individually has been shown to drive the TTM.

Thus, although the visually evoked escape response is likely to be started by the TTM, it is not mediated by the GDN. This finding suggests that during the escape response several pathways may act in parallel and neural integration may occur at the motorneuron level.

13.8

cAMP MEDIATES ACTION POTENTIAL BROADENING, AS WELL AS INCREASED EXCITABILITY, PRODUCED BY 5HT IN *APLYSIA* SENSORY NEURONS

B.A. Goldsmith* and T.W. Abrams Dept. of Biology & Institute of Neurological Sciences, Univ. of Pennsylvania, Phila., PA 19104.

Action potential (AP) broadening in *Aplysia* sensory neurons contributes to the enhancement of transmitter release produced by the facilitatory transmitter serotonin (5HT). Recent experiments have found that cell-permeable cAMP analogs are effective in mimicking the decrease in accommodation produced by 5HT, while producing only minimal AP broadening (Baxter and Byrne, 1990), suggesting that broadening is cAMP-independent. However, we have found that injection of the PKA peptide inhibitor, Walsh-PKI (5-24), blocked 76% of the AP broadening produced by a 2 min exposure to 5HT (broadening = 28±5.8% in controls vs. 7±1.5% with Walsh-PKI (5-24), $p<.01$). Injection of Walsh-PKI (5–24) also blocked 91±5.7% of the anti-accommodation effect produced by 5HT. Control injections of the autoinhibitory domain of PKC (19-36) did not significantly reduce the broadening or anti-accommodation produced by 5HT. In addition, the forskolin analog L-85 8051 applied with the phosphodiesterase inhibitor RO 20-1724 produced significant AP broadening (26±5.8%, $p<.01$). 1 mM chlorophenylthio-cAMP also increased the AP duration significantly (28±3.0%, $p<.01$). Thus, a cAMP-dependent mechanism appears to mediate AP broadening at early time points, but may require higher levels of cAMP than anti-accommodation. In contrast, at late time points, Sugita et al (1992) found that PKC may mediate AP broadening.

13.9

cAMP MODULATION OF MULTIPLE K^+ CURRENTS CONTRIBUTES TO BOTH ACTION POTENTIAL BROADENING AND INCREASED EXCITABILITY IN *APLYSIA* SENSORY NEURONS.

T.W. Abrams* and B.A. Goldsmith Dept. of Biology & Institute of Neurological Sciences, Univ. of Pennsylvania, Phila., PA 19104.

Low concentrations of cAMP analogs increase excitability and reduce I_{K-S} in *Aplysia* sensory neurons without causing spike broadening or modulation of the highly voltage sensitive, rapidly activating K^+ current ($I_{K-V,early}$) described by Baxter and Byrne (1989,1990). However, Goldsmith and Abrams (preceding abstract) found that more powerful activation of cAMP-dependent phosphorylation effectively broadens the sensory neuron action potential. This suggested that a low level of activation of PKA reduces I_{K-S}, while a high level of activation of the same kinase is required to modulate $I_{K-V,early}$. We compared modulation of these two currents by 50 uM and 500 uM chlorophenylthio-cAMP (CPT-cAMP), added with 0.5 mM RO 20-1724. I_{K-S} was measured at the end of a 300 ms step to -20 mV, while $I_{K-V,early}$ was measured at its peak during a step to +40 mV; V_{hold} = -70 mV. Exposure to 50 uM CPT-cAMP reduced I_{K-S} substantially with little or no effect on $I_{K-V,early}$. 500 uM CPT-cAMP produced a further reduction in I_{K-S} and reduced $I_{K-V,early}$. 500 uM CPT-cAMP also produced a slowing of the rate of activation and inactivation of $I_{K-V,early}$, similar to that observed with 5-HT. Thus, it appears that a single second messenger system may modulate multiple K^+ currents at different levels of kinase activation.

13.10

DIFFERENTIAL CYCLIC AMP DEPENDENCE OF SEROTONIN-INDUCED FACILITATION AT DEPRESSED AND RESTED *APLYSIA* SYNAPSES.

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Enhancement of synaptic transmission by serotonin (5HT) at sensory neuron synapses of *Aplysia* contributes to sensitization and dishabituation of defensive withdrawal reflexes. Earlier work implicated elevation of cyclic AMP (cAMP) in the sensory neurons in this facilitation. More recently, it was found that facilitation at synapses depressed by repeated activation, as occurs in dishabituation, involves different processes from facilitation at rested synapses, a mechanism underlying sensitization. The present study was designed to determine whether the two forms of facilitation differ in their utilization of cAMP.

Radioimmunoassay confirmed earlier findings that 5HT increases cAMP in sensory neurons. Forskolin also increased cAMP. Moreover, forskolin potentiated the effect of 5HT by 2 to > 10-fold. Effects of forskolin on postsynaptic currents (PSCs) were then examined at rested and depressed sensory-motor synapses in culture. Forskolin doubled the PSC at rested synapses, while it had no effect at depressed synapses. Furthermore, facilitation by 5HT at depressed synapses was unchanged with addition of forskolin.

These results imply that facilitation by 5HT at depressed synapses of *Aplysia* depends on factors other than cAMP, while facilitation at rested synapses may utilize cAMP. Extension of these findings to the behavioral level suggests that different biochemical mechanisms underlie the reflex enhancement in sensitization and in dishabituation.

13.11

SEROTONIN-INDUCED INCREASES IN EXCITABILITY AND ACTION POTENTIAL DURATION IN MECHANOSENSORY NEURONS: SIMILARITY ACROSS GANGLIA AND BETWEEN SPECIES.

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Two robust effects of the neuromodulatory transmitter serotonin (5HT) on the firing properties of mechanosensory neurons (SNs) in *Aplysia* are increases in excitability and action potential (AP) duration. In order to begin an evolutionary analysis of these two effects we have (a) established the baseline effects of 5HT on SNs of different ganglia (tested under identical conditions), and (b) compared this baseline to the effects of 5HT on putative SN homologues in the related species *Bursatella leachii pleii*. Standard intracellular recording techniques were used. 5HT (10 μM) was bath applied through a constant drip perfusion system.

Aplysia. 5HT produced similar effects on the firing properties of tail SNs in the pleural ganglion and siphon SNs in the abdominal ganglion. Increases in AP duration were indistinguishable (mean % increase ± s.e.m.: Tail SNs, 64.1±21.5, $t=2.98$, $p\leq0.05$, $n=10$; siphon SNs, 41.5 ± 5.8, $t=7.18$, $p\leq0.001$, $n=8$). Increases in excitability (1 to 12.6 APs in tail SNs, $p\leq0.001$; 1 to 10.2 APs in siphon SNs, $p\leq0.001$) were similar , although significantly greater in tail SNs (Mann-Whitney U-test, $p\leq0.05$).

Bursatella. Recordings were made from cells in the left pleural ganglion whose location, relative size, and physiological properties suggested that they are evolutionarily homologous to the tail SNs of *Aplysia*. As in *Aplysia*, 5HT produced an increase in AP duration (mean % increase 45.5 ± 15.5, $t=2.95$, $p\leq0.05$, $n=9$) and excitability (mean number of action potentials increased from 1 to 5.2 APs, t-test, $p\leq0.01$, $n=9$). Although the increase in AP duration was indistinguishable from that in *Aplysia*, the increase in excitability was significantly smaller than the increase observed in *Aplysia* ($t=6.2$, $p\leq0.001$).

We conclude from these data that under identical conditions, 5HT produces (a) very similar physiological effects on siphon versus tail sensory neurons in *Aplysia* and (b) qualitatively similar effects on tail SN homologues in the closely related genus *Bursatella*.

13.12

INDUCTION OF EGG LAYING BEHAVIOR IN *APLYSIA* BY EGG LAYING HORMONE.

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The marine mollusc *Aplysia* displays a regular pattern of behavior during egg laying. Egg laying and this accompanying behavior are initiated by an all-or-nothing synchronous burst of impulse activity in the neuroendocrine bag cells of the abdominal ganglion. In previous behavioral experiments, egg laying was elicited by electrical stimulation of the bag cells or by injections of bag cell extract. To identify the particular factor responsible for inducing the overt aspects of the behavior we injected animals with egg laying hormone (ELH), one of the neuropeptides secreted by the bag cells. We found that ELH causes a behavior pattern similar to that found in previous experiments. This behavior includes inhibition of locomotion and a pattern of head movements consisting of waves and undulations followed at the beginning of egg deposition by weaves and tamps. A similar pattern occurred in a second group of animals injected with atrial gland homogenate, which is presumed to cause a bag cell discharge and thus elicit normal egg laying behavior. The pattern did not occur in control animals injected with artificial sea water. These results further implicate ELH in regulation of egg laying behavior. We propose three possible regulatory mechanisms mediated by ELH: hormonal action of ELH released from the bag cells on neuronal circuits in the head ganglia, parasynaptic action of ELH released locally by ELH cells in the head ganglia, or reflex action in response to ELH-induced egg release from the ovotestis.

14.1

LINEAGE ANALYSIS REVEALS HETEROGENEITY IN CELL FATE OF NEURAL PLATE PRECURSOR CELLS. **P. Cochard, C. Soula, J. Smith* and A.-M. Duprat. Ctre Biol. Developm., CNRS UMR 9925, Univ. Paul Sabatier, 31062 Toulouse Cedex, France.**

We have studied the lineage relationships of neurons and astroglial cells at the onset of central nervous system (CNS) ontogeny, i.e. immediately after neural induction, in an amphibian embryo, *Pleurodeles waltl*. The fluorescent dye lysinated-rhodamine-dextran (LRD) was iontophoretically injected into individual cells in various areas of the neural plate of stage-13 embryos. The phenotype of LRD-labeled, clonally-related cells was documented at larval stages, 12 days later. Neurons were identified by morphological criteria and astroglial cells were recognized using antibodies directed against glial fibrillary acidic protein, expressed early by cells of the astroglial lineage in this species.

Among the 40 clones analyzed in the rhombencephalon and spinal cord, 33 were composed of both neurons and astroglial cells, while 7 contained only neurons. These purely neuronal clones were not assigned to a specific CNS region. Interestingly, however, they always derived from cells located in the lateral area of the neural plate, thus suggesting an early regionalization. Most cells in each clone remained grouped, except for some neurons which frequently underwent tangential migrations away from the clonal cohort. Purely astroglial clones were never observed.

These results suggest that precursor cells in the neural plate do not form a homogeneous population. Some of these cells may be restricted early to a specifically neuronal fate.

14.2

CELL LINEAGE ANALYSIS IN CHICK DIENCEPHALON. S. A. Arnold-Aldea*, C. Cepko. Dept.of Genetics, Harvard Med. Sch., Boston, MA 02115

Our laboratory has been interested in lineage analysis in the central nervous system, and has carried out such work using retroviruses to mark progenitor cells and their progeny. This study focused on a detailed analysis of lineage in the diencephalon to establish whether hypothalamic versus thalamic identity and the more restricted hypothalamic nuclear identity is lineage dependent. To establish lineage relationships with a high degree of certainty, a new marking technique was utilized that can allow establishment of clonal relationships even when sibling cells migrate a long distance or if multiple clones are close together in a small area of the brain. A retroviral library was constructed by inserting DNA fragments of different lengths and with different restriction sites into viral vectors that already contained a histochemical reporter gene. Two such retroviral libraries were constructed, one encoding the lacZ gene and the other encoding the gene for human placental alkaline phosphatase. Over one hundred different DNA fragments were used. Initially, infected cells are identified with histochemical techniques and then, such cells are analyzed using the polymerase chain reaction to amplify the DNA tag and determine its length and restriction pattern. Clonal relationship can then be determined using these genetic tags as cells derived from the same progenitor share the same tag regardless of their pattern of migration. Retroviral libraries were injected into embryonic chicks and the animals were harvested and analyzed at two time points: days 8-10 and days 19-21. These studies have revealed several interesting patterns of cell lineage relationships amongst hypothalamic and thalamic cells.

14.3

CLONAL ANALYSIS OF GRANULE CELL MIGRATION PATTERNS DURING DEVELOPMENT OF THE CHICK CEREBELLUM. E.F. Ryder* and C.L. Cepko. Department of Genetics, Harvard Medical School, Boston, MA 02115.

During the development of the cerebellum, granule cells and their progenitors undergo complex migrations to achieve their appropriate locations. Granule cell progenitors migrate from the inner proliferative zone (VZ) onto the cerebellar surface, where they form another proliferative zone, the external granule layer (EGL). As the granule cells are generated within the EGL, they extend axons, and their cell bodies descend deeper into the cerebellum. Using a replication incompetent retrovirus vector to label dividing granule cell progenitors and their descendents in a clonal fashion, we have traced the paths followed by clones of cells during the initial part of this migration. This study confirmed that migration begins in the most caudal portion of the cerebellar VZ, and that initially, progenitor cells move directly rostrally onto the cerebellar surface. This rostral migration is followed by extensive medial and lateral migration which has not been previously described. Clones can cross the cerebellar midline, and often cover large expanses of the cerebellum. The simplest model consistent with the data is that cells which were thought simply to extend their processes in the deeper part of the EGL are actually migrating long distances medially and laterally within this layer. This extensive and stereotypic migration may be important in positioning cells appropriately, or in establishing proper connectivity within the cerebellum.

14.4

EXPRESSION OF MN20, A *NOTCH*-RELATED MOUSE GENE, IS CONFINED TO MITOTICALLY ACTIVE PRECURSOR CELLS OF THE EARLY POSTNATAL CEREBELLUM. ME Ross*and M Risken Univ of Minnesota, Mpls, MN, 55455

In *Drosophila*, the neurogenic locus *Notch* (*N*) plays a pivotal role in the differentiation of neuroectodermal cells as they adopt a neuronal or epidermal fate. Its encoded protein is thought to act through cysteine rich repeated units in the extracellular domain which are homologous to epidermal growth factor (EGF) and to EGF-like repeats in the cell lineage control gene, *lin-12* of *C. elegans*. Several other neurodevelopmental genes in *Drosophila* possess EGF-like repeats, suggesting that proteins containing EGF-like regions represent a class of developmentally important genes, many working via cell-cell interactions. To identify mammalian members of this gene family and determine their role in CNS development, we have used probes encompassing the EGF-like repeats of *N* to screen a mouse cDNA library made from postnatal day (P) 3 to 5 cerebellar granule neurons (Ross and Heintz, Soc. Neurosci. Abs., 17:707, '91). Among these clones, MN20 expression appears to be cerebellar specific and strongly developmentally regulated. Northern analysis indicates that at postnatal day (P) 6, MN20 is heavily expressed in cerebellum but is not found in forebrain. Moreover, MN20 message is down regulated nearly 10 fold between P5 and P11 and is virtually turned off between P11 and P17 in cerebellum, in parallel with proliferation of cells in the external germinal layer (EGL). In situ hybridization of P6 mouse brain reveals MN20 message is confined in cerebellum to the superficial, proliferative zone of the EGL. A further developmental time course is being pursued. Upon differentiation of the embryonal carcinoma cell line P19 by retinoic acid into a mixed population of neuronal, glial and fibroblast cells, no MN20 expression was detected either by northern analysis or RT-PCR. These data suggest that MN20 expression is not a general feature of differentiating neuroectoderm but rather may have a more specific role in granule neuron development.

14.5

IDENTIFICATION OF A TRANSIENT STAGE OF GRANULE NEURON DIFFERENTIATION. L. Feng[1*], S.G. Kuhar[1], C.A. Mason[2], M.E. Hatten[2], N. Heintz[1]. [1]Rockefeller Univ., New York, NY 10021, [2]Columbia Univ., New York, NY 10032

To elucidate the epigenetic mechanisms of cerebellar development, we have isolated cDNA clones expressed at different stages of granule neuron differentiation using an antibody subtraction strategy (Kuhar et al, Soc. Neurosci. Abs., 524.10, 1991). The transient expression of two genes in small cells in the Purkinje cell layer during first two postnatal weeks implies the existence of a previously unidentified stage of granule neuron differentiation. Using digoxigenin labeled riboprobes we have been able to obtain clear cellular localization of their messages. The distinction of their *in situ* expression patterns from those of calbindin and GFAP strongly suggests that these two genes are expressed in granule neurons at the end of their radial migration and before their terminal differentiation. In P8 mouse cerebellum, these cells occupy a zone of several cell thicknesses in the outermost IGL and do not express mature granule neuron markers such as GABA receptor. One of the two genes is brain specific, and shares 70% homology to heart fatty acid binding protein, and thus is named brain fatty acid binding protein (BFABP). The other has restricted tissue distribution and is the 10-formyl tetrahydrofolate dehydrogenase. Antibody raised against BFABP stains small cells just below PCL, extracellular matrices in ML and distal Bergmann glia fibers in P8 mouse cerebellum. Since BFABP also has significant homology to retinoic acid binding protein, this staining pattern raises the possibility that BFABP is released by granule neurons to regulate the function of some signalling molecules. During early embryonic development, these two genes are expressed in postmitotic cells in the ventricular zone and some cells migrating away from there. The study of the regulation and functions of these two genes will shed light on the mechanisms of neuronal differentiation.

14.6

DEVELOPMENTAL POTENTIAL OF NEURAL CREST CELLS IN THE MOUSE. George N. Serbedzija,* Marianne Bronner-Fraser and Scott E. Fraser. Division of Biology, Caltech, Pasadena, CA 91125 and Dev. Biol. Center, U. C. Irvine, CA 92717.

The neural crest, as a population, gives rise to a wide variety of cell types including both neuronal and non-neuronal cells of the peripheral nervous system. To determine the developmental potential of individual neural crest cells in the mouse an *in situ* cell lineage analysis was performed. Single neural crest precursor cells were labelled by inotopheretic injection of Lysinated Rhodamine Dextran (LRD) into neural tube cells of embryos in culture. The embryos were allowed to develop for 1 to 1.5 days before being analyzed. Injections into dorsal neural tube cells resulted in LRD labelled cells in the neural tube, the dorsal root ganglia, the sympathetic ganglia and between the dermamyotome and the ectoderm. While individual embryos contained labelled cells in a subset these locations, most of the embryos contained labelled cells both in the neural tube and at least one neural crest cell derivative. Injection into a lateral neural tube cell resulted in labelled cells in the neural tube and in the limb bud (which have the morphological characteristics of Schwann cells). These experiments suggest that in the mouse embryo, as in the chicken embryo, there is: 1) a common precursor for neural crest cells and neural tube cells; and 2) a neural crest precursor that is capable of giving rise to multiple neural crest cell derivatives.

14.7

REGULATIVE CAPACITY OF THE CRANIAL NEURAL CREST AND NEURAL TUBE. Talma Scherson, G. Serbedzija, S. Fraser* and M. Bronner-Fraser, Developmental Biology Center, Univ., of Calif., Irvine, Ca. 92717

Cranial neural crest cells are thought to arise from the neural folds. However, cell lineage experiments suggest a common origin for some neural tube and neural crest-derived cells. To examine this lineage relationship, we have ablated the neural crest and/or portions of the neural tube in the midbrain and hindbrain region and examined the subsequent regulative capacity of the residual neural tube. To determine the fate of the remaining cells, neural tissue bordering the ablated region was marked by a single focal injection of the vital dye, DiI, either ventral, rostral or caudal to the extirpated region. Ablations were performed on 4 - 6 somite embryos, which subsequently were examined by confocal microscopy 24 hours later. Removal of the neural folds results in DiI-labelled cells in normal neural crest locations. Similarly, removal of nearly half of the neural tube results in reformation of neural tube and neural crest structures from the remaining half neural tube. The neural tube ventral to the ablated region, but not the flanking neural crest, regulated to form the missing neural crest/neural tube cells. In embryos sectioned 9 hours after ablation, the neural tube appeared to have completely reformed and some DiI-labelled cells were observed emigrating from its dorsal margin. These results suggest a remarkable regulative capacity of neural crest/neural tube in the chick. This plasticity appears to be age limited, since ablations at the 12 somite stage no longer yield neural crest cells. (Supported by USPHS HD-25138)

14.8

SPATIOTEMPORAL RESTRICTION OF TRUNK NEURAL CREST CELL LINEAGE IN THE EMBRYONIC ZEBRAFISH. D.W. Raible* and J.S. Eisen, Institute of Neuroscience, University of Oregon, Eugene, OR, 97403

We developed a fate map of the premigratory trunk neural crest of the embryonic zebrafish by labeling individual cells intracellularly with rhodamine-dextran. Cells that migrated laterally between somite and ectoderm gave rise only to pigment cells. Cells that migrated medially between somite and neural tube generated different cell types depending upon the time at which they began to migrate. Cells of the sensory and sympathetic ganglia were only derived from neural crest cells that began to migrate early. Schwann cells and pigment cells were derived from neural crest cells that began to migrate at any time. The first cells to migrate medially gave rise to clones of progeny with several phenotypes. Later, migrating cells gave rise to clones of progeny restricted to a single phenotype. We can now test whether there are temporal changes in the ability of individual premigratory neural crest cells to produce specific types of progeny. NIH HD22486; MDA.

14.9

SPECIFICATION OF SEROTONERGIC PHENOTYPE IN NEURONS OF *C. ELEGANS* C. M. Loer* and C. J. Kenyon, Dept. of Biochemistry & Biophysics, Box 0554, University of California, San Francisco, CA 943143.

We are seeking to identify genes required for specification of neurotransmitter phenotype in the nematode *C. elegans* by two approaches. The first approach is genetic: by isolating and characterizing mutations that eliminate the expression of serotonin. Mutations reducing serotonin may alter genes required for serotonin synthesis and handling or genes that coordinately regulate their expression. As a first step, we have characterized a male mating behavior that requires specific serotonergic neurons and shown that the known serotonin-deficient mutants *cat-1*, *cat-4*, and *ad446* are defective in this behavior. We should be able to isolate additional serotonin-deficient mutants by screening for animals displaying these male-specific behavioral defects. Our second approach is molecular: we are isolating by homology genes required for serotonin synthesis to serve as markers of the serotonergic phenotype. These are the genes acted upon by regulators of serotonin expression. We have cloned and sequenced an aromatic amino acid hydroxylase gene (AAH-1) encoding a candidate serotonin synthetic enzyme. *In situ* hybridization and a reporter gene fusion suggest that AAH-1 is expressed in hypodermal cells of the worm; therefore, this gene may not encode a serotonin synthetic enzyme. It does suggest, however, that biogenic amines in the worm may be used not only as neurotransmitters, but also as cuticle cross-linking agents as they are in insects. Supporting this hypothesis, we have found that serotonin-deficient *cat-4* mutants are more sensitive to a variety of drugs, consistent with their having a more permeable cuticle. We are also analyzing the expression pattern of a candidate serotonin synthetic decarboxylase gene kindly provided by M. Marra and D. Baillie. Analysis of the expression of serotonergic markers in serotonin-deficient mutants should help us to understand how the serotonergic phenotype is regulated.

14.10

OLIGODENDROGLIAL TROPHIC FACTOR IS A NEURONAL PROTEIN B.M. Ances and B.Q. Kreider*, Neurology Research, Children's Hospital of Philadelphia, Philadelphia, PA 19104

Oligodendroglia trophic factor (OTF), isolated from salt-extracted brain membranes of 3 day old rats, is a mitogen for cells of the oligodendroglial lineage. In addition to increasing the rate of proliferation of oligodendroglial precursor cells and galactocerebroside-positive oligodendroglia in rat cerebral white matter cultures, OTF inhibits myelin basic protein gene expression by cultured oligodendroglia. OTF activity can be eluted from an SDS-PAGE gel in the region corresponding to a molecular weight of approximately 65 kD. Monoclonal antibodies (mAb) against this protein band were generated, and two of these mAbs at a 1:100 dilution of hybridoma supernatant blocked 90% of the OTF mitogenic activity when incubated for three days with OTF in cerebral white matter cultures. These blocking antibodies were used to determine the cellular localization of the mitogen. Peroxidase anti-peroxidase staining demonstrated the presence of OTF in the cell bodies of a majority of the large and small neurons in aceton-fixed dorsal root ganglion cultures from P15 rats, but not in the satellite or Schwann cells. There was no detectable binding of the blocking antibodies in rat sciatic nerve cultures (containing Schwann cells and fibroblasts) or rat cerebral white matter cultures (containing oligodendroglia, astrocytes, O2A cells and microglia). These data strongly suggest that the cell of origin of OTF is neuronal.

14.11

NEX: A NEURONAL HELIX-LOOP-HELIX PROTEIN OF THE DEVELOPING AND ADULT CENTRAL NERVOUS SYSTEM K.-A. Nave* and A. Bartholomä, Center for Molecular Biology (ZMBH), University of Heidelberg, D-6900 Heidelberg, Germany.

The molecular mechanisms underlying cell lineage decisions and subsequent differentiation in the mammalian nervous system are largely unknown. In invertebrates and in the mammalian myogenic lineage, cell determination has been linked to the expression of transcription factors of the helix-loop-helix (HLH) superfamily of proteins. We have used highly degenerate oligonucleotides against HLH consensus sequences, in order to PCR amplify homologous sequences from mammalian neuronal and glial cells. Using this strategy, we have identified and cloned a novel HLH protein, termed NEX (for neural helix-loop-helix), that is specifically expressed in the mammalian central nervous system. The predicted structure of NEX includes a basic DNA binding domain and a leucin-zipper flanking the HLH domain. Outside this homology region, NEX has no similarity with other HLH family members. By Northern blot analysis, NEX mRNA is remarkably abundant in the adult rat brain but undetectable in any other organ tested. NEX expression begins in the third week of gestation, coinciding with the generation of postmitotic neurons and the onset of neuronal differentiation and synaptogenesis. By in-situ hybridization, NEX is expressed throughout the embryonic brain and spinal cord. In the adult rat brain, hybridization remains most prominent in the neocortex and in pyramidal neurons of the hippocampus, two regions that have been implicated in neuronal plasticity. (Supported by a grant from the Bundesministerium für Forschung und Technologie to K.A.N.)

14.12

THE IDENTIFICATION OF NOVEL, NEURONAL PROTEIN TYROSINE PHOSPHATASES. K. M. Walton*, K. J. Martell and J. E. Dixon, Dept. of Biol. Chem., Univ. of Mich., Ann Arbor, MI 48109-0606.

The role of tyrosine phosphorylation in the control of cellular function, proliferation and development has been well documented. The receptor for nerve growth factor, for example, is a tyrosine kinase. This family of tyrosine kinase receptors regulates its own activity via autophosphorylation, followed by the phosphorylation of other protein substrates. To control these receptors efficiently and specifically, counteracting protein tyrosine phosphatases (PTPs) are directed at both the autophosphorylation site and the cellular substrates.

Many tyrosine kinases have been cloned over the past decade. Recently, attention has turned to the investigation of PTPs. Since the isolation of the first identified PTP in 1988 from human placenta, over thirty PTPs have been cloned from various tissues. Because many tyrosine kinases have specific functions and highly restricted tissue localization, it is believed that PTPs will ultimately show an analogous pattern. However, only a few of the cloned PTPs appear to be expressed in a restrictive fashion. Similarly, the role of only a few PTPs have been established.

We are attempting to isolate PTPs specific to neuronal tissue. We are using PCR directed at two conserved regions of previously cloned PTPs to isolate novel phosphatases from adrenal medulla. The adrenal medulla, derived from the neural crest, is the source of virtually the entire peripheral nervous system. We have identified six novel PTPs using this technique, and have assessed their tissue specificity by RNase protection. Two of the clones have a broad tissue distribution, while another is abundant only in lung and spleen. Three of the isolated clones are strongly expressed in brain; one of these is abundant in adrenal medulla but not adrenal cortex. By identifying PTPs specific to neuronal tissue, we have taken the first step towards determining the role they play in neuronal development and differentiation. Supported in part by grants from the NIH.

15.1

ENDOCYTIC TRAFFICKING OF CELL SURFACE ALZHEIMER'S PRECURSOR PROTEIN. L.M. Refolo, K. Sambamurti* and N.K. Robakis. Dept. of Psychiatry and Fishberg Center for Neurobiology, Mount Sinai Medical Center, New York, NY 10029.

Normal metabolic processing of the Alzheimer's Amyloid Precursor (APP) involves cleavage within the ß/A4 peptide region and secretion of the truncated form. At least two potential subcellular locations have been proposed as the site for this processing event: an intracellular post-Golgi compartment or the plasma membrane. To distinguish between these two sites we have investigated the processing of cell surface APP in the C6 glioma cell line. We have found that, radiolabeled, cell surface APP is cleaved and secreted as a 120 kD truncated form. After a 5 min chase low levels of the truncated form was detected in the media and reached the maximum level at 10 min. Secretion of cell surface APP was reduced by several treatments known to inhibit endocytosis including: methylamine, colchicine and a 18°C temperature block. These data suggest that endocytosis of cell surface APP is a prerequisite for its cleavage at an intracellular location . In addition, treatment of cells with the lysosomotrophic amine chloraquine resulted in an increase in the levels of cell surface APP and a concomitant increase in the levels of cell surface derived-secreted form . From these studies, we propose that cell surface APP is processed either by the secretase or by a lysosomal pathway after endocytosis.

15.2

PROCESSING OF THE ß-AMYLOID PRECURSOR PROTEIN: A COMPARISON OF THE REINTERNALIZATION AND SECRETORY PATHWAYS. C. Haass, E.H. Koo, A. Mellon, A.Y. Hung and D.J. Selkoe*. Center for Neurologic Diseases, Harvard Med. School, Brigham and Women's Hospital, Boston, MA 02115.

Progressive cerebral deposition of the ß amyloid peptide is an early and invariant feature of Alzheimer's disease. Because secretory cleavage of ßAPP inhibits amyloid formation, we searched for an additional pathway which might use the NPXY consensus sequence for coated pit mediated internalization found in the C-terminus of ßAPP. Upon addition of the lysosomal protease inhibitor, leupeptin, to human endothelial cells, we observed a perinuclear lysosomal/late endosomal staining pattern with antibodies against various regions of ßAPP. Incubation of living cells with an antibody against the extramembranous part of ßAPP leads to its binding to cell surface ßAPP and targeting to lysosomes. After cell surface biotinylation, we recover biotinylated full length ßAPP within cell extracts. Furthermore, purification of lysosomes from leupeptin-treated cells shows that they contain full length ßAPP, the 10 kDa fragment and an array of C-terminal fragments bearing the intact ß-peptide. Deletion of the NPTY motif or the complete C-terminus results in a two fold increase in secretion of soluble ßAPP, indicating that this alternative pathway could account for about 50% of ßAPP turnover in the analyzed cell types. We present a model proposing that the mutations found within the ßAPP gene in familial AD might either cause a stabilization of lysosomally derived fragments or might change the degree of reinternalization and lysosomal targeting of ßAPP.

15.3

THE ROLE OF LYSOSOMAL PROCESSING IN THE METABOLISM OF THE AMYLOID PRECURSOR PROTEIN. D.H. Gabuzda*, J. Busciglio, L.B. Chen and B.A. Yankner. Harvard Medical School, The Children's Hospital and Dana Farber Cancer Institute, Boston, MA 02115.

The cellular localization and route of processing of the amyloid precursor protein (APP) was examined in COS cells transfected with eukaryotic expressor plasmids encoding the 695 and 751 amino acid isoforms of APP. Immunoprecipitation was performed with antibodies which recognize the APP C-terminal, N-terminal and amyloid β protein domains. In cell lysates, the C-terminal antibody identified the intact differentially glycosylated forms of APP and a 9 kd fragment that previous work has shown represents the normal secretase cleavage product. There was no significant production of APP C-terminal fragments containing full-length β amyloid under normal conditions. Treatment with the lysosomal inhibitors chloroquine, leupeptin or low dose ammonium chloride did not change cellular level of intact APP suggesting that most of the intact APP is not initially processed in lysosomes. However, the lysosomal inhibitors prevented the degradation of the 9 kd C-terminal fragment. Indirect immunofluorescence of transfected cells showed C-terminal APP immunoreactivity in lysosomes but little lysosomal immunoreactivity for amino-terminal APP epitopes. These results suggest that most APP is initially processed in a non-lysosomal cellular compartment and that the C-terminal fragments are subsequently translocated to lysosomes and degraded. The demonstration that familial mutations in APP alter the degradation of C-terminal fragments in lysosomes suggests that lysosomal processing of APP fragments may be important in the pathogenesis of Alzheimer's disease.

15.4

LYSOSOMAL DEGRADATION OF AMYLOID β/A4-PRECURSOR (APP). G.M. Cole*, L. Bell, T. Saitoh, and A.L. Miller, Univ. California, San Diego, Dept. of Neurosciences, La Jolla, CA 92093-0624.

We have previously shown that N-terminal and C-terminal APP domains are elevated in cultured human neuroblastoma and PC12 cells by longterm treatment with lysosomal protease inhibitors (Cole et al.,Neurochem.Res.14:933-939,1989). Primary amines such as chloroquine and ammonium chloride are effective in inhibiting degradation of mature APP and fragments in pulse-chase immunoprecipitation studies with C-terminal antisera. Because these drugs have many non-lysosomal targets, we have conducted similar studies, comparing APP degradation in normal and chloroquine treated fibroblasts with cells from I-cell patients with defective lysosomal hydrolase targeting. Both the I-cells and the chloroquine treated normal fibroblasts showed delayed degradation of both mature APP and a set of small C-terminal fragments. We also confirmed the lysosomal degradative pathway in transfected B103 rat CNS neuroblastoma cells overexpressing APP695 or APP751. Having established a lysosomal degradative pathway for APP including the beta protein domain , we have sought to determine both intrinsic and extrinsic factors which may modulate the capacity for degradation of potentially amyloidogenic fragments *in vitro* and whether or not the lysosomal pathway is active in the normal and AD brain. We found endogenous metabolites could inhibit lysosomal degradation *in vitro* and confirmed that potentially amyloidogenic C-terminal fragments can be detected in both the normal and AD brain.

15.5

An Alternative Secretase Cleavage Produces Soluble Alzheimer Amyloid Precursor Protein Containing a Potentially Amyloidogenic Sequence

N.K. Robakis*, J.P. Anderson, Y. Chen, K.S. Kim, Department of Psychiatry and Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029

Cell culture studies have shown that the Alzheimer amyloid precursor protein (APP) is secreted after full length APP is cleaved by a putative secretase at the Lys^{16}-Leu^{17} bond (secretase cleavage I) of the amyloid peptide sequence. Since this cleavage event is incompatible with amyloid production it has been assumed that secreted APP cannot serve as a precursor of the amyloid depositions observed in Alzheimer's disease. Here we show that in neuronal PC12 cell cultures a portion of the secreted extracytoplasmic APP reacted specifically with both a monoclonal antibody recognizing amyloid protein residues Leu^{17} to Val^{24} and a polyclonal antiserum directed against amyloid protein residues Ala^{21} to Lys^{28}. Furthermore, this APP failed to react with antisera recognizing the cytoplasmic domain of the full length protein. Similar results were obtained in human embryonic kidney 293 cell cultures. These data indicate that in mamalian cells exists an alternative APP secretase cleavage site (secretase cleavage II), C-terminal to the predominant secretase cleavage I. Depending on the exact location of cleavage site II, potentially amyloidogenic secreted APP species may be produced.

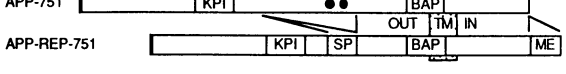
15.6

EXPRESSION OF AMYLOID PRECURSOR PROTEIN AND ITS ALTERED DERIVATIVES IN CULTURED CELLS SUGGESTS A BROAD SPECIFICITY FOR SECRETASE CLEAVAGE. S.R. Sahasrabudhe, M.A. Spruyt, H.A. Muenkel, A.J. Blume, M.P. Vitek and J.S. Jacobsen* Department of Molecular Pharmacology, Lederle Laboratories, American Cyanamid Company, Pearl River, NY, 10965.

The mechanism of abnormal proteolytic processing of amyloid precursor protein (APP) leading to the extraction and deposition of beta amyloid peptide (BAP) is thought to be central to the development of Alzheimer's Disease. The putative "secretase" activity normally cuts APP at residues within BAP leading to the release and secretion of an amino-terminal APP fragment, thereby precluding BAP formation and amyloidogenesis.

In order to better understand the features which make the APP molecule secretase cleavable, we have expressed a cDNA reporter construct representing the 751 amino acid isoform of APP (APP-REP) and its altered derivatives in cultured cells (see figure below).

Here, we show that (a) APP-REP is predominantly associated with the plasma membrane, (b) secretase cleavage of APP-REP, and its intracellular turnover is similar to that reported for intact APP molecule, (c) secretion appears unaltered by introduction of the glutamate to glutamine mutation found in the APP gene of patients suffering from HCHWA-D disease, (d) deletion of 19 juxtamembranous amino acids encompassing the secretase site also allows secretion, (e) the rate of accumulation of fragments secreted from altered derivatives, and their intracellular turnover is similar to that of APP-REP, (f) modulators of secretion have a similar effect on the APP-REP constructs tested. Our results indicate an unexpected broad substrate specificity for the secretase activity.



15.7

IMMUNOCYTOCHEMICAL ANALYSIS OF MUTANT AMYLOID PROTEIN PRECURSORS (APP) IN TRANSFECTED CELLS. M. Tabaton, T.E. Golde, P. Gambetti, and S. G. Younkin* Case Western Reserve University, Cleveland, OH 44106

Recent studies of several familial AD (FAD) kindreds have identified mutations in the membrane-spanning domain of the APP that cosegregate with the AD phenotype. In the three point mutations that have been identified, a valine in the middle of the membrane-spanning domain is converted to isoleucine, phenylalanine, or glycine respectively. Using 293 (human embryonic kidney), K562 (mononuclear leukemic), and M-17 (human neuroblastoma) cells, we have prepared stable cell lines transfected with normal APP695, 751, and 770, as well as the mutated forms of these proteins that have been identified in the various FAD kindreds. As part of effort to determine whether there is differential processing of the mutated and normal APPs expressed in these transfected cell lines, we are analyzing the APP in these cell lines immunocytochemically paying particular attention to the localization of fragments containing (i) the 20 residues at the carboxyl terminus of the APP or (ii) the BAP region. In general all of the transfected cells showed a moderate increase in immunoreactivity to a variety of APP epitopes when compared to cells transfected with vector alone. However, a small percentage of the K562 and M17 cells transfected with the FAD mutations also showed an extremely strong diffuse cytoplasmic immunoreactivity to anti-βAP and anti-carboxyl-terminal antibodies. These initial results indicate that the mutations cause marked accumulations of βAP and carboxyl- terminal epitopes in a small percentage of the cells. Work is in progress to localize these epitopes ultrastructurally, to correlate the immuncytochemical data with biochemical studies of these cells (see Golde et al. these meetings), and to determine why a small percentage of the cells show a marked accumulation of βAP and carboxyl-terminal epitopes in their cytoplasm.

15.8

SECRETORY PROCESSING OF THE ALZHEIMER AMYLOID ß-PROTEIN PRECURSOR (APP) IS INCREASED BY PROTEIN PHOSPHORYLATION. S. L. Gillespie, T. E. Golde, L. H. Younkin*, and S. G. Younkin Case Western Reserve University, Cleveland, OH 44106

Phorbol ester activation of protein kinase C increases the production of secreted forms of several membrane-bound proteins including the neutrophil MEL-14 adhesion protein, colony stimulating factor-1 receptor, the precursor of transforming growth factor a, and tumor necrosis factor receptor. Thus increased production of secreted APP derivatives could account, at least in part, for the decreased mature APP and the increase in a small COOH-terminal derivative that have been observed following treatment with phorbol esters (Buxbaum, et al., PNAS 87, 6003, 1990). To evaluate this possibility, we examined human embryonic kidney (293) cells transfected with a APP_{695} expression construct. Cells were labeled with ^{35}S-methionine and cysteine for 20 minutes. Treatment during the chase with 1.0 μM PDBu had a marked effect on APP metabolism. The most striking change was a marked increase in the level of the ~105 kD secreted derivative that began at 15 min and continued for every time point thereafter Accompanying this, there was a decrease in the mature holoprotein similar to that described by Buxbaum et al. We have observed this PDBu-induced increase in secretion of APP and the accompanying decrease in mature holoprotein in each of 15 additional experiments that we have performed on 293 and K562 cells, and this effect has been observed in cells transfected to express APP_{751} and APP_{770} or APP_{695}. Thus it appears that PKC activation may diminish the likelihood of amyloid deposition by increasing processing of APP in the secretory pathway. Before this concept can be accepted, however, it will, be important to more thoroughly evaluate the effect of PKC activation on the holoprotein that escapes cleavage by APP secretase and to evaluate in particular the effect of PKC on the βAP-bearing carboxyl-terminal derivatives produced in the endosomal/lysosomal pathway.

15.9

ALTERED PROCESSING OF MUTANT AMYLOID PROTEIN PRECURSORS IN TRANSFECTED CELLS. T. E. Golde*, M. Usiak, L. H. Younkin, and S. G. Younkin Case Western Reserve University, Cleveland, OH 44106

Our previous data (Golde et al., Science 255:728-730) indicate that normal endosomal/lysosomal processing of the amyloid protein precursor (APP) generates a complex set of carboxyl-terminal derivatives including potentially amyloidogenic derivatives containing the entire amyloid ß protein (βAP). To further examine APP processing we have stably transfected 293 human embryonic kidney cells, K562 leukemic cells, and a human neuroblastoma line with expression constructs encoding wild type APP695, 751, 770 cDNAs and APP695, 751, and 770 cDNAs that encode the mutations associated with familial AD (FAD) and the Dutch form of hereditary cerebral hemorrhage with amyloidosis (HCHWA-D). When transfected with wild type APPs, each of the cell lines produce a qualitatively similar set of augmented 8-12 kDa carboxyl-terminal derivatives; however, the neuroblastoma lines have a higher level of the βAP bearing derivatives than the peripheral lines. In addition we have also identified larger (15-50 kDa) carboxyl-terminal derivatives that are augmented in these transfected cell lines and appear to be generated by endosomal/lysosomal processing. In K562 cells, stable transfection of the mutant APPs associated with FAD and HCHWA-D resulted in increased accumulation of carboxyl-terminal derivatives that resemble the derivatives generated by normal endosomal/lysosomal processing. These results indicate that one of the effects of these mutations is to cause an increase in the steady state level of carboxyl-terminal derivatives of the APP. Significantly many of these derivatives contain the entire βAP and therefore are potentially amyloidogenic. We are currently employing a variety of experimental strategies to determine how the mutations alter the dynamics of APP processing and to determine whether the accumulation of these derivatives is due to altered processing of the mutant APPs in the lysosomal system.

15.10

ALTERED PROCESSING OF DISEASE RELATED MUTANT AMYLOID ß PROTEIN PRECURSORS. R.Bhasin*, I.A.Morozov, E.A.Barnes and D.Goldgaber, Dept. of Psychiatry, SUNY, Stonybrook, NY 11794.

Amyloid ß protein (AßP) is formed from a larger transmembrane glycoprotein called the amyloid ß protein precursor (APP). Normally, formation of AßP is prevented due to a proteolytic cleavage within the AßP region of APP. The larger N-terminal part of the cleaved molecule is secreted. Using the baculovirus expression system, we have expressed APPs with mutations found in Alzheimer's disease (AD) (Val^{717} to Ile) and hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D) (Glu^{693} to Gln) . We found that some APP molecules were secreted without proteolytic cleavage of the C-terminal part. The amount of these molecules increased in media of cells expressing mutated APPs. Thus, mutations found in AD and HCHWA-D increase the amount of secreted C-terminus containing APP. In addition, we analyzed media from fibroblasts of patients with the AD mutation (Val^{717} to Ile) and detected secreted APP molecules with the C-terminus intact. The secreted C-terminus containing APPs have both the N- and C- terminus ends of the AßP region accesible for proteolysis and could serve as substrates for extracellular amyloid formation. Based on the above data we hypothesize that the critical step that determines the fate of APP molecules might occur during translocation of newly synthesized APP molecules across the endoplasmic reticulum (ER) membrane. Mutations found in AD and HCHWA-D interfere with efficient retention of APP molecules in the ER membrane.

15.11

ABNORMAL PROCESSING OF AMYLOID PRECURSOR PROTEIN IN OLFACTORY NEUROBLASTS FROM ALZHEIMER DONORS: B. L. Wolozin*, C. Chen, D. Jablonska, L. Zhang, I. Basaric-Keys, I. Resau, R. S. Lebovics and T. Sunderland. Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892

We have been studying the expression of amyloid precursor protein (APP) in olfactory neuroblasts (ON) grown from Alzheimer (AD) and age matched neuropsychiatrically tested normal donors. Immunoblots of homogenates from the ON grown under basal conditions reveal that the 11.5 kDa C terminal derivative (CTD) of APP is more readily detectable in the AD cell lines than in the normal cell lines. Blockade of lysosomal metabolism by incubation of the ON with the lysosomal inhibitor chloroquine results in a 6.4 fold higher level of CTD's in Alzheimer ON (N=8) than in the ON from age matched normal donors (N=6). In contrast, lymphoblasts from AD and normal donors grown under similar conditions of lysosomal blockade do not show changes in CTD levels. The elevated levels of CTD's seen in ON from AD donors are associated with elevated levels of amyloidogenic CTD's. We have also found that the levels of CTD's can be decreased by activating protein kinase A or, to a lesser extent, by inhibiting protein kinase C. Thus, inadequate catabolism of APP within a nonlysosomal or chloroquine insensitive pathway leads to elevated levels of CTD's and may contribute to the formation of ß-amyloid in centrally derived ON cells but not in peripherally derived lymphoblasts of AD patients.

15.12

THE ALZHEIMER AMYLOID PRECURSOR PROTEIN IS A SERINE ESTERASE ENZYME CAPABLE OF CLEAVING ACETYLCHOLINE. U.S. Kayvali, @B.D. Greenberg, @M.B. Fairbanks*, and H. Potter. Dept. Neurobiology, Harvard Medical School, Boston MA 02115; @ Upjohn Co., Kalamazoo, MI 49001.

The precursor of the Alzheimer amyloid β/A4 protein, β-APP, is generated in at least three forms by alternative mRNA splicing. Two of the forms contain an extra domain with a Kunitz-type protease inhibitor (KPI) function. The secreted isoforms of the APP molecules that contain the KPI domain have been shown to be identical to the known protease inhibitor, protease nexin 2, and are therefore presumed to have as their function the inhibition of trypsin- and chymotrypsin-like proteases released during inflammation. However, no function for the APP695 protein, lacking the KPI domain, has been found. Here we report that the 695 amino acid isoform of β-APP has an esterase enzyme activity that is capable of cleaving acetylcholine and other ester substrates such as p-nitrophenylacetate. The fact that APP695 can be labeled with radioactive diisopropylphosphofluoridate (DFP) in a reaction inhibitable by excess substrate and then separated on SDS-PAGE indicates that the enzyme is an esterase/protease with a serine-based active site and that the esterase activity is not due to a contaminant in the preparation. The larger APP isoforms can also be shown to have an esterase activity that is normally inhibited by their KPI domain. Thus together, the various isoforms of β-APP constitute a family of serine esterases and inhibitors whose normal function may be to control an important physiological process in much the same way that antithrombin III controls the blood clotting cascade. The increase in acetylcholinesterase activity due to over-expression or aberrant processing of β-APP695 may also be significant to the pathology of Alzheimer's disease by reducing the level of the key neurotransmitter, acetylcholine.

15.13

PROCESSING OF ALZHEIMER AMYLOID PRECURSOR PROTEIN IS REGULATED BY MUSCARINIC ACETYLCHOLINE RECEPTOR ACTIVATION. Roger M. Nitsch*, Barbara E. Slack, Richard J. Wurtman and John H. Growdon. Dept. of Neurology, Massachusetts General Hospital, ACC 830, Boston, MA 02114 and Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Abnormal processing of the amyloid precursor protein (APP) is a central event in the formation of amyloid deposits in Alzheimer's disease brain. APP can be processed by a secretory and an alternate internal pathway, the regulatory mechanisms of which, however, are unclear. We investigated the possibility that APP processing is regulated by activation of cell-surface neurotransmitter receptors by stimulating human 293 cells transfected with the genes for human acetylcholine receptors with the cholinergic agonist carbachol, and measured the APP release from these cells using Western blot analysis and densitometric quantitation. 293 cells transfected with the muscarinic m1 and m3 receptor subtypes responded to receptor activation with a rapid 3-5 fold increase of APP release within a time-frame of minutes, indicating that preexisting APP is processed and released as a response to receptor stimulation. Receptor-coupled APP release from the m1 and m3 transfected cells was blocked by inhibition of PKC with staurosporine. Receptor-coupled activation of APP processing was subtype-specific as indicated by the failure of muscarinic m2 and m4 receptor subtypes (linked to adenylyl cyclase) to alter release of APP. These results show for the first time the APP processing can be regulated by cell-surface neurotransmitter receptors, presumably via receptor-coupled activation of PKC. Furthermore, the results reported here may provide an initial basis to tie together the abnormal APP processing and the cholinergic deficit in Alzheimer's disease brain. Supported by NIMH, NIA and the CBSM.

OPIOID AND SIGMA RECEPTORS

16.1

CLONING OF A cDNA ENCODING DELTA OPIOID RECEPTOR CHARACTERISTICS. C.J. Evans*, D. Keith Jr., H.M. Morrison#,and R.H. Edwards.# Brain Research Institute and Departments of Psychiatry and Neurology#, UCLA School of Medicine, Los Angeles, CA. 90024.

The endogenous opioid system has evolved a complex family of ligands and receptors. The known opioid precursors, POMC, proenkephalin and prodynorphin, in a processing dependent manner give rise to a wide spectrum of opioid peptides with very different properties, including differential affinity for the μ,∂ and κ receptor types. Based upon their physiological and pharmacological characteristics, all the opioid receptors belong to the G-protein coupled receptor family. The NG108-15 cell line is a rich source of delta opioid receptors. A size selected cDNA expression library (constructed in CDM8) was transfected into COS cells. Cells binding a radiolabelled delta peptide agonist (DADLE) were selected, the plasmids isolated and expanded in bacteria and re-transfected into COS cells. Following three such plasmid enrichments a clone of 1.8KB was obtained which upon transient transfection into COS cells exhibited DADLE binding to the intact cells. The DADLE binding showed stereospecific alkaloid displacement (dextrophan verses levorphanol), displacement by morphine, etorphine, diprenorphine, DISLET, DADLE and DPDPE but not with DAGO. The DNA sequence of this clone shows homology with other G-protein receptors.

Supported by grants from NIDA and the Keck Foundation.

16.2

EFFECT OF ICV ß-FUNALTREXAMINE (ß-FNA) ON μ AND δ OPIOID RECEPTORS IN THE RAT BRAIN: CONSIDERATION OF BINDING CONDITION. H.-H. Yang, J.U. Adams, & L.-Y. Liu-Chen*, Dept. of Pharmacology, Temple Univ. Sch. of Med., Philadelphia, PA 19140.

We demonstrated previously that icv ß-FNA (10 μg, ~20 nmole), while profoundly diminishing the antinociceptive effect of icv morphine, had a relatively minor effect on μ opioid receptor binding. In this study, we re-examined the effect of 24 h pretreatment with icv ß-FNA in rats on brain μ and δ opioid receptor binding under three different binding conditions (all at 25°C): (i) pretreatment of membranes with GDP and NaCl and binding in the presence of Mg^{++} in 50 mM Tris-HCl containing EGTA and leupeptin for 3-4 h; (ii) binding in 50 mM Tris-HCl containing bacitracin, leupeptin, chymostatin and bestatin for 3-4 h; (iii) binding in 50 mM Tris-HCl containing EGTA and leupeptin for 45 min. For μ receptor, comparison of three binding conditions revealed that control [^{3}H]DAMGO (1 nM) binding was much higher with **i** than with **ii** or **iii** and that there was no significant difference between **ii** and **iii**. ß-FNA (2, 6 or 20 nmole) significantly reduced [^{3}H]DAMGO binding with **i** but not with **ii** or **iii**. Saturation experiments with **i** showed that the reduction in [^{3}H]DAMGO binding following 20 nmole ß-FNA was due to a decrease in B_{max} (by 50%) with no change in K_d. For δ receptor, [^{3}H]DPDPE (2 nM) binding was much higher with **i** than with **ii** or **iii**. There was no significant change in [^{3}H]DPDPE binding with **i** after 2, 6, or 20 nmole ß-FNA. Opioid receptors are known to exist in multiple affinity states. Divalent cations such as Mg^{++} increase the number of high affinity sites for agonists when membranes are pretreated with GDP or GTP. ß-FNA binds irreversibly to μ opioid receptors only in the presence of Na^+, which induces the low affinity state. When binding is performed without Mg^{++} and pretreatment with a guanine nucleotide, changes in the receptors at the low affinity state can not be detected (**ii, iii**). It is only when the receptors at the low affinity state are converted to the high affinity state that the changes induced by ß-FNA pretreatment can be revealed (**i**). (Supported by NIDA grants DA04745 and DA07237)

16.3

BINDING OF [4'-^{125}I-PHE^3, Glu^4]DELTORPHIN TO δ OPIOID RECEPTORS OF RAT BRAIN. R.J. Knapp*, L. Fang, T. Matsunaga, S. Weber, T. Davis, V.J. Hruby, and H.I. Yamamura Univ. Arizona Pharmacol. & Chemistry Dept.s, Tucson, AZ 85724

Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2 ([Glu^4]deltorphin) is one of 3 amphibian peptides that contain a naturally occurring D-amino acid substitution in the 2 position and are highly selective for δ opioid receptors. Binding inhibition studies using [^{3}H]*p*-Cl-DPDPE (δ) and [^{3}H]CTOP (μ) give IC_{50} values of 0.7 and 17,000 nM at δ and μ opioid receptors, respectively.

Iodination of the 4' position of the Phe^3 aromatic ring reduces δ receptor binding affinity to 5.0 nM and increases that at μ receptors to 12,000. [4'-^{125}I-Phe^3, Glu^4]deltorphin was prepared from [4'-NH_2-Phe^3, Glu^4]deltorphin via a diazonium reaction to produce radioiodinated [Glu^4]deltorphin having a specific activity of 2,200 Ci/mmol. The site labeled in rat brain by ^{125}I-deltorphin was characterized by binding inhibition studies giving the following IC_{50} values:

Inhibitor	IC_{50} (nM ± SEM)	Hill Slope
δ Selective		
naltrindole	0.085 ± 0.012	1.12 ± 0.14
[Glu^4]deltorphin	0.558 ± 0.137	1.39 ± 0.22
pCl-DPDPE	0.423 ± 0.096	1.19 ± 0.25
DPDPE	3.61 ± 0.85	1.01 ± 0.11
DSLET	1.01 ± 0.08	1.00 ± 0.04
μ Selective		
PL-017	30200 ± 14400	1.20 ± 0.19
κ Selective		
U-69593	7000 ± 4300	1.72 ± 0.03

The major advantages of ^{125}I-deltorphin are high δ receptor affinity and selectivity, and high specific activity. Supported in part by NIDA grants.

16.4

CYCLIC AMP PROMOTES DESENSITIZATION OF THE OPIOID RESPONSE IN LOCUS COERULEUS (LC) NEURONS - EVIDENCE FOR HETEROLOGOUS DESENSITIZATION

G.K. Aghajanian* and M. Alreja. Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Membrane-permeant analogs of cAMP reverse morphine and clonidine-induced hyperpolarizations in LC neurons (Andrade and Aghajanian, 1985). This reversal may be due to heterologous desensitization since phosphorylation of the opiate receptor by protein kinase A decreases agonist-induced G-protein activation (Harada et al, 1990). Therefore, we investigated the role of cAMP and other phosphorylation pathways in acute desensitization of the opioid response in LC neurons.

Intracellular recordings were made from rat LC neurons in 500 μm thick slices. The effect of prolonged bath applications of 8-Br-cAMP, 8-Br-cGMP, or phorbol 12,13-diacetate (PDA) on the met-enkephalin-induced outward currents were studied in LC neurons voltage-clamped at -60 mV. In accord with a previous report (Harris and Williams, 1991), a maximal dose of met-enkephalin (200 μM) produced an outward current which partially desensitized during a 5 min. application period. Following bath application of 8-Br-cAMP (2 mM, 12-15 mins.) but not 8-Br-cGMP (2 mM) or PDA (100 μM), the opiate response showed a more rapid and pronounced desensitization.

We conclude that cAMP promotes desensitization of the opioid response in LC neurons possibly via receptor phosphorylation. Since the cAMP pathway is upregulated by chronic morphine, the resulting cAMP-induced heterologous desensitization of the opiate receptor may contribute to opiate tolerance.

16.5

ANATOMICAL AND SUBCELLULAR DISTRIBUTION OF "*Sigma*-1" AND "*Sigma*-2" SITES PRESENT IN RAT BRAIN. D. J. McCann*, A. D. Weissman, and T.-P. Su. NIDA Addiction Research Center, P. O. Box 5180, Baltimore, MD 21224.

Conditions were established for the selective labeling of two *sigma* ligand binding sites present in rat brain. At *sigma*-1 sites, labeled with 5 nM (+)[^{3}H]SKF-10,047 in the presence of 300 nM MK-801, rank-order affinities were as follows: (+)pentazocine > (-)pentazocine > DTG > (+)SKF-10,047 > (-)butaclamol > (-)SKF-10,047 > (+)butaclamol. At *sigma*-2 sites, labeled with 5 nM [^{3}H]DTG in the presence of 1 μM (+)SKF-10,047, rank-order affinities were as follows: DTG > (-)pentazocine > (-)SKF-10,047 > (+)pentazocine > (+)butaclamol > (+)SKF-10,047 > (-)butaclamol.

Rat brains were dissected into nine regions, and their corresponding homogenates were assayed using the selective conditions described above. *Sigma*-1 binding was enriched (> binding per mg protein than whole brain) in the hypothalamus, cerebellum, and pons-medulla. *Sigma*-2 binding also was enriched in the hypothalamus and cerebellum, but the pons-medulla yielded a level of sigma-2 binding which was among the lowest measured. Enrichment of *sigma*-2 without enrichment of *sigma*-1 binding was observed in both the frontal and posterior cortex, as well as the hippocampus. These findings strongly suggest that *sigma*-1 and *sigma*-2 sites are distinct entities which do not exist on the same receptor protein.

Experiments comparing the relative enrichment of *sigma*-1 and *sigma*-2 sites in subcellular fractions of rat brain homogenates are now underway.

16.6

THE EFFECTS OF SIGMA COMPOUNDS ON BOTH NMDA- AND NON NMDA-MEDIATED NEURONAL ACTIVITY IN RAT HIPPOCAMPUS. W.J.Martin*, J.S. Roth and J.M. Walker. Schrier Research Laboratory, Dept. of Psychology, Brown University, Providence, RI 02912.

The purpose of this study was: (1) to confirm the existence of a putative sigma/NMDA interaction by examining the effects of sigma ligands on NMDA-induced neuronal activity of CA_3 pyramidal neurons and (2) to examine the effect of sigma compounds on low frequency stimulus-evoked population potentials. *In vivo* extracellular recordings of dorsal hippocampus CA_3 pyramidal neurons in rat hippocampus were carried out in urethane-anesthetized rats. NMDA was microiontophorically-applied to elicit baseline levels of excitation. Low doses of DTG (1-3 μg/kg i.v.) and haloperidol (10 μg/kg i.v.) were administered, and their effect on NMDA-induced activity was determined. This study also examined the effect of DTG (6μg/kg i.v.) on CA_3 population potentials. DTG potentiated NMDA-induced responses (5.50 ± .77 vs. 2.62 ± .24 spikes/nC: $p < .02$; $n = 6$), but had no effect on the amplitude of non NMDA-mediated CA_3 population potentials. These results indicate that the potentiating effect of DTG is specific to NMDA activity, and not reflective of a generalized enhancement of neuronal activity. The present study confirms the results reported by Monnet et al. (Eur. J. Pharmacol., 179: 441-445, 1990) and suggests an important role of sigma receptors in hippocampal physiology.

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16.7

A NOVEL SIGMA BINDING SITE THAT MAY NEGATIVELY MODULATE EXCITATORY AMINO ACID NEUROTRANSMISSION. R. R. Matsumoto*, W.D. Bowen, B.R. de Costa and J.C. Houk. Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago, IL 60611 and Lab. of Med. Chem., NIDDK, Bethesda, MD 20892.

Bath application of sigma ligands affected the burst duration of red nucleus (RN) neurons in the *in vitro* turtle brain in a dose-dependent manner. Some sigma ligands [DTG, haloperidol, BD1031, BD1052, BD1069] increased burst duration while others [dextrallorphan, (+)-pentazocine, (-)-pentazocine, BD1047, BD1063] decreased burst duration. The opposite effects appear to be mediated through different populations of sigma sites. Scatchard analysis showed that the turtle brain contains sigma sites that can be labelled with [3H]DTG and [3H](+)-pentazocine. The two radioligands appear to label different populations of sites because 1) competition studies show no correlation between the IC50 values of a number of cold ligands against the two radioligands, and 2) preliminary autoradiographic studies in the turtle brain suggest different patterns of labelling for the two radioligands. Competition studies under regular and masking conditions suggest that [3H]-DTG predominantly labels sigma-2 sites in the turtle brain. Furthermore, the rank order of IC50 values from [3H]DTG binding correlates with the rank order of ED50s for compounds that increase the burst response in the electrophysiological experiments. The sigma compounds that increase the burst response in the electrophysiological experiments also represent the subset of sigma ligands that produce dystonia when microinjected into the RN of rats and this behavior is also thought to be mediated through sigma-2 receptors. In contrast, [3H](+)-pentazocine appears to label a novel (non-sigma-1 or sigma-2) site in the turtle brain as suggested by its greater Bmax value (3X that of [3H]DTG) in Scatchard experiments and different drug selectivity pattern than would be expected from the typical sigma-1 binding. The rank order of IC50 values from [3H](+)-pentazocine binding correlates with the rank order of ED50s for compounds that decrease the burst response in the electrophysiological experiments. Since the burst response in turtle RN neurons is mediated through excitatory amino acid (EAA) neurotransmission, this novel site may be involved in the negative modulation of EAA neurotransmission.

16.8

CHARACTERIZATION OF A NOVEL SIGMA-LIKE BINDING SITE FOR [^{3}H](+)-PENTAZOCINE IN CLONAL CELL LINES. B.J. Vilner*, B.R. de Costa, and W.D. Bowen. Unit on Receptor Biochemistry and Pharmacology, Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892.

We have previously reported that NB41A3 and N1E-115 neuroblastomas, C6 glioma, and NG108-15 hybrid cells contain sigma-2 sites as determined by binding of [^{3}H]DTG (*Soc. Neurosci. Abstr.* 17: 593, #236.15, 1991; in: Multiple Sigma and PCP Receptor Ligands: Mechanisms for Neuromodulation and Protection?, J.-M. Kamenka and E.F. Domino, eds., 1992, in press). However, the presence of sigma-1 sites was difficult to detect, as binding of the sigma-1-selective ligand, [3H](+)-pentazocine was anomalous. Here we characterize [^{3}H](+)-pentazocine binding in these cells and demonstrate a novel sigma-like site which is distinct from sigma-1. Ligands were incubated with membranes (100 ug protein) in 250 ul of 50 mM Tris-HCl, pH 8.0 at 25ºC for 120 min. Non-specific binding was determined with 10 uM (+)-pentazocine. Saturation analysis revealed the presence of two sites (range of values in the four cell types): Kd1 = 3.0 - 7.0 nM, Bmax1 = 31.4 - 94.8 fmol/mg protein; Kd2 = 247 - 360 nM, Bmax2 = 946 - 5,431 fmol/mg protein. The high affinity site may represent sigma-1, while the low affinity, high capacity site is novel. Competition studies using 10 nM [^{3}H](+)-pentazocine revealed the following rank order of potency: haloperidol > (+)-pentazocine = dextrallorphan > (+)-3-PPP > DTG > (-)-pentazocine > fluphenazine = (+)-SKF 10,047 > (-)-SKF 10,047 >>> MK-801, naloxone, naltrexone. Although this site shows some of the characteristics of sigma-1 sites (enantioselectivity for (+)-benzomorphans) and sigma-2 sites (haloperidol Ki = 29 - 65 nM; (+)-3-PPP Ki = 132 - 436 nM), the affinity for (+)-pentazocine (Ki = 98 - 376 nM) and DTG (Ki = 434 - 1,028 nM) clearly distinguish this site from either sigma-1 or sigma-2. Preliminary data suggests that human SK-N-SH cells, human placenta, and turtle brain (see Matsumoto *et al.*, this meeting) also contain this putative sigma subtype.

16.9

BIPHASIC EFFECTS ON NMDA RESPONSE OF TWO ANTIDEPRESSANTS WITH HIGH AFFINITY FOR SIGMA SITES. R. Bergeron*, G. Debonnel and C. de Montigny, Neurobiological Psychiatry Unit, Department of Psychiatry, McGill University, Montréal, Québec, Canada.

We have previously shown, using *in vivo* extracellular unitary recordings, a selective potentiation of the NMDA-induced activation of rat CA_3 dorsal hippocampus pyramidal neurons by low doses of sigma (σ) agonists and the suppression of this effect by σ antagonists such as haloperidol and BMY-14 802 (*Monnet et al., JPET, 261:123-130, 1992*). The present studies were undertaken to compare the effects of two antidepressants with high affinity for σ sites sertraline and clorgyline, to those of paroxetine and tranylcypromine, two antidepressants devoid of affinity for σ sites.

Male Sprague-Dawley rats were anesthetized with urethane. Five-barrelled micropipettes were used for extracellular recording of CA_3 dorsal hippocampus pyramidal neurons and microiontophoresis of NMDA, QUIS and ACh. All other drugs were administered intravenously.

Sertraline and clorgyline, but not paroxetine nor tranylcypromine, potentiated selectively and dose-dependently, with a bell-shaped curve, the excitatory effect of NMDA. A maximal potentiation, with both σ ligands, was obtained at a dose of 250 μg/kg i.v. At higher doses, the potentiation progressively decreased and completely disappeared at 500 μg/kg. The potentiation was reversed by haloperidol (10 μg/kg), suggesting that it is mediated via σ receptors. To determine if this biphasic effect was specific for sertraline and clorgyline, the σ ligands DTG, JO-1784, (+)pentazocine, BD-737, L-687-384 were tested at the same doses. All σ ligands presented a similar bell-shaped dose-response curve. These data suggest that, at high doses, the σ agonists might act on a different site, for which they would have a lower affinity, the activation of which would neutralize the potentiating effects obtained with the low doses. The basis for this phenomenon remains to be elucidated.

16.10

ELECTROPHYSIOLOGICAL EVIDENCE FOR THE EXISTENCE OF SUBTYPES OF SIGMA RECEPTORS IN THE RAT DORSAL HIPPOCAMPUS: I. ANTAGONISTIC EFFECTS OF HIGH DOSES OF SERTRALINE, CLORGYLINE AND L-687-384. G. Debonnel*, R. Bergeron and C. de Montigny. Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada.

We have shown (*Bergeron et al., Neurosci. Abst. 1992*), using *in vivo* extracellular unitary recording of rat CA_3 dorsal hippocampus pyramidal neurons, that the high affinity sigma (σ) ligands sertraline, clorgyline and L-687-384 potentiate at low doses the neuronal response to NMDA and that this potentiation disappears when high doses are administered. The present study was undertaken to determine if high doses of these sigma ligands would antagonize the potentiation of the NMDA response by low doses of the three prototypical σ agonists DTG, (+)pentazocine and JO-1784. All drugs were administered intravenously.

Sertraline (1 mg/kg) prevented and reversed the potentiating effects of DTG (1 μg/kg) and (+)pentazocine (10 μg/kg), but not that of JO-1784 (4 μg/kg). Clorgyline (1 mg/kg) prevented and reversed the potentiating effect of JO-1784 and (+)pentazocine, but not that of DTG. Finally, L-687-384 (100 μg/kg) prevented and reversed the potentiating effect of DTG, but not those of JO-1784 and (+)pentazocine. Haloperidol (10 μg/kg) antagonized the potentiating effects of all three agonists.

The different antagonistic profiles of sertraline, clorgyline and L-687-384 suggest that at least three subtypes of σ receptors are involved in mediating the potentiation of the NMDA response by low doses of σ agonists in the rat CA_3 dorsal hippocampus. These data provide the first electrophysiolgical evidence for multiple σ receptors, the existence of which had been postulated from radioligand binding studies (*Quirion et al., TIPS, 13: 85-86, 1992*).

16.11

ELECTROPHYSIOLOGICAL EVIDENCE FOR THE EXISTENCE OF SUBTYPES OF SIGMA RECEPTORS IN THE RAT DORSAL HIPPOCAMPUS: II. EFFECT OF LESIONING THE MOSSY FIBER SYSTEM. F.P. Monnet*, G. Debonnel and C. de Montigny. Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada and Institut de Recherche Jouveinal, France.

The existence of different subtypes of sigma (σ) receptors is now accepted (*Quirion et al., TIPS, 13:85-86, 1992*). However, there is little functional evidence for the existence of these subtypes. We have previously shown that, *in vivo*, high affinity σ agonists selectively potentiate NMDA-induced activation of pyramidal neurons in the rat CA_3 dorsal hippocampus, and that these effects are reversed by the σ antagonist haloperidol (*Monnet et al., JPET, 261:123-130, 1992*). The present studies were undertaken to evaluate, in this model, the involvement of the mossy fiber system, the major input to CA_3 pyramidal neurons, using extracellular unitary recording, following a unilateral destruction of the dentate gyrus by a local injection of colchicine (10 µg in 2 µl). (+)Pentazocine (5 µg/kg, i.v.) did not affect the NMDA response in the lesioned side, while it produced a robust potentiation in the contralateral side. JO-1784 (5 µg/kg, i.v.) induced a similar potentiation of NMDA in both sides. Conversely, DTG (1 µg/kg, i.v.) reduced in the injected side and potentiated in the contralateral side the NMDA response. The effects of both JO-1784 and DTG were reversed by the subsequent injection of haloperidol (10 µg/kg, i.v.). Applied iontophoretically in lesioned rats, JO-1784 and DTG induced effects similar to those obtained when administered intravenously. These results support the existence of several types of σ receptors. The σ receptor responsible for the potentiating effects of (+)pentazocine and DTG is likely located on the mossy fiber terminals, whereas the potentiating effect of JO-1784 and the inhibitory effect of DTG are mediated by different subtypes of σ receptors not located on mossy fibers.

16.12

NOVEL PHENYLAMINOTETRALINS AS POSSIBLE AGONISTS AT SIGMA-TYPE NEUROMODULATORY RECEPTORS. R.G. Booth*, N.S. Kula, A.M. Myers, S.D. Wyrick, & R.J. Baldessarini. Medicinal Chemistry Division, Sch. of Pharmacy, Univ. of North Carolina, Chapel Hill, NC 27599; Harvard Med Sch. & Mailman Res. Ctr, McLean Hosp., Belmont, MA 02178

Effects of the phenylaminotetralin (±)*trans*-1-phenyl-3-dimethylamino-6-Cl-7-OH-tetrahydro-naphthalene (PAT-6) and its analogs on tyrosine hydroxylase (TH) activity were assessed in rat striatal minces. Also, the binding profile of ^{3}H-PAT-6 was evaluated under σ assay conditions with membranes from guinea pig brain. PAT-6 stimulated striatal TH activity by 28% at 100 nM; this effect was blocked selectively by the putative σ antagonist BMY-14802. Similar results were obtained with other PAT analogs. Activation of TH by PAT-6 was not additive with a greater increase (95%) of TH activity produced by 1 µM forskolin. ^{3}H-PAT-6 showed very high affinity for cerebral membranes (K_d = 50 pM). The pharmacology of this binding was not consistent with dopamine, serotonin, adrenergic, or opioid receptors, nor with P-450 or MAO enzymes. BMY-14802 had moderate affinity (Ki = 64 nM) for the ^{3}H-PAT-6 site, but other σ ligands showed little affinity. Theses results suggest that PAT analogs may act as agonists at a novel neuromodulatory σ receptor subtype that mediates stimulation of brain catecholamine synthesis, perhaps via stimulation of adenylate cyclase and TH phosphorylation.

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CATECHOLAMINES

17.1

NEURAL CONTROL OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE AND DOPAMINE β-HYDROXYLASE. D.L. WONG*, B. SIDDALL, W. WANG AND A. LESAGE. Dept. Psychiatry & Beh. Sci., Nancy Pritzker Laboratory, Stanford Univ. Sch. Med., Stanford, CA 94305

Activation of the splanchnic nerve to the adrenal gland increases the synthesis of the catecholamine biosynthetic enzymes, phenylethanolamine N-methyltransferase (PNMT) and dopamine β-hydroxylase (DBH). To examine whether this response involves gene transcription, temporal changes in PNMT and DBH mRNA, activity and protein have been followed after acute (1 injection, 10 mg/kg i.p.) or chronic (4 injections on alternate days) reserpine treatment. PNMT and DBH mRNA were measured by solution hybridization, activity by radioenzymatic assay and protein by immunotitration. PNMT mRNA increases very rapidly, peaking at 7.5-fold control 6 hr after a single injection. With additional reserpine treatment, a second, slower rise occurs (2.8-fold control after 3 injections). Protein and activity changes lag behind and are of lesser magnitude. Activity rises 2.0-fold 12 hr after acute treatment and after 4 injections as well; protein increases 1.2-fold and 1.4-fold simultaneously. In contrast, an acute dose of reserpine does not change either DBH mRNA or activity. However, chronic treatment increases both indices in parallel with a plateau at 2-fold basal values. Interestingly, adrenal corticosterone (CORT) also responds to reserpine, peaking at 4-fold normal 3 hr after a single dose, declining slightly at 12 hr and then, resting at 4-fold normal thereafter. Changes in PNMT and DBH mRNA and activity/protein appear consistent with gene activation, although in the case of PNMT, magnitudinal differences in activity and protein suggest that other posttranscriptional controls may exist. Furthermore, although hormonal and neural stimuli have been shown to control PNMT and DBH degradation and synthesis respectively, the concommitment rise in CORT suggests an interplay between these regulators.

17.2

NOREPINEPHRINE AND ANXIETY-LIKE BEHAVIOR: ELECTROPHYSIOLOGY STUDIES IN MAUDSLEY RATS. J.S. Verbanac*, R.L. Commissaris, H.J. Altman and D.K. Pitts. Dept. Pharmaceut. Sci & Psychiatry, Wayne St. Univ., Detroit, MI 48202.

The Maudsley rat strains are purported to be a genetically-inbred animal model for anxiety. Consistent with this, Maudsley Reactive (MR) rats exhibit greater anxiety-like behavior than do their Non-Reactive (MNRA) counterparts in many behavioral tests. Norepinephrine (NE) neuronal transmission, particularly that eminating from the nucleus locus ceruleus (LC), has been proposed to be involved in the expression of anxiety-related behavior. In the present study, extracellular single-unit recording and behavioral conflict testing were conducted in the same subjects to determine the relationship between LC neuronal discharge and anxiety-like behavior in MR and MNRA rats. Consistent with their presumably lower level of anxiety, MNRA rats accepted significantly more shocks than did MR rats in the conflict task. The characteristics of LC neuron electrophysiology in conflict-experienced rats were found to be strikingly similar to those observed in naive MR and MNRA rats. That is, LC neuron basal discharge rates were lower in MR compared to MNRA rats. In addition, LC cells from MNRA rats were less sensitive to the rate decreasing effects of IV clonidine. Preliminary data suggests that there is no significant correlation between LC firing rates and conflict behavior in either MR or MNRA rats. Supported by MH47181 (RLC) and MH47857 (DKP).

17.3

THE EFFECT OF THE DURATION OF STREPTOZOTOCIN-INDUCED DIABETES ON TURNOVER OF CENTRAL BIOGENIC AMINES IN MICE. C.C. Chen, J.C. Yang and C.Y. Wen* Dept. of Pharmacology and Anatomy, College of Medicine, National Taiwan University, Taipei, Taiwan 10018, R.O.C.

The relationship between central catecholaminergic and serotonergic neurons and the development of streptozotocin (STZ)-induced diabetic mice was examined over a periods of 3,14,50 to 100 days. The accumulation of 3,4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP) were measured after central decarboxylase activity was inhibited by m-hydroxybenzylhydrazine (NSD-1015). Results indicated that DOPA accumulation in the hypothalamus was not altered during the entire time course of the experiment studied. On the other hand, DOPA accumulation in the striatum was decreased in 50 and 100 day diabetic mice. The DOPA levels in pons-medulla were not increased until 100 day diabetics. The accumulation of 5-HTP was decreased in the hypothalamus of 14 day diabetic mice and was also present at 50 and 100 days, that in the striatum and pons-medulla was not decreased until 50 day diabetics and persisted to 100 days in the the striatum. These data showed that both DOPA and 5-HTP accumulation in the striatum and pons-medulla were changed only in the long term diabetic mice, and suggested that changes in catecholamine and serotonin turnover rates are not generalized, but restricted only to some particular brain regions and time courses.

17.4

FLUORINE-18 LABELED BIOGENIC AMINES: POTENTIAL RADIOTRACERS FOR ADRENERGIC NEURONAL MAPPING BY PET. M.E. Van Dort, Y-W Jung, M.R. Kilbourn, P.S. Sherman, T.L. Pisani, D.M. Wieland*. Div. of Nucl. Med., Univ. of Michigan, Ann Arbor, MI 48109.

The overall goal in this work is to develop fluorine-18 labeled analogs of biogenic amines for mapping cardiac adrenergic neurons by Positron Emission Tomography (PET). The purpose of this study was to 1) evaluate the synthetic feasibility of replacing the benzylic hydroxyl group in biogenic amines with fluorine-18, and 2) determine the in vivo disposition and stability of these fluorine-18 labeled biogenic amines. Accordingly the ß-hydroxyl functionality of two model biogenic amines, (1R,2S)(-) ephedrine and (1S,2S)(+) pseudoephedrine was stereospecifically replaced with fluorine-18 by a novel cyclic sulfamate route to yield 1-[^{18}F]fluoro-1-deoxy-psuedoephedrine and [^{18}F]fluoro-1-deoxy- ephedrine, respectively, in high radiochemical yields. Preliminary biodistribution studies in mice indicated excellent in vivo stability towards defluorination and high blood-brain barrier permeability. These radiofluorinated analogs displayed time-radioactivity curves in mouse brain similar to that observed with carbon-11 labeled methamphetamine. Extension of this work towards the synthesis of a fluorine-18 labeled analog of the adrenergic neuronal mapping agent meta-hydroxyephedrine is currently in progress.

17.5

PHARMACOLOGICAL ACTIVATION OF QUINPIROLE MEDIATED PATHWAYS IN BABOON BRAIN STUDIED WITH PET. J.S. Perlmutter* & L.L. Lich Washington Univ. School of Medicine, St. Louis, MO 63110.

Previously, we measured regional cerebral blood flow responses to dopamine agonists in sedated baboons. Acute quinpirole produced a dose-responsive reduction of pallidal flow. We now demonstrate the specificity of this response and evaluate the effects of sedation. We surgically implanted a customized skull cap that fits into a headholder to permit precise fixation and correlation between PET and MR images. Flow was measured with 40-sec PET scans after bolus [O-15]water before & after quinpirole (.2mg/kg i.v.). Regional changes in flow were identified by subtraction image analysis. In sedated baboons (ketamine for induction, atropine to decrease secretions, nitrous oxide for sedation & gallamine to permit careful control of pCO2), pallidal flow decreased an average of 16%. Pretreatment with the selective D2 antagonist eticlopride (4mg/kg) eliminated the quinpirole-induced response. We also trained a monkey (operant conditioning using positive reinforcement, only) to lie awake in the PET. The qualitative decrease in pallidal flow (15.5%) after quinpirole was not significantly different from sedated baboons. These data demonstrate the specificity of the local flow response to quinpirole and validate the use of the sedated baboon. Pharmacologic activation using PET permits repeated evaluation of the function of receptor mediated pathways.

17.6

DOPAMINE SYNAPTIC ORGANIZATION IN MONKEY PREFRONTAL CORTEX J.F. Smiley* and P.S. Goldman-Rakic, Section of Neurobiology, Yale Sch. Med., New Haven, CT

The ultrastructural features and synaptic organization of dopamine axons were analyzed in individual layers of monkey prefrontal cortex. Dopamine immunoreactivity was visualized with a silver preciptiation technique, recently developed in our lab, which allowed clear resolution of internal structures and cell membranes of labeled axons. Dopamine varicosities contained numerous clear vesicles, and always contained dense core vesicles. This description contrasts to our finding in the anterior caudate of monkey, where dopamine axons lacked dense core vesicles, and suggests the presence of colocalized neuropeptides in the cortex. Complete serial sectioning through labeled varicosities showed that 40% of all varicosites formed synapses. However, dopamine synapses were small and symmetric, typically seen in only one or two serial sections. We therefore conclude that these are sometimes not visualized, and it is possible that all dopamine varicosities form synapses. In all layers dopamine synapses were onto spines and small dendritic shafts, some of which were seen to form spines. We conclude that the majority of dopamine synapses are onto distal dendrites of pyramidal cells. The homogeneous appearance of dopamine axons and their postsynaptic targets suggests that they arise from a single population of cells in the midbrain.
Supported by MH44866.

17.7

EFFECTS OF THE 5-HT_{1A} AGONIST 8-OH-DPAT ON BRAIN CATECHOLAMINERGIC NEUROTRANSMISSION IN THE RAT. S. Ahlenius[1]*, V. Hillegaart[1] and A. Vijkström[2]. Departments of Behavioral Pharmacology and Bioanalysis[2], Astra Arcus AB, S-151 85 Södertälje, Sweden.

The activity of 8-OH-DPAT at brain DA and NA receptors was studied by measuring compensatory changes in catecholamine synthesis, as estimated by DOPA accumulation in NSD-1015 (100 mg kg^{-1} IP) treated rats. Corresponding measurements of 5-HTP accumulation were made in parallel. In normal rats, there were no effects on the DOPA accumulation in the ventral or in the dorsal region of the neostriatum after the administration of 8-OH-DPAT (2.4 µmol kg^{-1} SC), whereas a significant increase was found in the frontal neocortex. Since NA is the predominant catecholamine in this latter area, the results suggest α_2-receptor blocking properties of 8-OH-DPAT. The two 5-HT_{1A} agonists 8-OH-DPAT and flesinoxan both produced a marked decrease in forebrain 5-HTP accumulation. Since an increased neocortical DOPA accumulation was only obtained by 8-OH-DPAT, and not by flesinoxan (0.6-10.0 µmol kg^{-1} SC), administration, it appears that 8-OH-DPAT may have a direct action as an α_2-receptor antagonist in the rat brain. In reserpine treated rats, 8-OH-DPAT produced a decreased DOPA accumulation in the ventral, but not the dorsal, neostriatum, indicating a selective stimulation of DA receptors in this limbic forebrain area. Taken together, these observations suggest that in addition to its demonstrated ability to stimulate brain 5-HT_{1A} receptors, 8-OH-DPAT possesses α_2-receptor blocking properties and also produces a region-selective stimulation of brain DA receptors.

17.8

EFFECT OF DAU 6215, A NOVEL 5HT3 ANTAGONIST, ON (+)- SKF 10,047-INDUCED DOPAMINE RELEASE IN STRIATUM AND NUCLEUS ACCUMBENS. A COMPARATIVE STUDY WITH HALOPERIDOL AND CLOZAPINE. M. Volontè*, A. Ceci, E. Esposito and Franco Borsini. Boehringer Ingelheim Italia S.p.A. Milan, Italy.

The potential antagonist effects of haloperidol and clozapine and of a new selective 5HT3 antagonist, DAU 6215 (3-alfa-tropanyl 1H-benzimidazolone-3-carboxamide) (Turconi et al. 1991), were evaluated on dopamine release stimulated by the sigma agonist (+)- SKF 10,047 (N-allylnormetazocine). Dopamine release was estimated in the striatum and nucleus accumbens of the same animal by trans-cerebral dialysis of freely moving rats. (+)- SKF 10,047 (2.5, 5 and 10 mg/kg s.c.) increased in dose-related manner the release of dopamine, with preferentially effect in nucleus accumbens versus striatum. Saline or (+)- SKF 10,047 (5 mg/kg s.c.) was administered 45 min after vehicle, DAU 6215 (10 µg/kg), haloperidol (50 µg/kg) and clozapine (10 mg/kg). All compounds were given s.c. at the volume of 1 ml/kg. Clozapine and DAU 6215, which per se did not modify dopamine release in both areas, antagonized (+)- SKF 10,047-induced dopamine release in nucleus accumbens but not in striatum. Haloperidol, which per se increased dopamine release in both areas, induced addictive effects with (+)- SKF 10,047 (5 mg/kg). Our results suggest that DAU 6215, similarly to clozapine, may have potential antipsychotic activity with low incidence of extrapyramidal side-effects.

Reference

Turconi M. et al., Eur. J. Pharmacol., 203, 203, 1991

17.9

EXTRACELLULAR LEVELS OF MONOAMINES IN STRIATUM OF BABOON AND RAT FOLLOWING ORAL PHENYLALANINE. B.Arvin, A.G Chapman & B.S.Meldrum. **Spon: Brain Res. Assoc.** Inst Psychiatry London SE5 8AF U.K.

L-phenylalanine (PHE) has been suggested to reduce seizure thresholds in some models of epilepsy by modulating brain dopamine (DA) metabolism. In this study we compared the effects of oral PHE on extracellular levels of DA and metabolites in baboon striatum with that of i.v amphetamine (amph 0.5mg/kg) in 8 conscious adult baboons (Papio papio) bilaterally implanted with guide cannula. Microdialysis samples (20µl/20min) were collected over 3 periods, basal (0-60min), 0-140min post PHE and 0-60min post amph. Mean plasma PHE levels were 49nmol/ml (control) and 930nmol/ml 100min after PHE 450mg/kg. In a parallel study transverse dialysis was done in the striatum of 10 Wistar rats using a similar protocol as above. There was an apparent (1.5 in baboon and 3 fold in rat) but non-significant increase in DA 100min post PHE (450 but not 100mg/kg). No change was seen in other metabolites after either dose of PHE. Amph caused a large increase in DA (5-30 fold) in both studies. In summary, oral PHE did not significantly change extracellular levels of DA and metabolites in baboon or rat striatum.

18.1

IS HUMAN TRICEPS SURAE ELECTROMYOGRAM A VESTIBULOSPINAL REFLEX RESPONSE? I. S. Storper and V. Honrubia*, Victor Goodhill Ear Center, UCLA School of Medicine, Los Angeles, 90024

Interest in understanding the human vestibulospinal reflex has increased enormously over the past three decades, as this reflex is the primary effector of maintenance of posture and balance. On a posture platform, forces exerted by the triceps surae (TS) and tibialis anterior (TA) muscles are measured to calculate center of mass (COM) sway. We wished to determine whether the TS response is a direct component of the vestibulospinal reflex.

Ten healthy human beings were stimulated with sinusoidal galvanic currents delivered over their mastoid processes. Sway response on a posture platform and TS electromyogram (EMG) were recorded for the following conditions: (1) standing unrestrained; (2) standing completely restrained above the leg; and (3) sitting unrestrained.

Results were similar for all subjects. Computer-aided analysis for case (1) reveals that TS EMG and horizontal body sway responses are generated at the same frequency as the stimulating current, with a phase lag of 90 degrees. For case (2), body sway response and any component of the TS EMG over the unstimulated condition were absent in all subjects. For case (3), body sway persisted but no TS EMG above the unstimulated condition was recorded.

As the TS EMG disappears when the standing subject is restrained from swaying or in the unrestrained subject, we conclude that the TS EMG response is compensatory to motion of more superior portions of the musculoskeletal system; it is not part of the vestibulospinal reflex.

18.2

POSTURAL FORCE FIELDS OF THE HUMAN ARM. R Shadmehr*, FA Mussa-Ivaldi and E Bizzi. Brain & Cognitive Sciences, MIT, Cambridge, MA 02139.

When a perturbation displaces the hand from equilibrium, arm muscles respond by producing restoring forces. Displacements given at various directions from the same equilibrium position result in restoring forces which describe a *postural force field.* We ask whether these forces are related to forces generated when a reaching movement is executed: if a movement is a consequence of a shift of the equilibrium position of the hand toward the target, then, from the postural force field, predictions can be made regarding the elastic forces generated during a movement. We have measured the postural force field over relatively large distances and compared these forces with the static forces generated at the hand during a reaching movement.

A robot was used to displace the hand at various directions from an equilibrium position. The measured restoring forces were fitted to a non-linear model to define a postural force field for that equilibrium position. This field was used to predict elastic forces generated when the subject attempted to move the manipulandum from a point on the circumference of a circle to a target at its center—the center corresponded to the equilibrium position where the postural field was measured at. In some of the movement trials, the manipulandum was locked during approximately the first 120 msec of the program for motion and the resulting static *evoked forces* measured. We found that: (1) the evoked forces did not point to the target, but were a function of the configuration of the arm and rotated with the shoulder joint, and (2) the magnitude of the evoked forces varied systematically, even though the movements were of the same magnitude. These patterns were remarkably similar to those observed in the postural forces. Our results provide experimental evidence linking maintenance of posture to the generation of movement in a multi-joint system. The evidence is consistent with the hypothesis that the CNS programs a reaching movement by shifting the equilibrium position of the hand toward the target.

This work was supported by NIH grants NS09343 and AR26710, ONR grant N00014/K/0372, and a McDonnell-Pew Post-Doctoral Fellowship.

18.3

EFFECTS OF NORMAL AND MAGNIFIED VISION ON HEAD OSCILLATIONS. J. Goldberg* and L. Weider. Clayton Lab., Dept. of Otolaryngology, Baylor College of Medicine, Houston, TX 77030.

Involuntary oscillations of the head on the trunk contain frequency components up to 15 Hz, beyond the limitations of visuo-motor reflexes. Thus, visual feedback can be expected to increase oscillation intensity.

To test this hypothesis in 3 dimensions, we recorded angular head velocity in roll, pitch, and yaw axes in 16 seated volunteers. They wore a headband carrying velocity sensors and a light source for projecting an arrow onto a screen 4m away. Root mean square (RMS) velocity was computed over 30 seconds and served as an index of oscillation intensity for each axis. Each subject was instructed to hold his/her head still and "look" at a horizontal or vertical line on the screen under four conditions presented in random order. These included: (i) normal 1x vision with arrow off (1xV), (ii) normal vision pointing (1xP) - keeping the arrow on the target line, (iii) 4x magnified vision pointing (4xP) - keeping the arrow on the line viewed through 4x magnifying spectacles, and (iv) baseline - vision occluded by opaque goggles, eyes open (0xV).

Analysis of variance on repeated measures indicated that visual feedback affected RMS velocity in the pitch and yaw axis during horizontal line viewing ($p<0.01$) and in yaw, pitch and roll axis during vertical line viewing ($p<0.05$). For the horiz. line, the largest increase relative to baseline 0xV was observed in pitch RMS, condition 4xP ($p<0.005$). For the vert. line, yaw RMS showed the largest increase, again in condition 4xP ($p<0.005$). For both line targets, RMS increased significantly with vision (1xV vs 0xV) in yaw ($p<0.005$) but not pitch.

These preliminary results show that visual feedback causes an increase in head oscillations, which appears to depend on feedback gain.

Supported by the Clayton Foundation for Research and EY06394

18.4

ASYNCHRONY BETWEEN 2-D BIMANUAL HAND MOVEMENTS AS A FUNCTION OF CEREBRAL DOMINANCE. P. Viviani, N. Stucchi, and J.Requin* Dept. of Psychobiology, Faculty of Psychology, University of Geneva, 1211, Geneva, Switzerland and Dept. of Psychobiology, CNRS, Marseille, France.

Reaction times for bimanual movements aimed simultaneously at visual targets are poor discriminators of cerebral dominance (Bartlett & White, 1965). By contrast, we found that temporal phase during periodic movements of the two hands depends on subject's handedness. Right- and left-handers were asked to trace continuously with both hands simple, closed, geometrical patterns (ellipses). Movements were performed in the frontal plane. Four types of motor tasks were considered which resulted from combining the two possible directions of rotation in each hand. Each type was executed at six different tempos (40 to 90 cycles/min). The trajectories of the outstretched index fingers were recorded with the ELITE system (spatial accuracy: $\leq$ 1 mm; sampling frequency: 100 Hz). By measuring the passage times at homologous points of the trajectories, we measured the instantaneous asynchrony between the two hands throughout several cycles of movement. It was found that, although motor performances in both hands are comparable for all subjects, the dominant hand leads the non-dominant one by about 30 msec. This temporal delay is affected neither by the type of movement nor by its rhythm. Thus, it cannot be construed as the result of a strategy in which the non-dominating hands tracks the other one. The variability of the delay across cycles depends on the location within the trajectory. The variability profile has well-defined maxima and minima. The profiles for movements engaging homologous muscles differ markedly from those engaging non-homologous muscles. We argue that the results are compatible with the hypothesis that bimanual periodic movements are timed by a functional module residing in the dominant hemisphere, and that the observed asynchrony is due to the necessity of transmitting time-keeping information to the other hemisphere, possibly through callosal pathways (Filbey & Cazzaniga, 1969).

18.5

CHARACTERISTICS OF GAIT INITIATION IN NORMAL AND ABNORMAL OLDER PEOPLE. R.J. Elble* and C. Moody. Southern Illinois Univ. Sch. of Med., Springfield, IL 62794-9230.

Electromyograms of the thigh and leg muscles, foot-floor reaction forces and motion of the body were recorded from 16 normal adults, ages 18 to 83, during initiation of a rapid forward step in response to a visual cue. These measures were compared with those from elderly patients with Binswanger disease (2), Alzheimer disease (2), Parkinson disease (3), sensorimotor neuropathy (1) and communicating hydrocephalus (1). During normal gait initiation the body rotates about the ankles like an inverted pendulum. The ventral muscles of the lower extremities fire synergistically in the initiation of gait to create a forward moment about the ankles, propelling the body into forward motion. The dorsal muscles fire subsequently as the center of mass is displaced laterally over the stance foot, in preparation for toe-off. This smooth, stereotypic sequence of postural shifts was exhibited by all of our healthy volunteers. The Alzheimer patients exhibited normal patterns of gait initiation, but their reaction times were approximately twice normal. Start hesitation in the remaining patients resulted from disordered patterns of muscle activation, which produced highly erratic, ineffectual postural shifts. Specific disturbances in the control of posture and movement, not a delayed formulation of the motor plan, probably underlie start hesitation in such patients. (Supported by NIA P30 AG08014)

18.6

MODULATION OF RISE IN PREMOVEMENT CORTICAL EXCITABILITY IN A CHOICE REACTION TIME PARADIGM. A. Pascual-Leone * and M. Hallett. Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892

In a simple reaction time (RT) paradigm, the excitability of the motor structures targetting agonist and antagonist muscles for the requested response movement increases progressively leading up to movement onset. Here we studied this premovement facilitation in a choice RT paradigm.

We studied 7 right-handed normal volunteers (5 men and 2 women, aged 23 to 50 years). A monitor displayed a yellow dot (warning signal) for 500 ms followed randomly, after a variable foreperiod (1-3 s), either by a red or a green dot (go-signal). In different sets of 200 trials, the subjects had to respond as fast as possible with right thumb abduction (sRT1) or with right ankle extension (sRT2) regardless of the color of the go-signal, or with right thumb abduction to the green and right ankle extension to the red go-signal (cRT). Surface EMG was recorded simultaneously from right abductor pollicis brevis (APB) and right anterior tibialis (AT). Transcranial magnetic stimulation was applied at different times following the go-signal at an intensity subthreshold for evoking motor potentials (MEPs) at rest.

In sRT1 and 2, the probability of evoking MEPs and their amplitude began to increase 110 ms before voluntary EMG onset and raised steadily thereafter but only for the muscle involved in the requested response (APB in sRT1 and AT in sRT2). In cRT, the probability of evoking MEPs and their amplitude began to increase 120 ms before voluntary EMG onset and raised steadily thereafter in parallel for APB and AT regardless of the eventual response. This pre-movement facilitation diverged only 20-30 ms before voluntary EMG onset when MEPs in the muscle involved in the eventual response became further facilitated while those in the other muscle ceased to grow or even decreased in amplitude.

The results suggest that in a two-choice RT paradigm, the motor structures targetting muscles involved in both responses are activated in parallel and only very shortly before execution of the response do the incorrect response structures get inhibited in favor of the correct ones.

18.7

H-REFLEX MODULATIONS DURING VOLUNTARY MOVEMENTS FOLLOWING PERINATAL BRAIN DAMAGE. C. T. Leonard*, Motor Control Research Laboratory, The Univ. of Montana, Missoula, MT 59812

An adaptation of H-reflex testing procedures has been developed that allows the continuous monitoring of human alpha motoneuron activity during movement. The technique differs from previously used H-reflex testing techniques in that it utilizes a 5 Hz stimulation to elicit the H-reflex. This allows for continuous and concomitant H and M wave recordings during a movement. This is an improvement over single stimulus techniques in that more data points are collected in less time, and subject anticipatory reactions are less of a factor. The procedure has proven particularly useful for the testing of neurologically impaired patient populations.

The technique has been used to assess agonist muscle alpha motoneuron activation and reciprocal inhibition of antagonist musculature during various voluntary movements of non-disabled individuals and of individuals with spastic-type cerebral palsy (CP). During voluntary dorsiflexions of the ankle, non-disabled individuals show an inhibition of the antagonist soleus alpha motoneurons prior to activation of the tibialis anterior (TA) muscle. The inhibition increases and remains during the time the tibialis anterior is actively contracted. Individuals with CP do not show inhibition of the antagonist soleus either prior to or during activation of the TA. These results suggest that the supraspinal component to reciprocal inhibition is absent in individuals with CP and explains, in part, the abnormal muscular co-contraction present during voluntary movement in individuals with CP.

18.8

LOCOMOTOR-LIKE ROTATION OF EITHER KNEE OR HIP STRONGLY INHIBITS SOLEUS H REFLEXES. J.E. Misiaszek*, J.D. Brooke and J. Cheng. Human Neurophysiol. Lab., School of Human Biology, University of Guelph, Ontario, Canada, N1G 2W1.

Human soleus H reflexes are depressed during active and passive pedalling of the ipsilateral leg, compared to control reflexes when sitting. We investigated whether this inhibition arises from peripheral events associated with rotation of either the knee or hip. H reflexes were recorded from four subjects during 1. passive pedalling rotation of the ipsilateral leg at 60 rpm, 2. rhythmic rotation about the ipsilateral knee, with maximum velocity and range as in pedalling ($p>0.05$), 3. similar movement of the hip and 4. stationary sitting. Rotation of either knee or hip significantly reduced ($p<0.05$) the magnitude of mean H reflexes compared to sitting control, arriving at values close to those when the whole limb was moved. [mean H reflexes: sitting 71.09%, whole leg 9.92%, knee only 13.19%, hip only 13.58%, as % Mmax from each subject]. Thus, somatosensory receptor discharge, from movement of the knee and also the hip, appears to be a foundation for leg movement induced inhibition of H reflexes in humans. Supported by NSERC CANADA Grant #A0025.

18.9

MAINTAINING LATERAL EQUILIBRIUM DURING STANDING. W.A.Lee*, A.M. Wing, J.R. Jenner. Physical Therapy, Northwestern Univ., Chicago IL USA 60611; MRC-APU, Cambridge Univ.; Addenbrooke's Hospital, Cambridge UK

How subjects maintain standing equlibrium against external perturbations has been less investigated in the frontal than the sagittal plane. We studied the properties of normal adult responses to abrupt step changes in force applied laterally to the hips.

A servo-controlled actuator coupled to a semi-rigid belt around the hips delivered forces of up to 15% body weight and measured hip displacement. Subjects were told to stand straight and resist force steps of increasing magnitude (maximum force = load sustained without stepping). Push and release trials (i.e., force increased from or decreased to zero) were alternated; force direction (right, left) was blocked. Trials were 5.0 s; the load was applied at 0.5s. Natural visual surround but not hip motion information was available to the subjects.

Hip displacement increased rapidly in the direction of the force to a final steady posture, measured during the last 0.5s of the trial. The initial peak and final displacements increased linearly with force, until subjects had to step. The form of the hip-force functions was consistent with a second-order system, implying that control of lateral hip displacement in this task can be modelled as an inverted pendulum with constant stiffness springs. (Partial support of W.L. by NSF BNS-9021486)

18.10

TRIGEMINAL STIMULATION AND ACTIVITY IN NECK MOTONEURONS. V.C. Abrahams*, F.J.R. Richmond, P.K. Rose, A.A. Kori and G.E. Loeb, MRC Group in Sensory-Motor Physiology, Department of Physiology, Queen's University, Kingston, Ontario K7L 3N6.

Most work on the control of head movement has been concerned with gaze, in which head movement is largely subordinated to the requirements of vision. There are, however, many head movements in which the requirements of vision are not pre-eminent. In the cat these include movements of grooming, tactile and olfactory exploration and reflex movements such as inertial feeding and head aversion. The neck muscle activity underlying head aversion following tactile stimulation of the face was examined in videotaped unrestrained cats instrumented with EMG electrodes. Typically a light tactile stimulus results in an upward and backward movement associated with simultaneous contraction of deep and superficial dorsal neck muscles. Studies in the ketamine/chloralose anaesthetized cat have shown that a similar pattern of muscle activation can be produced by applying a mechanical stimulus to the face. Light touch of receptor-rich glabrous skin around the nostrils is particularly effective. Brief stimulus trains delivered to the infraorbital nerves at or near threshold causes a compound action potential in dorsal neck muscle nerves at a latency similar to that of muscle contraction in the conscious cat and raises the possibility that this trigemino-neck reflex (TNR) arises from the same neural substrate as head aversion. Intracellular recording from neck motoneurons showed small EPSPs at a minimum latency of 2.5 msec following weak stimulation of the infraorbital nerve. However, the first action potentials arose from a large, later EPSP at a latency consistent with the observations made in the conscious animal and observation of the TNR. The TNR has been shown to be abolished by a low section of the descending tract of V, suggesting that an interneuronal system responsible for the TNR and perhaps head aversion may be located intraspinally.

18.11

FREQUENCY ANALYSIS OF THE POSTURAL SWAY OF NORMAL AND VISUALLY IMPAIRED CHILDREN. C.V. Portfors-Yeomans and C.L. Riach*. Department of Physical Education, McMaster University, Hamilton, Ont., Canada L8S 4K1

Thirty-six normal (N) children aged 4-12 and 12 congenital visually impaired (VI) children aged 5-12 stood quietly on a force plate (AMTI) under 4 conditions: normal surface, eyes open (NEO); normal surface, eyes closed (NEC); foam surface, eyes open (FEO); foam surface, eyes closed (FEC). Centre of pressure excursions (LAT and A-P) were sampled at 10 Hz and then analyzed using fast fourier transformation. The frequency data was analyzed between 0-4 Hz. Total power was calculated for each trial by integrating the frequency function. The frequency data was logarithmically transformed before linear regression analysis. The slope of the regression line was used to compare the relative amounts of low and high frequency sway. The N children were grouped by age (4-5,6-7,8-9,10-12) for ANOVA. The Mann-Whitney test was used to compare N and VI children. Young N children had higher power and relatively more high frequency sway in EO and EC. Total power and relative sway at high frequencies decreased with age with significant decreases after 7 years in the A-P direction. Young children may use a preprogrammed ballistic strategy of control as seen by increased high frequency sway while older children may integrate multi-sensory feedback control to produce slower but more accurate corrections. No observable changes were seen with age in VI. VI children had significantly greater total power and relatively more low frequency sway than N children. The postural control system of VI children may adapt at a young age to rely more on vestibular and somatosensory inputs to stabilize sway.

18.12

SYNERGISTIC MUSCLE COMPENSATION DURING FATIGUE. J. Mao, R.B. Stein* and J.W. Osborn. Faculties of Dentistry and Medicine, Univ. of Alberta, Edmonton, AB, CANADA T6G 2N8.

We have previously shown that when jaw closing muscles sustain a maximum incisal bite force (isometric) for as long as possible, the direction of the bite force changes. The goal of this study was to test possible associated changes in the tensions generated by these muscles. Three healthy human subjects sustained maximum voluntary bite forces on a custom developed 3-D force transducer placed horizontally between upper and lower central incisors. The magnitude of the bite force was displayed on a computer screen. Subjects could therefore verify that they were maintaining constant, maximum bite forces. Surface EMG of bilateral superficial masseter and anterior temporal muscles was measured. EMG of each muscle was plotted against time. The results showed that the masseter EMG steadily decreased during the test while the temporalis EMG was roughly maintained or slightly increased. These results seem to account for the change in bite force direction from anterior to nearly vertical in the previous study. The changes in tensions of these muscles may be attributed to (1) subjects learning to use combinations of muscles more effectively and (2) one muscle (and probably others) increasing its activity to compensate for a loss in the tension of a fatiguing synergistic partner.

18.13

H-REFLEX MODULATION BY BALANCE PERTURBATION.
M. Sabbahi,* H. Qureshy, F. Lin, and A. Overby. Texas Woman's University, School of Physical Therapy, Houston, TX 77030

Postural and balance control are often set by volitional and reflex components of movement. The gain of the stretch reflex has been found to be modulated during locomotion and balance (Brooke & Collins, 1991). This study evaluates the excitability of the H-reflex in response to perturbation.

Twenty young adult subjects stood comfortably on the footplates positioned on the platform of the Balance System with eyes open and eyes closed while forward-backward linear perturbations (LP) were elicited for 1.5 inches at a speed 0.8 inches/sec. The platform was then moved into angular dorsiflexion (ADP) or plantarflexion (APP) for 8^{o} excursion at a speed of 0.5^{o}/sec. Soleus H-reflexes were recorded using surface electrodes after tibial nerve stimulation (0.5 ms., 0.2 pps at 70% of maximum reflex). Reflex was recorded halfway into the perturbation and was monitored by the M-response. Responses were amplified 1000x using 10Hz - 10KHZ bandpass and four sweeps were averaged. A MANOVA was employed to compare the H-reflexes during different testing conditions. Results showed significant H-reflex inhibition during ADP with eyes open and closed; and H-reflex facilitation during APP with eyes open and closed. No measurable changes were recorded during LP. Results indicate that reflex modulation may occur during angular and not slow linear perturbation of balance. Visual feedback appears to have minimal effect on H-reflex modulation and motoneuron excitability.

OCULOMOTOR I

19.1

EYE POSITION AND VELOCITY INTEGRATORS ARE IN SEPARATE COMPARTMENTS OF THE GOLDFISH HINDBRAIN.
A. M. Pastor, R. R. de la Cruz, and R. Baker*. Dept. of Physiology and Biophysics, NYU Medical Center, New York, NY 10016.

Two neural integrators are required for generating horizontal eye position and extending the time span for oculomotor compensation of self or visual world motion. The location of these integrators was established in goldfish by single-unit recordings and lidocaine inactivation of brainstem areas while monitoring eye movements. Four regions, called Areas I-IV, were identified in the region extending from the obex to the caudal pole of the vestibular nuclei. Areas I and III processed signals related to eye position while Areas II and IV were related to eye velocity. Neuronal responses in Areas I and II were strongly correlated with eye position and velocity. Bilateral, 1nl injections of lidocaine into Area I produced, after spontaneous saccades, a centripetal exponential drift in eye position with a time constant of 240ms. Unilateral Area I inactivation resulted in time constants of about 1s. Unilateral Area II inactivation produced a nystagmus with constant velocity 20°/s slow phases directed towards the contralateral side. Bilateral Area II inactivation caused no velocity bias, but reduced the time constant of the per-rotatory responses to head velocity steps from a control value of 10s to a minimum of 270ms. Up to 10nl of lidocaine in Areas III and IV produced only small changes in eye movement that were likely due to spread into adjacent areas. Given the specificity and extent of horizontal eye movement deficits, we conclude that Areas I and II represent the 'velocity to position' and 'velocity storage' integrators, respectively. The uniformity of neuronal activity within each compartment suggests that integration is a local, one-step process that could be the consequence of the intrinsic membrane properties of individual neurons.

19.2

ROLE OF OMNIPAUSE NEURONS IN SACCADE-VERGENCE INTERACTIONS.
L.E. Mays* and P.D.R. Gamlin. Dept. of Physiological Optics, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Saccades are high velocity movements in which the two eyes move as if yoked. Looking between targets at different distances requires ocular vergence, in which the two eyes move slowly in opposite directions. Most gaze shifts between targets at different distances also require changes in direction mediated by saccades. When a saccade occurs during a vergence movement, the amplitudes of the saccades in the two eyes may be markedly unequal. This observation has led to speculation that primates retain circuitry which allows relatively independent control of the movements of two eyes. Instead, Fitzgibbon et al. (ARVO 1992) have proposed that the accelerated vergence movements might be the result of the release of omnipause neuron (OPN) inhibition of vergence burst cells during saccades. We report an experiment that is a critical test of this hypothesis.

Two rhesus monkeys were trained to track visual targets that could elicit symmetrical vergence, saccades, or mixed saccade-vergence movements. Eye movements were measured with search coils. Microelectrodes were localized in the pontine OPN region by recording the characteristic activity of these cells and by noting that electrical microstimulation halted all saccades. If OPNs partially inhibit vergence burst cells, then increasing the activity level of the OPNs should further depress the activity of vergence burst cells, resulting in a reduction of vergence velocity. We found that 1 sec stimulation trains in the OPN region during symmetrical vergence reduced the velocity of these movements by about 50%. Brief stimulus trains produced transient decreases in both convergent and divergent velocity with a mean latency of 24 msec. These findings strongly support the hypothesis that the acceleration of vergence during saccades is due to the cessation of the OPN inhibition. (Supported by NEI EY03463, EY07558, and P30 EY03039).

19.3

EYE-POSITION SIGNAL OF THE ABDUCENS MOTONEURONS DURING GAZE HOLDING AND VESTIBULOOCULAR REFLEX IN THE ALERT CAT.
E. Godaux* and G. Cheron (SPON : European Neuroscience Association). Lab. of Neurophysiology, University of Mons, 7000 Mons, Belgium.

Eye movements are triggered by velocity signals that must be converted into position signals by a brainstem network, the oculomotor neural integrator (NI). Theoretical and indirect experimental evidences suggest that the integration of all the eye-movement commands is made by a common network (Robinson, 1975). In a first step of our attempt to test this hypothesis by a direct way, we studied the output signal of the horizontal NI at the level of the abducens motoneurons.

The discharge of 32 antidromically identified abducens motoneurons were recorded in the alert cat during spontaneous saccades made in the light and during vestibuloocular reflex (v.o.r.) elicited in complete darkness by sinusoidal stimuli. During intersaccadic fixation, the firing rate of the motoneurons changed only with eye position (as eye velocity was null throughout). As a result, eye position sensitivity was obtained by establishing the firing rate – eye position relationship and by measuring the corresponding slope. During the slow phases of the v.o.r., the firing rate of the motoneurons consisted of two portions : one was related to eye velocity, the other to eye position. The problem was to take them apart. For this purpose, each time the eye passed through a chosen position during a slow phase, the firing rate was measured. Then, for that chosen eye position, the firing rate – eye velocity relationship was established. From this linear relationship, the firing rate of the neuron at null velocity was interpolated. Such a procedure was applied for a set of chosen eye positions. Finally, the sensitivity to eye position was obtained from the relationship between the interpolated firing rate and eye position.

If the NI was really common, each individual abducens motoneuron should have the same sensitivity to eye position in both studied movements. We found it to be the case.

19.4

VERTICAL EYE MOVEMENT-RELATED NEURONS IN THE INTERSTITIAL NUCLEUS OF CAJAL PROJECT TO THE VESTIBULAR NUCLEUS.
Y. Iwamoto*, S. Chimoto, K. Yoshida Dpt. Physiology, Inst. Basic Med. Sci., Univ. of Tsukuba, Tsukuba, Ibaraki, 305 Japan

Both the vestibular nucleus (VN) and interstitial nucleus of Cajal (INC) contain neurons with activity related to vertical eye movement. To examine a possible interaction between these two structures, projection of INC neurons to VN was studied in the alert cat.

Sixty-two INC neurons with activity related to vertical eye position were tested for the response to stimulation of the ipsilateral VN. Forty-one had a downward and 21 an upward on-direction. Thirteen of 41 downward neurons were antidromically activated following stimulation of VN with thresholds 36 to 220 μA. In some neurons, the latency jumped to a shorter value as the stimulus increased, suggesting branching in VN. None of 21 upward neurons showed antidromic responses. The stimulation sites were in the ventral part of VN at the level of the caudal edge of the abducens nucleus.

About half of INC neurons examined (9/13 upward, 13/23 downward) responded orthodromically to stimulation of the contralateral vestibular nerve. In particular, all the downward INC neurons antidromically activated from VN were excited at disynaptic latencies (1.3 to 2.0 msec). Thus, they not only projected to VN but also received excitatory connections from VN, most likely from secondary neurons carrying vertical eye position signals, which we have recently shown to project to INC. Such interconnections between VN and INC may form an important part of the neural circuitry responsible for the generation of vertical eye position signals.

19.5

EFFECTS OF ORBITAL POSITION OF THE EYE ON SACCADES ELICITED BY ELECTRICAL STIMULATION OF SINGLE AND PAIRED FRONTAL EYE FIELD SITES. E. C. Dias*, G. S. Russo and C. J. Bruce. Section of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The direction and amplitude of saccades elicited by electrical stimulation of the frontal eye field (FEF) can be systematically influenced by the position of the eye in the orbit during stimulation, with the magnitude of this orbital perturbation effect varying across FEF sites. Here we analyze the effect of simultaneous electrical stimulation of two different FEF sites on the orbital perturbation of elicited saccades. Electrical stimulation consisted of 70 ms trains of 350 Hz biphasic pulses given through glass-coated elgiloy electrodes with exposed tips of 20-40 μm. Stimulation was delivered through one, the other, or both electrodes while the monkey fixated a spot of light on one of a matrix of 15 locations on a tangent screen. All 45 stimulation conditions were tested 5-10 times in pseudo random order.

We tested 17 different pairs of FEF sites of one macaque monkey. All sites had low thresholds (<50μA) for eliciting saccades, and all site pairs were separated by at least 2 mm. The orbital perturbation of elicited saccades was quantified by linear regressions of fixation position on the direction and the amplitude of elicited saccades. In most cases orbital perturbations for paired stimulation was larger than the mean of the perturbations for stimulation of each site separately. This supports our hypothesis that large orbital perturbations of electrically elicited saccades could reflect multiple saccade representations being activated simultaneously through one electrode.

Paired stimulation usually elicited saccades at a higher probability and shorter latency than stimulation of either constituent site. Saccade dimensions from paired stimulation were often the weighted average of the saccade vectors from each stimulation site, with the shorter latency site having a larger weight. Saccade latency and probability also varied systematically with fixation position, with centripetal saccades having shorter latencies and higher probabilities, and centrifugal saccades having longer latencies and lower probabilities. Saccade latency and probability may reflect the orbitally-dependent weighting underlying the above hypothesis.

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19.6

NEUROETHOLOGY OF SACCADIC EYE MOVEMENTS IN CHICKENS. J-C. Letelier and J. Wallman*. Biology Department, City College, City Univ. of NY, New York, N.Y. 10031.

The saccadic behavior of birds is peculiar in that the eye proceeds toward the target not in a straight line, but in a slow (180 msec) trajectory containing extravagant (10-12 deg, 28 Hz, 600 deg/sec) oscillations mostly in the torsional plane. These oscillations are conjugate even during non-conjugate saccades.

Extracellular recording shows two classes of motoneurons in the trochlear nucleus. One type is like mammalian extraocular motoneurons in that the neurons fire during fixations with a rate dependent on position, but unlike mammalian motoneurons in that they pause for about 35 msec at the start of all saccades. The other type is silent between saccades, but active during saccades in all directions, firing bursts time-locked to each intorsional phase of the saccadic oscillation.

This separation into two classes of units, one with responses related to eye position and the other related to the saccadic oscillations, was also found in the afferents to the trochlear nucleus in the medial longitudinal fasciculus, using intracellular recording and labeling with HRP. Motoneurons with the pulse-step responses characteristic of mammals were never encountered. The functional segregation seen in avian motoneurons is an exception to the uniform activation found in most vertebrate motor pools and suggests that different vertebrates may employ different oculomotor strategies. Supported by NSF BNS - 8510945

19.7

SPATIAL ORGANIZATION AND NEUROTRANSMITTER UTILIZATION OF PREMOTOR NEURONES RELATED TO VERTICAL SACCADIC EYE MOVEMENTS IN THE CAT. S-F. Wang and R. F. Spencer*, Department of Anatomy, Medical College of Virginia, Richmond, VA 23298.

The rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) is the location of premotor neurones that control vertical saccadic eye movements. Toward the goal of delineating an intrinsic organization of neurones in the riMLF in relation to upward and downward eye movements, microinjections of HRP and biocytin have been made in different regions of the oculomotor nucleus and the riMLF, respectively. The results of these complimentary retrograde and anterograde labelling experiments demonstrate a caudal-upward and rostral-downward relation of neurones in the riMLF to the vertical motoneurones targeted in the ipsilateral oculomotor and trochlear nuclei. Two populations of biocytin-labelled riMLF synaptic endings are observed in the ipsilateral oculomotor and trochlear nuclei: (a.) presumed inhibitory synaptic endings that contain pleiomorphic synaptic vesicles, establish symmetrical synaptic contacts, and are GABA-immunoreactive, and (b.) presumed excitatory synaptic endings that contain spheroidal synaptic vesicles, establish asymmetric synaptic contacts, and are immunoreactive toward glutamate or possibly aspartate. Both populations of synaptic endings overlap in the same motoneurone subgroups, and retrograde HRP-labelled neurones that are immunoreactive toward GABA or glutamate/aspartate coexist within the riMLF. This ipsilateral inhibitory and excitatory organization of riMLF projections to vertical motoneurones differs from the reciprocal inhibitory and excitatory synaptic connections that characterize the vertical vestibulo-ocular and horizontal eye movement systems.

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19.8

A MODEL OF HUMAN OCULAR PURSUIT: INTEGRATION OF SMOOTH PURSUIT AND SACCADES. Ph.W. Koken and C.J. Erkelens (SPON: European Neuroscience Association)*. Utrecht Biophysics Research Institute, Dept. of Medical and Physiological Physics, University of Utrecht, 3584 CC Utrecht, the Netherlands.

We studied human ocular pursuit while a target was tracked either by the hand and the eyes together or by the eyes alone. Eye movements were measured by using the magnetic-field induction-coil technique. Hand movements were measured by recording the position of a hand held stick. The target was externally generated and moved horizontally in the fronto-parallel plane.

Ocular pursuit was smoother during simultaneous tracking with the hand of predictable target movements than during tracking by the eyes alone. However, gain and delay of ocular pursuit (smooth pursuit plus saccades) were not affected by simultaneous tracking with the hand. Tracking with the hand did not affect ocular pursuit when the target motion was unpredictable (Koken and Erkelens 1990, 1992a). Although the vergence system can track predictable target movements with a short delay comparable to the delay of smooth pursuit system (Koken and Erkelens 1992b), simultaneous tracking with the hand did not affect vergence pursuit.

From the obtained results we conclude that the ratio of smooth and saccadic components of ocular pursuit is task-dependent when a predictable target motion is tracked. We propose a model of ocular pursuit in which the predicted target motion results in input signals which are distributed (depending on the tracking condition) over the smooth pursuit and the saccade control systems.

19.9

SYNAPTIC INPUT TO OCULOMOTOR NEURONS OF THE FROG. S.L. Cochran*. University of Texas Medical Branch, Department of Otolaryngology, Rt. J63, Galveston, TX 77555-1063.

This study was undertaken to investigate the physiology and pharmacology of afferent synaptic transmission to the frog oculomotor nucleus. Intracellular recordings were obtained from these cells in the isolated brain bathed with Mg^{+2}-free saline. The neurons were identified by their location medial to the exit of the nerve 250-450 μm from the ventral brain surface. Some could be activated antidromically from either the ipsi- or contralateral nerve stump. Electrical stimulation of the VIIIth cranial nerves, the optic chiasm, vestibular nuclear complexes, medial longitudinal fasciculi, and optic tecti, evoked complex patterns of EPSPs and IPSPs in these cells. Two populations of cells appear to exist. One exhibits disynaptic EPSPs from stimulation of each VIIIth nerve. A second shows EPSPs from the contralateral and disynaptic IPSPs from the ipsilateral VIIIth nerve. Bath application of 3 mM kynurenic acid nearly abolished all evoked EPSPs (N=22), IPSPs that were other than monosynaptic, and spontaneous or bicuculline-induced rhythmic oscillations in the cells' resting potentials. Bath application of 5 μM bicuculline (but not 50 μM strychnine) abolished the IPSPs (N=4). These findings implicate glutamate and related compounds as the major excitatory transmitters employed by afferents to this nucleus, while GABA would seem to be the principal inhibitory transmitter.

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19.10

Predictive Components of Smooth-Pursuit Responses on Neurons in the Dorsomedial Frontal Cortex. S.J. Heinen* Smith-Kettlewell Eye Res. Inst., San Francisco CA.

The dorsomedial frontal cortex (DMFC) has been shown to be involved in saccadic eye movement generation (Schlag and Schlag-Rey, 1987). This area receives input from MST, an area which has neurons activated during smooth pursuit and for visual motion (Komatsu and Wurtz, 1988). Similar responses have been found on neurons in the DMFC, although these responses are weaker in terms of strength and tuning (Heinen, 1992). These cells were also sometimes modulated before tracking targets began to move, and often showed an increased discharge before the end of the trial, suggesting a more complicated role of these neurons than what would be expected from a cell conveying a simple motor signal. Single-units were recorded from neurons in the DMFC of two *Macaca fascicularis* monkeys. Eye position was recorded using the search coil. The monkeys task was to pursue sinusoidal and triangle-wave stimuli with amplitudes of either 10, 20 or 30 deg, and with frequencies of .3 or .5 Hz. It has been demonstrated that monkeys can predict periodic stimuli such as these, thereby producing a much smaller phase lag between the eye and the target than would be expected from the physiological latency (Deno et al. 1990). Seven of 14 cells tested with these stimuli showed peaks of activity that occurred an average of 104 ms before target turnaround. These results suggest that neurons in the DMFC carry a signal that could be used by the oculomotor system to predict target turnaround and reduce phase lag in periodic tracking tasks.

(Supported by Shannon Award 1 R55 EY09260-01, and the Smith-Kettlewell Eye Research Foundation)

19.11

POSSIBILITIES FOR CONVERGENCE OF FRONTAL EYE FIELD AND CEREBELLAR FASTIGIAL EFFERENT PROJECTIONS WITHIN BRAINSTEM PREOCULOMOTOR NUCLEI IN THE MONKEY. G.R. Leichnetz*, A. Gonzalo-Ruiz, R.F. Spencer, and D.J. Smith. Department of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

The frontal eye field (FEF) and fastigial nucleus (FN) of the cerebellum are both thought to play a role in the ocular motility. We have been interested in identifying: (1) areas of potential convergence between FEF and FN efferents within brainstem pre-oculomotor nuclei, and (2) areas where FEF efferents terminate in relation to sources of FN afferents. The research is based upon studies of anterograde and retrograde horseradish peroxidase (HRP) labeling following fluid injections or solid polyacrylamide HRP gel implants into the FEF, FN, or oculomotor-related brainstem nuclei in capuchin (Cebus) monkeys. It was found that the FEF and FN efferent projections probably converge in the supraoculomotor periaqueductal gray matter above the oculomotor complex, paramedian pontine reticular formation (PPRF) and nucleus raphe interpositus, and the nucleus prepositus hypoglossi. FEF efferents project to the nucleus reticularis tegmenti pontis, and dorsomedial and dorsolateral basilar pontine nuclei which are sources of FN afferents. The functional predisposition of the brainstem nuclei involved and their connections seem to support the interaction of FEF and FN in the control of both saccadic and smooth pursuit eye movements. The lack of a significant fastigiotectal projection suggests that convergence of FEF and FN efferents does not occur in the superior colliculus.

GENESIS OF NEURONS AND GLIA I

20.1

CONTACT WITH SELECTED CELL TYPES CAN STIMULATE DIVISION OF E14 RAT CORTICAL PROGENITOR CELLS. S. Temple* and A.A. Davis Dept. of Pharmacology and Toxicology and Div. of Neurosurgery, Albany Medical College, Albany NY 12208.

We have investigated how cell-cell contact affects the proliferation of rat cortical progenitor cells. At E14 most cortical cells are in division, but when they are dissociated and plated as single, isolated cells *in vitro*, division largely ceases and the cells differentiate. In contrast, when E14 cortical cells remain in contact with each other as small clusters of cells, division can continue and differentiation yields both neurons and glial cells. The incidence of progenitor cell division increases with cluster size. BrdU uptake over the first 24 hours of culture reveals that only 11% +/-3{SD} of cells in clusters of 2-3 cells are dividing compared to 93% +/-3{SD} of cells in clusters of 8-15 cells.

Cell-surface contact appears important for the continuation of proliferation. Single, dil-labelled, E14 cortical cells placed close to, but not in contact with, an unlabelled cluster of E14 cortical progenitor cells cease division, whereas if they are placed on top of such clusters they are stimulated to divide. The cell-contact effect appears to be cell-type specific: while single, dil-labelled, E14 cortical cells can also be maintained in division by neonatal cortical astrocytes, contact with meningeal fibroblasts reduces the chance of division even more than contact with poly-l-lysine coated plastic. These data suggest that close apposition between ventricular zone cells may be key to maintaining cell proliferation in the embryonic cortex, and that changing cell contacts may play a role in the differentiation of cortical cells. (Aided by the Klingenstein Fellowship Award in the Neurosciences, and by the Basil O'Connor Starter Scholar Research Award No. 5-FY91-0636 from the March of Dimes Birth Defects Foundation).

20.2

CHANGES IN STRUCTURAL AND FUNCTIONAL PROPERTIES OF RAT TELENCEPHALIC CELLS DURING EMBRYONIC DEVELOPMENT. J.-M. Mienville* and J.L. Barker. Lab of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

We have recorded whole-cell from embryonic (E12-E21) rat telencephalon and cerebral cortex with "in-situ patch-clamp" techniques (Edwards *et al.*, Pflügers Arch. 414, 1989), using a standard NaHCO3 buffer bubbled with carbogen at room temperature, and a K-free intracellular solution. As revealed by Lucifer Yellow spread from recorded cells and by low input resistances (down to ~ 50 MΩ), younger cells were consistently coupled in clusters whose size (up to ~ 100 cells) and occurrence decreased with development (Lo Turco and Kriegstein, Science 252, 1991). Furthermore, specific differences were noted when recording from ventricular vs. pial sides. In the absence of extracellular Ca, cells sealed to the electrode tip could be pulled away from the cluster, displaying sharp increases in resistance toward single cell values. Hence, there was close correspondence between apparent resistance and extent of coupling. The apparent resistance of the majority of coupled cells was increased at low intracellular pH or in mM heptanol and octanol. Although they were only partially reversible, these effects suggest involvement of gap junctions in coupling.

Recordings from spontaneously uncoupled cells revealed immature, TTX-sensitive (0.5 μM) action potentials, consistent with a low density of Na currents (I_{Na}). Density and occurrence of I_{Na} increased during development but, even at E21, the former remained much below adult values. Thus, telencephalic embryogenesis is characterized by a progressive decrease in junctional coupling and an increase in electrical excitability, the latter being present in the earliest period of post-mitotic differentiation.

20.3

DEVELOPMENTAL FATES AND MIGRATION PATTERNS OF JUVENILE RAT SUBVENTRICULAR ZONE CELLS. C. Chuang, S.W. Levison, and J. E. Goldman*. Dept. Pathology, Columbia University College of P&S, New York, NY 10032.

The subventricular zone (SVZ) is a germinal center of the mammalian forebrain that develops late in gestation. Using a murine retrovirus containing the E.Coli *LacZ* gene we have shown that immature cells infected in the P2 rat SVZ migrate dorsally and laterally into hemispheric gray and white matter (WM) and become both oligodendrocytes and astrocytes. To determine if SVZ cell fate changes with time, we injected the vector stereotactically into the forebrain SVZ of P14 rats. Infected cells were visualized histochemically after various intervals and their morphologies characterized. Two days post-injection (2dpi), most of the labeled cells were within the SVZ and displayed the same apolar or unipolar appearance as earlier SVZ cells. The remainder were located in WM and consisted of immature oligodendrocytes. At 21 dpi, only 8% were in the SVZ; the rest had developed into oligodendrocytes in WM. In contrast to SVZ cells labeled at P2, these had largely migrated medially towards the callosum, occasionally appearing in the WM on the contralateral side. Cells rarely migrated into gray matter and only few developed into astrocytes. Thus, both the migratory patterns and the developmental fates of SVZ cells change during the first 2 weeks of postnatal life. Supported by NS 17125 and MH 15174.

20.4

PCNA EXPRESSION IN CEREBRAL CYTOGENESIS. V.S.Caviness, jr*. and T.Takahashi, Dept. of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, 02114

Expression of proliferating cell nuclear antigen (PCNA, auxiliary protein of DNA polymerase-∂) fluctuates and is highly regulated during the cell cycle in normal as well as in tumor cells. The spatial distribution of cells expressing PCNA in the developing mouse cerebral wall has been determined for the first time. The alcohol fixed tissue from E14 mouse was stained immuno-cytochemically using an antiserum against PCNA. Only nuclei where PCNA was bound within the nucleus were stained.

Neocortical cytogenesis occurs within the ventricular zone (VZ). Progenitor cells within this proliferative zone undergo DNA synthetic (or S-) phase in the outer half of the VZ (S-phase zone). PCNA positive nuclei are concentrated at the outer margin of the VZ. A tritiated thymidine (^{3}H) /PCNA double labeling experiment (animals were sacrificed 1hr after ^{3}H injection) partitioned the total PCNA+ population into two subpopulations (i.e., PCNA+/^{3}H- and PCNA+/^{3}H+). PCNA+/^{3}H- (26% of total PCNA+ cells) should be in late G1-phase while PCNA+/^{3}H+ (74%) should be in early S-phase (within the S-phase population, PCNA+ represents only 30% of the total). Both PCNA+/^{3}H- and PCNA+/^{3}H+ cells have their peak distributions at the outer margin of the S-phase zone, evidence that the transition from late G1 (PCNA+/^{3}H-) to early S-phase (PCNA+/^{3}H+) occurs at the outer margin of the VZ.

These observations suggest that the G1/S transition of the progenitor cells of the cerebral cortex occurs at the interface between the proliferative zone (i.e., VZ) and the zone of the postmitotic migrating cells and ingrowing axons (i.e., intermediate zone). PCNA evidently participates in this transition and its pattern of expression may be sensitive to mechanisms which govern neocortical cytogenesis. Supported by NIH grant NS12005.

20.5

CELL CYCLE KINETICS OF THE VENTRICULAR ZONE IN THE FETAL MONKEY TELENCEPHALON. D.R. Kornack* and P. Rakic. Section of Neurobiology, Yale School of Medicine, New Haven, CT 06510.

Cell cycle kinetics in cerebral germinal zones may potentially influence the development of the unique size and configuration of primate neocortex, and may also play a role in the pathogenesis of particular genetic and acquired cortical abnormalities. However, the duration of the cell cycle and its phases has not been examined in the nervous system of any primate species.

We have begun to examine cytokinetic parameters in the cerebral ventricular zone (VZ) of the macaque monkey fetus using a cumulative S-phase labeling protocol that employs bromodeoxyuridine (BrdU) immunohistochemistry (Nowakowski *et al.*, '89, *J. Neurocytol.*, 18:311; Takahashi *et al.*, '92, *J. Neurocytol.*, 21:185). BrdU, a thymidine analog, is injected into the pregnant animal and incorporated into DNA of proliferative cells during the S-phase. This procedure allows the determination of (1) the duration of the cell cycle (Tc), (2) the length of the DNA-synthetic phase (Ts), and (3) the proportion of cells that comprise the proliferating population. Three pairs of animals were injected at embryonic days (E) 40, 60, and 80, corresponding to the beginning, middle, and end of the period of corticogenesis.

Our preliminary results indicate that at the onset of corticogenesis (E40), VZ cells in dorsomedial telencephalon have a Ts of 6.3 hrs; this duration is identical to that reported in a BrdU study of mouse VZ at a comparable developmental stage (E13)(Caviness *et al.*, '91, *Soc. Neurosci. Abstr.*, 17:29). By contrast, Tc in monkey lasted 24.1 hrs, substantially longer than the 14.7 hrs reported for mouse. Consequently, Ts may be conserved in mammalian VZ at early stages of corticogenesis, whereas the duration of at least one other phase of the cell cycle may vary across species. Experiments in progress focus on analyzing Tc and Ts in other cerebral areas at the middle and end of corticogenesis to determine whether cell cycle kinetics in primate VZ vary regionally and temporally. Supported by NS14841.

20.6

CYTOGENESIS IN THE SECONDARY PROLIFERATIVE POPULATION OF THE MURINE CEREBRAL WALL. T. Takahashi[1]*, R.S.Nowakowski[2] and Verne S. Caviness, jr.[1],[1]Dept. Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, [2]Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854

The secondary proliferative population of the developing cerebral wall (SPP) is generally thought to be the source of most cortical glial cells. The present analysis, based upon sequential S-phase labeling (^{3}H-TdR, 1hr; BUdR, 30 minutes; sacrifice), on embryonic days 14-17 (E14-E17) is concerned with the magnitude, distribution and rate of cell production within this population. The cells of the SPP are distributed from the ventricular zone (where they overlap with the proliferating pseudostratified ventricular epithelium, the PVE) outward. They extend through the subventricular zone and across the intermediate zone to the level of the external sagittal stratum, a fiber plane marking the inferior limit of the future cortex. Assuming that the length of the cell cycle is the same in PVE and SPP we calculated that: (1) The absolute size of the SPP increases by almost 500% by E16 but then declines approximately 25% between E16 and E17. (2) The relative size of the SPP as a proportion of the total proliferative population advances from 11 to 46% between E14 - E17 and then to 100% of the proliferative population on E18 after exhaustion of the PVE. From estimates of the rate of daughter cell production within this population, and with corrections for growth-caused tangential expansion of the proliferative zone, the proportion of SPP cells re-entering S-phase of the next cell cycle is estimated to be nearly 100% from E14 through E16. Over the next 24 hr, this proportion drops sharply to less than 30%. The waxing and waning of the SPP observed here precedes the seeding with glial cells of the outer cerebral wall and neocortex in the perinatal period. Supported by NIH grants NS12005 and NS28061 and NSF grant BNS8921020.

20.7

A COMMON PROGENITOR FOR TYPE 1 AND TYPE 2 ASTROCYTES EXISTS IN NEWBORN RAT SUBVENTRICULAR ZONE. S.W. Levison*, B.J. Abramson, and J.E. Goldman. Dept. of Pathology and Center for Neurobiology and Behavior, Columbia University College of P & S, New York, NY 10032.

We recently demonstrated that both oligodendrocytes and protoplasmic astrocytes are generated in vivo from immature cells of the subventricular zone (SVZ). To determine the developmental potential of these glial progenitors in vitro, SVZ cells of P2 rats were labeled by stereotactic injection of recombinant retroviral vectors. Two days later, forebrains were dissociated and cultured in MEM with 5% FBS. Labeled cells were visualized with X-gal and phenotyped using triple label immunocytochemistry with a panel of antibodies. After 1 div most of the labeled cells were apolar or unipolar. Sixty percent were ganglioside GD3+, 50% were nestin+, and 30% were vimentin+, with overlapping expression. Less than 5% expressed GFAP. When examined from 4-21 div, several types of clones were observed: 1. GD3-, GFAP+,Vim+, flat, "Type 1" astrocytes; 2. GD3+,GFAP+,Vim+, process-bearing, "Type 2" astrocytes; and 3. clones of cells with mixed morphologies and antigenic phenotypes, often containing both "Type 1 and 2" astrocytes. Large, flat, GD3+ astrocytes were not observed in any clones. Thus postnatal germinal matrix cells in the SVZ can produce both "Type 1" astrocytes and O-2A lineage cells. Supported by NS 17125 and AG 00189.

20.8

VARIATIONS IN CELL PROLIFERATION IN SUBDIVISIONS OF DEVELOPING MOUSE HIPPOCAMPAL FORMATION. W.A. Gregory*, N.L. Hayes, and R.S. Nowakowski. Department of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

We have used cumulative S-phase labeling with bromodeoxyuridine (BUdR) to measure the cell cycle in different subdivisions of the proliferative ventricular epithelium (PVE) of the developing mouse hippocampal formation. Cell proliferation was assessed at embryonic day 16 by injecting BUdR at two hr intervals over a period of 12 hr. Sections midway through the hippocampus were drawn for each time point using a drawing tube, and sectors 100-150 μm in length were defined along the ventricular surface. Counts of labeled and unlabeled cells in each sector were used to calculate the labeling index, from which the lengths of the cell cycle (T_C) and the S-phase (T_S) were determined. For the entire proliferative population, the average length of the cell cycle (T_c) was 13.8 hr, and the average length of the S-phase (T_s) was 5.2 hr. However, these parameters were not uniform in all hippocampal subdivisions. The cell cycle was longest in the portion of the PVE giving rise to the dentate gyrus (T_C=17.4 hr; T_S=6.5 hr) and shortest in the middle of three sectors along the length of the PVE of the hippocampus (T_C=9.9 hr; T_S=2.9 hr). These significant variations in the lengths of the cell cycle and the S-phase could contribute to known differences in the development of the hippocampal subdivisions.

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20.9

INTERKINETIC MOVEMENT OF NUCLEI IN THE PROLIFERATIVE VENTRICULAR EPITHELIUM OF THE DEVELOPING MOUSE HIPPOCAMPAL FORMATION. R.S. Nowakowski*, N.L. Hayes and W.A. Gregory. Department of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The developing archicortical structures of the hippocampal formation are produced in a single proliferative zone, the proliferative ventricular epithelium (PVE), that corresponds approximately to the cytoarchitectonically defined ventricular zone (VZ). In contrast, in the developing periarchicortex and neocortex there are two proliferative zones, the PVE and an adjacent secondary proliferative population (SPP). We have used cumulative labeling with the S-phase label bromodeoxyuridine (BUdR) to study the changes in the distribution of nuclei as they progress through the cell cycle. For this study, the hippocampal formation of the mouse on embryonic day 16 was divided into four 100-150 μm wide sectors and the PVE was divided into 10 μm deep bins. The number of labeled and unlabeled cells was counted in each bin and sector, and the proportion of labeled cells per bin (i.e., the labeling index) was computed. The results demonstrate that the PVE cells of the hippocampus enter the S-phase at the outer-most border of the VZ and move inward toward the ventricular surface as they progress through S, G2 and M, and that the entire outward movement of the nuclei takes place in G1. Thus, despite the fact that the hippocampal PVE is only half the thickness of the neocortical PVE, the behavior of the constituent cells of the two PVE's is similar.

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20.10

CLUSTERING OF NUCLEI AND SYNCHRONY OF CELL PROLIFERATION IN THE PROLIFERATIVE VENTRICULAR EPITHELIUM OF THE DEVELOPING MOUSE HIPPOCAMPAL FORMATION. N.L. Hayes*, W.A. Gregory, and R.S. Nowakowski. Department of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

During the cell cycle the nuclei of the cells of the proliferative ventricular epithelium (PVE) enter the S-phase at the outer-most border of the cytoarchitectonically defined ventricular zone and move inward toward the ventricular surface as they progress through S, G2 and M. The nuclei retrace their path during G1 during which the entire outward movement of the nuclei takes place. We have used bromodeoxyuridine (BUdR) immunohistochemistry and basic fuchsin staining to examine the distribution of cells in S-phase and of mitotic figures in the mouse hippocampal formation at embryonic day 13. It was noted that cells with BUdR label confined to small clumps of chromatin were located preferentially in small clusters at two levels in the outer two-thirds of the PVE. This position of cells along the radial dimension of the PVE and this pattern of labeling are consistent with the cells having either entered the S-phase just prior to sacrifice or having left the S-phase shortly after exposure to BUdR. Reconstructions from serial sections of the distribution of mitotic figures demonstrated that they also are located in clusters at the ventricular surface. These findings indicate that 1) cells in similar phases of the cell cycle tend to be located adjacent to one another and 2) small clones of PVE cells remain in approximate synchrony as they progress through the cell cycle.

Supported by NIH grant NS28061.

20.11

BIRTHDATES OF DEFINED AND MUTUALLY EXCLUSIVE SUBSETS OF TRIGEMINAL GANGLION CELLS. F.A. White* and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

Many trigeminal (V) ganglion cells respond to neonatal transection of their peripheral axons by dying and previous results from this laboratory (e.g. White et al., J. Comp. Neurol., 300:249-262, 1990) suggested that V ganglion cells that contained substance P- or calcitonin gene-related peptide- (CGRP) like immunoreactivity were less likely to be lost after neonatal infraorbital nerve lesion than a distinct subpopulation of cells that bound the lectin Bandierea simplicifolia-I (BS-I). To determine whether this differential effect of nerve damage might be related to the times at which BS-I- and CGRP-positive ganglion cells were born, we combined immunocytochemistry and histochemistry with [^{3}H]-thymidine labelling. We also determined the birthdates of the V ganglion cells expressing neurofilament protein (NFP). The distributions of birthdates for the three subpopulations were significantly different. Nearly all V ganglion cells are born between embryonic days (E-) 10.5 and 14.5. Cells containing NFP constituted 22.2% of all cells born on E-10.5, 10% of the cells born on E-11.5, 15.8% of those born on E-12.5, 4.4% of those born on E-13.5, and 1.8% of the cells born on E-14.5. The values for CGRP-containing cells were E-10.5: 0.1%, E-11.5: 0.5%, E-12.5: 1.0%, E-13.5: 14.9%, E-14.5: 14.4%. Those for BS-I-positive cells were E-10.5: 6.0%, E-11.5: 5.9%, E-12.5: 3.8%, E-13.5: 28.7%, E-14.5: 13.7%. These data show that a substantial fraction of the BS-I- and NFP- populations are born prior to CGRP-positive cells. These results are consistent with the conclusion that the latest born of these three subpopulations of V ganglion cells is the most likely to survive neonatal axotomy. NS 28888, DE 07734

20.12

MAPPING OF CELL PROLIFERATION PATTERNS IN EARLY EMBRYONIC RHOMBENCEPHALON USING A DOUBLE PULSE-CHASE PARADIGM WITH IODO- AND BROMO-DEOXYURIDINE. I. Maric*, D. Maric, W. Ma and J.L. Barker, Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892

We investigated the spatio-temporal proliferation patterns of cells in E11-E14 rhombencephalon using two thymidine analogues, bromodeoxyuridine (BrdU) and iododeoxyuridine (IrdU). Within a period of 60 minutes timed pregnant E11 Sprague-Dawley rats received multiple intraperitoneal (i.p.) injections of IrdU (50 mg/kg b.w.) in order to achieve maximum labelling of the embryonic proliferative cells. The animals were divided into four groups and sacrificed 2, 24, 48 and 72 hours later. Thirty minutes before sacrifice each rat was given a single i.p. BrdU (50mg/kg b.w.) injection. The E11-E14 embryos were removed from their mothers and the developing rhombencephalic regions either processed for immuno-histochemistry or acutely dissociated into single cell suspensions and further fractionated into germinal zone and mantle zone cells using discontinuous Percoll gradients. Both the cells and corresponding 12um thick coronal tissue sections of rhombencephalon were stained with FITC-conjugated mouse anti-Br-3 and IU-4 MAbs, specific for BrdU and BrdU/IrdU, respectively. In addition, the dissociated cells were counterstained for total DNA content with propidium iodide and their cell cycle profile quantified with a flow cytometer. The results show that at E11 92.8% of rhombencephalic cells proliferate and are located exclusively in the germinal zone. During the period of E12-E13 there is a progressive migration of IrdU labelled cells from the germinal to the mantle zone. The great majority of these IrdU$^+$ cells generally do not incorporate BrdU, indicating a terminally divided cell population. However, by E14 there is a dramatic 89.8% depletion of IrdU-labelled cells in the mantle zone suggesting excessive pre-programmed cell death in this region. At this embryonic age there were equal numbers of IrdU-labelled and BrdU-labelled cells in the germinal zone of the developing rhombencephalon.

CELL SHAPE AND DIFFERENTIATION I

21.1

DIFFERENTIATION THERAPY POTENTIATES CHEMOTHERAPY AND HYPERTHERMIA CYTOXICITY IN HUMAN AND CANINE GLIOMA CELLS IN VITRO. P.S. Ebert & M. Salcman*. University of Maryland School of Medicine, Baltimore, MD 21201.

Human glioblastoma (U-87MG) and canine glioma (CBT) cell lines were tested in vitro for their sensitivity to sequential treatment with differentiating agents and chemotherapy or hyperthermia. Both cell lines responded to the inducer combination 1 mM dibutryl cyclic adenosine monophosphate/1 mM Na butyrate, by the formation of cytoplasmic processes attaining approximately 90% morphological differentiation within 2 days of exposure. Clonogenic viability was assessed by the formation of macroscopic colonies originating from single cell suspensions plated after cultures were exposed to various regimens. The clonogenicity of both lines gradually decreased with lengthening exposure (up to 7 days) to the inducers, but differentiation alone did not eliminate clonogenicity. BCNU at 5 μM for 1 hr administered to both lines before or after 3 days of treatment with inducers potentiated their antiproliferative effects. Sequential treatment of CBT cells at 44°C for 30 min followed by inducers produced an additive potentiation of cell killing, while the reverse sequence did not. This study demonstrates that differentiation therapy can potentiate the therapeutic effects of hyperthermia and chemotherapy in glial tumors and can thereby reduce the concentration of cytotoxic drugs required to achieve effective cell killing.

21.2

PROCESS OUTGROWTH OCCURS MORE RAPIDLY IN N1E-115 CELLS AS PASSAGE NUMBER INCREASES. M.Heil & P.Cobbett* Dept.Pharmacol./Toxicol.& Neuroscience Prog., Michigan State Univ., East Lansing, MI 48824.

Cells of lines which are derived from the murine sympathetic neuroblastoma designated C1300 have been used as models to study neurite outgrowth. These cells proliferate when cultured in serum containing medium (SCM) but differentiate morphologically (i.e., become process bearing) when transferred to serum free medium (SFM). The maximal proportion (≈60%) of differentiated C1300 derived Nb2A clonal cells was attained within 3hrs of being placed in SFM (Gurwitz & Cunningham, 1988). Similarly, cells of the related C1 clone are 90% differentiated within 24hrs (Schubert *et al.*, 1971). In contrast, Cosgrove & Cobbett (1991) reported that differentiation of N1E-115 cells in SFM started within 2 days but was incomplete even after 8 days. We have now examined whether N1E-115 cells of different passage age differentiate at different rates. Cells of passages 12, 17 and 23 were plated at the same density in SCM which after 24hrs was replaced by SFM. Photographs of the cells were taken after 0.5, 1, 3 and 6 days in SFM. The fraction of cells that were process bearing and the mean process length were then determined. We found that the fraction of cells which were process bearing and the mean process length were smaller for cells of passage age 12 compared to cells of passages 17 and 23 after 1 day in SFM. This pattern continued through days 3 and 6. The data suggest that passage age is an important determinant in differentiation of N1E-115 cells and perhaps of other model neuronal cells.

Supported by the Pharmaceutical Manufacturers Association Foundation.

21.3

PC12 CELLS GROWN IN SPINNER CULTURES RETAIN CHARACTERISTIC CYTOLOGICAL MARKERS AND MORPHOLOGICAL RESPONSES TO NERVE GROWTH FACTOR. M. Palmatier*, B. Frydel, C. Barcus, J. Embree, T. Wright, F. Kaplan, CytoTherapeutics, Inc. Providence, RI 02906

The PC12 cell line is derived from a rat pheochromacytoma, a tumor of the adrenal chromaffin cells. Under attached growth conditions, PC12 cells express cytological markers, thy 1.1, tyrosine hydroxylase and chromogranin, consistent with the species and cell type of origin. They also are morphologically responsive to NGF and subsequently stain for neurofilament, indicative of the development of the chromaffin cell from the neural crest.

We grew PC12 cells in suspension to determine if the cells retained the expression of cytological markers and morphological responses to NGF of attached PC12 cells. PC12 cells were grown in spinner cultures at 30-100 rpm, 37°C, and 5-7% CO_2. The cultures were seeded at 1-5 X 10^4 cells/ml and grown to 0.5-1 X 10^6 cells/ml in PC12 medium (RPMI-1640 with 2 mM glutamine, 5% fetal bovine serum, and 10% donor horse serum).

For cytological staining, PC12 cells from spinner cultures were fixed immediately or plated onto collagen-coated slides in PC12 medium and allowed to attach for 12 to 72 hours. The cells were fixed in 4% paraformaldehyde and stained for thy 1.1, tyrosine hydroxylase, or chromogranin. For NGF responsiveness, PC12 cells from spinner cultures were plated onto collagen-coated slides in PC12 medium supplemented with 0, 10, 50, or 100 ng/ml mouse NGF. The medium was changed every 4 days. At the end of 2 weeks, the cells were scored for neurite outgrowth, fixed in 4% paraformaldehyde, and stained for thy 1.1, neurofilament, or chromogranin.

PC12 cells grown in spinner cultures stained for thy 1.1, tyrosine hydroxylase, and chromogranin. Greater than 80% of the spinner PC12 cells responded to NGF and stained for neurofilament. PC12 cells retain characteristic cytological markers and morphological responses when grown in spinner cultures.

21.4

BIOCHEMICAL, IMMUNOLOGICAL, AND MOLECULAR CHARACTERIZATION OF HCN-1A CELLS. M. Charlton*†, M. Connelly, J. Lancaster, A. Snowman, L. Williams*, and P. Sweetnam. *Dept. of Biology, Georetown University, Washington DC 20057; *NINCDS, Bethesda, MD 20892; NovaScreen®, Baltimore, MD 21224.

Ronnett, *et al.*, (Science, 1990) recently described the establishment of a human cerebral cortical neuronal cell lines (HCN-1A) using tissue obtained from patient with unilateral megalencephaly. These cells were shown to differentiate in response to a mixture of NGF, IBMX, and dibutryl cAMP. Several other agents have now been examined (EGF, bFGF, PDGF, and ILGF-1) and none appear to induce differentiation. Following differentiation, HCN-1A cells exhibit a neuronal morphology and neurofilament, tetanus, and synaptophysin-like immunoreactivity, but not R-mAB, O1, or GFAP-like immunoreactivity. The cells express a variety of neurotransmitters and neurotransmitter receptors. They stain positively for GABA and GAD but do not express the GABA uptake site as determined by [^{3}H]GABA labeling. PCR analysis has revealed that these cells express several $GABA_A$ receptor subunits but not the GABA uptake site. In addition, the +MK801 binding site, and the endothelin (ET_B) receptor have been identified by radioligand receptor binding. These studies confirm that the HCN-1A cells express numerous neuronal elements following differentiation.

21.5

DESTABILIZATION OF THYMOSIN BETA-10 mRNA WITH AN ANTISENSE DEOXYOLIGONUCLEOTIDE INHIBITS GROWTH OF NEUROBLASTOMA CELLS. A.K.Hall.,T Lysz*., J.J.Seebode and R.J.Irwin Jr., Dept of Surgery, UMDNJ-NJ Medical School, Newark, NJ 07103, U.S.A.

Thymosin beta-10, a 5Kda, retinoid-responsive, putative G-actin sequestering protein, is known to be expressed at high levels in the embryonic CNS and proliferating neuroblastoma cells [see J.Mol.Neurosci 2:229-237,1991]. It was thus reasoned that as retinoic acid-induced regulation of this gene precedes differentiation, the thymosin beta-10 protein may function as an independent oncogenic factor per se. As a first pass at studies designed to focus on this possibility, we made use of synthetic antisense deoxyoligonucleotides to neutralize expression of the endogenous beta-10 gene in neuroblastoma cells. An antisense deoxyoligonucleotide [made complementary to the first 20 bases starting at the AUG initiation codon of the beta-10 mRNA] at a concentration of 30uM induced a >40% reduction of steady-state beta-10 mRNA levels; this effect was dependent upon medium serum concentration. Partial neutralization of beta-10 gene expression significantly inhibited neuroblastoma cell proliferation. These findings, together with previous observations suggest that thymosin beta-10 (and perhaps other related proteins) may function as a novel class of oncogenes.

21.6

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

Human neuroblastoma cell line SHSY5Y expresses both the 75 kDa low-affinity nerve growth factor (NGF) receptor and the *trk* protooncogene and is responsive to NGF. Treatment of these cells with NGF resulted in neurite extension, but not in cessation of cell growth. SHSY5Y cells were treated with NGF and a pulse of cell cycle blockers aphidicolin, hydroxyurea or thymidine. Most of the cells extended long neurites (>400 μm). The response of the neuroblastoma cells was synergistic, i.e., differentiation was much greater for cells treated with NGF and aphidicolin than for cells treated with either drug alone. If maintained in NGF, the differentiated cells were stable for at least a month and were mitotically inactive. The sequence of events leading to differentiation was assessed. After about 1 day of treatment of SHSY5Y cells with NGF and aphidicolin, the protooncogene *c-myc* is downregulated. The cells cease to proliferate and commit to differentiation after about 3 days treatment. These two events could not be temporally distinguished and may be the same event. In preliminary studies, expression of the *c-ret* protooncogene and the *id-2* gene were upregulated during differentiation. mRNAs for *cdc2, cdk2*, cyclin A and cyclin B were not downregulated.

21.7

"DIFFERENTIATION INDUCTION" IN NEUROBLASTOMA CELLS. N. F. Schor*. University of Pittsburgh, Pittsburgh, PA 15213

Neocarzinostatin (NCZ) is a chemotherapeutic agent which induces morphological maturation in human and murine neuroblastoma (nb) cells. In murine cells, this is associated with an increase in cell surface neural cell adhesion molecules (N-CAM's), and cell death within 14 days. Unlike maturation, however, there is no change in expression of GAP-43 or MAP-1, and no detectible message for or morphological evidence of neurofilament proteins in these cells. Nerve growth factor (NGF) does not alter the effects of NCZ upon murine cell growth rate, morphology, or longevity. Treatment of murine nb cells with NCZ results in loss of their tumorigenicity. Treatment of low passage number SK-N-SH human nb cells with NCZ results in marked elongation and enlargement of these spindle-shaped cells, with bipolar extension of processes. The percentage of the cells which undergo this morphological change is dose-dependent, as is the decrement in the rate of cell culture growth. Unlike murine cells, low passage number human cells maintained in the presence of NGF (100-1000 ng/ml) are "protected" from the cytostatic and morphologic effects of NCZ. Furthermore, in contrast to low passage number human cells, higher passage number cells (no exposure to NCZ) die within 24 hours after exposure to NCZ without undergoing morphological change. This change in response to NCZ is not a function of altered sensitivity to the drug. Concomitant with this change in response to NCZ with increasing passage number, human cells lose their avid binding to tissue culture plastic. Western blot assay for N-CAM using a polyclonal antibody revealed that low passage number cells contain large amounts of two reactive proteins centered at 80 kD molecular weight, and smaller amounts of a 120 kD molecular weight reactive protein. In contrast, homogenates of up to ten times as many high passage number cells contained no detectible anti-N-CAM antibody-reactive proteins. These studies indicate that the effects of NCZ on nb cells are highly cell line and passage number dependent, and differ significantly from the normal induction of neuronal differentiation.

21.8

MORPHINE AFFECTS THE MORPHOLOGY OF DEVELOPING TYPE 1 ASTROCYTES BY INCREASING INTRACELLULAR CALCIUM. **A. Stiene-Martin*, M.P. Mattson and K.F. Hauser. Depts. of Clin. Sci., Sanders-Brown Ctr. on Aging, and Anat. and Neurobiol., Univ. Kentucky Med. Ctr., Lexington, KY 40536-0080.**

Opiate drugs can suppress the rate of proliferation while increasing morphologic differentiation in developing type 1 astrocytes *in vitro*. To address whether Ca^{2+} has a role in opiate-dependent astrocyte growth, astrocyte-enriched cultures derived from newborn ICR mouse cerebra were treated with Ca^{2+}-free DMEM media containing Ca^{2+} concentrations of 0, 0.3, 1.0, and 3.0 mM. Additional cultures were treated with DMEM containing the Ca^{2+}ionophore A23187. The cultures were grown for 5-6 days, fixed, and stained immunocytochemically for glial fibrillary acidic protein (GFAP). Areas of single, GFAP+, type 1 astrocytes were measured using computer-assisted image-analysis. There was a positive correlation between astrocyte area and $[Ca^{2+}]$. Morphine (1 μM) had an additive effect at calcium concentrations of 0.3 and 1.0 mM. Morphine had no effect on astrocyte area at high $[Ca^{2+}]_o$, in the absence of Ca^{2+}, or in the presence of 1 μM A23187. Fluorescent microscopic measurements of intracellular Ca^{2+} ($[Ca^{2+}]_i$) in individual identified cells using Fura-2 indicated that 1 μM morphine significantly increased $[Ca^{2+}]_i$ in type 1 astrocytes within 1 min following exposure and this effect was still apparent at 48 h. Morphine-dependent increases in astrocyte area, and in $[Ca^{2+}]_i$, were completely blocked by 3 μM naloxone. The data suggest that morphine affects the morphologic differentiation of type 1 astrocytes by increasing $[Ca^{2+}]_i$, and that Ca^{2+} normally mediates cellular differentiation in developing type 1 astrocytes.

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21.9

CELLS CULTURED FROM ADULT RAT BRAIN CAN DIFFERENTIATE INTO NEURONS OR OLIGODENDROCYTES

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Cells derived from adult rat cerebral cortex proliferated *in vitro* when supported by a modified N2 medium including nerve growth factor (NGF), basic fibroblast growth factor (bFGF) and 20% FCS. Initial antigenic characterization revealed the coexpression of neuronal markers (200 kDa and 160 kDa MW neurofilament proteins) with glial markers (S100 protein and the oligodendrocyte marker MBP. Vimentin was also strongly visualized. The RB3A cells also demonstrated positive reactivity to antibodies directed against the neurotransmitter synthetic enzymes tyrosine hydroxylase (TH) and glutamate decarboxylase (GAD) was visualized. Serotonergic and cholinergic markers were not found. When exposed to N2 medium lacking growth factors and serum, approximately 50% of the RB3A cell population formed MBP+/GC+/NF- aggregates. Single putative oligodendrocytes were less frequently observed (3-4%). The remaining cells were morphologically and antigenically disparate. Attempts to induce astrocyte differentiation by culturing cells in DMEM/10%FCS were unsuccessful. However, these cultures retained the RB3A parental antigenic profile. It was found that 90% of cells maintained solely in DMEM/10%FCS and then transferred to N2 medium containing NGF, bFGF and dibutyryl cAMP underwent neuronal differentiation. Such cells possessed phase-bright rounded somas that extended neurites strongly reactive to antibodies directed against the NF triplet and unresponsive to both anti-vimentin and anti-MBP. TH-immunoreactivity was intensified while anti-GAD reactivity was diminished. This study suggests that a reservoir of cells persists in adult rat brain retaining the capacity to differentially generate neurons or oligodendrocytes *in vitro*.

21.10

IDENTIFICATION OF NEURONS AND GLIA IN CULTURE; DIRECT NEURONAL LOCALIZATION WITH NEUROTAG™ D.S. Grega*+, N. Nousek-Goebl and T.J. Cavanagh. R&D Division, Boehringer Mannheim Corp. and +Indiana Univ. School of Medicine, Program in Medical Neurobiology, Indianapolis, IN 46250

Neurons and glia can be distinguished based upon their unique cellular epitopes. Tetanus toxin, in addition to being toxic, has been shown to bind to gangliosides in neuronal membranes, which make it a good, if somewhat dangerous neuronal marker. Tetanus toxin C fragment (TTC, papain digestion digestion of the native toxin) retains the binding properties of the tetanus toxin with a reduced hazard. Recombinant TTC functions indistinguishably from the natively derived TTC with no risk of contamination by the native toxin. Neurotag™ is recombinant TTC conjugated to either fluorescein (Green) or rhodamine (Red) which can be used to directly label neurons in culture.

We report labeling data using NGF-treated, mixed spinal cord/dorsal root ganglia (SC/DRG) cultures derived from fetal rat. Glial fibrillary acidic protein (GFAP) is the principle (and characteristic) component of intermediate filaments of the cytoskeleton within astrocytes. Neurons and astrocytes were localized in these cultures using Neurotag™ and anti-GFAP respectively. Neurotag™ labeled neuronal surfaces, permitting the visualization of soma and processes. Even very fine branches and processes were visible. Anti-GFAP labeled the cytoskeleton of stellate astrocytes, whose morphology and distribution were distinct from the neurons. Good cellular resolution was observed with both markers, permitting excellent discrimination of these two important neural cell types.

21.11

ULTRASTRUCTURAL AND IMMUNOCYTOCHEMICAL ANALYSIS OF DIFFERENTIATION IN A HUMAN CORTICAL NEURONAL CELL LINE (HCN-1A). R.L. Moses* and J.W. Haycock. Depts. Anat. and Biochem. & Molec. Biol., LSUMC, New Orleans, LA 70119

A neuronal cell line (HCN-1A) cloned from the cerebral cortex of a megalencephalic patient undergoes process formation when treated with a combination of cAMP and nerve growth factor (Ronnett et al., *Science* 248, 603 (1990)). In the present studies, treated and untreated HCN-1A cells were characterized by phase-contrast and immunofluorescence light microscopy (LM) as well as by scanning (SEM) and transmission (TEM) electron microscopy.

After treatment, the normally epithelioid cells became highly branched via a process of cytoplasmic contraction (*vs.* neurite extension). Several orders of branching were observed by both LM and SEM. By TEM, processes were heterogeneous in terms of membranous organelles and cytoskeletal elements. Microtubules and intermediate filaments were both present in the majority of processes and were oriented parallel to the long axes of the processes. Larger processes contained a variety of membranous organelles including RER. The very thin processes often contained only cytoskeletal elements and small vesicles. Although varicosities along their lengths contained a wider variety of membranous organelles, no immunocytochemical (*e.g.*, synapsin) or ultrastructural hallmarks of synaptic profiles were discerned. Moreover, although some neurons appeared to progress toward a more stable and polarized phenotype, distinction of axonal *vs.* dendritic processes was not apparent either immunocytochemically or by TEM.

[Cells were provided through The Johns Hopkins University Office of Technology Licensing.]

21.12

CHARACTERIZATION OF STRIATALLY-DERIVED IMMORTALIZED CELL LINES. M. Giordano*, H. Takashima, M. Poltorak, H.M. Geller[a] and W.J. Freed. NIMH Neuroscience Center at St. Elizabeths, Washington DC 20032; [a]Department of Pharmacology, UMDNJ-RWJMS, Piscataway NJ 08854.

From primary cultures of fetal rat (E14) striatal primordium, immortalized cell lines were produced by infection with a retroviral vector containing the temperature-sensitive tsA58 mutant of the SV40 large T antigen. Cultures were maintained in chemically-defined medium or DMEM:F12 containing 10% FCS for 5-21 days and then exposed to geneticin (200 ug/ml). Clones were initially screened for expression of SV-40 large T antigen at the permissive temperature (33°C). The 40 clones initially obtained were classified according to cellular morphology at 33°C: (a) small with no processes; (b) multipolar with short processes; (c) elongated or fibroblast-like, usually unipolar or bipolar; or (d) large cytoplasm without processes. Cells with type "b" and "c" morphology differentiated and developed processes when exposed to dibutyryl cAMP, IBMX, and NGF at the non-permissive temperature (39.5°C), while cells of type "a" and "d" did not change. Type "a" cells were positive for vimentin whereas most clones of the "b" and "c" types were vimentin-negative. Four cell lines (one clone, and three subclones derived from a second clone) of the multipolar "b" type were found to be positive for MAP-2, expressing immunoreactivity on short cytoplasmic extensions and on some, but not all, of their processes. These cells were selected for further characterization.

21.13

STIMULUS EVOKED CHANGES IN CALCIUM LEVELS ARE DEVELOPMENTALLY REGULATED IN CULTURED CEREBELLAR GRANULE NEURONS. J. Holliday*, and D.L. Gruol. Department of Neuropharmacology, The Scripps Research Inst., La Jolla, CA 92037.

The development of cultured cerebellar granule cells has been well studied and has been found to be dependent upon calcium influx through either voltage or ligand gated channels (see Gallo et al., 1987). In order to determine how calcium levels are controlled during differentiation and subsequently regulate further development, we have studied calcium responses to a variety of stimuli using fura-2 imaging. Neurons were cultured as either purified or mixed cell types or as organotypic slice cultures from 8 day rats. This will allow us to assess the effects of interactions between various cell types on the maturation of granule cell calcium responses.

In mixed cell cultures, virtually all granule neurons responded to KCl depolarization from the first day in culture. The magnitude of the response increases during the first week in culture, from less than 3 to at least 10 times the resting concentration of calcium. The response was inhibited by the voltage-gated calcium channel antagonist, nifedipine. In contrast, the percentage of cells responding to the neurotransmitter, glutamate, increased during the first week in culture and developmental changes in the amplitude of the response were less evident than observed with KCl depolarization. The developmental profile of caffeine responses was similar to that of glutamate suggesting that granule cells become better able to amplify calcium responses through the calcium dependent release of calcium from intracellular stores during the first week in culture. After 12 days in culture, all responses appear to be more robust in the organotypic cultures. Supported by #MH47680.

21.14

MORPHOGENESIS AND SURVIVAL OF MOTONEURON SUB-POPULATIONS *IN VITRO*. C.E. Henderson*, H. Ansanay-Alex, E. Bloch-Gallego, B. Buisson, W. Camu, H. El M'Hamdi, A. Gouin and C. Mettling. Biochimie CNRS-INSERM, Montpellier, France.

Soon after motoneurons (MN) are born, their axons grow out through the rostral half of each somite while dendrites develop within the spinal cord. From E6 on in the lumbar cord, half the MN die. At brachial levels, cell death begins later and occurs more slowly. We used cultures of purified MN sub-populations to analyse the extrinsic and intrinsic factors regulating these phases of development.

Explants of rostral and caudal somite from E3 embryos were grown for 3 days until they had formed monolayers; they were still labeled appropriately by markers such as peanut lectin and M7412 antibody (gift of H. Tanaka). Astrocyte monolayers were prepared from E6 chicken spinal cord and neonatal rat cortex. Purified MN were seeded on the monolayers. After 3 days, their morphology differed markedly: on astrocytes, they were highly multipolar, on rostral somite they grew well but were pseudo-unipolar, and on caudal somite they failed to grow, suggesting that the signals for MN morphogenesis are to a large extent present at the surface of cells in their environment.

Using a modified metrizamide method that enriches for MN with an acute requirement for trophic support, MN were purified from brachial and lumbar regions of E5 neural tube. Extracts of wing and leg at E6 showed identical survival-promoting activity, whatever the motoneurons used. However, brachial MN died more slowly in the presence of any given extract than did lumbar MN, suggesting that cell death *in ovo* is at least partly regulated by factors intrinsic to the motoneurons.

This work was supported by AFM and IRME.

21.15

Serum affects neuronal cell growth and channel expression

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Neuroblastoma cells (N1E-115 cells), which were derived from undifferentiated murine neuroblastomas, were normally maintained in DMEM (Dulbecco's modified Eagle medium) with 10% FBS (fetal bovine serum) and antibiotics (penicillin, neomycin and strentomycin). Fetal bovine serum usually provides nutritional needs for cell growth. When the neuroblastoma cells were cultured in DMEM with 10% FBS, they grew fast with few neurites. Data from our experiments show that ^{3}H-thymidine incorporation was serum dependent with maximal response at 1.25% FBS in the media (cell density $5x10^6$/ml). However, when the cells were cultured in DMEM without FBS, the cells grew slowly and neurites appeared from the 2nd day of culture. The percentage of the cells with neurites significantly increased and the density of the cells was markedly reduced. When neuroblastoma cells were cultured normally with 10% FBS, the T type Ca^{2+} channel current was observed. However, when the cells were cultured without FBS for approximately 1 month, the T channel current disappeared. These cells expressed an inward Ca^{2+} channel current which showed slow inactivation. This current was sensitive to Bay K 8644 and completely blocked by 2 mM La^{3+}. Therefore, these results suggest that serum-free media may promote neurite growth and alter the channel expression in neuroblastoma cells.

21.16

NEURONAL PROPERTIES OF IMMORTALIZED RAT SEPTAL CELL LINES. J. Kwon[1], E. M. Eves*[1], and B. H. Wainer[1,2]. Depts. [1]Pharm. & Phys. Sci., and [2]Path., The University of Chicago, Chicago, IL 60637.

The immortalization of embryonic day 14-15 rat septal neuronal progenitor cells via retroviral transduction and their initial characterization was described previously (Soc. Neurosci. Abs. 17:37, 1991). Agents that act to increase intracellular cyclic AMP levels, such as forskolin and dibutyryl cAMP, induced a morphological change from a flat epithelioid morphology to a highly refractile neuron-like morphology within a period of 4 hours. Subsequent studies have shown that 20μM norepinephrine and 100μM dopamine produce a similar rapid morphological response while other transmitter agonists such as carbachol, galanin, l-glutamate, histamine, serotonin, substance P, vasopressin, and VIP do not. Basic fibroblast growth factor also induces a similar morphological change but with a time course of 48 hours. Several cell lines express measurable levels of either choline acetyl transferase or GABA. The data indicate that the septal transductant cell lines express characteristics displayed by developing septal neurons. Furthermore, the cell lines may provide a good model for the study of cellular functions that are regulated by the activation of specific receptors for neurotransmitters and growth factors. (Supported by NS 25787, the IDPH Alzheimer's Disease Research Fund, and GM 07151-17.)

21.17

DIFFERENTIATION OF PRIMARY BASAL FOREBRAIN NEURONS IN COCULTURE WITH HIPPOCAMPAL AND SEPTAL CELL LINES. M. Downen*, C. Christian, E. Eves, & B. H. Wainer, Dept. of Pharm. & Physl. Sciences, U. Chicago, Chicago, IL 60637

A "bilaminar" culture system was used to investigate the survival and differentiation of developing basal forebrain (BF) neurons. Dissociated E16 BF cells on coverslips were inverted above a cell feeder layer. The cultures were treated with an antimitotic agent at 2 days to arrest glial proliferation. The ability of immortalized progenitor cell lines from E17 hippocampus and E15 septum to support the maturation of developing BF neurons was compared to that of primary forebrain glia. The feeder layer consisted of primary glial cells, a hippocampal glial line (H19-5) or neuronal line (H19-7), a septal neuronal line (AS583-6), a neuroblastoma line (N18TG2), a glioma line (C6) or a fibroblast line (NIH 3T3). Survival of BF neurons was assessed using the intravital dye, fluorescein diacetate. The hippocampal lines were as effective as primary glia in the maintenance of viability of BF neurons, while the septal line was somewhat less effective. N18TG2 cells were only 50% and C6 glioma cells were only 20% as effective as glia in maintaining viability. BF neurons did not survive in the presence of NIH 3T3 cells. Differentiation of primary BF neurons was determined by immunodetection of the low affinity nerve growth factor receptor (LNGFR). The proportions of LNGFR+ BF neurons were 5.8% over primary glia, 5.6% over H19-5, 9.2% over H19-7, and 2.6% over AS583-6. These data indicate that immortalized cell lines derived from the hippocampus can support survival of BF neurons as efficiently as primary glia. Expression of the LNGFR in BF neurons in bilaminar culture appears to be differentially modulated by different feeder layers: it is equivalent in coculture with H19-5 cells and primary glia, increased in coculture with H19-7 cells, and decreased in coculture with AS583-6. (Supported by NS 25787; IDPH Alzheimer's Disease Research Fund)

21.18

CHARACTERIZATION OF GABA-ERGIC NEURONS IN HIPPOCAMPAL CULTURES. D.L. Benson,* F.H. Watkins, Q. Steward, and G. Banker, Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, Virginia 22908.

Neurons normally develop in a complex environment where genetically determined patterns of development can be influenced or modified by epigenetic factors. When embryonic hippocampal neurons are dissociated and grown in culture, they must develop in comparative isolation, and it remains to be determined how closely they resemble their counterparts in situ. To examine this question, we analyzed the development of a specific subpopulation of hippocampal neurons, identified by their immunoreactivity for GABA. After two weeks in culture, 11-15% of the total number of neurons are GABA-immunoreactive, which is similar to the proportion of GABA cells in the hippocampus in situ. This proportion remains relatively constant between 4 and 35 days in culture. GABA-immunoreactive axons arborize extensively throughout the cultures, contacting both GABA-negative and GABA-positive neurons. Most of the cultured GABA-immunoreactive neurons can be separated into three broad categories based on their morphology: 1) large cells, frequently triangular in shape, with beaded axons and only two or three major dendrites, 2) medium to small cells that are fusiform in shape and 3) medium sized multipolar cells, some of which have large straight spines. These three subclasses correspond in many respects to distinct classes of non-pyramidal neurons that have been previously identified in situ. The results of the present study would suggest that the basic form of GABA neurons is endogenously determined. *Supported by NIH grant NS23094; DLB was recipient of postdoctoral fellowship NS07199.*

21.19

EFFECTS OF POSTNATAL MOSSY FIBER DEVELOPMENT ON CA3 APICAL DENDRITES IN DIFFERENTIALLY REARED MONKEYS. S.J. Siegel *1, P.R. Hof 1,2, G.W. Kraemer 3, W.T. McKinney 3, and J.H. Morrison 1,2. 1 Fishberg Res. Ctr. for Neurobiology and 2 Dept. of Geriatrics and Adult Development, Mt. Sinai Sch. of Med., New York, NY 10029, 3 Dept. of Psychiatry, Univ. of Wisconsin Sch. of Med. Madison, WI 53792.

The pattern of pyramidal and granule cell development in the primate hippocampal formation has been examined with respect to the effect of the mossy fiber system on apical dendrites of CA3 pyramidal cells. Other investigators have previously demonstrated in monkeys that postmitotic pyramidal cells in the CA3 subfield only develop specialized dendritic appendages called thorny excrescences following ingrowth of mossy fibers during the first six postnatal months. Based on our earlier observation of neurofilament changes in the granule cell somata of socially deprived monkeys (Siegel et al. Soc. Neurosci. Abstr. 17, 895 1991), studies of the development of the mossy fiber system and apical dendrites within CA3 have been undertaken. Antibodies to MAP2, which label CA3 pyramidal cells, calbindin, which label axons within the entire stratum lucidum, and phosphorylated neurofilament, which label axons within a portion of stratum lucidum have been used to characterize mossy fiber development within stratum lucidum and the coincident organization of CA3 apical dendrites. We postulate that mossy fibers have an organizing effect on dendritic spacing and bundling of apical dendrites within CA3. Additionally, our results suggest that this organizing effect is delayed or disrupted in monkeys which have been raised in social deprivation. Supported by the John D. and Catherine T. MacArthur Foundation and the Brookdale Foundation.

21.20

MORPHOLOGIC STUDY OF THE HYPOTHALAMIC NUCLEI IN THE NUDE MICE DURING ITS POSTNATAL DEVELOPMENT. I. de la Rosa A., G. G. Ortiz Feria-Velasco, F. Martínez-Sandoval*. Universidad Autónoma de Guadalajara, México., Unidad Invest. Biomed. Occte., I.M.S.S. Guadalajara, Jal., Unidad Invest. Biomed. Centro Médico Siglo XXI., I.M.S.S., México, D.F.

Mutant mouse strain (Balb/c, An Bom nu/nu) is characterized by the absence of the thymus and some endocrine deficiencies such as alterations in the growth and maturation of some tissues, mainly the CNS, particulary the cerebral and cerebellar cortices, although the remaining SNC structures develop prenatally in rodents. This study reports our comparative morphological observations in some hypothalamic nuclei of 40 male mice: 20(nu/nu) and 20 non-mutant mice (nu/-), at postnatal age 15,30,60 and 90 days (n=5). Frontal sections were used for Hematoxylin and Eosin stain. Identification of the nuclei was based upon the nomenclature of Paxinos and Watson. Selected nuclei were the Suprachiasmatic (SCh), Periventricular (Pe), Paraventricular (Pa), Anterior Hypothalamic Area (AHA), Supraoptic (So), and Medial Preoptic Area (MPA). In the non-mutant mouse (nu/-) of all ages ovoid cells with basophil cytoplasm and central nucleolus were identified in (SCh), (AHA), and (MPA). A monolayer of flattened cells, with a thin cytoplasm was seen lining the wall of the III ventricle in the area corresponding to Pa and Pe. Additionally, an abundant and well distributed package of cells was seen in all nuclei. In the mutant mouse (nu/nu) of 15 postnatal days, the periependymal zone had a spongy-like appearance in the area corresponding to (Pe) and (Pa) larger, irregular from flattened to cuboidal cells were arranged into two or three layers lining the cavity of the third ventricle. The cells were larger than those observed in the control group and showed a thin acidophilic cytoplasm, hyperchromatic nucleus, 2 or more nucleoli, and cilia-like structures in the apical region of some cells. At 30,60 and 90 postanatal days (MPA) showed scattered cells; SCh had cells with hyperchromatic nuclei and pink granules in their cytoplasm. (So) and (AHA) depicted irregular shaped cells with a thin cytoplasm, hyperchromatic nucleus, and 2 or more nucleoli. These morphologically differences between (nu/-) and (nu/nu) strains suggest the presence of clear signs of delay in cell migration probably due to a deficiency in the growth and development of the SNC of this nude mouse that may be associated with some neuroendocrine alterations previously reported.

AXON GUIDANCE MECHANISMS AND PATHWAYS I

22.1

SIGNAL TRANSDUCTION BY RETINAL GROWTH CONES NAVIGATING *IN VIVO*: THE ROLE OF FILOPODIA AND SECOND MESSENGERS. C.-B. Chien*, D.E. Rosenthal, W.A. Harris, and C.E. Holt. Dept. of Biology, UC San Diego, La Jolla, CA 92093-0322.

During initial development, *Xenopus* retinal axons navigate along a stereotypical path to their target using cues seen by their growth cones. To study the signal transduction mechanisms involved, we expose the embryonic optic tract by removing the skin, dura, and eye from one side of the head, and then bath-apply various drugs. Effects on pathfinding are assayed by HRP-filling the optic projection, and then correlated with effects on growth-cone dynamics, assayed by high-magnification time-lapse imaging of dil-stained growth cones.

To test this pharmacological strategy, we have first used cytochalasins, which disrupt microfilaments. Experiments in grasshopper limb bud [Bentley and Toroian-Raymond (1986) Nature, 323:712] have shown that cytochalasins disrupt pathfinding, presumably by their removal of growth cone filopodia. Using time-lapse video, we find that 0.15 µg/ml cytochalasin B (CB) removes nearly all filopodia and slows the advance of the growth cone, while leaving lamellipodia intact and active. Both effects are reversed by washing out the drug. After 18-24 h in CB, HRP-filled retinal axons display a characteristic pathfinding error, missing their normal posterior turn towards the tectum and instead continuing dorsally within the diencephalon. Though this could be an indirect effect, the most parsimonious explanation is that intact filopodia are required in order to make the posterior turn, perhaps playing a sensory role.

We are now screening a variety of drugs (*eg* cAMP and cGMP analogs) that could perturb signal-transduction pathways involved in pathfinding, and will report on any results.

22.2

THE ORGANIZATION OF F-ACTIN AND MICROTUBULES IN GROWTH CONES EXPOSED TO A BRAIN-DERIVED COLLAPSING FACTOR. J.Fan, S.G.Mansfield, T.Redmond, P.R. Gordon-Weeks & J.A.Raper*. Dept. Neurosci., U. Penn., Phila., PA 19104

Growth cones from dorsal root ganglia grown in culture can be induced to collapse by a brain derived collapsing factor (Raper & Kapfhammer, 1990). We studied the organization of F-actin and microtubules during collapse induced by enriched collapsing factor. Growth cones before or during collapse were analyzed by time-lapse videomicroscopy and stained for F-actin with Rhodamine-Phalloidin and for tyrosinated α-tubulin with FITC-YL1/2.

Actin: Time-lapse video studies showed that the behavior of growth cones in response to 500x enriched collapsing factor was similar to their behavior in response to 2 µM cytochalasin B, suggesting that collapsing factor inhibits actin polymerization. We measured the spatial pattern of F-actin in control and collapsing factor treated growth cones by comparing the distribution of Rhodamine-Phalloidin to a fluorescent marker for all proteins. In control growth cones, the concentration of F-actin in the leading edges is about twice as high as in growth cone centers. In growth cones treated with collapsing factor for 5 min, the concentration of F-actin in the leading edges decreases dramatically, while the concentration in the centers remain unchanged. These results suggest that collapsing factor causes net depolymerization of actin.

Microtubules: Growth cones treated with 10x enriched collapsing factor for 0, 5, 10, or 60 min were stained with FITC-YL1/2. Growth cones were also analyzed using time-lapse videomicroscopy before and during collapse, and then stained with FITC-YL1/2. We found no evidence that collapsing factor stimulates polymerization or depolymerization of microtubules.

22.3

ROLES OF SECOND MESSENGERS IN CONTROL OF PIONEER NEURITE OUTGROWTH AND PATHFINDING IN THE GRASSHOPPER LEG. K. L. Lankford* Dept. of Mole. & Cell Biol, University of California, Berkeley CA 94720

To assess the roles of specific second messengers in growth cone steering and neurite outgrowth, cultured grasshopper embryos or dispersed neurons were exposed to specific second messenger agonists or antagonist at different developmental stages. Transient (3 hour) calcium elevating treatments could inhibit axon initiation almost completely in 28-29% stage embryos or increase axon initiation by an average of 67% in CNS cultures from 35-45% stage embryos. Chronic calcium elevation or lowering also reduced the rate of outgrowth in embryos by an average of 26% and calcium elevation noticeably decreased axon diameter. Calcium manipulations had no significant effects on pathfinding however, suggesting that global calcium levels did not play a critical role in steering. Whole mount TEM micrographs showed losses of actin filaments in growth cones after calcium elevation, suggesting a possible site of calcium action in control of neurite outgrowth. In contrast to calcium manipulations, 1 mM 8-bromo cGMP had no effect on axon initiation or rate of outgrowth, but induced a variety of axonal steering errors and produced curved axons in dispersed culture. Drugs affecting intracellular pH inhibited the rate neurite outgrowth as well as inducing steering errors. Many manipulations, including cAMP and protein kinase C agonists affected the epithelial development without detectable effects on neurons.

22.4

EFFECTS OF TYROSINE KINASE INHIBITION ON GROWING RETINAL GANGLION CELL AXONS. T. L. Worley and C. E. Holt.* Dept. of Biology, Univ. of Calif., San Diego, La Jolla, CA 92093.

We are investigating the role of tyrosine kinases (TKs) in the growth and guidance of retinal ganglion axons in *Xenopus laevis*. Immunostaining of wholemount embryonic brains with an anti-phosphotyrosine antibody (PY20) has revealed positive immunoreactivity in the fibers crossing the optic chiasm and in the ventral to mid-optic tract. A similar pattern of staining was obtained using a *Xenopus* anti-pp60src antibody (T. Unger and R. Steele), which is specific for the plasma membrane associated TK src. To test the possible role of TKs *in vivo* we have directly applied TK inhibitors to the developing optic tract using an exposed-brain preparation. Lavendustin A and genistein were bath-applied for 10-14 hours and the optic projections subsequently assayed with HRP. We found that treated projections are delayed, have an altered growth cone morphology and display possible pathfinding defects. Similarly, retinal ganglion axons treated with TK inhibitors in culture show a reduction in neurite length as well as an altered growth cone morphology. These results suggest that TKs are important for the normal growth of retinal ganglion axons in the developing optic tract. (Supported by grants from NIH, the March of Dimes and the Pew Foundation.)

22.5

EXPRESSION OF ENDOGENOUS CELL SURFACE ALKALINE PHOSPHATASE ACTIVITY IN GRASSHOPPER LIMB BUD EPITHELIA DURING OUTGROWTH OF PIONEER AXONS. Wesley S. Chang* and David Bentley. Department of Molecular & Cell Biology, University of California, Berkeley, CA 94720.

Alkaline phosphatases (ALPs) are expressed in highly localized patterns during embryogenesis of many different organisms. In grasshopper embryos, endogenous ALP activity is expressed in the epithelia of segmented appendages, as revealed by enzyme histochemistry. In limb buds, ALP activity is present during initial epithelial outpocketing and later becomes restricted to a pattern of circumferential bands of cells. Growth cones of the Ti1 pioneer neurons contact ALP-expressing cells as they pathfind along a highly stereotyped route towards the central nervous system. Double-labeling studies show that the Ti1 axons reorient from proximal to ventral growth immediately proximal to a band of ALP expression that spans the femur-trochanter segment boundary (Fe-Tr). Thus, the change in Ti1 growth cone orientation occurs at a location in the limb that is specified by the transition from expression to non-expression of ALP activity by substrate cells.

Analysis of the embryonic grasshopper ALP indicates that it is active at neutral pH, dependent on divalent cations and resistant to inhibition by levamisole or tetramisole. It can be inhibited by millimolar quantities of nucleotide phosphates, such as AMP and IMP. Treatment of embryos in vivo with phosphatidylinositol-specific phospholipase C (PI-PLC) releases the ALP activity, suggesting that it is due to a cell surface enzyme(s) attached to the membrane via a glycosyl-phosphatidylinositol (GPI) anchor.

22.6

ACTINOMYCIN D BLOCKS CHANGES IN AXON PATHWAY SELECTION IN THE GRASSHOPPER EMBRYO: CHANGES IN THE EXPRESSION OF SURFACE MOLECULES. R. von Bernhardi* and M. J. Bastiani. Dept. of Biology, University of Utah, Salt Lake City UT 84112.

Actinomycin D (A-D) administered at early stages of the development of the grasshopper nervous system inhibits the ability of growth cones to switch pathways at pathway choice points (von Bernhardi & Bastiani. Soc. Neurosci. Abst. 17:743). Our findings raise two main questions: Is the expression of molecules that may participate in pathfinding modified by the A-D and, at the low doses of A-D used (0.05-0.1 µg/ml), how much is RNA synthesis affected?

We examined the effects of A-D on the immunolabeling patterns of 6 different antibodies (Ab) that recognize surface molecules expressed during the period of early axon outgrowth. A-D affected the labeling of 4 of them, including the Ab recognizing Fasciclin I and Lachesin (Kalstrom & Bastiani, Soc. Neurosci. '92). In the presence of A-D, Fasciclin dramatically decreased over time, and Lachesin failed to be expressed in the leg sensory neurons Ti1 and CT1. These changes in immunolabeling suggest that the expression of these surface molecules is closely related in time with the synthesis of their messages. It is also evidence that the effect of A-D may depend on its suppression of surface molecules required for pathfinding. To explore the effect of A-D on RNA synthesis, we measured the incorporation of ^{3}H-Uridine in total RNA. Paradoxically, we found that embryos treated with A-D showed a 30-200% increase in the incorporation of ^{3}H-Uridine over the control. The effect of A-D on the Ab labeling pattern suggests that the synthesis of mRNA and probably rRNA is being blocked. Our hypothesis is that the increased incorporation may be the result of an increased synthesis of tRNA. Assays of ^{32}P-Uridine incorporation will allow us to identify the RNA species that may be responsible for this increased incorporation.

Supported by NIH grant NS25378, the McKnight Foundation and a ASO training fellowship to R.v.B.

22.7

A MULTI-STEP PROCESS INVOLVING CARBOHYDRATE RECOGNITION MEDIATES THE FORMATION OF PRESYNAPTIC PROJECTIONS OF LEECH SENSORY NEURONS. J. Song and B. Zipser*. Dept. of Physiology, Neuroscience Program, Michigan State Univ., E Lansing, MI 48824.

We are using leech sensory afferents as a model system to test the hypothesis that the interactions of carbohydrate-binding proteins with their cognate saccharides mediate pathfinding and target recognition. A given sensory afferent expresses two different carbohydrate epitopes on 130 kD peripheral membrane proteins, the mannose-containing Lan3-2 epitope (fullset epitope) and one of several mixed carbohydrate epitopes (subset epitopes) which distinguish subsets of sensory afferents transducing different modalities. Sensory afferents form their presynaptic projections in the CNS neuropile in a multi-step process. First, they defasciculate into the neuropile after having grown through peripheral nerves in tightly bundled tracts. Previously, we presented direct experimental evidence that this axonal defasciculation is mediated by mannose-specific recognition involving the fullset epitope. Second, each axonal subset, identifiable by its subset epitope, forms a unique presynaptic projection. The formation of unique presynaptic projections by the different subsets correlates with the late expression of the subset epitopes. The subset epitopes appear two days after the fullset epitope is expressed. Third, the presynaptic projections of these different neuronal subsets are stabilized. The stabilization of presynaptic projections correlates with the late expression of the fullset epitope on integral membrane proteins. It remains to be seen whether the late onset of subset epitopes and of integral membrane proteins during the formation of presynaptic projections are activation-dependent processes as are those found during the multi-step process of lymphocyte homing.

22.8

CHARACTERIZATION OF TWO LEECH LECTINS: GALACTOSE-BINDING PROTEINS THAT SHARE EPITOPES WITH THE 130 KD GLYCOPROTEIN ON SENSORY NEURONS. R.N. Cole* and B. Zipser. Dept. of Physiology, Neuroscience Program, Michigan State Univ., East Lansing, MI 48824.

Previously we presented evidence that carbohydrate recognition mediates the projection of sensory neurons in the synaptic neuropile of the developing leech CNS (J. Neurosci. 11: 3471). This event involves the Lan3-2 carbohydrate epitope (identified by mAb Lan3-2) that is present on the 130 kD glycoprotein expressed on sensory neuron surfaces and a putative carbohydrate-binding protein (CBP) that must be present in the CNS. Presently we are characterizing two CBPs isolated from the leech which have apparent MWs of 35 and 67 kD, termed Leech Lectin 35 (LL35) and Leech Lectin 67 (LL67). Approximately 10 µg of LL35 and LL67 can be isolated from one leech by passing Triton X-100 extracts of crude whole leech membranes through an asialofetuin affinity column and eluting the CBPs with lactose. Both lectins specifically bind to galactose derivatives and not to the corresponding glucose and mannose derivatives. Both lectins share common epitopes since polyclonal antibodies generated against the LL35 gel band (anti-LL35) bind to both LL35 and LL67. LL35, however, can be separated from LL67 because LL35 binds to α-methyl-galactose and LL67 does not. In addition, LL35 is more abundant than LL67 in the CNS. Peptide maps of these lectins indicate that LL35 and LL67 are different proteins. Interestingly, both lectins share the anti-LL35 epitopes and Lan3-2 carbohydrate epitope with the 130 kD glycoprotein on sensory neurons. Anti-LL35 recognizes the 130 kD sensory glycoprotein and Lan3-2 binds to both leech lectins. Preliminary data suggests that anti-LL35 stains axons, glia, and muscles in whole mounts of leech CNS.

22.9

EFFECT OF GROWTH ASSOCIATED GANGLIOSIDES ON AXONAL OUTGROWTH. R. Mendez-Otero*, J. Friedman and M. Constantine-Paton. Instituto de Biofisica Carlos Chagas Filho, Univ. Federal do Rio de Janeiro, RJ, 21941, Brazil and Dept. Biology, Yale University, New Haven, Ct. 0651.

Gangliosides constitute a major group of cell surface carbohydrate molecules that have been implicated in numerous cellular functions in the developing and adult mammalian nervous system.We have investigated the role of 9-O-acetylated gangliosides, identified by the Jones monoclonal antibody (Mab)(Constantine-Paton et al., Nature, 324:459,1986), in the migration of growth cones extended by sensory neurons of embryonic rat dorsal root ganglia(DRG) explants grown on laminin substrate. The behavior of individual growth cones was recorded using a time-lapse video-enhanced imaging system before and after the addition of antibodies that recognize especific gangliosides known to be expressed on these growth cones. It was possible to demonstrate that the advance of growth cones on laminin was halted in the presence of Jones Mab. The onset of effects was rapid and signaled by an immediate cessation of elongation, a loss of lamellipodia and a retrieval of axoplasm. This effect was partially reverted by washing the explants for several minutes with culture medium. Mab A2B5 which also recognizes gangliosides expressed on these growth cones does not induce any change on the growth rate. Our findings shown that membrane associated molecules, such as 9-O-acetyl gangliosides, may play an important role on the adhesion of growth cones to different substrates and consequently influence navigation and pathway finding during development.

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22.10

EXPRESSION AND ROLE OF PLASMINOGEN ACTIVATORS IN DEVELOPING RAT NERVOUS SYSTEM. Y. Sumi, M.A.R. Dent and R.J. Morris*, Norman and Sadie Lee Research Centre, Laboratory of Neurobiology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.

Tissue plasminogen activator (tPA) is expressed at high level in just one site - the floor plate - of developing nervous system (from embryonic day (E) 10.5 onwards), as assessed by *in situ* hybridisation, immunohistochemistry and enzymatic assay. A model is presented whereby this selective protease could influence the direction of growth of commissural axons. Messenger RNA for urokinase-type plasminogen activator (uPA) is expressed by many of the neurons of developing brain. It appears on them at a very early developmental stage. For instance, high levels of expression are seen by *in situ* hybridisation on motor and sensory (DRG) neurons at E13.5, and persist throughout embryonic development while these cells undergo their main period of axonal growth. This protease, therefore, could assist axonal migration by regulating adhesion.

22.11

MISEXPRESSION OF FASCICLIN II LEADS TO ALTERED GROWTH CONE GUIDANCE IN THE DROSOPHILA EMBRYO. D. Lin, D. Van Vactor, M. Tiemeyer*, and C.S. Goodman, HHMI, MCB, U.C. Berkeley, CA 94720

In principle, there are two key experiments to test the function of a molecule such as fasciclin II in growth cone guidance. First, as reported last year, we would like to use genetic analysis to remove the function of the molecule, to determine if identified growth cones stall at specific choice points. Second, we would like to misexpress the molecule on cells that normally don't express it, to determine if identified growth cones can be selectively mis-routed. To this end, we have utilized the GAL4 enhancer trap method (A. Brand and N. Perrimon) to obtain lines expressing GAL4 in specific subsets of neurons and muscle during embryogenesis (GAL4 is a yeast transcription factor that can activate its specific binding site, UAS_G, in Drosophila). One such transformant line expresses GAL4 in all PNS neurons. In separate transformants, we generated flies that carry a P element containing the fas II cDNA under the control of the UAS_G. By mating these flies, we generate embryos that specifically misexpress fas II in all PNS neurons. Normally, fas II is expressed by many motoneuron growth cones, including aCC, but by virtually none of the PNS. As a result of ectopic fas II PNS expression, dramatic misrouting of the aCC growth cone is seen. At a specific choice point, the aCC growth cone extends abnormally along the axons of the PNS neurons. In many segments, the aCC growth cone appears to stall at these PNS neurons, while in others it extends abnormally across segment boundaries, sometimes fasciculating with motorneurons from neighboring segments instead of extending towards their targets. These striking phenotypes demonstrate that misexpression of fas II is sufficient to alter growth cone guidance.

To improve our ability to visualize specific axons of interest in these and other experiments, we are testing a variety of different reporter constructs (which in principle will mark either the cell surface or the cytoskeleton) in this type of ectopic expression system in order to visualize specific axons.

22.12

MOLECULAR CHARACTERIZATION OF EMBRYONIC GLIA IN DROSOPHILA. V.J. Auld* and C.S. Goodman, HHMI, Dept of Molecular and Cell Biology, U. of California, Berkeley, CA 94720

The growth cones that pioneer many of the major CNS axon tracts and nerve roots, and many PNS axon pathways, in the Drosophila embryo extend towards and along specifically positioned glial cells. A large-scale enhancer trap screen was conducted previously (see Auld et al., 1991) to obtain new molecular lineage markers that selectively label different subsets of glia, and to identify new genes expressed in these glia that might function in the formation or maintenance of axon pathways. For example, two lines (AE2 and rS136) are expressed in embryonic PNS glia, and a third line (rQ286) is expressed in the exit glia (just outside the CNS); all three of these genes mutate to lethality. Embryos mutant for AE2 appear uncoordinated and are unable to hatch. Although all three genes are being cloned and further characterized, the most is known about AE2. This gene encodes a new transmembrane protein with a serine esterase-like domain that is related to neurotactin and glutactin, two other Drosophila proteins; in all three cases, the serine esterase-like domain is simply a structural motif and appears to lack esterase enzymatic activity. Glutactin is found in basement membranes throughout development; neurotactin is a transmembrane protein that is expressed in the embryonic and larval nervous system and appears capable of mediating cell aggregation in a heterophilic fashion. We are currently generating antibodies against the AE2 transmembrane protein to localize its expression, sequencing the rS136 and rQ286 genes, and characterizing the defects that lead to lethality in mutations in all three of these genes expressed in embryonic PNS or exit glia.

22.13

THE PATHWAY AND MOLECULAR ENVIRONMENT OF DEVELOPING CALLOSAL AXONS FOLLOWING EMBRYONIC CORTICAL LESIONS. K. Morigiwa*, C. Doller and J. Silver. Dept. of Neurosciences, Case Western Reserve University, Cleveland, OH 44106

In the rat, callosal axons project toward the cerebral midline around embryonic day 18 (E18). They course through the deeper layers of the intermediate zone (IZ) and form a distinct bundle above the subventricular zone (SZ), which appears to be a non-preferred growth region. We are interested in the pathfinding of callosal axons laterally within the hemispheres, particulary after perturbation, and whether they are capable of coursing through a lesioned area and of redirecting themselves toward their correct pathway. We mechanically lesioned the cortical area above the ventricle of embryonic rats from E17 to E21 using an angled dissection needle passed through the uterus. At different survival times, the cytoarchitecture of the lesioned region was examined with 1 µm plastic and EM sections. DiI and/or DiA were applied to the cortical surface medial and/or lateral to the lesioned site in fixed whole brains and in 100 µm vibratomed slices. These sections and 10 µm frozen sections were stained with antibodies to ß-tubulin, vimentin, GFAP, chondroitin-6-sulfate proteoglycans (CSPG), and tenascin/cytotactin (TN). In the lesioned brain, the cortex appears to develop parallel to the lesion rather than to the pial surface. Radial glial cells change their geometric pattern and appear to arch toward the lesion, but with no evident gliosis as seen in adult lesions. The axons can go around but also directly through the lesions in E17-18 brains. The majority of the axons course along the lesion, are allowed into SZ, and form pseudo-glomeruli. In some regions, however, axons also appear to cross through the ventricular zone (VZ) to the other side of the lesion. In superficial lesions, they course around the lesion through SZ and VZ. No dramatic upregulation of CSPG or TN was seen as in adults following lesions. These results suggest that during embryogenesis axons are capable of coursing through normally non-preferred regions when forced, but they change their overall fascicular pattern dramatically. However, once passed the lesion, they reorient themselves to their appropriate pathway. Supported by Gliatech, Inc. and NS25713.

22.14

IN VITRO DEMONSTRATION OF TRANSIENTLY EXPRESSED ECM MOLECULES, TENASCIN AND A PROTEOGLYCAN. Monte A. Gates, Andreas Faissner, and Dennis A. Steindler*, Dept. Anat. & Neurobiol., Univ. of Tennessee, Memphis; Dept. of Neurobiol., Univ. of Heidelberg.

Extracellular matrix (ECM) molecules play important roles during pattern formation of the central nervous system. We have made use of culturing techniques of embryonic and neonatal explants to examine the expressions of two developmentally regulated ECM molecules, tenascin and a chondroitin sulfate proteoglycan (CSPG). Previous *in vivo* and *in vitro* studies of these glycoconjugates have shown that they are concentrated in boundaries which surround developing neural units, and they may play a role in the complex events which bring about growth, migration and organization of functional neuronal aggregates. The tenascin glycoprotein, itself, has been shown to be synthesized and released by young astrocytes, and may alone produce diverse effects on cellular attachment and process outgrowth of developing neurons.

Here we report that tenascin and a CSPG are expressed in long term cultures of embryonic and neonatal neostriatum. We have defined culturing conditions (e.g. using plasma clot substrates and sera-containing media as previously described by Gähwiler) in roller tubes which maximize the expression of glycoconjugates involved in glia-neuron interactions during development. Mono- and polyclonal antibodies to tenascin and a CSPG (previously, 473 proteoglycan) have been used to observe intense expression of such glycoconjugates in neostriatal explants, on and around astrocytes (labeled with GFAP) with various morphologies. We are attempting antibody perturbations of these glycoconjugates to study their effect on the development of substantia nigra-neostriatal co-explants where we know that the expressions of ECM molecules like tenascin and the 473 proteoglycan are, to some extent, afferent-dependent. Supported by NIH grant NS 20856 and NSF grant BNS 8911514.

22.15

DISTRIBUTION OF PROTEOGLYCANS IN THE DEVELOPING VISUAL PATHWAY OF CHICK. B.D. McAdams and S.C. McLoon*. Dept. of Cell Biology & Neuroanatomy, Univ. of Minnesota, Minneapolis, MN 55455.

Previous investigations have suggested that proteoglycans can have an inhibitory effect on neurite extension *in vitro* and are found *in situ* in regions of the CNS and PNS that may act as barriers to axon growth. Most prominent among these presumptive inhibitory proteoglycans are keratan sulfate and chondroitin-6-sulfate. The present study utilized immunohistochemistry to examine the spatial and temporal distribution of keratan sulfate and chondroitin-6-sulfate glycosaminoglycans in the embryonic chick retina, optic nerve, chiasm, tract, and tectum to determine whether their localization might be relevant to the pathfinding of retinal ganglion cell axons through these regions. Keratan sulfate immunoreactivity was first observed in the optic chiasm and on radial cells in the optic tectum at E6, when the first optic axons were just reaching the tectum. By E10, it was expressed by cells in the optic nerve, tract and tectum where optic axons were also observed. Chondroitin-6-sulfate was found in the inner retina at E2. At E3, it was coextensive with retinal axons in the retina and was found in the optic stalk and chiasm. Chondroitin-6-sulfate was expressed in the outer layers of the tectum throughout the period of invasion by optic axons. In the neonate, the entire retinotectal pathway was immunoreactive for this glycosaminoglycan. These results reveal a distribution of keratan sulfate and chondroitin-6-sulfate that is not consistent with a role as a barrier molecule, except possibly in the chiasm. The functions of these glycosaminoglycans are also being investigated by disrupting their synthesis *in vivo* and by looking at their effects on growing neurites *in vitro*.

22.16

DIFFERENTIAL LOCALIZATION OF EXTRACELLULAR MATRIX MOLECULES AND CELL ADHESION MOLECULES IN THE PATHWAY OF DEVELOPING PRIMARY AFFERENT FIBERS. T. Shiga[1]*, H. Tanaka[2], M. Grumet[3], G.M. Edelman[4], R.W. Oppenheim[5] and T. Shirai[1]. Dept. Anat., Yamagata Univ., Yamagata[1], Dept. Neurosci. & Immunol. Kumamoto Univ., Kumamoto[2], Dept. Pharmacol., New York Univ. Medical Centr., NY[3], Rockefeller Univ., NY[4], Dept. Neurobiol. & Anat., Wake Forest Univ., NC[5].

To investigate the role of extracellular matrix and cell adhesion molecules in the pathway formation of primary afferent fibers (PAFs), we examined immunohistochemically the distribution of laminin, fibronectin, type IV collagen, Ng-CAM, and SC2 (cell surface molecule specific for PAFs) in chick embryos. The pathway formation of PAFs could be divided into three stages. At stage I, PAFs extended through migrating neural crest cells and abundant extracellular space, from dorsal root ganglion (DRG) to spinal cord. At stage II, PAFs ran longitudinally in the dorsal funiculus, forming fascicles. At stage III, PAFs entered the gray matter without fasciculation. Laminin was localized in the pathway of PAFs at stage I, although it was never found within the spinal cord throughout the stages examined. At stage I, DRG cells and PAFs weakly expressed Ng-CAM, but not SC2. At stage II, strong immunoreactivity of both Ng-CAM and SC2 was found on the fasciculated PAFs running in the dorsal funiculus. At stage III, Ng-CAM was still expressed on PAFs both in the dorsal funiculus and dorsal horn. In contrast, PAFs were SC2 negative in the gray matter and weakly positive in the dorsal funiculus. These results suggest that Ng-CAM and SC2 may be involved in the outgrowth and fasciculation of PAFs, respectively. The disappearance of SC2 may play some role in the entry of PAFs into the dorsal horn from dorsal funiculus.

22.17

EXPRESSION OF T-CADHERIN IN THE FLOOR PLATE CORRELATES WITH COMMISSURAL AXON GROWTH ACROSS THE VENTRAL MIDLINE OF THE SPINAL CORD S.S. Kanekar* and B. Ranscht, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Commissural (C) neurons in the dorsal and ventrolateral chick spinal cord extend axonal projections ventrally through the motor neuron pool and then medially across the ventral midline through the floor plate (FP). Immediately after crossing the FP, C axons alter their trajectory from a transverse to a longitudinal plane. The FP has been implicated in providing both chemotrophic and contact guidance cues for C axons. T-cadherin is a glycosyl phosphatidylinositol-anchored membrane protein that belongs to the cadherin family of cell adhesion molecules and mediates calcium-dependent, homophilic cell-cell adhesion. In the developing chick spinal cord, T-cadherin immunoreactivity is detected at Hamburger/Hamilton stage 15 in dorsal and lateral, but not ventral neuroepithelial cells. Staining in the FP is first distinguished in its dorsal aspect at stage 17, when the first C axons (identified with monoclonal antibody 3A10 from J.Dodd, Columbia Univ.) cross the FP. By stage 20, T-cadherin immunoreactivity is also seen along the ventral boundary of the FP, where the C axons project. Dense staining in the FP is detected between this stage and stage 26, when most of the C axons have crossed over to the contralateral side of the spinal cord. At all of these stages, however, T-cadherin expression is not restricted to the FP, but is also detected at lower intensity in the dorsal and ventral spinal cord, including the motor neuron pool. By stage 27, T-cadherin staining in the FP is diminished, but is dense in the ventromedial funiculus, which defines the longitudinal projections of C neurons. The spatial and temporal distribution of T-cadherin in the FP correlates with C axon growth through this region and raises the possibility that T-cadherin may contribute to the establishment of the commissural axon trajectory. (Supported by NIH (HD25938), the MOD and the McKnight Foundation)

22.18

THE DEVELOPMENT OF SENSORY PROJECTIONS IN THE CHICK HINDLIMB IN THE PRESENCE OF ANTIBODIES AGAINST L1. M.G. Honig* and U. Rutishauser, Dept. of Anatomy and Neurobiology, Univ. of Tennessee-Memphis, Memphis TN 38163 and Dept. of Genetics, Case Western Reserve Univ., Cleveland, OH 44106.

We are interested in understanding how sensory neurons in the chick hindlimb choose the correct pathways to grow along and, in particular, how these choices are influenced by interactions between sensory growth cones and the axons they encounter as they grow. To study this, we have injected antibodies that block the function of various cell adhesion molecules (CAMs) into the developing limb. The injections were done at St. 25 since interfering with CAM function at this stage should be too late to alter motoneuron pathway selection, but may still perturb the subsequent growth of sensory axons. The embryos were allowed to develop for 2 additional days and then the pattern of nerve projections was assessed. Here, we have focused on the action of the L1 adhesion molecule because of its known importance in mediating axon-axon interactions. In the presence of αL1, sensory axons projected along muscle nerves and along cutaneous nerves in relatively normal numbers and the segmental pattern of projections was generally appropriate. However, there were significant alterations. In normal embryos, the medial femoral cutaneous nerve contains axons from sensory neurons situated in segments LS1-3; the axons from LS1 have to cross from the anterior part of the crural plexus to a posterior position in order to project along this nerve. In the presence of αL1, the projection from LS1 was decreased and the projection from more posterior segments (LS2 or LS3) was increased. In other αL1-treated embryos, we found that the sensory projection to the sartorius muscle was dominated in above-normal proportions by neurons from a single segment. These results suggest that L1-mediated interactions may influence the ability of sensory growth cones to navigate through the axon-rich environment of the plexus.

22.19

AXONIN-1 HETEROLOGOUSLY EXPRESSED IN MYELOMA CELLS IS FUNCTIONALLY ACTIVE. C. Rader, E.T. Stoeckli*, T.H. Hasler, B. Kunz, R.A. Zuellig, K. Karjalainen+ & P. Sonderegger. Institute of Biochemistry, University of Zurich, CH-8057 Zurich, Switzerland, and +Basel Institute of Immunology, CH-4058 Basel, Switzerland.

The 135-kd chicken axonal glycoprotein axonin-1 is involved in neurogenesis. Molecular cloning of axonin-1 revealed its membership in the group of Ig/FNIII-like proteins and its homology to rat TAG-1. Recently, we have shown that axonin-1 does mediate neurite outgrowth-promoting activity by a heterophilic interaction with NgCAM, the putative chicken homologue of mouse L1. Here we report the heterologous expression of axonin-1 in myeloma cells. A stable expression system designed for the large-scale production of biologically active mammalian proteins has been used (A. Traunecker, F. Oliveri & K. Karjalainen, TIBTECH 9, 109-113, 1991). This system is based on the mouse myeloma cell line J558L and vectors including an Ig V_κ promoter, an Ig κ enhancer, and an Ig C_κ exon which constitutes the 3' end of the transcript encoding the recombinant protein; it has successfully been applied to the production of soluble lymphocyte-derived cell-surface receptor proteins. We transfected myeloma J558L cells with two different vector constructs. One (pMAX) representing the entire axonin-1 coding sequence, the other (pSAX) containing a termination codon where the putative GPI cleavage site is encoded and therefore representing a truncated, soluble version of axonin-1. Transfection was performed by protoplast fusion or electroporation. Clones were selected in the presence of histidinol and screened by a sandwich dot blot and by immunofluorescence analysis. Both constructs gave rise to high-level expression of a 135-kd protein which exhibits axonin-1 immunoreactivity. Like axonin-1, the product of pMAX occurs both as a membrane-bound GPI-anchored and as a soluble form. In contrast, the product of pSAX was detected in the supernatant only. Functional analysis revealed that recombinant axonin-1 has biological activity: as native axonin-1, it promotes neurite outgrowth and interacts with NgCAM.

22.20

HUMAN AXONIN-1: cDNA-CLONING, STRUCTURAL CHARACTERIZATION, AND EUCARYOTIC EXPRESSION. T.H. Hasler, C. Rader, R.A. Zuellig, H.-C. Bauer*+, and P. Sonderegger. Institute of Biochemistry, University of Zurich, CH-8057 Zurich, Switzerland, and +Institute of Molecular Biology, OAW, A-5020 Salzburg, Austria.

Axonin-1 of the chick and TAG-1 of the rat are homologous members of the immunoglobulin superfamily expressed either as membrane-associated GPI-linked or as soluble forms, and they are believed to play an important role in the axon guidance mechanisms in developing nerve tissues. We have now cloned the human homologue from a fetal brain cDNA library (Stratagene). At the amino acid level, human axonin-1 has a homology of 91% and 75% to rat TAG-1 and chicken axonin-1, respectively. Of the six immunoglobulin C2 and the four fibronectin-type-III domains, the highest degree of conservation is found in the second IgC2 domain: 99% identity with TAG-1 and 83% with axonin-1. The least conserved domain is the first IgC2 domain: 81% identity with TAG-1 and 66% with axonin-1. Chicken axonin-1 undergoes a heterophilic interaction with NgCAM and this interaction is a prerequisite for the process outgrowth on immobilized axonin-1 (Kuhn et al., J. Cell Biol. 115, 1113-26, 1991). Recently, human L1, most likely the homologue of chicken NgCAM, has been cloned (Hlavin and Lemmon, Neuroscience Abstracts, 1991). The overall homology of human L1 and chicken NgCAM is considerably lower than for human and chicken axonin-1: 40% identity at the amino acid level. We have now been able to transiently express the human axonin-1 in monkey COS 1 cells and to select stable human axonin-1 expressing cell lines with a myeloma-based expression system (Rader et al., Neuroscience Abstracts, 1992). The expressed protein shows a strong immunocrossreactivity with polyclonal anti-chicken axonin-1 antibodies. Considerable amounts of the expressed protein are also released into the cell culture medium. With this tool in hand we should soon be able to investigate a possible heterophilic interaction between human axonin-1 and human L1.

22.21

GENOMIC CLONING OF THE AXONALLY SECRETED CELL ADHESION MOLECULE AXONIN-1. R.J. Giger, R.A. Zuellig, and P. Sonderegger*. Institute of Biochemistry, University of Zurich, CH-8057 Zurich, Switzerland.

Axonin-1 is a 135-kd glycoprotein of the chick. It is secreted from the neurites of cultured neurons (Stoeckli et al., Eur. J. Biochem.180, 249-258, 1989) and occurs also as a membrane-bound form (Ruegg et al., J. Cell Biol.109, 2363-2378, 1989). Axonin-1 of the chick is presumably the species homologue of TAG-1 of the rat. The cDNA of axonin-1 has recently been cloned (Zuellig et al., Eur. J. Biochem. 204, 453-463, 1992). The deduced amino acid sequence contains six immunoglobulin (Ig)-like domains and four fibronectin-type-III (FN III)-like domains. In addition, extrapeptides of 23 amino acids and 35 amino acids are located at the N- and C-terminus, respectively. Fragments from a cDNA encoding axonin-1 were used to isolate genomic axonin-1 clones from a lambda EMBL-3 chicken liver library. Sequence analysis of the aligned lambda clones revealed a genomic structure of 23 exons and 22 introns spanning over approximately 20 kb. Each Ig and FN III domain is encoded by two exons. The introns between two domains are exclusively phase I introns, suggesting that axonin-1 had been generated by exon shuffling. Introns interrupting a domain are either phase I or II. The leader sequence is encoded by two exons, exon 2 and exon 3. The first exon, only 77 bp long, is located several kb upstream from the rest of the axonin-1 gene. Exon 2 encoding the first part of the signal peptide and exon 3 are separated by an intron of approximately 3.6 kb. The introns separating exon 3 through 23 are relatively short, ranging from 89 to 700 bp. The last exon codes for the C-terminal 35 amino acids which are probably responsible for GPI-anchorage. At least three different transcripts of the axonin-1 gene were detected by Northern blot analysis. It is most likely that they are generated by usage of different polyadenylation signals.

22.22

HNK1 AND NCAM EXPRESSION IN CULTURES OF DEVELOPING XENOPUS NEURAL TISSUE. R.H.Nordlander* and P.L.Booth. Dept.Oral Biol., Ohio State Univ., Columbus, OH 43210.

The neural cell adhesion molecule (NCAM) is one of many adhesion molecules with subclasses that bind the antibody HNK1. HNK1 recognizes a carbohydrate epitope which has been implicated in cell-cell interactions during neurodevelopment. Studies of Xenopus embryonic CNS in wholemounts and sections have revealed coincident immunoreactivity (IR) of neurons with both HNK1 and anti-NCAM, but marking of non-neuronal elements only with anti-NCAM (Nordlander, '89, Dev Br Res 50; Nordlander and Liu,'90 Abst Soc Neurosci 17).

To further define the cellular expression patterns of the HNK1 and NCAM antigens we examined antibody marking patterns in mixed cultures from the developing Xenopus CNS. Cells with distinctly neuronal morphologies were almost always labeled by both antibodies. Double labeling of individual neuron-like cells showed nearly point to point correspondence, but with HNK1 being somewhat more distinct. HNK1 was consistently localized in vesicles of the golgi apparatus and axoplasm, and on the surface membranes of soma, axon and growth cone. HNK1-IR in these cells was especially dense at sites of axon or growth cone contact with other cells. Type I astrocytes were usually NCAM-IR but not HNK1-IR. These observations confirm and expand our earlier findings that the two antibodies mark with overlapping, but differing patterns.

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22.23

DISRUPTION IN VIVO OF THE DEVELOPING HIPPOCAMPAL MOSSY FIBER CIRCUIT BY ANTIBODIES TO THE LIMBIC SYSTEM ASSOCIATED MEMBRANE PROTEIN. M. F. Barbe* and P. Levitt. Dept. of Physical Therapy, Temple Univ., Phila. PA 19140 and Dept of Anatomy and Neurobiology, Med. Coll. of Penn., Phila., PA 19129.

The limbic system associated membrane protein (LAMP) is expressed in limbic-associated areas from the time of inception of the neurons. Results of previous in vitro explant studies, in which the addition of anti-LAMP prevented the de novo formation of the cholinergic septo-hippocampal circuit, suggested that LAMP may be a target recognition molecule (Keller et al., Neuron 3: 551,1989). In an attempt to demonstrate the role of LAMP in circuit formation in vivo, we examined the effects of antibody treatment on a connection that forms postnatally in the rat, the mossy fiber projections from the dentate granule neurons to the CA3 pyramidal cells. Five µl of either saline or purified Fab fragment of anti-LAMP (2 mg/ml) was injected into the cisterna magna postnatal days (P) 0, 2, 4, and 6. The brains were prepared and stained for zinc using the Timms' method on P8 in order to identify the terminal distribution of the developing mossy fibers. The mean area fraction of the suprapyramidal mossy fibers occupying the CA3 region was measured using a Bioquant Imaging System. The mean area fraction of suprapyramidal mossy fibers in the saline injected animals was 0.60±0.09, which was three fold higher than the mean area fraction of mossy fibers in the anti-LAMP injected animals (0.22±0.04). The remaining mossy fibers appeared to distribute more diffusely throughout the molecular layer. Qualitative examination of cresyl violet stained sections indicates that there is not a noticeable decrease in the number of granule or CA3 pyramidal neurons. These results suggest that LAMP provides chemospecific cues that are necessary for proper axonal pathfinding and synaptogenesis. (Supported by NIMH Grant MH45507).

22.24

ACTIVELY-SCANNED MICROELECTRODE ARRAYS. T. K. Whitehurst, C. W. Storment, C. R. Belczynski* and G. T. A. Kovacs. Stanford Univ. Dept. of Electrical Engineering, Room 130, Stanford, CA 94305-4070.

Numerous cell culture systems incorporating microelectrodes have been developed in order to study populations of electrically active cells *in vitro*, by means of multi-site, simultaneous recording and/or stimulation. Since a prime objective of such devices is to allow electrical access to numerous microelectrodes in contact with cells, large numbers of electrical interconnections are necessary. Typically, either large arrays of external amplifiers (and/or current sources and other circuits) are employed, or small numbers of such instruments are used at one or two microelectrodes at a time, defeating the purpose of the multi-site arrays. The goal of this work is to overcome this problem by developing silicon-substrate microelectrode arrays that incorporate *on-chip* signal processing and multiplexing circuitry. Since multiplexing circuitry is included, the number of connections to external circuits would be greatly reduced (for example, a large array of recording sites could be scanned using only five external connections). The current focus of this project is the implementation and testing of an actively-scanned, two-dimensional array of microelectrodes. This device should provide improved access to the electrical activity of living cells *in vitro*, allowing simultaneous recordings to be made from a large number of microelectrodes. The basic approach is to fabricate the active microelectronic circuits using a standard BiCMOS process and follow this with additional processes for fabrication of the microelectrodes and application of an insulation (or passivation) layer for protection of the active circuits from biological fluids. We are performing numerous evaluations of the active arrays and other test circuits in saline and cell culture media, to verify that the technology proposed can be used to protect active microcircuits under continuous immersion in biological fluids.

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22.25

DEVELOPMENT SPECIFIC CELL-SURFACE ANTIGENS OF THE MAMMALIAN CNS. M.J. Riggott* and W.D. Matthew Dept. of Neurobiology, Duke University Medical Center, Durham, N.C. 27710

Molecules that direct axonal growth in the developing CNS may be in low abundance. In order to generate immunological probes to these molecules, we used a chemical tolerization technique to bias the immune response. Monoclonal antibodies were made from mouse spleens after the mice had been tolerized to a homogenate of adult rat brain, and then immunized with a homogenate of brain from E16, P0 and P7 brains. From this set of fusions, we generated 60 antibodies that bind developing CNS tissues. Of these, 28 label neuronal or glial cell surfaces.

The functions of cell-surface antigens were explored using antibodies in bioassays testing neuronal cell aggregation as well as adhesion and neurite outgrowth on cryostat sections of developing brain. We found that seven of these antibodies promoted cell aggregation and one inhibited aggregation. Eight antibodies promoted neuronal cell adhesion to cryostat sections in culture; at least one of these promotes neurite growth. Further studies will determine whether these antibodies affect axonal outgrowth in living slices of brain tissue in culture. Such antibodies may provide probes for molecules that direct axonal growth in the normal developing CNS.

22.26

COVALENT BINDING OF LAMININ FRAGMENTS TO TEFLON-FEP MEMBRANES PROMOTES THE SELECTIVE ATTACHMENT AND DIFFERENTIATION OF NEURAL CELLS. R. Bellamkonda*, J. P. Ranieri, P. A. Tresco, T.G. Vargo[1], J.A. Gardella[1] and P. Aebischer. ABC Section, Brown University, [1]Dept. of Chem., SUNY at Buffalo.

Cell adhesion proteins contain minimal peptide sequences which are specifically recognized by cell surface receptors and can be covalently immobilized on synthetic materials. In the present study we tested the possibility of guiding axonal outgrowth on a patterned surface on which fragments of the laminin molecule, such as YIGSR and IKVAV, have been covalently immobilized. Fluorinated Ethylene Propylene (FEP) films were chemically modified in a patterned fashion (300µ stripe modifications by 500µ land spacings) using a radio frequency glow discharge (RFGD) process in a low vacuum chamber containing both hydrogen and methanol gases. This modification allows for the replacement of the surface fluorine atoms with hydroxyl functionalities while maintaining the desirable topographic and surface energy properties of the base FEP material. The laminin fragment YIGSR, was then covalently bound to the FEP surface hydroxyl groups via an ester bond to the carboxylic terminus of the peptide using a nucleophilic substitution reaction (SN_2) in an aprotic solvent (DMSO) containing potassium carbonate as an acid acceptor. Covalent binding of the YIGSR peptide was verified with both Time of Flight-Secondary Ion Mass Spectroscopy (TOF-SIMS) and Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR). Rat E15 septal cells plated onto these patterned membranes in DMEM containing 10% FBS demonstrated selective attachment to the YIGSR regions. The outgrowth of the neurites remained primarily on the YIGSR stripes with few neurites extending onto the unmodified regions. No attachment was observed when IKVAV or hexaglycine was immobilized. These results demonstrate that a non-adherent substrate can be modified with signaling peptides to induce both attachment and differentiation in a controlled manner.

22.27

AN IMPROVED METHOD FOR CREATING WELL-DEFINED, PATTERNED SUBSTRATES FOR STUDIES OF NEURONAL ADHESION AND GUIDANCE

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We are developing techniques for patterning tissue culture substrates with adhesive molecules to study fundamental issues in neuronal development such as cell adhesion and guidance. Kleinfeld et al. (*J. Neurosci.* 8:4098, 1988) combined silane chemistry and photolithographic techniques to pattern neurons on quartz and silicon substrates. That strategy eliminated imprecise boundaries and topographical features inherent in previous methods (e.g., evaporation and filtration techniques), but it required 36 hours and utilized expensive facilities and materials. We report here a faster, inexpensive method of patterning culture substrates while maintaining submicron precision and complete control of the surface features. With our approach, well-defined, patterned substrates can be made in 10-12 hours in a conventional biological laboratory. Patterns of amines, peptides, proteins, lipids, and/or carbohydrates can be made on borosilcate glass. We have focused on patterns of amines (e.g., ethylenediamine) and lipids (e.g., hexadecane) for biological studies (see Soekarno et al., these abstracts). Using surface analysis techniques (X-ray photoelectron spectroscopy) we have confirmed that surfaces covalently bound with amines are of monolayer thickness (10-20 Å) and therefore provide no topographical features. We have also used photolithographic techniques to pattern extracellular matrix molecules (fibronectin, laminin, collagen) on glass. These patterning techniques may be useful for studies of cell-substrate interactions, analysis of pathfinding strategies, and formation of neural networks *in vitro*.

This research was funded by MH-10217 and by a Biomedical Engineering Grant from the Whitaker Foundation.

22.28

DIGITAL IMAGING MICROSCOPY OF CELL GUIDANCE ON WELL-DEFINED PATTERNED SUBSTRATES

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Department of Physiology and the Institute for Neuroscience, Northwestern University Medical School, Chicago, IL 60611.

We are studying locomotion of mouse neuroblastoma cells (N1E-115) cultured on photolithographically patterned substrates. Substrates were patterned with monolayers of adhesive amines (ethylenediamine) and non-adhesive alkanes (hexadecane) allowing us to study cell guidance in the absence of topographical cues (cf. B. Lom et al., these abstracts). Cell behavior on amine-coated lines (10 µm in width) was visualized using digital imaging microscopy. The leading edge or nuclei of undifferentiated cells (i.e., those with fibroblastic morphology) migrated along lines at speeds one-half the rate of growth cones or nuclei of differentiated cells. Regardless of the state of differentiation, the rate of locomotion slowed considerably at junctions between lines where cells invariably explored their choices. Undifferentiated cells displayed random behavior when choosing between different directions of movement. Growth cones, on the other hand, displayed a statistical preference for moving straight ahead at junctions. Interference reflection microscopy (IRM) was used to assess differential cell adhesion to the substrates, as well as to visualize the pattern which is not visible using either phase-contrast or Hoffman optics. IRM of neurites and cell bodies revealed primarily "close" contacts (30 nm separation) with the substrate and few "focal" contacts (10-15 nm). Although most cells preferred to grow on amines over alkanes, IRM showed that the preference is not due to differences in the type of adhesion on the two substrates. Fluorescent imaging of rhodamine-phalloidin demonstrated little correspondence between actin filaments and focal contacts beneath cells.

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FORMATION AND SPECIFICITY OF SYNAPSES I

23.1

REGENERATION OF ALLOTRANSPLANTED NEURONS IN THE CRAYFISH.

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We are studying the transplantation, between different animals, of the neurons innervating the superficial flexor muscle system of the crayfish Procambarus clarkii. The isolated ganglion containing the somas of these neurons can be succesfully transplanted from one crayfish to another: the neurons survive, grow and form new synaptic contacts with a denervated muscle target (Krause et. al., Soc. Neurosci. Abstr. 16: 489, 1990). Some initial small-size, long duration junction potentials (jp's) eventually become normal sized, short duration jp's, yet the synapses do not acquire the normal facilitation characteristics of mature synapses (Krause & Velez, Soc. Neurosci. Abstr. 17: 216, 1991). Further experiments using this system have shown that (1) there is regrowth by more than one neuron, (2) there are many "holes" in the connectivity maps, i.e. places where no jp's are detected, and (3) most synapses keep their immature jp's characteristics. It appears that the allotransplanted neurons are not able to regenerate normal connections with the target area. The lack of synaptic input into the isolated ganglion could be affecting the ability of these cells to regenerate normal connections. We are presently backfilling the transplanted neurons with cobalt blue in order to study the dendritic arborization of these cells to see whether the lack of synaptic input could also generate detectable changes in dendritic morphology.

23.2

DAY/NIGHT AND CIRCADIAN RHYTHMS AMONG CELLS OF THE LAMINA IN THE HOUSEFLY'S OPTIC LOBE.

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In the first visual neuropile (lamina) of the optic lobe of the housefly, *Musca domestica*, photoreceptor terminals (R1-R6) inervate monopolar interneurons within discrete cartridges. Two large cells in each cartridge, L1 and L2, receive input synapses from R1-R6; L2 in turn feeds back synapses upon R1-R6. The frequencies of both synaptic contacts oscillate under day/night (LD) and constant darkness (DD) conditions.

We have now detected that the axon diameters of L1 and L2 also reveal circadian changes. In flies reared in DD, L1 and L2 are both larger in subjective day than in subjective night, indicating a circadian rhythm. Under LD, L2 is larger during the day than the night, whereas L1 is of similar size in both day and night, indicating that L1's circadian rhythm is offset by the effect of light. The difference in response to light between L1 and L2 is confirmed in flies reared in continous light, when L2 is larger during subjective day but L1 larger during subjective night. The changes in L1 and L2 thus reflect two mechanisms: functional (light exposure) and circadian. To untangle the relative influence of these two, we reverse reared flies from day/night to night/day. Cell size does not change, indicating that circadian influence is stronger than light exposure. On the other hand, flies exposed to light for 1h after rearing from pupae in DD show a 60% increase in cell size, indicating a strong direct action of light. Cell size changes in LD are not induced by others factors like water deprivation. Although both mechanisms, functional and circadian, in L1/L2 size changes are unknown, they correlate in the lamina with day/night changes in serotonin-like immunoreactivity of a wide-field optic lobe cell, LBO5HT. Under LD, the lamina fibres of this cell are large in the day and exhibit more varicosities than during the night. L1/L2 were also smaller in the optic lobes of two flies, on the side ipsilateral to that receiving stab wounds to ventral protocerebrum designed to interrupt the axon of LBO5HT.

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23.3

EXPRESSION OF INSULIN AND INSULIN RECEPTORS AT NEUROMUSCULAR JUNCTIONS OF *DROSOPHILA*

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We have been using the body wall muscle preparation of *Drosophila* larvae as a model system to study the development of neural connections. Our aim is to take advantage of molecular and genetic techniques in this organism to identify molecules that are essential for the development of specific patterns of innervation. One such molecule whose role in regulating neural development has been suggested is insulin. Both an insulin-like peptide and an insulin receptor-like gene have been characterized in *Drosophila*. In this study immunocytochemistry and receptor binding assays were employed to examine the expression of insulin and the insulin receptor at the body wall neuromuscular junction.

Insulin-like immunoreactivity was expressed at a subset of synaptic boutons in one identified muscle fiber per hemisegment. Insulin receptor-like immunoreactivity, as determined by 2 different monoclonal antibodies directed against the receptor alpha subunit, was present at all neuromuscular junctions and surrounded synaptic boutons near the nerve branch point of each muscle fiber. Similar localization was found using FITC-conjugated bovine insulin in unfixed preparations. Receptor binding assays using a wheat germ agglutinin protein extract from larval membranes showed the presence of a high affinity insulin binding activity. We are now screening for mutants of the insulin receptor gene to determine whether insulin and the insulin receptor can regulate the development of neuromuscular junctions. Supported by NIH grant NS30072 and a Sloan Fellowship

23.4

ULTRASTRUCTURAL CHARACTERIZATION OF NEUROMUSCULAR JUNCTIONS AT *DROSOPHILA* LARVAL BODY-WALL MUSCLES.

X. Jia and V. Budnik* Department of Zoology, University of Mass, Amherst, MA 01003

The body-wall muscles of *Drosophila* larvae are innervated by 3 morphologically distinct subsets of terminals. Mutations that increase the activity of motorneurons increase the number of synaptic boutons in one of these subsets. To examine the structural basis for this morphological plasticity we have initiated an ultrastructural analysis of nerve terminals. Motor axon terminals were examined with TEM by serial transverse thin sections at identified body-wall muscles in third instar wild-type larva.

Three types of motor axon terminals were found. One type of terminal contained clear synaptic vesicles 30-50 nm in diameter. It was surrounded by a well-developed subsynaptic reticulum -- a complex membrane system formed by invagination of the sarcolemma. Synaptic specializations at these terminals were characterized by electron-dense T-shaped bodies. The second type of terminal contained 80-120 nm dense-core vesicles and was devoid of clear vesicles and subsynaptic reticulum. Preliminary results showed the presence of septate junction at these terminals. The third type was a "mixed terminal" containing both clear and dense-core synaptic vesicles. As in dense-core vesicle terminals, the subsynaptic reticulum in mixed terminals was not well-developed. Both dense core and mixed terminals usually lied on grooves on the surface of the muscle cell, whereas clear vesicle terminals were more deeply invaginated on the muscle. We are in the process of performing serial reconstructions in order to compare the structure of these terminals to those seen at the light microscopic level and to the structure of terminals in mutants with altered excitability. Supported by NIH grant NS30072.

23.5

NERVE/MUSCLE INTERACTIONS DURING DEVELOPMENT OF INSECT ADULT LEG MUSCLES IN VIVO AND IN VITRO.
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During metamorphosis in the hawkmoth, *Manduca sexta*, larval leg muscles die and are replaced by new adult muscles, while the same motoneurons persist to innervate the adult leg muscles (Kent and Levine, 1988). We are interested in the role leg motoneurons play in the development of adult leg muscles. Markers for muscles and muscle precursors, including peanut agglutinin, phalloidin, and an antibody to the *Drosophila* twist protein (courtesy F. Perrin Schmidt), were used to follow the differentiation of adult leg muscles *in vivo* during metamorphosis. 5-bromo-deoxyuridine (BUdR) incorporation was used to determine the time course of muscle cell proliferation. As reported for other muscles in holometabolous insects, we find that innervation is crucial for the development of adult leg muscles. Formation of adult leg muscles was prevented by cutting the leg nerve prior to the onset of metamorphosis.

Similar results were obtained *in vitro*. Dissociated tissue from developing adult legs included spindle-shaped cells that proliferated, fused, and differentiated into contractile networks only when co-cultured with neurons. Development of putative muscle cells was similar regardless of whether thoracic or abdominal ganglia were the source of the neurons.
Supported by NIH NS 28495

23.6

MOLECULAR HETEROGENEITY OF EMBRYONIC *DROSOPHILA* MOTONEURONS AND MUSCLES.
E. W. Harkins*, A. Chiba, and H. Keshishian. Biology Department, Yale University, New Haven, CT 06511.

To test the idea that molecular recognition is a guiding factor in connectivity at embryonic neuromuscular junctions, we asked whether molecular specialization exists among muscle and motoneuron populations. Through an enhancer trap screen (Soc. Neurosci. Abstr. 16:135.9), we identified expression patterns which include subsets of motoneurons and/or muscles during the time of synaptogenesis (stage 16). Previously undescribed patterns are presented here.

Five lines label subsets of the RP motoneurons, which have identified targets. These lines distinguish RP2 and RP3 from each other and from RP1 and RP4; however, none distinguish between RP1 and RP4, which coinnervate muscle 13. Four lines distinguish motoneuron aCC from its non-motoneuronal sibling, pCC.

Ten lines have expression in a subset of muscles. None represent an obviously position-dependent pattern, in either the dorsoventral or anteroposterior axis. All are segmentally repeated. Embryonic expression patterns are not necessarily maintained in larvae. Line 3250 labels a subset of embryonic muscle fibers including 7 but not 6. Differential labelling of 7 and 6, which is also seen with *Toll* (Nose & Goodman, Soc. Neurosci. Abstr. 17:297.10; Halfon et al., this volume), is interesting because these muscles are dually innervated by the same motoneurons. Line 1849 labels only one of approximately 6 nuclei in muscle 5. The mechanism for this subcellular localization is not known but is intriguing given that heterogeneity between nuclei within a single muscle fiber has recently been described in vertebrates. These and previous results (Soc. Neurosci. Abstr. 17:91.10) show that there are molecular differences between individual cells of both the motoneuron and muscle populations, and perhaps even between nuclei of a given muscle fiber.

23.7

TARGET CONTACT REGULATES THE EXPRESSION OF SYNAPTIC MARKERS DURING SYNAPTOGENESIS.
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Identified buccal neurons of the snail Helisoma display differing abilities to become the presynaptic element of a functional chemical synapse. Buccal neuron 5 (B5) rapidly forms synapses after contacting targets and does not require new protein synthesis. In contrast, B19 must contact its specific synaptic target (SLT muscle) and undergo protein synthesis for synaptogenesis (Doyle et.al.,,1990, Soc. Neurosci. Abst.).

We have generated monoclonal antibodies with the goal of identifying presynaptic molecules that are necessary for synaptogenesis. Antibody SIN-1 is directed against a presynaptic marker of Helisoma chemical synapses. Immunoreactivity is present in the neuropil of ganglia and at varicosities on muscle fibers as seen in frozen sections. In cell culture the antigen is localized in varicosities of neuron B5 independent of target contact, while immunoreactivity is lacking in the neurites of neuron B19 when cultured alone. However, following contact between neuron B19 and its synaptic target, SLT muscle, immunoreactivity is seen at neuron-muscle contact sites. These contact sites occur either at terminal varicosities or regions along the neurite of B19. Thus, SIN-1 recognizes a synaptic antigen whose expression is retrogradely regulated by synaptic target contact.

23.8

SELECTION OF TRANSMITTER RESPONSES AT SITES OF NEURITE CONTACT DURING SYNAPSE FORMATION.
S. Ching, S. Catarsi and P. Drapeau#, McGill University, Center for Research in Neuroscience, 1650 Cedar Ave., Montreal, P.Q. H3G 1A4.

Pressure sensitive (P) cells of the leech receive an inhibitory Cl-dependent synapse from serotonergic Retzius (R) cells; P cells also have an extrasynaptic, excitatory response to 5-HT that is reduced upon contact with the R cell. By allowing cultured neurons to extend neurites, we have observed that this contact mediated loss of response is highly localized to only those neurites and growth cones of a P cell contacted by similar structures of the R cell. In P cell bodies and uncontacted P cell neurites and growth cones, we resolved a depolarizing response to focal application of 5-HT, as measured by intracellular recording at the soma. The excitatory response was markedly reduced in P cell growth cones contacted by R cell growth cones and neurites but not at uncontacted processes from the same cells. Similarly, single channel patch clamp recording identified a cationic channel activated by 5-HT in uncontacted P cell growth cones whose modulation by 5-HT contrasted with the lack of modulation of cationic channels from contacted growth cones. The nature of this loss of channel modulation is described in the accompanying Abstract. We conclude that neurite contact selectively eliminates extrasynaptic responses at sites of future synapses.

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23.9

ELECTROPHYSIOLOGY OF DEVELOPING SYNAPSES BETWEEN FILIFORM HAIR SENSORY NEURONS AND GIANT INTERNEURONS.
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Each cercus of the newly-hatched cockroach (*Periplaneta americana*) has two filiform hair sensory neurons, one medial (M) and the other lateral (L). Both axons arborize within the terminal ganglion where the L afferent forms a strong monosynaptic connection onto giant interneuron (GI) 3 but does not synapse with GI2. The M afferent evokes large monosynaptic EPSPs in GI2 and small EPSPs in GI3. To determine the initial specificity of the synapses, I recorded intracellularly from the cell bodies of the filiform hair sensory neurons and GIs 2 and 3 at different stages of embryonic development. Action potentials were first detectable in both sensory neurons at 52% of development, at which stage L spikes evoked small EPSPs (0.5 mV) in 5/13 GI3s. Spikes in M did not produce EPSPs in GI2 until the 56% stage. Small M–GI3 EPSPs were detectable at the 59% stage, by which time both sensory neurons showed some spontaneous activity. At no stage did L spikes elicit EPSPs in GI2, suggesting that synapse formation is specific from the outset. Synaptic strengths increased rapidly up to about the 67% stage, when L–GI3 EPSPs had a mean stabilized amplitude of 8.7 mV, M–GI2 EPSPs 3.3 mV, and M-GI3 EPSPs 0.8 mV. The L–GI3 EPSPs subsequently declined to 4.4 mV at 81%, while the others remained constant. The properties of the synapses remained unchanged from 60% up to the first instar; with repeated stimulation at rates above 0.2 Hz the EPSP amplitude stabilized at half its initial value. At greater than 100 Hz the synapses showed modest facilitation (F values of 0.5–1). Supported by NIH Grant NS07464.

23.10

SYNAPTIC TARGET-INDUCED REGULATION OF NEURONAL CALCIUM HOMEOSTASIS.
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Synaptogenesis establishes an orthograde flow of information from the presynaptic neuron to its postsynaptic target. This cellular communication between synaptic partners usually begins as a neuronal growth cone encounters the target cell. We report a retrograde signaling in which contact with a specific muscle target causes a transformation of calcium homeostasis in the growth cones and neurites of an identified presynaptic neuron during early synaptic interactions in cell culture.

Motoneuron B19, isolated from the buccal ganglion of *Helisoma* and plated into cell culture, is highly restricted in its synaptogenesis. Previous studies of interactions between neuron B19 and its normal synaptic partner, the supralateral radular tensor(SLT) muscle, have shown that contact with SLT muscle induces an enhancement in the responsiveness of neuron B19's secretory machinery to internal calcium levels. Through this retrograde signaling, the neuron acquires the ability to couple action potentials to the release of neurotransmitter, thus becoming a competent presynaptic partner only following appropriate target contact.

Resting calcium levels in presynaptic neuron B19 were monitored during initial interactions with synaptic targets. Within minutes of muscle target contact, rest calcium levels are locally elevated in the presynaptic neuronal growth cone. Following 24h of contact with SLT muscle fibers calcium levels were elevated throughout the entire neuron. Estimated calcium levels in muscle-contacted neurites of neuron B19 were 192±66nM (mean±s.d.; n=21) as compared to resting levels of 107±49nM (n=18) in neurites of non-contacted parallel control neurons. These elevated levels were maintained after severing target contacts. Similar elevations were achieved using extracted SLT muscle membranes. This shift in rest calcium set-point, induced by synaptic target contact, is a sustained and integral change in neuronal physiology associated with the onset of synaptic transmission.

23.11

H-7 PREVENTS TARGET INDUCED ENHANCEMENT OF ACTION POTENTIAL-EVOKED CALCIUM ACCUMULATION IN THE PRE-SYNAPTIC NEURON DURING SYNAPTOGENESIS L. R. Funte*, and P. G. Haydon. Dept. of Zoology and Genetics, Iowa State University, Ames, IA 50011.

During synapse formation between neuron 19, B19, and its *in vivo* target the supralateral radular tensor muscle, action potential - evoked calcium accumulation is enhanced at sites of neuron-muscle contact. We have examined the time course of this enhancement using the calcium-sensitive dye Fura-2 to monitor changes in action potential evoked calcium accumulation. Neurons B19 were plated into culture alone and the action potential - evoked calcium accumulation was found to be 247 ± 86 nM (n = 5). Individual muscle fibers were manipulated into contact with B19's neurites. The action potential - evoked calcium accumulation at sites of contact was monitored over time. After contact, action potential - evoked calcium accumulation increased to 461 ± 55 nM after 10 minutes of contact, and to 849 ± 133 nM after 30 minutes of contact. Contact with non-synaptic novel targets did not cause such an enhancement.

We have begun to investigate the role of protein phosphorylation in the enhancement of action potential-evoked calcium accumulation. The addition of the protein kinase inhibitor H-7 (40 - 100μM) to the culture medium prevented the muscle induced enhancement of calcium accumulation (n = 6). However, the addition of H-7 to the bath following sustained contact with muscle (greater than 14 hours) did not reduce the already enhanced calcium accumulation, (n = 3) These data indicate that protein phosphorylation is involved in the induction of the enhancement of action potential evoked calcium accumulation, but is not necessary for its maintenance.

ION CHANNELS: DEVELOPMENT

24.1

APPEARANCE OF VOLTAGE-DEPENDENT CURRENTS DURING EMBRYONIC CEREBELLAR DEVELOPMENT. M.A. Holman*, S. Bakalis, K. Kosik, G.R. Strichartz. Anesthesia Research Labs and Division of Neurology, Brigham & Women's Hospital, Boston, MA 02115

During embryonic brain development, neurons differentiate and migrate considerable distances before they achieve their final location and begin process differentiation. They extend processes, develop polarity and continue growth until they synapse on an appropriate target. The focus of this study was to correlate the development of electrical excitability with the distinctive morphological changes that cerebellar neurons undergo in culture.

Embryonic cerebella were taken at day 15 of gestation (E15), dissociated, and plated. The development of ionic current was monitored by standard whole cell voltage clamp techniques. Resting potentials (Em) of newly plated cells (3-7 hr) under our recording conditions were generally between -5 and -15mV, with cells developing an average Em of -21mV by 24-48 hr, and -30mV after 72 hr. From initial plating to 24 hrs, cells had only a voltage dependent K^+ current (I_{K+}) with current density at +50mV = 12.6pA/pF. This I_{K+} was completely abolished by 10mM external TEA or 140mM internal Cs^+. These cells had no detectable inward current. 24hr or more after plating, when morphological polarity was clearly evident, an inward Na^+ current had developed with midpoint potentials for activation ($E_{0.5}$) of -18mV and inactivation (E_h) of -58mV, and a current density of 9.2pA/pF. In addition, the TEA sensitive I_{K+} in these cells had a current density of 16.9pA/pF at +50mV.

These data suggest that E15 cerebellar neurons are electrically unexcitable initially, but develop Na^+ based excitability within 48 hours of plating. This induction of ionic current is independent of synapse formation and occurs after the cells develop a discrete axon.

24.2

DEVELOPMENT OF MEMBRANE EXCITABILITY IN THE RAT CORTEX BEGINS AT EMBRYONIC DAY 11. D. Maric*, J. Maric, S.V. Smith and J.L. Barker. Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892

In this study we examined the development and maturation of ion channels and membrane receptors in E11-E22 rat cortical cells. Experiments were carried out on single cell suspensions obtained from embryonic cortex by gentle enzymatic digestion with papain. The cellular responses to a Na^+ channel agonist, veratridine, and to steroids, GABA, kainate, glutamate, NMDA, glycine, β-alanine and taurine were detected using a fluorescence-activated cell sorter and a negatively-charged fluorescent indicator dye (oxonol) that partitions into the cells according to their transmembrane potential. At E11-E13 we have observed consistent depolarizing responses to veratridine, GABA and a progesterone metabolite 5α-pregnan-3α-ol-20-one (5α,3α), which were not blocked by the commonly used antagonists tetrodotoxin, bicuculline and picrotoxin, respectively. In fact these antagonists at E12 and E13 by themselves acted as depolarizing agents. Furthermore, at this early embryonic period there were detectable hyperpolarizing responses to 5β-pregnan-3β-ol-20-one (5β,3β), kainate, glutamate and glycine. Over E14-E19 the depolarizing responses to most of above agonists progressively increased, although some hyperpolarizing responses, particularly to kainate, glutamate and GABA persisted. However, all responses became sensitive to their respective antagonists at E14. In addition, 5β,3β became a very effective blocker of 5α,3α-, GABA- and veratridine-induced cell depolarizations. From E19-E22 the depolarizing responses to GABA, taurine and some steroids gradually decreased whereas those to kainate, glutamate, NMDA, β-alanine and glycine were enhanced. The results reveal a complex development of membrane excitability at the level of the cell body during the earliest period of postmitotic differentiation of the cortex.

24.3

A Novel Potassium Channel cDNA Clone is Developmentally Regulated and is Expressed in Specific Cell Types in the Mouse Cerebellum. E. Chan, D. Goldman-Wohl, and N. Heintz*. Rockefeller University, New York, New York 10021.

In a screen to identify genes whose expression is either specific to Purkinje cells or whose expression is dependent upon the presence of intact Purkinje cells, a novel potassium channel cDNA was isolated. This gene (Kv3.3P?) is highly homologous to and may be an alternatively spliced form of the previously identified Shaw-related potassium channel gene Kv3.3 (Ghanshani et al, Genomics, 1992). The Kv3.3P gene is developmentally regulated, with expression beginning at P8-P10 and increasing as the animal matures to adulthood. It is clearly expressed at much higher levels in the cerebellum than in any other tissues examined thus far. *In situ* hybridization using the colorimetric detection technique demonstrates that Kv3.3P is expressed specifically in Purkinje cells and in deep nuclei in the cerebellum. Because of the developmental expression of this gene and the cell types in which it is expressed, the possibility exists that this novel potassium channel gene plays a pivotal role in connection formation in the cerebellum.

24.4

EXTRINSIC FACTORS INFLUENCE THE EXPRESSION OF K+ CURRENTS IN CHICK CILIARY GANGLION NEURONS. M.M.Dourado* and S.E.Dryer, Department of Biological Science, Program in Neuroscience, Florida State University, Tallahassee, FL 32306.

Mature neurons of the chick ciliary ganglion are known to express several calcium-dependent potassium currents ($I_{K(CA)}$) and two groups of voltage sensitive potassium currents (I_A, I_{DR}). A study of the normal development of these neurons showed that I_A and $I_{K(Ca)}$ are barely detectable at early stages, but increase dramatically between stages 30 and 35 (embryonic days 7 to 9), a period that coincides with synapse formation with target tissues. In order to determine whether extrinsic factors are required for the normal development of ionic currents, cells were removed at stage 35 (embryonic day 9) and maintained *in vitro* for 4 days. These neurons, when compared with those acutely isolated from stage 39 (embryonic day 13) embryos, expressed very low amounts of I_A and $I_{K(Ca)}$. When the neurons were cultured as ganglion explants, rather than as dissociated cells, they expressed subnormal but detectable levels of I_A, suggesting that cell-cell interaction with satellite cells plays a minor role in the expression of I_A but not $I_{K(Ca)}$. In contrast, coculture with striated muscle results in the normal expression of I_A but not $I_{K(Ca)}$. Addition of whole chick embryo extract to the culture medium also supports the expression of I_A, but not $I_{K(Ca)}$ suggesting the existence of soluble factors regulating I_A expression. However CNTF, a target-derived trophic factor that supports the survival of these neurons, has no effect on the expression of these currents *in vitro*.
Supported by NIH Grant NS-27013

24.5

MECHANISM OF AXOTOMY-INDUCED SPIKE BROADENING IN BULLFROG SYMPATHETIC NEURONES. B.S. Jassar and P.A. Smith. Dept. Pharmacol. Univ. Alberta, Edmonton, Canada, T6G 2H7.

Axotomy of peripheral and central neurones produces different changes in the shape of the action potential (a.p.) in different neuronal types (Titmus & Faber, Prog. Neurobiol., **35**:1,1991). The question therefore arises as to whether axotomy affects the same ion channels in all neuronal types and that the differential response simply reflects the different ion channels which are involved in a.p. generation in different cell types. To start to address this question, we used the whole-cell patch-clamp technique to compare Ca^{2+}, Na^+ and K^+ currents from control bullfrog sympathetic B-neurones with those recorded from neurones 14-15days after *in vivo* axotomy. Axotomy of these cells results in an increase in the width and height of the a.p. and a decrease in the amplitude and duration of the afterhyperpolarization which follows the a.p. (Gordon *et al.*, J. Physiol., **392**:213,1987). We studied whether the increase in spike width resulted from decreases in Ca^{2+} current and concomitant reduction of the voltage-dependent $G_{K,Ca}$ which repolarizes the a.p. (I_C; Adams *et al.*, J. Physiol., **330**:537,1982) or whether it resulted from slowing of I_{Na} inactivation. We have already established (Jassar & Smith, Soc. Neurosci Abs., **17**:67, 1991) that axotomy causes about a 53% reduction in Ca^{2+} current. Axotomy produced a 55% decrease in I_C, a marked (about 178%) increase in average I_{Na} amplitude (at +10mV), and *increased* the rate of I_{Na} inactivation ($P<0.05$, for most voltages). These changes in I_{Na} may explain the increased spike height seen after axotomy but cannot explain the increased spike width. The latter effect is attributable to changes in I_C. It remains to be determined whether this decrease in I_C simply reflects the decrease in I_{Ca} or whether axotomy produces additional, direct effects on I_C channels. Supported by the MRC of Canada.

24.6

ACTIVATION OF VOLTAGE- OR LIGAND-GATED CALCIUM CHANNELS INDUCES NEURITE RETRACTION. Luís E. Politi*, Evan B. Dreyer, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA.

Our laboratory previously showed that NMDA (100 μM) induced retraction of neurites in rat retinal ganglion cells (RGCs; Offermann et al., *Soc. Neurosci. Abstr.* 1991;17:927). To investigate further the ionic channels involved in this process, glutamate (GLU; 0.1-1mM) or NMDA (100 μM) in Mg^{2+}-free Hanks' solution was 'puffed' onto rat RGCs or hippocampal neurons (HNs) in culture in the presence or absence of various ion channel antagonists, and the responses were recorded by computer-enhanced video microscopy. Following application of GLU or NMDA, ~75% of RGCs (n = 59) and 100% of HNs (n = 14) retracted their neurites within 10 s to 4 min; when agonist was discontinued, the neurites regrew. In RGCs, NMDA-induced retraction was blocked significantly by the NMDA open-channel blocker MK-801 (12 μM) or by intracellular Ca^{2+} chelation using BAPTA-AM (50 μM), but not by nimodipine (1 μM). Compared to GLU or NMDA, depolarization with 55 mM KCl resulted in a slightly smaller percentage of cells retracting their neurites (56% for RGCs; 78% for HNs; n = 25). In RGCs, nimodipine (1 μM) but not ω-CgTX (10 μM) blocked the K^+-induced retraction of neurites, suggesting that L-type voltage-dependent Ca^{2+} channels can also be involved in the retraction process. The concentrations of GLU, NMDA & KCl shown to cause neurite retraction all evoked similar increases in $[Ca^{2+}]$ in the RGC body (mean 495 ± 85 nM, n = 5). Thus, Ca^{2+} entry via either NMDA-operated channels or L-type Ca^{2+} channels may be associated with neurite retraction.

24.7

PRE-SYNAPTIC CALCIUM CHANNELS SUGGEST DIFFERENCES IN EPILEPTIC MOUSE BRAIN: ω-CONOTOXIN BINDING AND SYNAPTOSOMAL DATA

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With a loss of calcium homeostasis suggested as part of the pathophysiology of epilepsy, the VSCC plays a critical role in action potential propagation and neurotransmitter release. ω-conotoxin is a 27 amino acid neuropeptide toxin believed to mark pre-synaptic transmitter release related VSCC. Vesicles derived from pre-synaptic membranes of neurons, called synaptosomes allow the entry of ^{45}Ca through VSCC when they are depolarized. Esplin et.al.,1991, have described a unique pattern of ω-conotoxin binding site development in DBA audiogenic seizure mice. Audiogenic seizure susceptibility in DBA mice is greatest at juvenile ages 16 to 30 days and then gradually dissipates with age, whereas C57 mice are resistant to audiogenic seizures at all ages. Audiogenic seizure mice represent a serviceable model of generalized epilepsy, particularly for human photosensitive and absence seizures.

Binding was done in synaptosomal membrane preparations from whole brain. The DBA show a slow gradual increase in ω-conotoxin binding from birth to postnatal day 7 when seizure susceptibility onsets and plateaus through day 16 when seizure susceptability peaks. Binding in C57 shows a rapid period of increase of 40 - 50% during postnatal days 10 - 15. This corresponds with critical period in non-epileptic mice as described by Himwich (1962) during which the central nervous system appears to be electrically activated. Synaptosomes were prepared from fresh whole brains taken from adult mice (80 days of age). Data shows at least a two-fold increase in calcium fluxes in the DBA audiogenic seizure mice as compared to the C57 mice. This data suggests pre-synaptic calcium channels in DBA mice are functionally more susceptible to depolarization and may underlie the pathophysiology of audiogenic seizures in these mice. Developmental synaptosomal studies are currently under way. Pre-synaptic calcium channel mechanisms allowing for neurotransmitter release may underlie receptor and calcium homeostatic changes described in various seizure models. (This work was supported in part by NIH grant # HD00886.) We gratefully acknowledge the late Dr. DM Woodbury's contributions.

24.8

CALCIUM CHANNELS IN NEURONS DIFFERENTIATING *IN VITRO* FROM THE NEURAL CREST ARE PREDOMINATELY DIHYDROPYRIDINE AND ω-CONOTOXIN RESISTANT. Steven G. Matsumoto.* Dept. Biol. Struct. & Funct. Oregon Health Sciences University, Portland, OR 97201.

The neural crest gives rise to all peripheral autonomic and spinal sensory neurons. These neurons possess high threshold calcium channels that display varying degrees of sensitivity to dihydropyridine (DHP) and ω-conotoxin (ω-CgTX) blockade. Sympathetic neurons display the grestest sensitivity to ω-CgTX blockade with a small component of DHP-sensitive and DHP/ω-CgTX resistant calcium current. Dorsal root ganglion cells possess a more equal distribution of DHP/ω-CgTX sensitivity and a significantly larger proportion of calcium current that is resistant to both agents (Regan et al., 1991. Neuron **6**: 269). Studies have also shown that the the pharmacology of calcium channels may change during development (eg. Gray, et al., 1992 Neuron **8**: 715).

The neurons that differentiate *in vitro* from mouse neural crest cells possess characteristics, such as neurotransmitter expression, that are a composite of many types of peripheral sensory and autonomic neurons. In the present study we have begun to examine the pharmacology of high threshold calcium currents in these neurons in an attempt to determine their state of differentiation. Virtually all of the neurons recorded from under whole cell voltage clamped conditions displayed Cd-sensitive Ba currents that were resistant to both calcium channel antagonists. The addition of NGF and/or CNTF which increases the survival rate of these neurons had no effect on their calcium channel pharmacology. Current studies are designed to examine the potential role of synaptic target interactions on the differentiation of calcium channels. (supported by NIH grant NS25644-06)

24.9

PRIMARY CULTURE FROM DISSOCIATED PUPAL NERVOUS SYSTEM OF *DROSOPHILA MELANOGASTER*. **Yi-an Sun and R. J. Wyman*.** Dep't. Biology, Yale University, New Haven, CT 06511

Here we report the first primary culture from dissociated pupal nervous system. The method was basically similar to that of Wu et al. (J. Neurosci. 3:1888-1899, 1983). Pupae were staged according to Bainbridge et al. (J. Embryol. exp. Morph. 66:57-80, 1981). After plating all neurons were round or oval with no neurite or an unbranched neurite of 5-40μ. The diameter of the cell bodies ranged from 3-12μ. A few cells were as large as 20-25μ. Just after plating there were almost no bipolar or multipolar neurons. In a typical culture plated from an individual ~50 hr. after pupation and counted 84 hours later, there were 1520 monopolar, 1180 bipolar and 2240 multipolar neurons (total cell count: 4940). After 7 days, the average length of the neurites was ~103μ (range 20-323μ). The neurites were well developed and extensively branched; most had 3d or 4th order branches. Growth cones had various morphologies: some had clear c- and p-domains. The filopodia were relatively long (10-30μ) compared to the size of the growth cones. The neurons became intricately networked, but we do not yet know if they form real synapses or are coupled electrically. In addition to neurons, the cultures included at least two different kinds of glial cells and several unidentified cells. Preliminary whole cell clamp recordings show action potentials. Some cells even show repetitive firing during current injection. Voltage clamp shows inward, outward and A-type currents. Identification of channels, synaptic function, and neurotransmitters is now ongoing. Support: NIH NS 07314; NSF BNS 85-10678.

25.1

REDUCTION OF EARLY NEURONAL DEATH IN THE CERVICAL SPINAL CORD OF THE CHICK EMBRYO. S.E. McKay*, C.J. Cardwell, and R.W. Oppenheim. Dept. Neurobiology & Anatomy, Wake Forest Univ., Bowman Gray Medical School, Winston-Salem, NC 27157.

We have examined the extent of cell death in a population of neurons in the ventral horn of the cervical spinal cord under conditions which reduce motoneuron death in other regions of the spinal cord in order to determine if survival of the cervical neurons is regulated like that of somatic motoneurons. Embryos were treated in ovo with pharmacological agents that reduce motoneuron cell death at later stages in lumbar and brachial spinal cord. The drugs were injected on embryonic days (E) 3 and 4 of incubation. Chicks were sacrificed on E4.5 (stage 24-25). The tissue was embedded in paraffin, cut into serial sections and stained with Hematoxylin. Degenerating neurons were counted in every fifth section of cervical segments 9 through 12. Preliminary results indicate that cervical neurons were protected from cell death by application of the protein synthesis inhibitor, cycloheximide as well as by curare and protein extracts from skeletal muscle, all agents known to prevent motoneuron death in other regions. Unlike motoneurons, however, the cervical neurons also appear to be sensitive to NGF. Further studies are in progress to confirm the sensitivity of these neurons to NGF as well as to identify other neurotrophic agents to which these neurons are responsive.

25.2

NOTOCHORD DEPENDENT CELL SURVIVAL IN THE VENTRAL HALF OF THE CHICK NEURAL TUBE. S. Homma* and R. W. Oppenheim, Dept. of Neurobiol. and Anatomy, Bowman Gray Sch. of Med., Winston-Salem, NC 27103

We previously reported that three pycnotic cell loci are observed in the chick embryo neural tube (NT) at stage 17-19: (1) the dorsal part of NT including the roof plate; (2) the area between prospective lateral motor column and the floor plate; and (3) the mid-portion of the floor plate.

Recently, we have begun to examine the factor(s) that control the extent and the distribution pattern of pycnotic cells in the NT. One candidate is a signal(s) from the notochord (NC). Apparently, the zone of polarizing activity (ZPA) specifies the pattern and extent of cell death in the chick embryo limb bud as well as the polarity in the anterior-posterior axis (Hinchliffe,'81). The NC is analogous to the ZPA in the chick embryo spinal cord, acting to specify polarity in the dorso-ventral axis (Yamada et al., '91). These data led us to test the hypothesis that the NC might specify the extent and distribution pattern of pycnotic cells in the NT.

We used the method described by Yamada et al., ('91) to remove the NC. The NT at the operated (NC-less) site (lumber segments, stage 20) was embedded in plastic and serial semi-thin sections were cut and stained with toluidine blue. Our preliminary results indicate that ventral NT structures appear to be absent as described by Yamada et al., ('91). In addition, embryos lacking the NC appear to exhibit considerably more pycnosis in the ventral NT compared to controls. Finally, there was also an apparent rostral-caudal gradient of pycnotic cells in the NC-less NT. In the caudal operated regions, more pycnotic cells were observed along the ventral midline, whereas at the rostral regions increased dying cells were located more dorsally.

25.3

DEVELOPMENT OF NUCLEUS AMBIGUUS (NA) AND HYPOGLOSSAL NUCLEUS (XII) IN THE RAT: A HISTOLOGICAL, DiI, AND IMMUNO-CYTOCHEMICAL STUDY D.R. Friedland, A.R. Eden*, J.T. Laitman, Depts of Cell Biol./ Anat. and Otolaryngol., Mt. Sinai Sch. of Med., NY 10029.

Our previous studies have focused on the assessment of motoneuron cell death (MCD) in NA and XII by routine histological methods. Current studies continue this approach, and add examination of nucleus formation and cellular identity using fast-DiI and immunocytochemical methods. Rats at gestational ages 15 days (E15), E16, E17, E19 and postnatal days 1 (P1) and adult were studied. MCD was assessed on paraffin embedded tissue, serially sectioned, and stained with H&E. For fast-DiI and immunocytochemical studies, rats were perfused through the umbilical vein with fix. DiI was put on vagus and hypoglossal nerves just distal to the brainstem. Standard immunocytochemical methods against ChAT and GABA were employed.

Histology shows organization of both nuclei by E16 although NA is not fully distinct until E17. Cell loss in NA between E17 and adult is approximately 48% ($P \leq 0.01$). In XII, MCD occurs between E16 and E19 (34% loss, $P \leq 0.001$) with no loss through P1 or adult. Fast-DiI studies indicate nucleus formation earlier than that seen histologically. Traces of E15 vagus nerves reveal a well defined NA with no migrating cells apparent outside the nucleus. Thus, MCD in NA may begin prior to E17 but can not be assessed by routine histological methods. DiI traces of the hypoglossal nerve show little change in XII over this time period. This appears to be due to cells of XII undergoing little, if any, migration after neurogenesis. Immuno-cytochemical studies show few GABAergic interneurons among the motoneurons in NA and XII consistent with findings in adult rats by Mugnaini and Oertel, 1985.

Precise characterization of nucleus formation, cellular identity, and MCD in NA and XII will define critical periods in the development of upper respiratory tract control, and lend insight into the myriad of postnatal neuromuscular pathologies in this region.

25.4

EXPRESSION OF CELL CYCLE GENES IN POSTMITOTIC NEURONS DURING PROGRAMMED CELL DEATH. R. S. Freeman*, P. A. Lampe, and E. M. Johnson, Jr. Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

We are interested in characterizing the molecular mechanisms that cause programmed cell death (PCD) in rat sympathetic neurons upon withdrawal of NGF. The hypothesis we are testing is that cell cycle genes may function as regulators of PCD. In several model systems, PCD is characterized by (1) a requirement for transcription and translation, (2) an initial setting of the cell cycle to a G_0/G_1-like phase, (3) ultrastructural changes reminiscent of those that accompany mitosis, (4) and increased expression of specific genes that are similarly induced during the cell cycle. We have used northern blot analysis to characterize cell cycle gene expression in neuronal cultures prepared from rat superior cervical ganglia and maintained in the presence or absence of NGF. Several members of the cyclin gene family (cyclins C, D, and E) are expressed in our cultures with cyclin D expression increasing about 3-5 fold concomitant with PCD. These cyclins are believed to function during the G_1-phase of the cell cycle as opposed to cyclins A and B that act during the S- and M-phases. We have not detected expression of the A- and B-type cyclins nor have we observed expression of the cdc2 gene. Three genes encoding extracellular-signal related protein kinases (ERKs or MAP kinases) are abundantly expressed although little or no change in expression accompanies PCD. These results suggest that certain cell cycle genes are expressed and, therefore, may function in postmitotic neurons. We are currently using *in situ* hybridization to determine exactly which cells are expressing these genes and whether the enhanced expression of cyclin D during PCD is neuron-specific. Supported by the Wash. U. ADRC(AG 5P01-A605681-08) and an NIH training grant (2-T32-NS07071-13).

25.5

CHANGES IN DNA BINDING TO TRANSCRIPTION FACTORS DURING PROGRAMMED NEURONAL CELL DEATH. S. Wang*, A. J. DiBenedetto, and R. N. Pittman. Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104

Double stranded oligonucleotides representing consensus sequences for Sp1, CRE, Oct, HSVOct, and E2F are being used in mobility gel retardation assays to determine if changes in the binding of transcription factors to these sequences correlate with early events in neuronal cell death. Two neuronal populations undergoing programmed cell death are being used. The first population consists of PC12 cells in which 90-95% of the cells become dependent on NGF for survival and undergo RNA-dependent programmed cell death following removal of NGF (Pittman et al., 1992; Soc. Neurosci. Abstr.). The second population consists of whole rat SCG in suspension culture at 37°C. In the absence of NGF, neurons in the ganglia die within 12 hrs, while addition of NGF or Actinomycin D to the medium rescues a large number of cells during the first 6 hrs. Initial observations indicate that changes in gel shift patterns occur in both neuronal populations and that NGF can delay and/or reverse these changes. This work was supported by the Univ. of PA Research Foundation.

25.6

ISOLATION OF GENES UP- OR DOWN-REGULATED IN CELLS UNDERGOING PROGRAMMED NEURONAL CELL DEATH. A.J. DiBenedetto,* S. Wang, and R.N. Pittman, Department of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

PC12 cells grown under culture conditions that render them dependent on NGF for survival undergo RNA and protein synthesis-dependent cell death upon NGF deprivation. A differential hybridization screen of a cDNA library made from PC12 cells committed to die upon removal of NGF resulted in the isolation of several clones expressed more highly in cells committed to die, and others expressed strongly in control cells but not detectable in dying cells.

Current studies include sequence analysis of newly isolated genes and homology searches for known functional motifs. The time course of differential expression of these genes following removal of NGF is being determined by Northern blot analysis. The newly isolated genes are also being examined for their expression in neurons before, during and after the period of naturally occurring cell death in embryonic rat tissues. In addition, sets of pre-selected genes are being screened for differential hybridization to cDNAs derived from control and dying cells. This work was supported by the University of PA Research Foundation.

25.7

THE APOPTOSIS INHIBITOR, AURINTRICARBOXYLIC ACID PREVENTS EXCITOTOXIN-INDUCED NEURONAL DEATH *IN VIVO*. J. M. Roberts-Lewis*, V. R. Marcy, Y. Zhao, T. Fedora, M.E. Lewis, R. Siman and J. L. Vaught. Cephalon, Inc., West Chester, PA 19380

It is well established that a precipitous or sustained elevation in intracellular calcium levels can trigger a sequence of events leading to neuronal death. Such a disruption of calcium homeostasis can be brought about by excessive excitatory amino acid (EAA) receptor stimulation. EAAs have been directly linked to ischemia-induced neuronal degeneration in animal models, and may be involved in a host of other neurodegenerative diseases or injuries as well. The lethal effect of elevated intracellular calcium is likely to be mediated by one or more intracellular calcium-dependent enzymes, such as phospholipases, protein kinases, proteases or endonucleases. Programmed cell death, or apoptosis, is characterized by the internucleosomal cleavage of DNA by a calcium-dependent endonuclease. Previous investigations, using *in vitro* systems, have demonstrated that apoptotic cell death could be prevented with the endonuclease inhibitor, aurintricarboxylic acid (ATA). To begin to explore whether there might be some commonality between apoptosis and excitotoxin-induced neuronal death *in vivo*, we administered ATA to NMDA-treated rats in order to evaluate the neuroprotective potential of ATA in this model. ATA treatment virtually abolished NMDA-induced spectrin proteolysis in the hippocampus, indicating a robust neuroprotective action of this compound against excitotoxin-mediated neuronal death *in vivo*. We are presently examining the neuroprotective activity of ATA in cerebral ischemia. Preliminary results have shown that ATA is also neuroprotective in a transient, global ischemia model.

25.8

GENES ASSOCIATED WITH NEURONAL APOPTOSIS APPEAR TO DIFFER FROM THOSE IN OTHER CELLS. S.R. D'Mello* and C. Galli. Inst. of Neurobiology, CNR, 00137 Rome, Italy.

Programmed cell death (apoptosis) occurs naturally during embryogenesis and can be induced *in vitro* in a variety of cell types. Similar morphological and biochemical changes including increased transcription of specific genes accompanies apoptosis in different cell types, suggesting a common mechanism for this mode of degeneration. Cultured rat sympathetic neurons deprived of NGF die within 48-72 hours by a process resembling apoptosis. As a first step towards understanding neuronal apoptosis, we examined in sympathetic neurons, the expression of 4 genes whose expression is increased in other biological systems. The levels of mRNA encoding sulfated glycoprotein 2 (SGP-2), RP-8, 14-Kd lectin, and ubiquitin have been reported to be dramatically induced in apoptotic prostate, thymocytes, leukocytes, and hawkmoth intersegmental muscle, respectively.Interestingly, in apoptotic sympathetic neurons SGP-2, RP-8, and ubiquitin mRNA levels are not significantly altered, whereas 14-Kd lectin mRNA is reduced 2 fold within 24 hours.

Like sympathetic neurons, NGF-differentiated PC12 cells undergo apoptosis and die in 4-5 days following NGF deprivation. In these cells, SGP-2, RP-8, and ubiquitin mRNA levels are unchanged for up to 48 hours of deprivation. However, 14-kd lectin mRNA decreases 2-3 fold in 24 hours. Taken together, the lack of induction of these genes in neurons raises the possibility that the molecular mechanisms involved in neuronal apoptosis may differ from those operating in other cell types. We are presently attempting to identify genes, if any, that are induced in neurons during apoptosis.

25.9

A DEATH-ASSOCIATED mRNA IN THE OLFACTORY SYSTEM OF THE RAT: AN IN SITU HYBRIDIZATION STUDY. T.J. Mahalik', E. W. Johnson and G.P. Owens. Dept. of Cellular and Structural Biology and Rocky Mountain Taste and Smell Center. U. of Colorado Health Sciences Center. Denver, CO. 80262.

In the vertebrate CNS, programmed neuronal death plays an important role in development, little is known about the mechanisms that underlie and control programmed neuronal death, however. To better understand these processes, we examined the expression of a **death-related** mRNA **RP8** (Owens et al.Mol. and Cell. Biol. 11:4177:91) in the rat olfactory epithelium with in situ hybridization.

RP8-labeled cells were primarily located near the basal layer of the olfactory epithelium. This pattern of labeling corresponded to the labeling pattern observed with a GAP43 probe which labels immature olfactory neurons (Verhaagen et al. J. Neurosci. 9:683:89). Virtually no RP8 labeling was observed in regions of the olfactory epithelium containing mature receptor neurons.

Given that the RP8 message is truly a cell death gene, its expression in the olfactory epithelium suggests that newly formed olfactory receptor cells normally undergo programmed cell death.

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25.10

EXPRESSION OF A DEATH-ASSOCIATED mRNA IN THE CEREBELLAR CORTEX OF WEAVER MICE. G.P. Owens, T.J. Mahalik and W.E. Hahn*Dept. Cell. & Struct. Biology, U. of Colorado School of Medicine, Denver, CO 80262

Despite the numerous examples of programmed cell death (PCD) in the developing nervous system, little is known about the molecules and mechanisms regulating this process. We have recently isolated a cDNA clone, RP-8, that is expressed in immature thymocytes programmed to die. (Owens *et al.* Mol. Cell. Biol., 11:4177, 1991). To determine if this gene is expressed in dying cells in the brain, we probed the cerebellar cortex of *weaver* mice by *in situ* hybridization. In *weavers* precursors to granule neurons fail to differentiate and die postnatally.

RP-8 positive cells were scattered throughout the inner region of the external germinal layer (EGL) where death of progenitor cells is usually observed. Expression of RP-8 largely ceased by postnatal day 17 and it was not observed in the EGL of normal (control) cerebellae. RP-8 labeling was also detected in the globus pallidus indicating that this may be another brain region where cell death is elevated in the weaver mutant. The infrequent appearance of labeled cells may reflect the rapidity of death and efficient removal of cellular debris. These data suggest RP-8 functions in PCD or is a marker of the process.

Supported by Grants NS HHS NS09199 AND NS10813.

25.11

BCL-2 INHIBITS APOPTOSIS IN MULTIPLE NEURAL CELL TYPES. L.T. Zhong, S.P. Mah, R.H. Edwards and D.E. Bredesen* CEL, UCLA, Los Angeles, CA 90024-1769.

The proto-oncogene bcl-2 (Tsujimoto et al., Science 1984;226:1097) was recently shown to inhibit apoptosis in B lymphocytes (Hockenbery et al., Nature 1990;348:334). We found that PC12 pheochromocytoma cells do not express endogenous Bcl-2 ; however, expression of Bcl-2 in PC12 cells via a retroviral expression vector led to a marked inhibition of apoptosis (Mah et al., submitted for publication). This inhibition occurred in both undifferentiated and nerve growth factor-treated PC12 cells.

We now report that the expression of Bcl-2 in two temperature-sensitive immortalized central neural lines also inhibits apoptosis. In a nigral neural line (Durand et al., Soc. Neurosci. Abs. 1990;16:40), apoptosis was inhibited at both the permissive and restrictive temperatures. Furthermore, apoptosis induced by either serum and growth factor withdrawal or by the calcium ionophore A23187 was inhibited by the expression of Bcl-2. In a cerebellar neural line (Greenberg et al., PNAS 1984:969), a more modest inhibition of A23187-induced apoptosis was observed at both permissive and restrictive temperatures; serum withdrawal did not induce apoptosis in this cerebellar neural line.

Analyzing the mechanism(s) by which Bcl-2 expression in central neural cells inhibits apoptosis may advance the understanding of certain forms of neural degeneration.

25.12

INDUCED NEURON DEATH IN A THALAMIC NUCLEUS INVOLVED WITH VOCAL LEARNING IN ZEBRA FINCHES.

F. Johnson* & S.W. Bottjer. USC, Los Angeles, CA 90089

A discrete network of forebrain nuclei underlies vocal learning and production in male zebra finches. This network includes three nuclei that form a neural pathway important for vocal learning in juveniles: Area X (X) in the avian basal ganglia projects to a thalamic nucleus (DLM), which in turn projects to a nucleus in the neostriatum (IMAN). Dramatic morphological changes occur in these nuclei over the course of vocal learning. While X increases in volume and neuron number, DLM decreases in volume but not neuron number, and IMAN decreases in both volume and neuron number. We are interested in determining whether access to afferent inputs and efferent targets is necessary for the normal development of these nuclei.

In an initial study, IMAN was electrolytically lesioned in male birds at various stages of vocal development (20, 40, 60 days of age and adult) and birds were sacrificed either 2, 4, or 6 days post-lesion. Electrolytic lesions of IMAN remove the efferent target of DLM and axotomize the distal portion of DLM axons. Although DLM does not normally lose neurons during development, IMAN lesions in 20 day-old birds result in the appearance of numerous pyknotic cells in DLM by 4 days post-lesion and the near-complete loss of DLM neurons by 6 days post-lesion. In contrast, identical lesions in adult birds have little or no effect on neuronal survival in DLM. Analysis of 40 and 60 day-old birds shows an age-related decrease in the ability of IMAN lesions to induce neuron death in DLM. These data suggest that the survival of DLM neurons may depend on access to a factor(s) supplied by their efferent target, but only during a restricted period of development that coincides with the acquisition of learned vocal behavior.

25.13

NATURALLY OCCURRING CELL DEATH DURING POST-NATAL DEVELOPMENT OF THE SUBSTANTIA NIGRA PARS COMPACTA (SNpc). E Janec and RE Burke*. Dept. of Neurology, Columbia University, NY, NY 10032.

We have previously shown in rat that a striatal axon-sparing injury, due either to hypoxia-ischemia or to the excitotoxin quinolinate, during development results in a diminished adult number of SNpc dopaminergic neurons (Neuroscience, 1992). This decrease occurs in the absence of direct injury to the SNpc. We have proposed that this effect may be due to diminished striatal target-derived trophic support for the developing SNpc, leading to an augmented developmental regressive event. If this proposal is correct, then it should be possible to demonstrate naturally occurring cell death during normal SNpc development. Therefore, we examined the post-natal development of this region using Nissl and silver staining, to identify pyknotic cells. Both stains revealed pyknotic cells in SNpc from post-natal day (PND) 2 to 20. Rare pyknotic cells were observed on PND 28, and none on PND 70. Pyknotic cells were most abundant on PND 2 (mean 9.6+1.2 [SEM] pyknotic cells per section), and rapidly decreased to 1.2+0.2 by PND 12. However, on PND 14 a second peak of cell death occurred, with a mean of 4.5+0.3 pyknotic cells. The number then gradually declined to 0.2+0.1 by PND 28. We conclude that programmed cell death does occur during post-natal development of the SNpc. The second peak of cell death may correspond to a period of synaptogenesis (PND 13-17) in the rat striatum. NS26836, PDF, UCP.

25.14

IS THERE A NEURON-KILLING FACTOR IN THE *MANDUCA SEXTA* NERVOUS SYSTEM? M. K. Choi and S. E. Fahrbach*. Department of Entomology and the Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, IL 61801

The emergence of the *Manduca sexta* moth is accompanied by the death of half of the neurons in the abdominal nervous system. In the case of the MN-12 motoneurons, this cell death is regulated both by hemolymph concentrations of a steroid hormone, 20-hydroxyecdysone, and by actions exerted by adjacent ganglia (Truman and Schwartz, 1984; Fahrbach and Truman, 1987). To characterize this signal, we have developed culture conditions in which the extent and pattern of cell death parallels that seen in intact animals. The culture condition that produced a profile of neuronal degeneration most similar to the intact control ganglia of the same age was the supplementation of Grace's insect medium with 20% heat-inactivated FBS. We have replicated, *in vitro*, the sparing effect on MN-12 of cutting the ventral nerve cord anterior to the first unfused abdominal ganglion (A3). This effect was most readily seen when the cultures were initiated between 21 and 24 h after adult ecdysis. There was a significant increase in the percentage of MN-12 motoneurons dying in A3 when the pterothoracic ganglion was co-cultured with the isolated abdominal nerve cord instead of being removed entirely after transection. We also systematically varied the length of connectives left attached to A3 in our cultures. Longer connectives were always associated with a higher proportion of dying MN-12 neurons. Heterochronic cultures have been used to study the developmental expression of the ability of the pterothoracic ganglion to kill neurons in A3. A rather broad range (Day -1 to Day +1) of the killing effect was detected. These results suggest the existence of a soluble neuron-killing factor in the pterothoracic ganglion of the *M. sexta* nervous system.

25.15

NORMAL MORPHOLOGY AND ELECTRICAL ACTIVITY DEMONSTRATED IN A MOTONEURON SPARED FROM DEVELOPMENTAL CELL DEATH IN THE MOTH, *MANDUCA SEXTA*. A.W. DeLorme[1], K.A. Klukas[1], K.A. Mesce *[1,2], S.E. Fahrbach[3]. Department of Entomology[1] and Graduate Program in Neuroscience[2], University of Minnesota, St. Paul, MN 55108 and Department of Entomology and Neuroscience Program[3], University of Illinois at Urbana-Champaign, Urbana, IL 61801.

Motoneuron 12 (MN-12), located in the abdominal ganglia of *Manduca sexta*, undergoes developmental cell death within four days of adult ecdysis. Histological studies have demonstrated that MN-12 can be spared from neuronal death by isolating the abdominal ganglia from adjacent anterior neuronal input (Fahrbach and Truman, 1987, J. Neurobiol., 18: 497-508). We wanted to determine the degree of this sparing effect by examining the electrical activity and morphology of control and spared MN-12 neurons. To accomplish this, we recorded from and dye-filled MN-12 neurons using Neurobiotin-filled electrodes. Adult male moths were ligated above the abdomen just prior to adult ecdysis to spare the MN-12 cells from death. MN-12s were recorded from intracellularly four or more days later. Control motoneurons, from adult males, were impaled before the occurrence of cell death. The morphology of Neurobiotin-filled MN-12 cells was visualized by binding the Neurobiotin with a monoclonal anti-biotin antibody conjugated to the cyanine flourophore, Cy-5. MN-12 cells were subsequently examined with a laser scanning confocal microscope equipped with a krypton/argon laser. Even though the spared MN-12s had no muscle target and experienced the normal decline in steroid levels that 'triggers' such death, both intracellular recordings and morphological data showed no discernable differences between spared and control MN-12s. This is the first demonstration that a neuron can be fully spared from developmental cell death and maintain its normal morphological and electrical properties. We are currently examining the functional role that MN-12 plays in the performance of adult behavior.

NEURONAL DEATH: TOXINS

26.1

A DECOMPOSITION PRODUCT OF THE CONTAMINANT IMPLICATED IN L-TRYPTOPHAN EOSINOPHILIA MYALGIA SYNDROME KILLS SPINAL CORD NEURONS BY STEREOSPECIFIC, INTERLEUKIN-1 DEPENDENT MECHANISMS. E.M. Sternberg*, S.W. Page, M. Schultzberg, F.S. Thomas, P. Zelazowski, R. Avidor and D.E. Brenneman Clinical Neuroendocrinology Branch, NIMH; Center for Food Safety and Applied Nutrition, FDA; National Institute of Child Health and Disease; Bethesda MD 20892 and Washington D.C.20204; Clinical Research Center, Division of Basic Science in Dementia, Karolinska Institute, Huddinge Hospital, S-141 86 Huddinge, Sweden.

The L-Tryptophan eosinophilia myalgia syndrome (L-TRP-EMS), related to ingestion of impure L-tryptophan (L-TRP), occurred in epidemic proportions in the United States in 1989. The most frequent cause of death during the acute phase of the illness was respiratory failure in the context of a degenerating axonal neuropathy. In the present study, two diastereoisomeric metabolites of 1,1[1]-ethylidenebis[L-tryptophan] (EBT), the impurity most highly associated with development of L-TRP EMS, were compared for evidence of neurotoxicity *in vitro*. In one month old spinal cord cultures derived from fetal mice, synthetic (-)-(1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (1S-β-C) produced a 30-35% loss in numbers of neurons. Toxicity was not apparent after treatment with the R-isomer of the same compound or with the parent compound, EBT. Co-treatment of cultures with 1S-β-C and neutralizing antiserum to interleukin-1 alpha (IL-1 α), or with 1S-β-C and neutralizing antiserum against the murine IL-1 receptor, prevented neuronal cell death associated with 1S-β-C. Recombinant IL-1 α also produced neuronal killing that was not additive to that observed with the 1S-β-C treatment. These studies strongly implicate 1S-β-C as one potential causative neurotoxic agent in the axonal degenerating neuropathy of L-TRP EMS and suggest that the mechanism of this effect is receptor-mediated and linked to IL-1 α.

26.2

REDUCTION OF INTRAMUSCULAR NERVE BRANCHING RESULTS IN DECREASED MOTONEURON SURVIVAL J. Tang* and L. T. Landmesser. Dept. Physiology & Neurobiology, University of Connecticut, Storrs, CT 06269.

Blockade of neuromuscular activity during the period of naturally occuring cell death increases intramuscular nerve branching, synaptogenesis, and survival of embryonic chicken motoneurons. Therefore enhanced motoneuron survival following activity blockade may result not only from increased production of trophic factor by the target, but also from enhanced ability of motoneurons to take up trophic factor via additional branches and/or synapses. Since removal of polysialic acid (PSA) by a PSA specific endoneuraminidase (Endo-N) during the period of nerve ingrowth and motoneuron cell death has been shown to reduce intramuscular nerve branching, we wished to determine if this would also affect motoneuron survival. When PSA was removed from St 30 to 35 by injecting Endo-N into the limb of chicken embryos, total motoneuron counts at both St 33 (day 8, middle of the naturally occuring cell death period) and St 36 (day 10, close to the end of the cell death period) were significantly decreased compared to controls. Therefore a reduction of intramuscular nerve branching and possibly in synapse formation as well (currently under investigation) affects motoneuron survival. Further, since removal of PSA does not appreciably alter spontaneous neuromuscular activity, these experiments avoid the complication of activity blockade which may alter motoneuron survival by affecting events other than / or in addition to intramuscular nerve branching.

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26.3

EFFECT OF CURARE ON NATURALLY OCCURRING CELL DEATH OF MUSCLE SENSORY NEURONS. P. Wenner*K. Sharma, F. Hory-Lee, and E. Frank, Dept. of Neurobiology, Anatomy & Cell Science, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Motoneurons that naturally die during embryonic development can be spared by chronic applications of d-tubocurarine (dTC) in developing chick embryos (Pittman & Oppenheim, '78). In contrast, the total number of sensory neurons is not changed by this treatment, as determined by counts of sensory axons in dorsal roots (Oppenheim & Chu-Wang, '83). It is not known, however, whether specific subpopulations of sensory neurons, such as those innervating skin or muscle, respond differently to the application of this drug. We have examined this possibility by labeling fibers in a muscle nerve in the hindlimb of developing chick embryos in control and dTC-treated embryos.

Curare was applied daily to the chorioallantoic membrane during the period of naturally occurring sensory neuron cell death between E5 and E11. The spinal cord, with attached peripheral nerves, was isolated and the obturator nerve was labeled with HRP. Counts of labeled neurons were made in 10 μm paraffin sections.

Preliminary results show that muscle sensory neurons projecting in the obturator nerve are reduced in number in dTC-treated embryos. Because the total number of sensory afferents is not changed in such embryos (Oppenheim & Chu-Wang, '83), these results suggest that the survival of different groups of sensory neurons may be differentially affected by curare.

Supported by NSF to EF.

26.4

DOES DOXORUBICIN INDUCED MUSCLE INJURY RESULT IN LOSS OF FACIAL MOTOR NEURONS? L.K. McLoon*, S. Cameron and J.D. Wirtschafter, Dept. of Ophthal., Univ. of MN, Mpls, MN 55455.

We have been developing a novel permanent non-surgical treatment for blepharospasm, hemifacial spasm and related facial dystonias. Doxorubicin injected directly into the eyelid can result in a permanent muscle loss of up to 90% of the orbicularis oculi muscle (McLoon et al.,'91). A first clinical trial has resulted in its successful use in human patients, where 10 out of 20 treated with this protocol had long term relief of their muscle spasms (Wirtschafter,'91). Doxorubicin can be retrogradely transported from an injection site to the brain. We examined the effect of injection of 2 mg doxorubicin in the eyelids of rabbits on the number of facial motor neurons. Five New Zealand white rabbits were injected with 2 mg doxorubicin into their right lower eyelids. One month later, both eyelids were injected with 400 ul horseradish peroxidase (30% in 2% DMSO in isotonic saline). Two days later, the rabbits were deeply anesthetized and perfused with 2% glutaraldehyde in phosphate buffer. The brains were removed, sectioned and processed for visualization of HRP using the metal intensified DAB technique. The number of HRP labeled facial motor neurons in both facial motor nuclei were quantified. Despite extensive and permanent muscle loss, there was no significant difference between the numbers of facial motor neurons on the treated and untreated sides. Thus, this dose of doxorubicin injection into the eyelids does not result in a significant loss of facial motor neurons.

Supported by NIH 07935 to LKM and JDW.

26.5

NMDA-RECEPTOR BLOCKADE RESULTS IN INCREASED SURVIVAL OF DEVELOPING CHICK SPINAL MOTONEURONS. F. Hory-Lee and E. Frank*, Dept. of Neurobiology, Anatomy & Cell Sci., Univ. of Pittsburgh Medical School, Pittsburgh, PA 15261.

Developing spinal motoneurons are dependent on their peripheral targets for survival. Neuromuscular blockade by d-tubocurarine (dTC) during the period of normally occurring cell death results in increased survival of motoneurons (Pittman and Oppenheim, '78). Developing motoneurons are spontaneously active during this period however, and this activity is also blocked by dTC (Landmesser & Szente, '86). Because high levels of neural activity can lead to cell death in the mature nervous system, we wondered if the blockade of spontaneous activity by dTC might be responsible for the drug's effects on motoneuronal survival. We are therefore examining the effects of reduced electrical activity on cell death, both in vitro and in ovo, by blocking receptors that mediate excitatory transmission in the CNS.

Established cultures of dissociated spinal neurons from E6 chick embryos were transferred to media without muscle extract, and with or without APV, a competitive antagonist of the NMDA receptor. Such cultures normally display high levels of spontaneous activity which is decreased by APV (O'Brien and Fischbach, 1986). Motoneurons were prelabeled on E5 by injecting fluorescent latex beads into limb buds in ovo. Surviving retrogradely labeled motoneurons were counted 2-3 days after the transfer. Preliminary results indicate that motoneuronal survival in cultures containing APV is higher than in cultures where the drug is absent. To test the effect of APV on naturally occurring cell death in ovo, we applied the drug daily to the chorioallantoic membrane of embryos from E5 to E11, as in the earlier experiments with dTC. Surviving motoneurons within the LMC of segments L3&4 were counted in transverse sections. APV results in a large increase in the number of surviving motoneurons, comparable to the rescue seen with dTC. These results suggest that at least some of the effects of dTC on motoneuronal survival may result from its central effects on neural activity.

Supported by NSF to EF.

26.6

POLY (ADP-RIBOSE) POLYMERASE: AN EARLY MARKER OF GLUTAMATE MEDIATED NEURONAL DEATH. C. Cosi*, H. Suzuki[1], L. Facci, D. Milani, and S.D. Skaper. Fidia Research Labs, Abano Terme, Italy and [1] Istituto di Chimica Biologica, Università di Verona, Verona, Italy.

Glutamate neurotoxicity (GNT) is correlated with an increase of cytosolic free Ca^{++}. Activation of Ca^{++}-dependent endonucleases can damage DNA and activate the enzyme poly (ADP-ribose)polymerase (ADPRP). We have investigated if ADPRP may be involved in GNT. Cerebellar granule cells, 12 days in culture, were treated with a toxic dose (100uM) of glutamate (Glu) and inhibitors of ADPRP (3-aminobenzamide, benzamide, luminol, 5mM) or endonucleases (aurintricarboxylic acid). Inhibitors added to the culture 60 min before and during the 30 min of Glu treatment prevented neuronal death by 60-100% as tested 24hr after Glu, without affecting Glu-induced Ca^{2+} influx. Using polyclonal antibodies against the polymer of ADPribose as an index of ADPRP enzyme activity the number of immunopositive cells progressively increased, reaching a maximum 30 min after Glu addition. Immunostaining was heterogeneous, decreasing to controls levels 60 min post Glu. Non-toxic levels of Glu were ineffective. Activation of the above cascade may represent an early index of GNT.

26.7

THE EFFECT OF [K+] DURING CULTURE ON THE APPEARANCE OF SPONTANEOUS $[Ca^{2+}]_i$ OSCILLATIONS IN RAT CEREBELLAR GRANULE CELLS. A.J. Irving, J.G. Schofield and G.L. Collingridge*. $Dept of Pharmacology Univ of Birmingham and *Dept of Biochemistry Univ of Bristol, UK.

The induction of epileptiform activity associated with spontaneous $[Ca^{2+}]_i$ oscillations can result in hippocampal cell death (Abele & Miller 1990, Neurosci.Lett. 115,195-200). We have investigated the appearance of $[Ca^{2+}]_i$ oscillations in cerebellar granule cells cultured under conditions that favour either cell survival (25 mM K+) or cell degeneration (5.4 mM K+). The $[Ca^{2+}]_i$ was measured in individual cell bodies using Fura-2 microfluorimetry.

When cells were cultured for beyond 5 days in 5.4 mM K+ large fluctuations in $[Ca^{2+}]_i$ (200-1500 nM) were observed on superfusion with Mg^{2+}-free, glycine supplemented media. However, similar activity was not observed in cells cultured for up to 7 days in 25 mM K+. The $[Ca^{2+}]_i$ oscillations were blocked with 1 μM TTX and were highly synchronised, indicating that they are synaptically generated. The fluctuations were also blocked by nitrendipine and AP5, but not CNQX (in 10 μM glycine), indicating the involvement of voltage-gated Ca^{2+} channels driven by NMDA receptor activation. These $[Ca^{2+}]_i$ oscillations were not mimicked by agents that mobilise intracellular Ca^{2+} stores. In conclusion the tendency of neurons cultured in 5.4 mM K+ to display epileptiform activity may underlie their enhanced cell death during culture.

26.8

LACK OF A PERSISTENT EPILEPTIC DEPOLARIZATION FOLLOWING LOW MAGNESIUM INDUCED SEIZURES RECORDED FROM THE GERBIL HIPPOCAMPUS IN VITRO. James D. Stittsworth, Jr.* and Thomas H. Lanthorn Neurological Diseases Research, Searle, Skokie, IL 60077.

It has been shown that ischemia and ischemia-like events will lead to a massive depolarization known as "anoxic depolarization" (AD). A similar event has been shown to occur with some types of severe seizure and has been termed "epileptic depolarization" (ED). It has further been suggested that AD and ED are preludes to neuronal death. The gerbil is widely used to study both ischemia and epilepsy. A population spike (mean amplitude = 7.4 ± 1.0 mV) was recorded from CA1 region of the gerbil dorsal hippocampus, in vitro, in response to stimulation of the Schaeffer collateral/CA3 region. Following a period of equilibration in the recording chamber a low magnesium aCSF was introduced. Both evoked and spontaneous epileptiform activity was observed in the gerbil slices. However, differences were observed between the gerbil and rat hippocampus when the glucose concentration of the aCSF was lowered to 2 mM. In the rat hippocampal slice, a spontaneous ED (14.4 ±1.9 mV) occurred within 4.9 ± 1.9 min., however a spontaneous ED was never observed in the gerbil hippocampal slice under the same conditions. In 1 of 6 gerbil slices studied an ED (12 mV) was produced following a high frequency stimulation with a concomitant loss of synaptic responses. Recovery from the depolarization in the gerbil slice was seen in 2 min and 75 % recovery of synaptic response was observed at 20 min post-ED. These results suggest that gerbil hippocampal slice is resistant to ED induced by low magnesium seizures.

26.9

CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE RAT HIPPOCAMPAL FORMATION AFTER CHRONIC ALCOHOL CONSUMPTION. A.Cadete-Leite, M.M.Paula-Barbosa*, A.Ribeiro-da-Silva** and N. Sousa. Dept. of Anatomy, Porto Medical School, Porto 4200, Portugal; ** Dept. of Pharmacology and Therapeutics, McGill University, Montreal, Canada.

Choline acetyltransferase (ChAT) activity is reduced in the aged brain and changes in cholinergic mechanisms are known to play a role in cognitive functions such as memory. As we demonstrated that the hippocampal formation (HF) is affected after chronic alcohol consumption (CAC) with correlated memory disfunctions similar to those observed in advanced age, we designed this study to evaluate whether ChAT activity in the HF was affected by CAC. Fibers and neurons were analysed in alcohol-fed rats and controls. Immunohistochemistry was performed with a monoclonal antibody against ChAT. We found a global reduction in the immunostaining of the fiber plexus, which was more evident in the supragranular zone and in the outer portion of molecular layer of the dentate gyrus. The areal density of ChAT immunoreactive (ChAT-IR) neurons was reduced too. Differences were particularly striking in the stratum lacunosum moleculare of regio inferior, the zone of the HF where ChAT-IR neurons are more abundant. We concluded that CAC leads to an impoverishment of cholinergic fiber plexus as well as to marked death of the acetylcholine secreting neurons. These findings might be of importance to explain the cognitive disfunctions observed after CAC.
(Supported by JNICT - Project PMCT/C/SAU/32/90).

26.10

INVOLVEMENT OF URIC ACID IN *IN VIVO* DOPAMINERGIC NEURODEGENERATION. W.H. Church[1*], V. Ward[1], and J.F.McGinty[2], Depts. of Chemistry[1] and Anatomy and Cell Biology, School of Medicine[2], East Carolina University, Greenville, NC 27858-4353

The effect of endogenous levels of uric acid on $FeCl_3$-induced dopaminergic neurodegeneration in male guinea pigs was investigated. Uric acid is an antioxidant present in brain which is a potent chelator of ferric ion ($K_s = 1.1x10^{11}$) and can inhibit Fe^{+3}-catalyzed DA autoxidation. Iron has been shown to increase the generation of free hydroxyl radicals and the rate of lipid peroxidation, events which lead to increased cell death. Post-mortem analysis of brain tissue from Parkinsonian patients has revealed an increase in iron content in the substantia nigra. Injection of $FeCl_3$ into the substantia nigra of rats can produce behavioral and biochemical parkinsonian-like effects. The iron chelator desferrioxamin, when injected into rats can retard 6-hydroxydopamine deneneration of nigrostriatal dopamine cells. Twelve male guinea pigs were divided into two groups; a) six animals with normal brain levels of uric acid and b) six animals with reduced brain levels of uric acid. Uric acid levels were reduced by injecting the animal with the xanthine oxidase inhibitor allopurinol. Both groups were then given injections of $FeCl_3$ (0.5μL of a 100 mM solution) into the substantia nigra. The animals were sacrificed three weeks later and tissue collected from the substantia nigra, striatum, and frontal cortex. Tissue samples (approx. 1 mg) were analyzed for uric acid, ascorbic acid, and dopamine by HPLC with electrochemical detection. Dopamine levels were used in conjunction with neuroanatomical staining methods to determine the extent of neurodegeneration. The results are discussed in relation to uric acid's role in dopamine neurodegeneration processes.

26.11

MULTIPLE EFFECTS OF MPP^+, A POSSIBLE INDUCER OF PARKINSON'S DISEASE, ON PC12 CELLS. Y. Nomura*, Y. Kitamura and Y. Itano. Dept. of Pharmacology, Fac. Pharmaceutical Sci., Hokkaido Univ., Sapporo 060, Japan.

To clarify cytotoxic effects of MPP^+ (1-methyl-4-phenylpyridinium ion, a proposed toxin to Parkinsonism) on PC12 cell, a model of dopamine (DA) neuron, we examined effects of MPP^+ on 1) the content of DA and its metabolites, 2) induction of tyrosine hydroxylase (TH) mRNA and 3) leakage of lactate dehydrogenase (LDH). MPP^+ (100 μM) treatment for 24 hr increased DA but decreased 3,4-dihydroxyphenylacetic acid, suggesting an inhibition of MPP^+ (100 μM) on monoamine oxidase (MAO). A result that MPP^+ (1 mM) decreased DA and protein content indicates that MPP^+ causes cell death at a high concentration. In contrast, Northern blot analysis showed that MPP^+ (100 μM) increased TH mRNA expression. Thus MPP^+(100 μM)-induced DA elevation is provably due to TH induction as well as MAO inhibition. Treatment with MPP^+ at 1 mM for 24 hr caused a significant leakage of LDH. The leakage was suppressed by NGF (100 ng/ml), dibutyryl cAMP (1 mM), cycloheximide (0.3 μM) or aurintricarboxylic acid (100 μM, a DNase inhibitor). It is suggested that MPP^+ does not exert cytotoxic effects when the cells are differentiated by NGF or cAMP and that unknown proteins involved in "programmed" neuronal death could be induced by MPP^+ stimulation in PC12 cells.

26.12

EXTRACELLULAR ATP INDUCED CYTOTOXICITY IN PC-12 CELLS. A.Y. Sun*, Y. Cheng, P. Wixom, H.D. Kim and G.Y. Sun. Depts. of Pharmacology and Biochemistry, Univ. of Missouri, Columbia, MO 65212

Extracellular ATP has been implicated in the regulation of norepinephrine (NE) uptake (Hardwick *et. al.*, J. Neurochem. 53: 1512, 1989) and voltage-dependent calcium influx (VDCI). Using PC-12 cells as a model of catecholaminergic neurons, we have examined the effect of heavy metal ions on the viability of these cells. We have found that Mn^{2+} and Fe^{2+} ions in micromolar concentrations induced lipid peroxidation and cell death, as measured by the amount of thiobarbituric acid reactive substance (TBARS) formed and the amount of lactic acid dehydrogenase (LDH) released to the medium, respectively. When 1 mM ATP was added to the culture medium, a 2.5-fold increase in cell lysis was observed, as indicated by LDH released, as compared with controls containing Fe^{2+} alone. By using fura 2 as a calcium probe, we also observed the increase in $[Ca^{2+}]_i$ upon addition of ATP. Whether ATP induced cell viability is due to the alteration of Ca^{2+} homeostasis or through other mechanisms remains to be seen. Nevertheless, our finding may have important implications for the effect of stimulated release of ATP on neurodegenerative processes. (Supported in part by AA02054 from NIAAA)

NEURONAL DEATH: AXOTOMY

27.1

BOTH SELEGILINE AND PARGYLINE REDUCE THE DEATH OF IMMATURE MOTONEURONS CAUSED BY AXOTOMY W.G Tatton, P.T. Salo*, P.H. Yu, D.P Holland, M.M. Kwan and K.S. Ansari Center for Research in Neurodegenerative Diseases, Univ. of Toronto, Toronto, Ontario and Neuropsychiatric Res. Unit, Univ. of Saskatchewan, Saskatoon, Saskatchewan. Selegiline reduces the death of murine dopaminergic substantia nigra neurons (dSNns) after MPTP exposure, even if selegiline administration is delayed until MPTP conversion to MPP+ is completed (J. Neurosc. Res. 30:666,1991) and also increases the survival of rat facial motoneurons (FMns) axotomized at age 14 days (J. Neurosc. Res. 31:394,1992). We counted ChAT immunopositive and Nissl stained FMn somata 21 days after a unilateral facial nerve transection carried out at 14 days of age. During the 21 days either saline, selegiline (10 or 0.01 mg/kg) or pargyline (10 mg/kg) were administered intraperitoneally every second day.

	Saline	Sel. 10 mg/kg	Sel. .01 mg/kg	Parg. 10 mg/kg
%Ipsilat. FMns (axotomized)	26.5+/-5.7	50.9+/-6.5 (p=.0009)*	54.0+/-5.8 (p=.0014)*	43.2+/-4.7 (p=.0015)*
%Contra.FMns (contralateral)	84.6+/-9.7	94.5+/-13.0 (p=.1530)*	100.4+/-8.1 (p=.0131)*	81.4 +/-8.8 (p=.5570)*

[% of unlesioned, saline treated controls, *U test comparisons to saline groups]
Both selegiline and pargyline can rescue the motoneurons, apparently by partially compensating for the axotomy-induced loss of target-derived trophic support in the immature motoneurons. Selegiline rescues MPTP-damaged murine dSNns at 0.01mg/kg, which is insufficient to inhibit whole brain MAO-B or MAO-A (Soc. Neurosc. Abstr.17:757, 1991) in accord with the 0.5 mg/kg ED50 for adult rodents. The 14 day old rats showed increased sensitivity to selegiline since there was inhibition of both MAO-B and MAO-A activity for the 0.01 mg/kg dose, i.e brainstem MAO-A and MAO-B activity were 74.3+/-21.4% and 19.9+/-5.5% respectively at 4 hours. Hence it is uncertain whether the FMn rescue can occur independently of MAO inhibition similar to the rescue of murine dSNns.

27.2

LEUKEMIA INHIBITORY FACTOR (LIF) PREVENTS THE DEATH OF AXOTOMIZED SENSORY NEURONS IN NEWBORN RAT DORSAL ROOT GANGLION (DRG) IN VIVO. S.S. Cheema*, L. Richards, M.Murphy and P.F.Bartlett. The Walter & Eliza Hall Institute, Parkville, Victoria. Australia 3052.

LIF acts as a survival factor *in vitro* for neonatal sensory neurons and is retrogradely transported *in vivo* into murine DRG (Murphy et al.,1991 P.N.A.S. 88:3498; Hendry et al., 1992 J.Neurosci. in press). To test if LIF is a neuronal survival factor *in vivo*, we examined its action on axotomised sensory neurons in the DRG of the newborn rat pup.

Newborn (P0) Wistar rat pups were anesthetised on ice and the left sciatic nerve exposed and cut .The sciatic nerve received a single dose of either the diluent or recombinant murine LIF . LIF was soaked in gelfoam (20 μg) or injected directly (5μg) into the sciatic nerve a few minutes prior to the axotomy. After 1-36 days the animals were perfused with buffered paraformaldyde. The L5 DRGs were dissected out, embedded in paraffin, cut at 8μm sections and stained in 0.1% cresyl violet. Neurons were counted in every 10th section and the T-test was used to determine statistical differences between groups.

In the control animals, 5% of the ipsilateral L5 DRG neurons were lost after 12 h and between 2-7 days the reduction was about 50% and up to 80% after 36 days. At 2 days, a single exposure to LIF resulted in a statisically significant reduction in the death of axotomised sensory neurons. In these animals, only 17% of the sensory neurons were lost compared to 50% in the controls. These experiments suggest that LIF, a survival factor for sensory DRG neurons *in vitro*, can act as a survival factor for the same population *in vivo*.

27.3

DISSOCIATION OF THE RESPONSES OF MOTONEURONS AND THEIR TARGET MUSCLES TO DEVELOPMENTAL AXOTOMY IN THE HORMONE-SENSITIVE SNB. J.L. Lubischer* & A.P. Arnold, UCLA Brain Research Inst. and Dept. Psychol., Los Angeles, CA 90024-1563

Neonatal axotomy results in motoneuron death and permanent muscle atrophy, in contrast to the virtually complete recovery seen after adult axotomy. Axotomy-induced cell death in neonates might be due to a greater dependence on muscle-derived trophic support at this age. In this view, loss of contact with the muscle results in motoneuron death, with muscle atrophy secondary. A second possibility is that the primary effect of axotomy in development is a severe denervation-induced muscle atrophy, reducing the trophic factor available to motoneurons and resulting in their death. We now report evidence arguing against the latter hypothesis.

Spinal nucleus of the bulbocavernosus (SNB) motoneurons innervate the levator ani (LA) and bulbocavernosus (BC) muscles through the pudendal nerve. This nerve was cut unilaterally in male rats at one of five ages from postnatal day 1 (P1) to P28. After perfusion at about P55, the spinal cord and LA/BC muscle complex were removed. SNB nucleoli were counted (reported previously) and LA/BC muscle complexes from axotomized and contralateral sides were weighed and compared.

The massive SNB cell death that follows axotomy at P1 or P7 is matched by severe LA/BC atrophy (80% at P1; 83% at P7). The lack of significant cell death after axotomy at P21 or P28 is accompanied by moderate muscle atrophy (26% at P21; 31% at P28). Interestingly, compared to these older ages, axotomy at P14 results in the same degree of muscle atrophy (37%), but extensive cell death. Thus, the extent of neonatal axotomy-induced motoneuron death is not determined by target muscle atrophy. P14-axotomized muscles are innervated by half as many motoneurons as are comparably-sized P21-axotomized muscles, suggesting an interesting alteration in the pattern of innervation after P14 axotomy. If fiber counts show equal numbers of fibers in these muscles, then SNB cells axotomized at P14 might have expanded motor units and/or less overlap between motor units.

Supported by NIH grant HD 15021 and an NSF Graduate Fellowship.

27.4

TRANSIENT APPEARANCE OF GRANULAR CELLS IN SPINAL GANGLIA AFTER NEONATAL AXOTOMY IN RATS. M. DeSantis and M. Dibble. Dept. of Biological Sciences and WAMI Medical Program. University of Idaho, Moscow, ID 83843, USA.

Cell bodies of neurons in spinal ganglia (SG) degenerate in massive number if their axons are cut in neonatal as opposed to adult mammals (e.g. Exp. Neurol. 1981. 74:597-604; 1983 82:568-580). Aldehyde fixed SG from the lesioned and unoperated sides of neonatal rats were incubated in osmium tetroxide. Cells with granules in them were apparent in aqueous mounts of frozen sections (30 um) of the SG from the experimental, but not the control, side. There were very few granular cells in SG at 3 or 11 days after a similar sciatic nerve resection in young adult rats. Our hypothesis is that the granular cells reflect the presence of dying neurons. In order to study the time course of their appearance, we counted the total number of granular cells in serial sections of the L4-6 SG between 8 and 288 hours after a unilateral resection of the sciatic nerve done on postnatal day 3. Values were compared to those for the contralateral side where the sciatic nerve had been either unoperated or sham operated. The number of granular cells in ganglia from the lesioned side exceeded that from the control side for all times from 20 to 260 hours after the lesion. They occurred in greatest numbers from 30 to 150 hours after resection. The number of granular cells in SG from the lesioned side fluctuated in a way which suggested that axotomy-induced neuronal death in SG may be episodic. (Supported by USPHS grant NS 27253).

NEURONAL DEATH: RETINA

28.1

EFFECTS OF OPTIC NERVE CUT OR CRUSH ON THE SHORT AND LONG-TERM SURVIVAL OF RAT RETINAL GANGLION CELLS. M. Berkelaar, A. Cohen, G.M. Bray*, A.J. Aquayo.
McGill University Centre for Research in Neuroscience, Montreal General Hospital Research Institute, 1650 Cedar Avenue, Montreal, Quebec, H3G 1A4.

To investigate further the conditions that influence the loss of axotomized retinal ganglion cells (RGCs) in mammals, we determined the density of RGCs at several times after an intracranial optic nerve (ON) cut or crush at one single distal level situated 8-10 mm from the posterior pole of the eye of adult rats. The RGCs were identified by retrograde labelling with Fluorogold(R) applied to the superior colliculus one week prior to ON injury.

An early and marked loss of RGCs was observed 2 weeks after both types of ON injury. This early loss was more than twice as severe following ON cut than ON crush: 42% and 18%, respectively. Although less pronounced, the rates of RGC loss observed between 2 weeks and 3 months after axotomy were persistently greater after ON cut than crush.

Thus, while the type of ON injury (cut or crush) affects neuronal survival soon after axotomy, these two lesions also have different effects on the long-term viability of RGCs. It is possible that the alterations in cut ONs, which differ from those in crushed ONs (Berkelaar et al., Soc.Neurosci.Abstr. 17:555,1991), affect RGC survival by mechanisms that may include a disruption of the local supply of trophic factors or the production of molecules that induce cell death.

28.2

Accelerated neuronal death and differentiation in the developing Quail retina S.W. Wang and L.E. Johnson *,Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, N.C. 27157-1010

The Japanese quail, *Coturnix coturnix japonica*, has a greatly accelerated ontogeny when compared with the domestic chick *Gallus domesticus* (ie. hatching occurs at approximately 16 days of incubation as compared with 21 days in the chick). The aim of this study was to determine the rate of developmental changes in neuronal death and differentiation in the quail retina.

Retinas were sectioned or whole-mounted for basophilic staining and the total surface area and volume of retinas were measured on embryonic days 4 through 15 (E4-E15). The number of mitotic figures, the number of pyknotic nuclei, the thickness of each retinal layer and the density of cells in the retinal ganglion cell (RGC) layer were also measured. In addition, RGC soma and dendritic arbors were retrogradely labeled from the optic nerve with the flourescent dye DiI.

Retinal development in the quail closely paralleled development in the chick during the first six days of incubation. The retinal ganglion cell layer was segregated from the more compact ventricular layer on E7. The accumulation of retinal ganglion cells rapidly accelerated reaching a peak at E-9 in the central retina with a very rapid subsequent loss of cells. At least three types of RGC could be distinguished by soma and dendritic properties. These results suggest that both the normal neuronal death and differentiation of RGC is rapidly accelerated in quail retinal development.

28.3

RETINAL GANGLION CELL DEGENERATION AFTER OPTIC NERVE LESION AND NERVE GROWTH FACTOR PROTECTIVE EFFECT IN THE DEVELOPING RAT.
S.A. Rabacchi*, M. Ensini, A. Gravina, M. Fagiolini, L. Bonfanti and L. Maffei.
Ist. di Neurofisiologia del CNR and Scuola Normale Superiore, Pisa (ITALY).

Section of the optic nerve in mammals induces degeneration of most ganglion cells both in the adult and developing retina. In neonatal rats ganglion cell death has been reported to be completed 2-3 days after the lesion (Perry et al, 1983). Our experimental model consists in performing the lesion *in vivo* both prenatally *in utero* (E18 approximately) and postnatally (P0), and analyse morphologically whole-mounted retinas stained with cresyl violet.

Our results show that, when the optic nerve is transected at P0, the first signs of degeneration (increase of pyknotic nuclei) appear 12-14 hours postoperatively, with a peak at 24 hours and a decline after 48 hours; no pyknotic nuclei were seen after 72 hours, because of macrophage endocytotic activity. Degeneration occurs much earlier when the optic nerve is transected prenatally (E16-19).

Previous studies have shown that Nerve Growth Factor (NGF) promotes the survival of retinal ganglion cells after optic nerve transection in the adult rat (Carmignoto et al, 1989). In this study, we confirmed that NGF exerts similar protective effects in neonatal rats. When the optic nerve is sectioned at P0 and the animals are left to survive for 24 hours, we found that intraocular injections of NGF (0.5ug in 250 nl) cause a reduction in pyknotic nuclei density: 199/mm^2 in NGF treated animals (30 animals) versus 236/mm^2 in the control (20 animals) group ($p<0.001$). This shows that in neonatal rats NGF exerts protective effects on retinal ganglion cells within a remarkably short time after the axotomy.

28.4

AURITRICARBOXYLIC ACID PROTECTS RETINAL NEURONS FROM INJURY-INDUCED DEGENERATION.
L. Colombaioni*. Istituto di Neurofisiologia del CNR, 56127 Pisa, Italy.

The purpose of this study has been to investigate the cellular events underlying the process of neuronal degeneration following injury. Several morphological modifications, including a widespread chromatin condensation, characterise neuronal death. To test the hypothesis that the activation of endonucleases (enzymes capable of producing DNA fragmentation) can initiate the process of neuronal degeneration, the protective effect of a general inhibitor of endonucleases, the aurintricarboxylic acid (ATA), was examined. Rat retinal neurons during the first postnatal days have been chosen as model system. The experiments were carried out *in vitro* on isolated eye cups cultured for periods variable from 4 hours to 2 days in serum-free medium. In this experimental condition the ganglion cells were axotomized, in fact, in control experiments, they showed the characteristic pattern of DNA fragmentation associated with cellular degeneration. When ATA was added to culture medium, at concentrations between 10-500 µM, the nuclear alterations were clearly reduced and an improvement of cell viability, assessed by trypan blue exclusion, was observed. The effect was dose-dependent. In retinal radial sections it was possible to observe that the increase in neuronal survival was a general phenomenon also extended to retinal neurons other than ganglion cells. It is therefore possible to speculate that, in nervous tissue, the activation of endonucleases plays a critical role in triggering cell degeneration process even if the possibility that ATA prevents cell death by other mechanisms cannot be excluded.

29.1

CHANGES IN CAUDATE NUCLEUS MORPHOLOGY AFTER NEOCORTICAL LESIONS IN CATS. L.D.Loopuijt*, J.R.Villablanca, D.A.Hovda and R.Scola, Ment. Retard. Res. Ctr., UCLA, Los Angeles, CA 90024.

After a small unilateral cortex lesion in fetal kittens both caudate nuclei increase in size (Soc. Neurosci. Abstr.16:983,90). To study if this effect occurs postnatally, 8-13 day old kittens received a small left frontal cortex lesion (NF, N=4) or hemidecortication (NH, N=7) while 4 adult cats also were hemidecorticated (AH). After longterm survival (>371 days), thionin-stained coronal sections of these brains (plus 7 intact controls, C) were used to stereologically measure caudate nuclei volumes and neuron/glial cell packing density.

The mean caudate n. volumes (±SD) at either side of the brain in NF and NH cats did not differ from controls (left: C 199.0 ± 35.0; NF 215.0 ± 41.7; NH 197.8 ± 21.0; right: C 199.3 ± 33.9; NF 206.3 ± 26.3; NH 186.0 ± 12.8 mm^3). However, in AH the caudate of the lesioned side was smaller (155.5 ± 18.5 mm^3, P< 0.05) with no difference contralaterally, (188.8 ± 19.4 mm^3). The neuron packing density on the lesioned side did not differ from controls (C 7.00 ± 3.3;NH 8.1 ± 3.1; AH 7.70 ± 2.7 cells/47300 μm^3; N=2) whereas the glial cell packing density tended to increase in AH (C 15.2 ± 5.5; AH 21.8 ± 5.2 cells/47300 μm^3, N=2) but did not show a consistent trend in NH. These data suggest that in AH there is a loss of neurons with an increase in glia in the caudate n. ipsilateral to hemidecortication which suggests transneuronal degeneration. Unknown events responsible for caudate hypertrophy in prenatal-lesioned may have contributed to sparing in neonatal-lesioned cats. Grants USPHS HD-05958 and 04612.

29.2

IBOTENIC ACID DESTRUCTION OF CAUDATE NUCLEUS AND LATERAL GLOBUS PALLIDUS ARE NECESSARY FOR SUBSEQUENT TRANSNEURONAL DEGENERATION IN SUBSTANTIA NIGRA RETICULATA. M. Saji*, M. Cohen, B.T. Volpe, Dept. of Neuro. and Neurosci., Cornell Univ. Med. School, at The Burke Institute for Medical Research, White Plains, NY 10605.

In rodents, neurotoxin lesions (ibotenic acid, IBO, or kainic acid) that destroy the corpus striatum initiate a mechanism, perhaps disinhibition, that leads to death of GABAergic neurons in the substantia nigra reticulata (SNr). In a test of the necessary striatal injury to cause SNr neuron loss, animals were injected unilaterally with IBO (10 $\mu g/\mu l$) in the caudate nucleus (CN, .5 μg, N=4) or in the lateral globus pallidus (LGP, .3 μg, N=4) or in the CN and LGP (.8 μg, N=4). Animals were sacrificed 3 weeks later. Histologic and immunocytochemical analysis of glial fibrillary acidic protein (GFAP) demonstrated that IBO lesions in the CN and LGP reproducibly caused neuron loss and gliosis marked by increased GFAP positive astrocytes in the ipsilateral SNr. The area of neuron loss in the SNr was topographically related to the location of the injury in the CN and LGP. Animals with IBO lesions in the CN or LGP alone had normal SNr. There was no SNr damage contralateral to the IBO injection. These data suggest that injury in the CN and LGP are necessary to cause delayed SNr degeneration. The predictable topography of the SNr neuron loss supports a transneuronal mechanism, perhaps unopposed excitotoxic stimulation or disinhibition or both.

29.3

A NEW TECHNIQUE FOR DEMONSTRATION OF LOCAL AND DISTANT CELLULAR DAMAGES AFTER AMYGDALOID KAINATE LESIONS IN THE RAT. A. Hajnal, A. Czurkó, B. Faludi, Z. Karádi and L. Lénárd (SPON: European Neuroscience Association) Institute of Physiology, Pécs University Medical School, Pécs, H-7643 Hungary

In order to determine the extent and time-course of local and distant neuronal damage produced by microiontophoretic administration of kainic acid (KA) into the amygdala (AMY), both "conventional" light microscopic staining and a specific silver impregnation method were used. The affected, so-called "collapsed" or "dark" neurons, were visualized by a modified version of the Golgi-like argyrophil III physical developing procedure according to Gallyas et al. (1990). Unilateral iontophoretic KA lesions of the central part of the AMY were accomplished by means of glass micropipettes. The KA microlesions affected neurons not only locally in the AMY but also in broad areas of the rat brain. The regions most vulnerable to KA were the claustrum, hippocampal formation, subiculum and entorhinal cortex, however, the extent and time-course of the degenerations varied. In contrast to the conventional light microscopic staining method that appeared not to be sensitive enough, the spatial and temporal distribution of neuronal degeneration, especially in the early postoperative period and in distant brain regions, could only be revealed by the silver impregnation technique for "collapsed" neurons.

29.4

TEN OR MORE WEEKS AFTER T-2 SPINAL CORD HEMISECTION, MOST SURVIVING IPSILATERAL SPINOCEREBELLAR NEURONS HAVE CONTRALATERAL PROJECTIONS TO THE CEREBELLUM. J.C. Morgan, T.M. Wallace, E.R. Feringa and R.L. McBride*, VA Medical Center and Medical College of Georgia, Augusta, GA 30910.

We determined if Clarke's column neuron survival following axotomy is influenced by contralateral projections terminating in the cerebellum. In one group of rats, we retrogradely prelabeled spinocerebellar neurons with True Blue, hemisected the spinal cord at T-2 and sacrificed the rats 10 weeks later. 40% of Clarke's column neurons from T-13 to L-2 ipsilateral to the hemisection survived, but only 25% between T-11 and T-12. In another group of rats, we postlabeled spinocerebellar neurons with Fluoro-Gold 12 or 17 weeks after T-2 hemisection. 28% of ipsilateral neurons between T-13 and L-2 were labeled, but only 17% between T-11 and T-12. Ascending axons or collaterals of these neurons must have crossed to the other side of the cord caudal to T-2. Most of the ipsilateral neurons surviving 12 or more weeks are not immediately adjacent to the dorsal funiculus and have contralateral projections, and therefore should not be considered Clarke's column neurons. Supported by the VA.

NEURONAL DEATH: SYMPATHETIC NEURONS

30.1

VERATRIDINE INDUCES NEURITE DEGENERATION BUT DELAYS CELL DEATH IN SYMPATHETIC NEURONS AND DIFFERENTIATING PC12 CELLS. T. Koike * and S. Tanaka, Department of Natural Science, Saga Medical School, Nabeshima, Saga 849, Japan.

It is well established that afferent inputs regulate neuronal death that occurs during development. Intracellular calcium or Ca2+ influx may be an universal cellular signal for promoting neuronal survival on one hand and causing neuronal death on the other. Since nerve cells exhibit enhanced expression of voltage-dependent Na+ channel upon differentiation, it has been considered that Na+ channel activation may be involved in regulating neuronal survival. However, it has not yet been critically tested whether there is a distinct pathway for Na+ influx-mediated neuronal survival independent of trophic support. Dissociated superior cervical ganglion cells from newborn rats were grown in the presence of NGF for 5-7 days, and used for experiments. We found that veratridine (1-2μM) displayed two contradictory effects on these cells: it significantly delayed the onset of cell death after NGF withdrawal, while it promoted neurite dilation and disintegration in control and NGF-deprived neurons. These two effects were blocked by 1 μM tetrodotoxin. The L-type Ca2+ channel antagonist nifedipine (2μM) improved the neurite dilation caused by veratridine, while it did not affect the former effect. In contrast, elevated K+ (35mM) prevented both neurite degeneration and cell death. Similarly, chronic depolarization with high K+ (>35mM) prevented both degenerative processes of differentiating PC12 cells after NGF deprivation, while veratridine (35-45μM) partially prevented degeneration; the effect was blocked by tetrodotoxin (1μM). This system may provide an opportunity to study relationships among voltage-dependent Na+, Ca2+ influxes, and cell survival.

30.2

BIOCHEMICAL CHARACTERIZATION OF DEGENERATION AND DEATH OF SYMPATHETIC NEURONS INDUCED BY NEUROTROPHIC FACTOR DEPRIVATION. T.L. Deckwerth* and E.M. Johnson, Jr. Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

During development of the nervous system, neuronal survival is regulated by the availability of target-derived neurotrophic factor. The molecular events that follow neurotrophic factor deprivation and ultimately lead to death were studied in established primary cultures of dissociated rat sympathetic neurons maintained by their physiological trophic factor, nerve growth factor (NGF). After an initial period of 12 h, during which no changes were visible light-microscopically, NGF deprivation caused the onset of degenerative changes of the cell body (50% of all somas were altered after 19 h). Half the neurons were committed to die by 22 h as measured by the ability of NGF-deprived neurons to respond to NGF with long-term survival and lost viability 5 h later as measured by staining with crystal violet. Looking for the molecular events capable of causing these changes, we decided to investigate general metabolic parameters. We found that the rate of glycolysis fell to 20% after 12 hours and that measures of mitochondrial function displayed a slow and steady decline, both indicating a reduced availability of energy for cellular processes. Rapidly decreasing protein synthesis (to 50% after 8 hours) and increasing protein degradation indicated a shift from anabolic to catabolic protein metabolism contributing to soma degeneration. Concurrent with the onset of commitment, the nuclear DNA became fragmented into oligonucleosomes, suggesting a possible role for DNA fragmention in the process of commitment.

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30.3

SYMPATHETIC TRUNK GANGLIA ARE NORMAL STRUCTURALLY BUT EXHIBIT ABNORMAL GROWTH PATTERNS IN EXPERIMENTAL (THOR-BR) CHICK EMBRYOS. P. Cauwenbergs*, J.T. Norton and D.W. Colburn CMCC, Toronto, Ontario, Canada M4G 3E6.

To investigate the development of sympathetic trunk ganglia (STG) in chick embryos the morphology and growth patterns of brachial STG were analysed in a total of 40 experimental and control embryos from embryonic day 8 (day 8E) to day 16E. Experimental (Thor-Br) embryos were prepared by transplanting a donor thoracic neural tube into the site of the extirpated brachial neural tube in host embryos at day 2E. Control embryos either received a brachial neural tube transplant (Br-Br) or remained unoperated (UC). To determine brachial ST morphology camera lucida tracings of thionine stained serial cross-sections were used to reconstruct the ST. In all experimental (Thor-Br) embryos analysed brachial ST morphology was comparable to that of control (Br-Br and UC) embryos.Tracings of individual brachial STG were then cut out and weighed to estimate the volume of STG at specific developmental stages. Results show that the growth of STG in experimental (Thor-Br) embryos was reduced significantly from that of control (Br-Br and UC) embryos. This affect of the experimental procedure was most pronounced caudally in the brachial ST and ranged from a 0 to 20% reduction in more cranial ganglia to about 55% in caudal ganglia. Ongoing investigations will determine if this differential growth response is reflected in the number of postganglionic neurons within individual STG. (Funded by CMCC).

30.4

PROMOTION OF SYMPATHETIC NEURONAL SURVIVAL BY CHRONIC DEPOLARIZATION AND INCREASED $[Ca^{2+}]_i$ IS A "THRESHOLD" PHENOMENON. J.L. Franklin*, A. Juhasz, E.B. Cornbrooks, P.A. Lampe, and E.M. Johnson, Jr., Dept. of Mol. Biol. and Pharm., Washington Univ. Sch. of Med., St. Louis, MO 63110

Sympathetic neurons from E21 rats were grown in culture in a medium containing NGF. After 5d NGF was removed causing >90% of the cells to die. This death was prevented by chronic depolarization with elevated $[K^+]_o$. Fura-2 measurements showed that high $[K^+]_o$ caused a sustained rise of $[Ca^{2+}]_i$ (100 nM maximal) that increased linearly when $[K^+]_o$ was increased from 5-50 mM. From 50-100 mM $[K+]_o$, $[Ca^{2+}]_i$ dropped to near control levels. Long-term survival was promoted only by $[K^+]_o$ between 25-80 mM. Corresponding $[Ca^{2+}]_i$ measurements showed that survival occurred only if >1/2 of the maximal sustained rise of $[Ca^{2+}]_i$ was achieved. Both survival and the rise of $[Ca^{2+}]_i$ caused by 50 mM $[K^+]_o$ were blocked by low nM concentrations of nifedipine. These data suggest that survival promotion by elevated $[K^+]_o$ is mediated by a sustained rise of $[Ca^{2+}]_i$ caused by Ca^{2+} influx through dihydropyridine-sensitive Ca^{2+} channels and that a "threshold" of $[Ca2+]_i$ must be exceeded before survival enhancement occurs. Since the Ca^{2+} binding protein, calmodulin, can be activated when $[Ca^{2+}]_i$ reaches a threshold level, these data suggest that the high $[Ca^{2+}]_i$ effect may be mediated by calmodulin. The calmodulin antagonist, calmidazolium, blocked survival promotion by elevated $[K^+]_o$ at concentrations that did not affect survival of cells maintained in NGF. However, calmidazolium also blocked the sustained rise of $[Ca^{2+}]_i$ caused by elevated $[K^+]_o$, probably due to Ca^{2+} channel block. An inhibitor of Ca^{2+} sequestration, thapsigargin, in combination with elevated $[Ca^{2+}]_o$ enhanced survival and caused a sustained increase of $[Ca^{2+}]i$. Nifedipine did not affect this rise. Thus, thapsigargin enhances survival by causing a sustained increase of $[Ca^{2+}]_i$ by a different mechanism than elevated $[K^+]_o$. We are now investigating the affects of calmodulin antagonists on the survival and rise of $[Ca^{2+}]_i$ caused by thapsigargin/high $[Ca^{2+}]_o$. Supported by W.U. ADRC# AG05681.

30.5

THAPSIGARGIN ENHANCES SURVIVAL OF SYMPATHETIC NEURONS BY ELEVATING INTRACELLULAR CALCIUM CONCENTRATION ($[Ca^{2+}]_i$). P.A. Lampe, E.B. Cornbrooks*, A. Juhasz, J.L. Franklin, and E.M. Johnson, Jr. Dept. of Mol. Biol. and Pharm., Washington Univ. Sch. of Med., St. Louis, MO 63110

Previous work from our laboratory has suggested that the rise of $[Ca^{2+}]_i$ in response to K^+ depolarization is responsible for depolarization-enhanced survival of NGF-deprived rat sympathetic neurons. As a potential alternative means of increasing $[Ca^{2+}]_i$ and promoting sympathetic neuronal survival in culture, we examined the effects of thapsigargin, an inhibitor of Ca^{2+}_i sequestration. It was observed that thapsigargin had modest neuronal protective activity with an EC_{50} of 10nM in a 5 day survival assay. Fura-2 measurements showed that these concentrations of thapsigargin also produced a modest sustained elevation of $[Ca^{2+}]_i$. In an effort to augment the survival promoting effect of thapsigargin, treatment with drug was combined with elevated extracellular calcium ($[Ca^{2+}]_o$). Elevating $[Ca^{2+}]_o$ alone did not promote survival and caused little increase of $[Ca^{2+}]_i$. However, treating cells with both thapsigargin and elevated $[Ca^{2+}]_o$ produced a much more profound effect on neuronal survival and much greater elevations of $[Ca^{2+}]_i$. This finding suggests that the source of the increased $[Ca^{2+}]_i$ induced by thapsigargin is Ca^{2+}_o. We have not yet determined the route of Ca^{2+} entry activated by thapsigargin, but it is thought that depletion of Ca^{2+}_i stores causes an increase of plasma membrane Ca^{2+} permeability. These data are consistent with our hypothesis that elevated $[Ca^{2+}]_i$ (within limits) promotes survival and makes sympathetic neurons trophic factor-independent. Supported by Washington University ADRC# AG05681.

30.6

THE ROLE OF THE PERFORANT PATHWAY IN THE DEVELOPMENT AND MAINTENANCE OF THE DENTATE GYRUS. D.C. Snyder*, C. F. Ide. Dept. of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118.

During neural development, afferent fibers have profound influences on both the differentiation and ultimate survival of target neurons. In the development of the cerebral cortex-hippocampal complex in the mouse, fibers from the entorhinal cortex (EC) first innervate the hippocampus between embryonic day 15 (E15) and birth (E20, P0). During early postnatal development (P5-7), fibers from this early pathway enter the hippocampal dentate gyrus to form the perforant path. Placing lesions in the fetal EC at E16 using exo utero surgery methods produced, in adults, massive degeneration in the dentate gyrus and CA3 region of the hippocampus. These data implied that a trophic influence exists between the EC and the hippocampus. However, whether this influence involved specific innervation of the hippocampus was not apparent. Thus, EC lesions were placed in 9 additional E16 embryos. These animals were reared by foster mothers to P20. Histological analysis revealed the absence of the perforant pathway entering the dentate gyrus in the region of the EC lesion. Additionally, granule cells of the lateral blade of the dentate gyrus showed edema, the onset of degeneration, and abnormalities in cell layering. In the most severe cases, the dentate appeared as a bilayered structure indicating altered morphogenesis of the dentate. Thus, specific afferent fibers from the EC are crucial for the development and survival of neurons of the hippocampus.

NEURONAL DEATH: CELLULAR RESPONSE

31.1

ISOLATION OF cDNAS FROM A POSTNATAL DAY 1 *wv/+* SUBTRACTED cDNA LIBRARY BY DIFFERENTIAL SCREENING. L.Sangameswaran*, K.R.Haddle, B. Ghetti, S.R. Dlouhy and M.E. Hodes. Depts. of Pathology and Medical Genetics, Indiana University Medical School, Indianapolis, IN 46202.

Cerebellar granule cell (GC) precursor degeneration is observed on postnatal day 1(P1) as a consequence of a mutation in the *weaver* (*wv*) gene. To obtain molecular probes for analysis of the cerebellum, a subtracted P1 *wv/+* cDNA library was constructed. P1 *wv/+* cerebellar cDNA was subtracted with mRNA from P31 *wv/wv* cerebellar vermis, so that the library should be enriched for cDNAs from GC precursors, *wv/+* GC and other differentiating cells. After several cycles of screening, 28 cDNA clones were isolated that appeared to be differentially expressed in the screening procedure. End-sequencing by PCR amplification was performed to determine redundancy among the clones and to search for sequence homology with all the entries in GenBank. Seven cDNAs were redundant; one of the clones had a high homology with T-cell receptor 4-1BB and another with mitochondrial genome. Altogether 19 unique cDNAs were found. Northern blot analyses have been done to determine the size, abundance and distribution of the transcripts, and to test for differential expression. The 4-1BB cDNA probe detects 6 transcripts that are expressed in a variety of different brain regions and nonneural tissues. Comparison of transcripts in *wv/wv and +/+* cerebella by northern analysis suggests that some of these cDNAs are GC positive. In addition, the cDNAs are being evaluated to find out whether any one of them is a candidate for the *wv* gene. (Supported in part by USPHS P01 NS 27613 and NS 14426)

31.2

IMMUNOHISTOCHEMICAL LOCALIZATION OF UBIQUITIN DURING DEVELOPMENT OF MURINE LUMBAR SPINAL CORD. S. Chaubet†, H. L. Stewart*, M. H. Droge, N. C. Mills. †Department of Biological Sciences, University of North Texas, Denton TX 76203. *Department of Biology, Texas Woman's University, Denton TX 76204.

We are investigating postnatal cell death in murine lumbar spinal cords using a monoclonal antibody against ubiquitin, a cell death-related protein. Transverse sections (10 µm thin) of freshly dissected lumbar spinal tissue from female Balb/C mice aged 0-14, 21, 28, 35 and 42 days were sliced and incubated with anti-ubiquitin 1° antibody for 24 hours. Additional slices were incubated with heat-inactivated antibody as a negative control. The detection system used was biotin-streptavidin (BSA) with alkaline phosphatase as the enzymatic marker. Standard histochemistry (toluidine blue and AChE) was performed on some sections of each age to distinguish neuronal from glial cell populations, and cholinergic neurons in particular.

Preliminary results indicate that increased levels of ubiquitin staining occurring at ages 6-8 days and at 10-12 days postnatally may be correlated with periods of postnatal neuronal cell death.

Further work will be done to correlate the spatial and temporal distribution of ubiquitin expression in the lumbar spinal cord with other developmental milestones. (NIH1R29 NS 25250-01)

31.3

HEAT SHOCK PROTEIN GENE EXPRESSION DURING NEURONAL PROGRAMMED CELL DEATH. S. Estus* and E. M. Johnson, Jr. Dept. Mol. Biol. and Pharm., Washington Univ. Med. Sch., St. Louis, MO 63110

For several years, our laboratory has investigated the mechanism of programmed cell death (PCD) induced in superior cervical ganglia neurons by NGF deprivation. Induced cell death is viewed increasingly as an active process involving the altered expression of specific gene(s). To evaluate a possible role of heat shock protein (HSP) gene expression, we are engaged in (i) quantitating the mRNA levels of inducible and constitutively expressed members of the HSP family in *in vitro* cultures of neurons undergoing PCD. and (ii) examining whether HSP induction protects neurons from PCD. Individual mRNA levels were assessed at various times after NGF deprivation by isolating total RNA and subjecting equivalent amounts to a sensitive and specific RT-PCR based technique. As the neurons die, the extracted RNA is increasingly derived from the small percentage of contaminating non-neuronal cells in the culture as demonstrated by the observation of large decreases in the levels of neurofilament M, tyrosine hydroxylase and neuron-specific enolase mRNAs relative to a minor decrease in the ubiquitously expressed mRNA for cyclophilin. Initial results indicate that HSP70 and BIP do not change during PCD. Although HSC73 decreases approximately 70% during PCD, the decrease occurs over a time course that parallels the decline in the neuron-specific messages and cyclophilin, suggesting that the decrease reflects differences in the basal expression between neurons and the non-neuronal cells. Future experiments will examine levels of HSP90 and HSP60 mRNA as well as whether HSP induction protects neurons from PCD. The results of these studies will be presented. Supported by the Washington Univ. ADRC (AG 5P01-A605681-08) and an NIH training grant (5T32-HL07275).

31.4

HSP 72 AND UBIQUITIN EXPRESSION IS NOT CORRELATED WITH NEURONAL SURVIVAL AFTER LOW DOSES OF NMDA IN THE RAT ENTORHINAL CORTEX W.M. Yee§, D.M. Frim*§, S.R. Bossi§, and O. Isacson§¶. §Neuroregeneration Laboratory, McLean Hospital, Belmont, MA 02178 ¶Program in Neuroscience, Harvard Medical School, Boston MA 02115

Stress protein induction is associated with neurodegenerative and neuroprotective events in the CNS. It remains unclear whether expression of certain stress-related proteins is protective for or merely a marker of neuronal injury. Excitotoxic damage mediated through the NMDA receptor, linked to neuronal injury in certain neurodegenerative conditions, is known to elicit a stress protein response. To assess neuronal sensitivity to NMDA in relation to stress protein expression, we stereotactically infused low doses of NMDA into the rat medial entorhinal cortex (EC). At 0.5 hrs to 168 hrs after a 1.25 µl EC infusion of 15 mM NMDA, 30 mM NMDA, or saline, we evaluated neuronal loss and expression of ubiquitin (Ub) and 72kD heat shock protein (HSP 72). Volumes of entorhinal Ub and HSP 72 immunoreactivity peaked between 18 and 48 hrs for both 15 and 30 mM NMDA infusions. Mean maximal volumes of HSP 72 and Ub immunoreactivity in the EC at 48 hrs after 15 mM NMDA infusions predicted the mean maximal volume of neuronal loss seen after 96 hours. In contrast, after infusion of 30 mM NMDA, the mean maximal volume of HSP 72 and Ub immunoreactivity was significantly larger than the maximal volume of neuronal loss, implying that a population of HSP 72 and Ub immunopositive cells survived the NMDA insult. These data show a specific neuronal stress protein response preceding neuronal death after low doses of NMDA. In addition, we have found that HSP 72 and Ub immunoreactivity are not necessarily correlated with neuronal survival in vivo.

31.5

GANGLIOSIDES RESCUE NEURONAL CELLS FROM DEATH AFTER NEUROTROPHIC FACTOR DEPRIVATION G. Ferrari *[1], A. Batistatou and L.A. Greene. Dept. of Pathology, Columbia Univ., New York. 1 Fidia Research Labs., Abano T. (PD), Italy

Serum-free cultures of PC12 cells have been used as a model system for studying neuronal death occurring after neurotrophic factor deprivation. In this system, NGF rescues cells from death and prevents apoptotic DNA fragmentation (Batistatou and Greene JCB 115, 1991). We report here that GM1 also promotes long-term survival of PC12 cells in serum-free medium and prevents internucleosomal cleavage of PC12 cell DNA. These effects of GM1 are concentration-dependent, with near maximal activity at 30 µM. Optimal promotion of survival is obtained with multiple additions of GM1. Asialo GM1 and sialic acid do not mimic these actions, indicating a requirement for the intact GM1 molecule. Prevention of serum-free PC12 cell death is also obtained with di-, tri-, and tetra-sialo gangliosides. The ganglioside effects on survival and DNA fragmentation appear to be independent of macromolecular synthesis. GM1 is also effective under conditions in which cellular PKC activity is down-regulated by pre-exposure to a high concentration of TPA. Preliminary experiments indicate that GM1 also prevents death of cultured rat sympathetic neurons after withdrawal of NGF. These findings complement prior observations that gangliosides protect cerebellar granule neurons from neurotoxicity caused by exposure to excitatory aminoacids (Favaron et al. PNAS 85, 1988) and extends the actions of gangliosides to rescue of neuronal cells deprived of support by neurotrophic factors.

31.6

PROTEIN SYNTHESIS INHIBITION REDUCES INFARCT VOLUME FOLLOWING FOCAL CEREBRAL ISCHEMIA: EVIDENCE SUPPORTING PROGRAMMED CELL DEATH IN STROKE.

MD Linnik*, RH Zobrist. Dept. of Pharmacology, Marion Merrell Dow Research Institute, Kansas City, MO 64134.

Cells within the core of an ischemic insult exhibit features consistent with necrotic cell death. However, cells on the perimeter of the ischemic zone can be preserved by various therapeutic regimes. Protein synthesis may contribute to the extent of ischemic injury. Thus, the present experiments examined the effect of protein synthesis inhibition on the volume of the ischemic zone 24 hours after permanent occlusion of the middle cerebral artery in spontaneously hypertensive rats. Continuous intracerebroventricular administration of the protein synthesis inhibitor cycloheximide (1mg/kg/24 hours) initiated prior to ischemia resulted in a significant attenuation ($p<0.01$) of ischemic brain volume (147 ± 11 mm^3) vs. matched saline controls (189 ± 7 mm^3). In addition, electrophoretic examination of cortical DNA from the ischemic zone revealed endonucleolytic degradation of the DNA into nucleosome ladders. These data demonstrate that inhibiting protein synthesis reduces the infarct size following focal cerebral ischemia, and is consistent with the possibility that programmed cell death contributes to the loss of neurons in this model.

31.7

INHIBITORS OF PROTEIN AND RNA SYNTHESIS PREVENT MOTONEURON CELL DEATH AFTER TROPHIC DEPRIVATION *IN VITRO*. J.X. Comella, C. Sanz-Rodriguez* and J. E. Esquerda. Unitat Recerca Neuromuscular, Fac. Med. Lleida, Univ. Barcelona, E-25006-Lleida, Spain.

During embryonic development, most neuronal populations undergo a process of programmed cell death, which reduces substantially the number of neurons previously generated. Death appears to result from the failure of neurons to acquire an adequate supply of a specific neurotrophic factor produced by their innervation target field. Several reports have provided evidence that neuronal death is not merely a passive consequence of general metabolic failure, but an active process which depends on the synthesis of new macromolecules. Recently Oppenheim et al. (1990) have shown that motoneurons die actively during programmed neuronal death *in vivo*. In order to study the direct effect of RNA and protein synthesis inhibitors on the process of motoneuronal death, we have established a pure culture of motoneurons and thus overcome the inherent problems of an *in vivo* system (indirect effects through alteration of muscle motility, presence of non-neuronal cells...). Motoneurons were cultured for 48 h in the presence of muscle extract. Next, they were deprived of muscle extract and RNA or protein synthesis inhibitors were added to the culture media. Cycloheximide, anisomycin and puromycin, three protein synthesis inhibitors acting through different mechanisms, significantly reduced cell death. Actinomycin D and dichlorobenzimidazole riboside, two RNA synthesis inhibitors, caused an important reduction. Chloroquine, PMSF and leupeptin, inhibitors of proteases, had no effect. We conclude that inhibiting transcription and translation directly blocks the suicide program in motoneurons deprived of muscle extract *in vitro*.

Quoted reference: Dev. Biol. 138:104-113(1990).

32.1

CLONING AND MOLECULAR CHARACTERIZATION OF LACHESIN, A NOVEL GRASSHOPPER AND DROSOPHILA SURFACE PROTEIN PRESENT ON CELLS FOLLOWING NEURONAL DEVELOPMENTAL PATHWAYS. Rolf O. Karlstrom* and Michael J. Bastiani. Dept. of Biology, University of Utah, Salt Lake City, UT 84112.

We have cloned, sequenced and characterized a novel surface molecule present during grasshopper neurogenesis. We call this molecule Lachesin (after one of the three Greek fates) to depict its potential role at points of cellular fate transitions. Lachesin is expressed on those cells which follow a neuronal developmental pathway; those cells which diverge from the neuronal pathway stop expressing the protein. Specifically, at the beginning of neurogenesis Lachesin is expressed throughout the neurogenic ectoderm. As neurogenesis proceeds, expression becomes restricted to neuroblasts and their progeny and then further restricted to a subset of neurons and axonal pathways.

PCR primers, based on protein sequence, were used to amplify a specific product from grasshopper and Drosophila cDNA. These PCR products were used to screen cDNA libraries and each pulled out a clone whose DNA sequence predicts the expected protein product. A search of the sequence data bank shows that Lachesin is a novel protein. Lachesin is a 38 kD protein containing immunoglobulin (Ig) motifs and is bound to cell surfaces by a phosphatidylinositol lipid tail. Sequence comparisons show Lachesin belongs to a family of proteins including Drosophila amalgam (ama) and the human polio virus receptor (pvr). These molecules all contain 1 C and two V type Ig domains and are expressed on neurons. It is known that ama expression is regulated during embryogenesis, but nothing is known of its function during development. A Drosophila Lachesin has been cloned which is distinct from amalgam. We are undertaking functional studies to determine the role Lachesin plays in insect neurogenesis.

Supported by NS25378, McKnight Foundation, and NSF graduate fellowship to R.K.

32.2

APLYSIA mRNAs EXPRESSED DURING NERVOUS SYSTEM DEVELOPMENT AND PLASTICITY. S.T. Lockhart, T.M. Hodgson* and C.W. Pikielny. Brandeis University, Waltham MA 02254.

We have developed an improved method which uses PCR to generate representative cDNA populations from very small amounts of RNA. We are using these cDNAs in screens and subtractive hybridizations looking for genes expressed during various forms of CNS plasticity. In a screen for genes regulated by conA, a known modulator of neuronal function, we have identified an mRNA which appears to be specific to glial cells and are currently using it as a marker to study the origin of glia and glia-neuron interactions in Aplysia development. We are also carrying on a screen for genes expressed during a critical stage of development where the structure of the Aplysia nervous system undergoes a radical change both in structure and in function. During this relatively late stage, a disproportional increase in the number of neurons and the volume of the neuropile coincides with the ability of animals to exhibit sensitization and its cellular analog, facilitation of non-decremented EPSPs.

32.3

STRUCTURAL AND FUNCTIONAL PROPERTIES OF MYELINATED AXONS IN POSTNATALLY DEVELOPING RAT MENTAL AND SURAL NERVES. C.S. Johansson, C.M. Bowe†, C. Hildebrand*, and N. Evans†, Dept. of Cell Biology, University of Linköping, S-581 85 Linköping, Sweden, †Section of Neurobiology, Dept. of Clinical Neuroscience, Brown University, Providence, RI, USA

This study examines the nodal spacing (L) and nodal-paranodal fine structure in the mental (MN) and sural nerves (SN) of developing and adult rats by light and electron microscopy. In addition, the effect of the potassium channel blocking agent 4-aminopyridine (4-AP), on the compound action potential waveform of the MN and SN is analyzed. In the MN, the L-values of conventional internodes increase from 100-500μm by two weeks after birth, to 150-600μm in the adult, as fibre diameter (D) ranges expands from 1-6μm to 2-10μm. In the SN the L values are 100-400μm by 2w and 200-1200μm in the adult. Corresponding D-values are 1-5μm and 2-9μm respectively. Thus, the largest internodes in the adult MN are half as long as those in the SN. Both in the MN and the SN of 2-3week-old rat pups some exceptionally short distorted myelin sheaths or very short internodes without signs of myelin debris are seen (L=15-80μm). The adult nodal-paranodal ultrastructure is essentially similar in the two nerves. Developing MN and SN exhibit a marked sensitivity to 4-AP. Although the physiological effects of 4-AP diminish with maturation in both nerves, the MN retains a greater sensitivity than the SN. We conclude that different cutaneous nerve trunks exhibit partly different structural and functional properties.

32.4

THE DISTRIBUTION OF BOTH THE AMPA AND KAINIC ACID RECEPTORS IS DEVELOPMENTALLY REGULATED IN THE RAT SPINAL CORD. M. W. Jakowec, A. J. Fox, S. Holloway* and R. G. Kalb Department of Neurology, Yale University, New Haven, CT. 06510.

In early postnatal life, the nervous system demonstrates plasticity during a circumscribed period in early post-natal life. Deprivation of stimulus-evoked neuronal activity during this critical period has a significant effect on neuronal phenotype while deprivation in adults has little effect. This phenomena is most compelling in the cat and primate visual system but it is likely to occur throughout the nervous system. We have shown that motor neurons have an activity-dependent developmental critical period. In the rat and hamster, monoclonal antibodies have identified a proteoglycan (Cat-301) whose expression is dependent upon activity in the spinal cord. Recently, we have shown that Cat-301 expression is dependent upon activation of the NMDA subclass of glutamate receptor and that NMDA receptors are transiently expressed in the ventral horn during development. Since activation of the NMDA receptor requires co-activation of non-NMDA receptors, we were interested in examining the distribution of a subclass of non-NMDA receptors. We have used receptor-ligand autoradiography to characterize the distribution of both the AMPA and Kainic acid sub-types of the glutamate receptors in the developing rat spinal cord. Tissue sections of rats at postnatal day 7 (P7), P14, P21, P28, and adult were examined. At P7 the expression of these binding sites is present throughout the gray matter of the spinal cord and over the next three weeks all these binding sites are lost except in the substantia gelatinosa. These results demonstrate synchronous expression of multiple glutamate receptor subtypes in the developing spinal cord. Transient expression of NMDA and non-NMDA receptors is likely to provide a critical mechanism for spinal neurons to mature neuronal features during a critical period in early postnatal life.

32.5

MOLECULAR EVIDENCE FOR NITRIC OXIDE SYNTHASE-MEDIATED DEVELOPMENT OF MOTOR NEURONS Robert G. Kalb* Yale University School of Medicine, New Haven, CT 06510

During a critical period in early postnatal life, patterned neuronal activity can regulate the acquisition of mature morphological and electrophysiological neuronal properties. We examined the molecular development of motor neurons and found that the expression of the cell surface proteoglycan recognized by monoclonal antibody Cat-301 depends on neuronal activity during a circumscribed period in early postnatal life. The development of Cat-301 expression on motor neurons requires activation of N-methyl-D-aspartate (NMDA) receptors at the spinal segmental level. A transient high level expression of NMDA receptors in the ventral horn occurs during this period suggesting the expression of NMDA receptors on motor neurons is essential for their appropriate activity-dependent development. The potential signal transduction pathways that follow NMDA receptor activation during motor neuron development was examined.

Immunohistological studies employing a polyclonal sera to PKC revealed labeling restricted to the substantia gelatinosa in both neonates and adults. Immunohistologic studies employing the 6G9 monoclonal antibody to Ca/Cam Kinase showed labeling only in the adult (but not neonatal) substantia gelatinosa. Histochemical localization of NADPH-diaphorase activity (an excellent marker for NOS) revealed intense labeling in the adult substantia gelatinosa. In the postnatal day 7 (P7) spinal cord, diaphorase activity is present in neurons throughout the spinal grey matter and motor neurons appear intensely labeled. Since both NOS and NMDA receptors appear to be transiently expressed by neonatal motor neurons, NOS may participate in NMDA receptor-mediated signal transduction. We evaluated the effect of the NOS inhibitor nitroarginine (NA) on the development of motor neuron Cat-301 expression and found that NA administered daily from P7 to P21 specifically inhibited the development of Cat-301 expression on motor neurons. These results suggest that NMDA receptor-mediated development of motor neurons employs the NOS signal transduction pathway. Supported by the NIH and the Muscular Dystrophy Association

32.6

Synaptophysin and chromogranin-A are expressed in fetal and adult female rat paracervical ganglion autonomic neurons and SIF cells.

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Paracervical ganglia(PG) in uterine cervical perimetrium consist of several subsets of autonomic neurons(AN) and small intensely fluorescent(SIF) cells based on their peptide and transmitter content. Small synaptic vesicles(SSV) and large dense core vesicles(LDCV), organelles present in most neurons and neuroendocrine cells, are components of two different secretory pathways which are involved in uptake, transport and exocytosis of bioactive molecules. In the present study, expressions of synaptophysin(SY), a SSV membrane protein, and chromogranin-A (CGA), a LDCV matrix protein, were used to examine development of AN and SIF cell secretory organelles in PG. Sections of PG were analyzed for SY-, CGA- and tyrosine hydroxylase(TH) immunoreactivities (-I) using ABC reactions or specific SY and CGA mRNA in situ hybridization(ISH) with ^{35}S-cRNA antisense probes.

Results reveal clusters of intensely SY-I cells in PG on fetal day(FD) 16; serial sections show coincident CGA- and TH-I. These characteristics correspond to SIF cells and remain prominent features in neonatal(NN) and adult PG. SY-I preganglionic terminals appear among AN in PG on FD 18 & 21. By NN days 1 & 5, SY-I terminals of various sizes and number develop axo-somatic relationships with most AN and SY-I postganglionic terminals appear in the uterine cervix. Terminal SY-I becomes more prominent in adult PG; in contrast, terminals are not CGA-I. Accumulating ISH data reveal expressions of specific mRNAs for SY and CGA in PG. Data imply that organelles (SSV & LDCV) associated with two different secretory pathways in neural cells develop earlier in SIF cells compared to AN in PG. Supported by: DFG-Ma1080/2-1 (A.M.); DAAD (H.T.).

32.7

EXPRESSION OF THE CHICK NEUROFIBROMATOSIS-1 GENE (cNF-1) IN THE DEVELOPING NERVOUS SYSTEM AND NEURAL CREST. A.I. Kavka & K.F. Barald*, Program in Neuroscience and CMB Program, University of Michigan Medical School, Ann Arbor, MI 48109-0616 and C.M.R.F., University of Sydney, P.O. Box 61, Camperdown 2050, NSW, Australia.

Von Recklinghausen's neurofibromatosis type 1 (NF1) is one of the most frequently inherited autosomal dominant disorders in humans. Although the symptoms and severity of the disease are variable, the majority of tissues affected in NF1 are of **neural crest** (NC) origin. The chick neurofibromatosis 1 (*cNF1*) 432-bp cDNA which we have cloned and sequenced is highly (at least 86%) homologous to the human *NF1* gene at the nucleic acid level. We are using this probe to establish the normal expression pattern of *cNF1* in early chick and quail embryos, and to examine the potential role of *cNF1* in neural crest development. In Northern blot analysis we have found two transcripts expressed: a large (12.6kb) *cNF1* transcript expressed as early as Hamburger & Hamilton stage 11 (40-45 hours *in ovo*) through to adulthood, and a smaller transcript (4kb) which also appears as early as stage 11 but is downregulated by stage 15 (50-55 hours *in ovo*). *In situ* hybridization experiments have shown *cNF1* to be ubiquitously expressed as early as stage 8; both earlier and later stages are being investigated to determine the onset and pattern of gene expression. Monoclonal and polyclonal antibodies (Gutmann et al, 1991) are also being used to examine protein expression during development. We have produced a *src*-transformed quail NC cell line with a temperature sensitive mutant of the Rous Sarcoma virus. In its undifferentiated state, the quail NC cell line does not express the *cNF1* gene; however, *cNF1* mRNA levels increase at least two orders of magnitude after differentiation of the NC cell line into neuron-like cells with retinoic acid. This model system can be used to examine the involvement of *cNF1* in NC development and differentiation. Support: NSF BNS8910987 & Fulbright Foundation (KFB); U of M International Research Partnership (KFB&AIK); NIH Developmental Biology & CMB Training Grants (AIK).

32.8

COLOCALIZATION OF NERVE GROWTH FACTOR RECEPTOR- AND AC3-IMMUNOREACTIVITY IN EMBRYONIC RAT CORTICAL SUBPLATE AND BASAL FOREBRAIN NEURONS. Z.-Y. Yu*, D. E. Anderson, and J. E. Bottenstein, Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77555

Double-immunostaining for nerve growth factor receptor (NGFR) and AC3 antigen has been performed in embryonic rat brain. NGFR- and AC3-immunoreactive cells were visualized in the same coronal section of embryonic rat brain. Mouse anti-rat NGFR monoclonal antibodies (192) bind to low affinity NGF receptors in embryonic through adult stages; AC3 monoclonal antibodies bind to cytoplasmic antigens in the cortical subplate, basal forebrain, and other regions during embryonic and early postnatal periods but not after P21 (Soc. Neurosci., Abstract 524.9, 1991). Neurons double-immunostained for NGFR and AC3 are present in the cortical subplate and adjacent cortical plate, basal forebrain (especially septum), and medial hypothalamus (especially median and medial preoptic nuclei). The NGFR localization we observed in embryonic rat brain is similar to that described by Koh and Loy (J. Neurosci., 9:2999-3018, 1989).

Colocalization of NGFR- and AC3-immunoreactivity suggests there may be a functional link between these two cell survival related proteins in the maintenance and development of neocortical, cortical subplate, and basal forebrain neurons.

32.9

A HUMAN HOMOLOGUE OF SC1 (DM-GRASP) IS EXPRESSED IN THE HUMAN EMBRYONIC NERVOUS SYSTEM. David Shelton*, Hideaki Tanaka, and Joni Sutherland. Dept. Neuroscience, Genentech, Inc. South San Francisco, and Gunma University, Maebashi, Japan.

SC1 is a 100 kD integral membrane glycoprotein, first identified by monoclonal antibodies generated to chick embryo spinal cord membranes. It has recently been cloned, and predicted amino acid sequence shows that it has five immunoglobulin-like domains, a transmembrane domain and a short cytoplasmic tail. *In vitro* expression studies have demonstrated that SC1 is capable of mediating intercellular adhesion in a homophilic manner, and also of mediating neurite outgrowth. Because none of the monoclonal or polyclonal antibodies to SC1 recognize any proteins of mammalian origin, molecular cloning techniques were used to identify the human homologue of this molecule. Probing of a fetal human brain cDNA library with a chicken SC1 probe at low stringency revealed clones which are highly homologous to chicken SC1. The overall nucleotide sequence identity is approximately 90%. Both the cloning of this molecule from a brain library and preliminary *in situ* hybridization analysis verify that this human SC1 homologue is expressed in the embryonic human nervous system.

32.10

EXPRESSION OF SCG10 AND P19/STATHMIN mRNAs IN THE ADULT RAT BRAIN AND THE EFFECT OF LESION. T. Himi*, T. H. McNeill, N. Mori Div. Neurogerontology, Andrus Gerontology Center, University of Southern California, Los Angeles, CA90089.

SCG10 and p19/stathmin are members of a newly identified gene family, which are expressed specifically or preferentially, respectively, in the nervous system. The brain expression of SCG10 and p19 mRNA is maximal in late gestation to early postnatal period and dramatically reduced in the adult rat brain. In this study, we investigated 1) the distribution of SCG10 transcripts in the CNS of the young adult rat in comparison with p19 transcripts, and 2) the change in the expression during neural degeneration and regeneration after partial brain lesion. To detect these transcripts by *in situ* hybridization, we used two kinds of RNA probes respectively: one was transcript from full-length cDNA and the other was transcript from 3'-noncording region.

1) In 3-month-old rat brains, the expression of both SCG10 and p19 is widespread through most brain regions. The precise distribution patterns at the cellular level are as follows: SCG10 mRNA is expressed strongly in mitral cell layer of olfactory bulb, pyramidal layer of hippocampal CA3 - 4 region, piriform cortex, cerebellar Purkinje cells and magnocellular motor neurons in midbrain and brain stem, and expressed moderately in olfactory tract, nucleus olfactory tract, hippocampal CA1 - 2 region, septum, rhonboid thalamic nucleus, hypothalamus, and substantia nigra. Whereas, p19 mRNA is expressed mainly in external plexiform layer of olfactory bulb, tenia tecta, olfactory nucleus, piriform cortex, thalamic nucleus, raphe and A7 in midbrain, however, weak or no signals were detected in hippocampus, cerebellum or brain stem. The result suggests that SCG10 and p19/stathmin are differentially expressed in young adult brain.

2) In order to investigate the effect on mitral cells and cells in piriform cortex, we tested SCG10 mRNA expression in partial lesion of the olfactory bulb. The expression of SCG10 in piriform cortex was reduced in the ipsilateral frontal side, which was maximal 3 days after lesion. It is suggested that the change of SCG10 expression is involved in neural degeneration and regeneration.

32.11

ONTOGENY AND SEX DIMORPHISM OF NEURAL-SPECIFIC mRNA'S IN RAT BRAIN. R.H. Lustig*, P. Hua, M.A. Wilson, and H.J. Federoff. Dept. Pediatrics, U. Wisconsin, Madison, WI 53792; Scripps Research Institute, La Jolla, CA 92037; and Dept. Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Growth-Associated Protein 43kDa (GAP-43) gene expression is estrogen-regulated in adult ventromedial hypothalamus (VMH) and sexually dimorphic (M:F=1.8:1) in adult cortex (CTX) (Mol Brain Res. 11:125,1991). Such effects intimate hormonal regulation of synaptic plasticity. This study examined ontogeny of expression of mRNA's encoding 3 neural-specific proteins: GAP-43, SCG10, and synaptosomal-associated protein 25 kDa (SNAP-25); and also glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Male and female rats were sacrificed at 1, 4, 11, 23, and 60 days, levels of specific mRNA's in VMH and CTX were quantitated on slot-blots by ^{32}P-cDNA hybridization (using cyclophilin (CYC) and poly-dT$_{50}$ (dT) as standards), and peak density (PD) determined by autoradiography and optical densitometry. PD ratios were compared across ages and between sexes.

PD ratios of CYC/dT remained constant in each region over time and sex. PD ratios for other mRNA's were congruous regardless of standard. In VMH, GAP-43 mRNA levels were high on days 1 and 4 with a 3-fold decrease by day 23; in CTX, GAP-43 mRNA increased by 2-fold by day 11, then decreased 2.5-fold by day 23. In VMH, SCG10 mRNA showed only small increases with time; but in CTX, there was a 5-fold drop from days 4 to 23. In VMH, SNAP-25 mRNA was low and changed only slightly; but in CTX there was a 5-fold increase between days 4 and 60. At birth, there was no sex dimporhism in either area, but all 3 neural-specific mRNA's were sexually dimorphic in adult CTX (M:F=1.76 for GAP-43, 1.46 for SCG10, 1.44 for SNAP-25); VMH was not sex dimorphic. GAPDH mRNA levels were regulated developmentally in VMH and CTX, but there was no sex dimorphism in either area.

These results are consistent with other ontogenic studies, show that neural-specific mRNA's are differentially regulated, and are coincidental with synaptic ontogeny in these areas. They also confirm a sex dimorphism of neural-specific mRNA's in adult CTX, and show that these dimorphisms are not present at birth, but rather become apparent at adulthood, suggesting that they may be hormone-dependent.

32.12

MOLECULAR CHARACTERIZATION OF A NOVEL CEREBELLAR cDNA, PRENATAL ONTOGENY, AND CELLULAR DISTRIBUTION IN THE BRAINS OF NORMAL, WEAVER, *PCD* AND REELER MUTANT MICE. *M. Kambouris, L. C. Triarhou,* L. Sangameswaran, S. R. Dlouhy, B. Ghetti and M. E. Hodes.* Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

Following the identification of several novel cDNAs by screening a neonatal *wv*/+ cerebellar cDNA expression library with an anti-granule cell antiserum (*Soc. Neurosci. Abstr. 17:* 556, 1991), we characterized and sequenced one cDNA; using *in situ* hybridization histochemistry, we also studied its expression during prenatal development and its cellular distribution in adult cerebellar mutant mice. This cDNA represents a single-copy gene with a mRNA of 2.4 kb. In normal adult mouse brain, strong hybridization signal is seen *inter alia* in cerebellar granule and Purkinje cells, in hippocampus and in substantia nigra. The message is already present in the CNS of +/+ embryos at E15. In adult *wv*/*wv* cerebellum, the signal is seen mainly in Purkinje cells. The transcript is markedly reduced in *wv*/*wv* substantia nigra. In *pcd*/*pcd* cerebellum, granule cells show hybridization signal, but overall expression is decreased owing to the absence of Purkinje cells. In *rl*/*rl* cerebellum, the strongest hybridization signal is found in a superficial layer, while diffuse grains are observed in the subcortical white matter. The *rl*/*rl* hippocampus appears disorganized. In all, the cellular localization in the mutants correlates with their known anatomical deficits. The nature and function of the polypeptide encoded by the cDNA is under study. (Supported by USPHS PO1-NS27613).

32.13

SEQUENCE AND CHARACTERIZATION OF A DEVELOPMENTALLY REGULATED PROTEIN DURING EARLY CORTICOGENESIS IN THE RAT. J.E. Minturn*, H.J.L. Fryer, D. Geschwind, and S. Hockfield. Section of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT, 06510

Using two-dimensional gel electrophoresis we previously identified an acidic, 64 kd protein (protein 310), that is up-regulated during neurogenesis in the developing rat cerebral cortex (J. Neurosci. 9:4304). Protein microsequencing of purified 310 has been carried out and 5 peptides reveal no significant homology to other previously described proteins.

Polyclonal antisera were raised to synthetic peptide fragments of protein 310 derived from partial sequence data. The antibodies recognize a 64 kd band on immunoblots of cerebral cortex homogenates; the band is eliminated when the antibodies are pre-incubated with their specific peptides. Developmental immunoblot analysis of cortex, spinal cord, liver and lung homogenates from animals of ages ranging from embryonic day 14 (E14) to adult reveals a neural specific expression beginning at E14, peaking at post-natal day 5 (P5), with markedly diminished staining in the adult.

Immunohistologically the protein is expressed by cells in the developing cortical plate but is not expresssed in the ventricular zone, consistent with an expression by early post-mitotic cells. The morphology of antibody-positive cells suggests that they are post-mitotic neurons during their migratory and post-migratory stages. Antibody positive cells are not stained by monoclonal antibody Rat-401, which is specific for progenitor and radial glial cells.

Antibody probes demonstrate that protein 310 1) increases in abundance during neurogenesis, 2) is expressed in post-mitotic neurons, 3) is down-regulated in the adult, and 4) is enriched in neural tissues. These results suggest that protein 310 may play an important role in cortical neurogenesis. [Supported by NS22807 and the Howard Hughes Medical Institute]

32.14

A NOVEL MARKER FOR SELECTED NEURONS IN EMBRYONIC RAT TEL- AND DI-ENCEPHALON. Z.-Y. Yu, D. E. Anderson and J. E. Bottenstein* Marine Biomedical Institute, University of Texas Medical Branch in Galveston, Galveston, Texas 77555

Our previous work (Soc. Neurosci.,Abstract 524.9, 1991) has shown that AC3 monoclonal antibody (mAb) is a novel marker for neurons in cortical subplate, medial septal and preoptic nuclei, and some subventricular zone cells in P0-P14 rat brain.The distribution of AC3 immunoreactivity (AC3 IR) was examined in serial coronal sections of embryonic (E15-E19) rat brain. **E15:** Strong AC3 IR is present in the olfactory tract (LOT), paraventricular hypothalamic nuclei (PHN), and suproptic nuclei; medium AC3 IR in septum, medial forebrain bundle (MFB), and medial and lateral hypothalamus; low AC3 IR is shown in the putamen. No AC3 IR is found in the neocortex or ventricular/subventricular zone. **E17:** Strong AC3 IR is present in the olfactory tubercule (TU), median preoptic nuclei (MnPO), and LOT; strong-medium AC3 IR in the cortical subplate, adjacent cortical plate, and septum; medium AC3 IR in the MFB and medial preoptic nuclei (MPO). **E19:** Strong AC3 IR is present in the TU, LOT, MnPO and optic tract; strong-medium AC3 IR in the cortical subplate, adjacent cortical plate, dorsoventral piriform cortex, septum, and dentate gyrus; medium AC3 IR in the hippocampus, MFB, diagonal band, MnPO, amygdala, PHN, and median eminence. AC3 immunoreactive subplate neurons are detected when the subplate zone first appears but not after P21. AC3 IR is found in the CA1 and CA3 fields of the hippocampus and in the dentate gyrus at E19 but disappears after birth. AC3 IR is not observed in the ventricular/subventricular zone at E15-E19 but is observed in the early postnatal period. No AC3 IR is observed in any brain area after P21.

Our results suggest that the AC3 monoclonal antibody can also be employed as a marker for studying neuronal development in the embryonic rat brain.

32.15

TWO MONOCLONAL ANTIBODIES WITH REGIONAL AND TEMPORAL SPECIFICITY IN THE DEVELOPING CHICK EMBRYO. A. M. SMITH*, L.C. ELLIS, and I.S. ALVAREZ. Department of Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.

An objective of our research program is to produce monoclonal antibodies specific for proteins which are regulated during early stages of chick development. In particular we are generating molecular markers specific for different classes of cells or different regions of the developing chick embryo. Using an immunization technique which enriches for the production of MAbs against rare antigens (1986, PNAS 83:4336-4340) we have produced two new MAbs, 2B8 and 1E10. MAb-2B8 is an IgM and stains the cytoplasm of several classes of cells in the developing embryo. At stage 4 only endoderm cells stain and this staining persists in the adult. By stage 10 isolated cells in the auditory placodes can be detected and by stage 11 positive staining of cells in the roof of the hindbrain can be detected. Also by stage 11 labelled cells aligned below the notochord are apparent. The MAb 1E10 was obtained by immunizing BALB/c with neural plate tissue from early chick embryos. The surface of cells is stained by this antibody. By stage 6 regions of the developing head region is stained by 1E10 and by stage 8 the neural folds stain. (Supported by USPHS grants no. NS 18112 and DC 00144)

SENSORY DEVELOPMENT: AUDITORY AND OLFACTORY

33.1

CHLORAMPHENICOL ENHANCES RIBOSOMAL DISSOCIATION IN DEAFFERENTED NEURONS OF THE CHICK BRAINSTEM. M. Hartlage-Rübsamen, D.E. Cunningham, E.W. Rubel* Virginia Merrill Bloedel Hearing Research Center, Univ. of Washington, Seattle, WA 98195.

Following cochlea removal approximately 30% of the neurons in the ipsilateral primary auditory nucleus, nucleus magnocellularis (NM), of the chick undergo cell death. Previous studies have shown that by 6 hours after receptor destruction NM neurons destined to die ceased protein synthesis. EM-analysis of these neurons reveals complete dissociation of cytoplasmic ribosomes. In addition, there is a generalized up-regulation of oxidative metabolism throughout the deafferented nucleus. All of these changes are due to the elimination of afferent activity. Prevention of up-regulation with chloramphenicol, an inhibitor of mitochondrial protein synthesis, causes increased cell death after deafferentation (Hyde & Durham, Soc. Neurosci. Abs., 16:A 984, 1990). In the present study we used the same drug to assess whether the increased cell death can be traced back to the same early degenerative events as observed in affected NM-neurons following cochlea removal alone.

Posthatch chicks (P12-14) were given hourly injections of chloramphenicol (250g/kg/6h). Thirty minutes after the first injection, animals were anesthesized and the right cochlea was removed. The birds were sacrificed 6 hours after surgery. EM-analysis of cytoplasmic morphology shows a 3-4 fold increase in the number of cells displaying complete dissociation of ribosomes compared to deafferented, vehicle-injected controls. This suggests that enhanced cell death involves the same cellular events in NM neurons as found after deafferentation alone. These results indicate that up-regulation of mitochondrial function plays a critical role in the survival of neurons following withdrawl of afferent input. *(Supported by N1H DC 00520)*

33.2

CLONAL RELATIONSHIP OF AVIAN BRAINSTEM AUDITORY NUCLEI. E.A. Lachica* & E.W Rubel, Dept. of Otolaryngology-Head & Neck Surgery, University of Washington, Seattle WA 98195.

In avians, nucleus magnocellularis (NM) receives a tonoptic projection from the eighth nerve. NM projects tonotopically onto n. laminaris (NL). Topographic connectivity between NM and NL appears to be established early, developing independent of physical or physiological cues conveyed by the ganglion cells of the peripheral sensory receptor. Hence, factors linked to the early development of NM and NL cells may underlie the ontogeny of topographic specificity. In this study we examined the clonal relationship of NM and NL cells by infecting embryos with a replication defective recombinant retrovirus during a period when NM, ***but not*** NL cells are proliferating. Embryos developed outside of the shell. When they acquired the features typical of 9 to12 day old embryos, they where exsanguinated, then fixed via transcardial perfusion of 1% acrolein + 4% formaldehyde. Coronal sections of the brainstem 50 μm thick were cut with a cryostat and processed for β–galactosidase (X-gal) histochemistry. X-gal labelled cells in NM and NL suggest that neurons in these two nuclei are clonally related. X-gal-positive cells were also present in the neuropil surrounding NL, and neuropil just subjacent to the floor of the IVth ventricle. GABA-ergic cells are known to reside in these areas. Labelled cells were also present in the ventral vestibular nucleus. Plots of X-gal labelled cells of E9 embryos show that clonally related cells are scattered throughout NM and NL. However, reconstructions of the auditory nuclei of E12 embryos indicate that clonally related cells lie in topologically congruent positions of NM and NL. Supported by PHS DC00040 to EAL & DC00395 to EWR.

33.3

The Role of Experience in Regulating Myelination in Nucleus Laminaris of the Barn Owl. C. T. Hue* and C. E. Carr. Dept. of Zoology, Univ. Maryland, College Park, MD 20742.

The adult barn owl uses interaural time differences to localize sound in azimuth. The timing information is processed in the auditory brainstem neural pathway involving nucleus laminaris (NL). Maps of interaural phase difference are formed dorsoventrally in NL by interdigitating axons from the ipsilateral and contralateral nucleus magnocellularis (Carr and Konishi, 1990).

Athough this circuit is present at hatching, the magnocellular axons in NL are unmyelinated. The physiological significance is that myelination is critical for their proper functioning in the adult NL. Myelination begins towards the end of the first week post-hatch and is completed at 1 month post-hatch. Thus myelination coincides with head growth, and with the appearance of stable interaural time differences. Schwab and Caroni (1988) showed that oligodendrocytes are nonpermissive substrates for neurite growth, and thus may be important in regulating CNS development. The hypothesis that myelination may be affected by auditory experience was tested by deafening young birds within days of hatching. Immunohistochemistry was used to detect oligodendrocyte proteins.

Preliminary observations from owls deafened at both 1 and 5 days after hatching showed a relatively normal time course of migration of oligodendrocytes into NL, and initial expression of myelin did not appear to be affected by deafening. In summary, myelination of NL circuit may not be regulated by auditory experience of interaural time difference cues, but could be developmentally regulated instead.

33.4

AN ORGANOTYPIC CULTURE OF THE VENTRAL AUDITORY BRAIN STEM. A. Hafidi* and D.H. Sanes. Center for Neural Science, New York Univ., New York, NY 10003.

While the organotypic culture technique has been successfully applied to several neural systems, the mammalian auditory brain stem nuclei have never been studied *in vitro*. We report here our preliminary observations on the study of the superior olivary complex (SOC) using a thick slice preparation (Stoppini et al., J Neurosci Meth, 1991). Gerbils (*Meriones unguiculatus*) of 5-7 days postnatal were used for this study. Gerbils were anaesthetized, the brain was carefully removed, and was cut on a vibratome at a 300 μm. Sections of the brain stem containing the SOC were collected in Dulbecco's modified Eagle medium, transferred to serum containing medium and placed in culture plate inserts that contained a microporous membrane. The tissue slices were incubated at 36° C with a 5% CO_2 enriched atmosphere, and transferred to fresh media daily. The slices were processed for electron microscopy after 3, 6 and 15 days in vitro (DIV).

After 3 DIV, SOC slice cultures showed a change in the cross-sectional shape of the nuclei, probably due to the near total deafferentation. Toluidine blue stained semithin sections indicated a great number of degenerative neurons after 3 DIV, but considerably less after 6 DIV. The slices became thinner ($\approx$ 80 μm) and contained many neurons with a healthy morphology. Preliminary electrophysiological observations confirm that neurons survived up to 10 DIV. This preparation will allow for direct observation and manipulation of developing neuronal connections. (Supported by NIH DC00540)

33.5

THE DEVELOPMENT OF SYNAPTIC INTEGRATION IN THE LATERAL SUPERIOR OLIVE. D. H. Sanes*. Center for Neural Science, New York Univ., New York, NY 10003.

The development of synaptic integration underlying a simple binaural computation, interaural level difference coding, was studied using an *in vitro* slice preparation through the gerbil (*Meriones unguiculatus*) auditory brain stem. The binaural properties of lateral superior olivary (LSO) neurons are available to intracellular analyses by delivering electrical stimuli to the afferent pathway from each ear (Sanes, J Neurosci 10; 3494, 1990). The ipsilateral pathway is largely excitatory, and the contralateral pathway is largely inhibitory. Long duration synaptic responses ($\approx$100 ms) were observed in recordings from neonatal animals (1-8 days postnatal). Short trains of electrical stimuli to either pathway commonly produced a temporally summated response, and membrane potential remained hyperpolarized or depolarized for the stimulus train duration. In neonatal animals, the IPSPs were often large enough to induce an action potential upon repolarization, suggesting the presence of a low threshold Ca^{++} response. The synaptic responses from 10-14 day animals, about the time when airborne sound is first transduced *in vivo*, remained of longer duration. When the latency of an ipsilaterally evoked action potential was successively advanced through a contralaterally evoked IPSP, by delaying the relative stimulus time, the duration of action potential blockade was found to be up to 10-fold longer than in 18-25 day animals. These results indicate that the temporal integration of synaptic potentials is severely compromised when animals first begin to process acoustic information. (Supported by NIH DC00540)

33.6

TONOTOPY IN THE INFERIOR COLLICULUS SHIFTS BY 1.5 OCTAVES DURING POSTNATAL DEVELOPMENT OF THE MONGOLIAN GERBIL *MERIONES UNGUICULATUS*. M. Schäfer* and R. Rübsamen. Dept. for Zoology and Neurobiology, Ruhr-University Bochum, D-4630 Bochum, FRG.

In a comparative study we addressed the question of functional and structural dynamics in the postnatal development of the central hearing system in different mammalian species. Here we report the ontogenesis of the tonotopic organisation in the auditory midbrain nucleus Inferior Colliculus (IC) of the anaesthetised mongolian gerbil *Meriones unguiculatus* as revealed by stereotaxic multi-unit recordings (ca.200 recording sites/animal) following pure tone stimulation.

At birth, gerbils are deaf. Starting at the 12th day after birth (DAB) a tonotopic organisation in the IC is established first in dorso-lateral parts of the nucleus for frequencies from 0.6 to 3-5 kHz with thresholds of 85-100 dB SPL. At this time other parts (56 Vol.%) of the nucleus do not respond to any stimuli presented. Within the following 6-8 days this partial excitability improves to a consistent tonotopy covering all three subdivisions (ICX, ICP and ICC) of the IC with a low-to-high frequency axis running from dorso-lateral to ventro-medial. Thereby, the formerly unresponsive areas successively become involved into auditory processing. At the 18-20th DAB the hearing range of the animals is adult-like and covers frequencies from 0.2 to 50 kHz with a threshold minimum of -6 dB SPL at 3.7 kHz. Between the 13th and 16th DAB the frequency-place code shifts by 1.5 octaves, e.g. the representation site of 5.6 kHz (13 DAB) shifts to 16.4 kHz (16 DAB). These developmental changes are restricted to frequencies above 5 kHz, i.e. 50 % of the IC volume devoted to the processing of 0.5-5 kHz remain unaffected by spatial rearrangement.

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33.7

THALAMOCORTICAL PROJECTIONS IN NEONATALLY DEAFENED CATS. S.G. Stanton*, R.V. Harrison, A. Nagasawa, and R. Mount. University of Toronto, Toronto, Ont., CANADA, M5G 1X8.

Standard microelectrode recording techniques were used to obtain cochleotopic maps of stimulus frequency representation in the primary auditory cortex (AI) of adult cats deafened at birth. In addition, retrograde tracers were used to examine the cochleotopically organized projection from the medial geniculate body (MGB) to AI. As a consequence of high frequency neonatal cochlear hearing loss, the physiologic map of AI becomes extensively reorganized, with anterior regions of AI normally devoted to high frequencies becoming tuned to a lower frequency. However, tracer injections into different regions in AI labelled separate cell populations in MGB in both deafened animals and controls, suggesting that the cochleotopic organization of the thalamocortical projection is not disrupted in deafened cats, despite extensive physiological reorganization of stimulus frequency representation in the cortical map.

33.8

OTOTOXICITY OF NEONATAL RATS TO SINGLE DOSE GENTAMICIN/FRUSEMIDE. S.J. O'Leary and D.R. Moore (SPON: Brain Research Association), University Laboratory of Physiology, Parks Rd., Oxford OX1 3PT, U.K.

Previous investigators have noted that neonatal rats cannot be deafened by aminoglycosides at doses which are ototoxic to older animals. However, these studies have used a treatment regime extending over >= 3 days and it has thus been difficult to correlate susceptibility to deafness with parallel morphological and physiological changes within the cochlea. We have, therefore, determined the susceptibility of agouti rats to ototoxicity with a single dose of gentamicin (200 mg/kg, i.m.) and frusemide (100 mg/kg, i.m.) given prior to the onset of hearing. In rats this occurs at postnatal day (P)11-12 (n=20), as determined by click-evoked brainstem responses. When animals were aged P24-28, brainstem response thresholds to condensation clicks were 26-33 dB peak SPL from untreated controls (n=5, mean=29dB, s=1.8) and from P6 (n=5, mean=29dB) and P7 (n=6, mean=29dB) treated animals. Thresholds were 30-48 dB from P8 (n=5, mean=38dB, s=8.5) treated animals and unrecordable at 87 dB from P10 (n=10) treated animals. These observations are consistent with the hypothesis that ototoxicity requires access to an active hair cell receptor, since susceptibility to the gentamicin/frusemide treatment correlates with the emergence of the cochlear microphonic on P8.

33.9

THE DEVELOPMENT OF THE OLFACTORY PLACODE IN THE EMBRYONIC ZEBRAFISH. E.J Collins and L.S. Ross*. Dept. of Biological Sciences, Ohio University, Athens, OH 45701.

We have examined the early development of the olfactory placode in the zebrafish using light, electron, and video microscopy. Three distinct cell types are seen in the developing placode, and each cell type is limited to a specific region within the placode. Darkly staining basal cells form a layer along the deep margin of the placode and a dense cluster in the ventral placode. Bipolar receptor neurons have thick apical dendritic processes extending toward the luminal surface and thin basal axonal processes projecting to the adjacent telencephalon. Receptor cells are found primarily in the central regions of the placode. Lightly staining supporting cells with prominent nucleoli are scattered throughout the placode, but are sparsely distributed in basal cell regions.

Differentiation within the olfactory placode follows a spatial gradient. The styryl dye DASPEI, a vital stain for mitochondria, was used to visualize olfactory cells in living embryos with video microscopy. The first labeled cells appear in the placode between 30 and 32 hours postfertilization. The distribution of DASPEI-labeled cells corresponds to the distribution of differentiated receptor cells in sectioned embryos. At 30-32 hours, the earliest labeled cells are restricted to the caudal region of the placode, directly adjacent to the eye. As the embryo develops, the distribution of labeled cells spreads anteriorly around the circumference of the placode. By 48 hours labeled cells are distributed throughout the placode. These data suggest that there is a caudal to rostral gradient of receptor cell differentiation in the olfactory placode of the embryonic zebrafish. Supported by OURC grant #861.

33.10

IN VIVO ZINC SULFATE ($ZnSO_4$) ADMINISTRATION INCREASES THE YIELD OF ADULT MOUSE OLFACTORY RECEPTOR NEURONS *IN VITRO*. J. Sosnowski, M. Gupta*, and F. Roisen. Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292.

Adult olfactory receptor neurons are replaced following their fatal damage by an injury-induced mitotic stimulation of resident stem cells (Graziadei and Graziadei, J Neurocytol 8, 1979). In the present study, an *in situ* $ZnSO_4$ insult was applied to the olfactory epithelium prior to its removal for tissue culture to determine if the insult would enhance the *in vitro* neuronal population. Young adult male C57BL/6 mice were treated with 1% $ZnSO_4$ by nasal irrigation and sacrificed by decapitation 5 days following the treatment. The olfactory epithelium was removed and dispersed with a 0.125% trypsin incubation at 37°C for 40 min. After removal of trypsin by centrifugation, the cells were resuspended in Minimum Essential Medium with 10% heat-inactivated fetal bovine serum and cultured on glass coverslips in Maximow chambers. Scanning electron microscopy was used to document chemically-induced degeneration as well as the enhanced regenerative process of the olfactory epithelium after 5 days *in situ*. Quantitative analysis of cultured cells revealed a significant ($P < 0.005$) increase in the number of bipolar neurons compared to the untreated sham controls. Immunofluorescent studies demonstrated the heterogeneous nature of the surviving cell population which contained cells in various stages of development. Keratin-positive stem cells that *in situ* overlie the basement membrane in the olfactory epithelium were present *in vitro* as nests of keratin-positive cells. The receptor neurons, which normally comprise the majority of cells in olfactory epithelium, appeared *in vitro* as bipolar cells that label positive solely for neurofilament protein or for both keratin and neurofilament protein. Some of the bipolar cells had partially developed olfactory vesicles on both poles, suggesting that these cells were maturing olfactory receptor neurons. Further immunofluorescent characterization is in progress. This study demonstrates that an *in situ* toxic insult of olfactory epithelium with $ZnSO_4$ can be used to produce an enriched population of bipolar neurons *in vitro*. Supported by PHS NS24291 to MG.

33.11

ODOR STIMULATION OF C-FOS mRNA EXPRESSION IN RAT OLFACTORY BULB DURING POSTNATAL DEVELOPMENT. K.M. Guthrie* and C.M. Gall. Dept. of Anatomy & Neurobiology, Univ. of Calif., Irvine, CA 92717

We have previously reported that odor stimulation increases c-*fos* mRNA expression by neurons in spatially discrete regions of the glomerular layer (GL), and in underlying fields of the external plexiform and granule cell layers (GCL) of rat olfactory bulb. Some mitral cells are also labeled. We have proposed that this columnar pattern of neuronal activation defines a basic functional anatomical unit of odor processing in the olfactory bulb. Using in situ hybridization of 35S-labeled c-*fos* cRNA, we examined changes in this response pattern in normal rats over the course of postnatal development. At postnatal day 4 (PN4), odor stimulation resulted in broad areas of hybridization throughout the developing mitral and granule cell layers. Labeling of spatially discrete regions of the GL was first apparent at the end of the first PN week. At this age, mitral cell labeling was reduced but hybridization in the GCL still occurred over broad regions. By PN14, mitral cell labeling was further diminished, cell labeling in restricted areas of the GL and underlying GCL was robust, and the spatial nature of the odor response was clearly evident. Air-exposed littermates exhibited low levels of hybridization, and unilateral naris occlusion significantly reduced hybridization in the deprived bulb at both PN4 and PN21, indicating that c-*fos* expression is odor dependent at all ages examined. To determine if gene induction is dependent on NMDA mechanisms (Cole et al., '89), additional rats (PN21) were treated with the NMDA antagonist MK-801 prior to odor stimulation. In these animals, c-*fos* mRNA levels were dramatically increased in all laminae throughout the entire olfactory bulb, suggesting a disinhibition of c-*fos* expression with this treatment. Supported by NIDCD grant DC 01534.

SENSORY DEVELOPMENT: SOMATOSENSORY

34.1

NEONATAL PERIPHERAL NERVE SECTION RESULTS IN A REARRANGEMENT OF THE CENTRAL TERMINALS OF ADJACENT AND SURVIVING AXOTOMIZED PRIMARY AFFERENTS IN ADULT RAT SPINAL CORD. P. Shortland, M. Fitzgerald & C.J. Woolf. (SPON: Brain Research Association). Dept. Anatomy & Neurobiology, St. Louis Univ. Sch. Med., St. Louis MO 63104; Dept. Anat. & Dev. Biol., Univ. Coll. London, London WC1E 6BT.

Neonatal peripheral nerve section results in the death of a substantial number of axotomized primary sensory neurons. In addition, adjacent intact peripheral nerve A and C fibres sprout into the denervated central terminal area (Fitzgerald, '85; Fitzgerald et al., '90). Here we examine the fate of the central terminals of axotomized and adjacent intact A-fibres in adult rats following day of birth sciatic nerve ligation and section. Bulk labelling of the sciatic (Sci N=7) or saphenous (Sa, N=6) nerve using B-HRP revealed alterations in the spinal cord somatotopic map. The intact Sa nerve territory expanded from mid L4 to sacral cord compared to the contralateral control Sa nerve. Labelling the axotomized Sci nerve revealed little change in the gross somatotopic organization of the A-fibres, although labelling was less intense overall compared to control nerves. However, invasion of substantia gelatinosa (SG) by axotomized Sci A-fibres was seen in segments L3-5 and a similar invasion of SG by intact Sa A-fibres was seen in L4-5. These reults demonstrate that (1) surviving axotomized Sci primary afferents generate a "normal" somatotopic map in the rostrocaudal plane; (2) surviving axotomized A-fibres sprout into SG; (3) intact Sa A-fibres sprout into SG as well as invading Sci territories; (4) considerable overlap between adjacent nerve territories occurs in the denervated area of the spinal cord. Supported by the MRC.

34.2

THE EARLY DEVELOPMENT OF ASCENDING SPINAL PATHWAYS IN THE BRAZILIAN OPOSSUM, Monodelphis domestica. Y.Q. Qin, X.M. Wang, X.M. Xu* and G.F. Martin. Dept. of Anatomy, The Ohio State Univ., Coll. of Med., Columbus, Ohio 43210.

Monodelphis, a marsupial, is born 14-15 days after copulation and is available for experimentation at stages corresponding to those which occur in utero in placental mammals. When injections of orthograde tracers were made into the caudal thoracic or lumbar cord prior to postnatal day (PD)3, few if any axons were labeled at supraspinal levels. By PD3, however, labeled axons were found adjacent to the nucleus gracilis, within the ventrolateral medulla and pons, and within the cerebellar anlage. Many of the axons forming the fasciculus gracilis arose from neurons within dorsal root ganglia as evidenced by the transganglionic transport of cholera toxin-HRP from the hindlimbs. By PD7, a few labeled axons had begun to penetrate the inferior olive. The subsequent growth of spinoolivary axons appeared to be restricted to subnuclei innervated at later stages of development and in the adult animal. It was not until PD16 that labeled axons within the cerebellum began to segregate into the longitudinal zones seen in the adult animal. (Supported by NS-25095 and 10165).

34.3

DISCREPANCIES BETWEEN THE MORPHOLOGICAL AND FUNCTIONAL REPRESENTATION OF THE SI FOREPAW MAP FOLLOWING DIGIT REMOVAL IN THE ADULT RAT. C.A. McCandlish*, C.X. Li, and R.S. Waters, Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Col. of Medicine, Memphis, TN 38163.

The representation of the forepaw in SI cortex of adult rats contains an organization of cell aggregates, or barrels, corresponding to the dorsal and ventral surfaces of the contralateral forepaw. This forepaw barrel map undergoes a critical period of development (McCandlish and Waters, 1989) whereby removal of afferent input, prior to the critical period, disrupts the normal barrel pattern. After the critical period deafferentation produces no change in the barrel pattern. This report addresses the relationship between structure and function by examining the consequences of deafferentation on the electrophysiological and morphological representation of the forepaw barrel subfield in adult rat.

Adult rats having undergone amputation of the third digit (D3) were compared to normal controls. Five days after surgery, both groups were anesthetized with Nembutal (40 mg/kg ip), fixed to a headholder, and the parietal cortex was exposed. Carbon-fiber electrodes were used to record single/multiunit responses following mechanical stimulation of the contralateral forepaw. The cortex was systematically explored at 50-75 μm intervals. Receptive fields recorded at each penetration site were used to generate a physiological map of the forepaw. Lesions marked selected recording sites. Animals were sacrificed, hemispheres removed, flattened and sectioned tangentially, and processed using SDH and Nissl-stain. Our results are as follows:

1. Morphological maps of deafferents showed a normal forepaw barrel subfield.
2. Physiological maps of deafferents showed enlargement of D2 and D4 which expanded into the cortical barrel representation formally served by D3.

These results suggest a mismatch between structure and function in the deafferented adult forepaw barrel subfield. (Supported by NSF Grant BNS 88-02766)

34.4

Cortical and Subcortical Components of Plasticity in Rat Barrel Cortex. K. Fox,* Dept. Neuroscience, Brown University, Providence R.I.02912.

Rats were raised with a single D1 vibrissa from P0 to P60. This procedure causes an expansion of the D1 domain in the barrel cortex (Fox, (92) J. Neuroscience 12). To estimate the proportion of plasticity due to cortical changes versus a passive relay of subcortical changes, the D1 barrel was ablated at P60 with 2 to 3 lesions (15 - 25μA, 10s). This procedure abolishes the D1 representation in the cortex of normal animals and would be expected to do the same in uni-vibrissa animals if all the plasticity were of cortical origin. In control animals, the volume of D1 ablated was directly proportional to the loss of D1 responses in adjacent barrels, in close correspondence to previous reports (Armstrong-James et al. (91) J. Comp. Neurol. 303). Inputs from principal and adjacent intact barrels were unaffected. In uni-vibrissae animals, the same linear relation was found between lesion size and reduction of D1 representation, but the slope was steeper. D1 lesions caused a greater proportional reduction in D1 representation in uni-vibrissae animals than in controls. Cells were far less likely to respond to D1 if the lesion was interposed between the cell and the D1 barrel. This *masking effect* was studied further in uni-vibrissa animals by leaving D1 intact, forming a wall of lesions between D1 and D2 and then recording in D2. This type of lesion was the most effective in abolishing D1 responses in the D2 barrel. However, cells in barrels surrounding D1 behind the wall of lesions exhibited the usual large D1 input. These experiments suggest that a large component of the plasticity induced by uni-vibrissae rearing is cortical in origin. Any subcortical plasticity that may occur does not appear to be transmitted to cortex under the conditions prevailing in these experiments. (NS-27759).

34.5

INFLUENCE OF THE THALAMIC PROJECTION PATTERN ON BARREL FORMATION IN RAT SOMATOSENSORY CORTEX. M.Möck*, I.Kehrer and J.Bolz, Friedrich-Miescher Labor der Max-Planck Gesellschaft, 7400 Tübingen, Germany

In rodent somatosensory cortex the distribution of the contralateral mystacial vibrissae and sinus hairs is represented by groups of neurons in layer 4 that are called barrels. At the time of birth the cortex is still immature and the development of the barrels occurs during the following days. In rat they can be first visualized around postnatal day 3 (P3). It is not yet known which factors are responsible for the formation of the barrels. There is strong evidence that thalamocortical afferents play a crucial role in the barrel development, but other subcortical or cortical influences could also be important. In order to examine the factors required for barrel formation we prepared slice cultures from immature somatosensory cortex alone and slice cultures that in addition contained the ventrobasal (VB) thalamus. We used antisera against the α_1 subunit of the GABA-A receptor as a convenient tool to identify the presence of barrels *in vivo* and *in vitro*. In the somatosensory cortex *in vivo* there was no immunostaining at P0 and P1. Immunoreactivity was first detectable at P2 when the antibody labelled a homogeneous band throughout layer 4, and after P3 the staining became patchy and labelled the barrel hollows. When slices from somatosensory cortex (P0-P2) were cultured alone, after about 4 DIV, immunoreactivity was visible as a continuous band in layer 4, but even after 10 DIV never showed the patchy distribution found *in vivo*. In contrast, cultures from somatosensory cortex of the same age co-cultured with VB thalamus, after 4 or more DIV, often revealed a patchy pattern of immunoreactivity which was very similar to that observed *in vivo*. These results suggest that the innervation of axons from the VB thalamus is necessary and sufficient for the formation of barrel-like patterns in the somatosensory cortex.

Supported in part by DFG.

34.6

TARGETING SPECIFICITY OF PRIMARY SENSORY THALAMOCORTICAL AXONS IN DEVELOPING RAT CORTEX.

J.A. De Carlos*, B.L. Schlaggar, and D.D.M. O'Leary.
Molecular Neurobiology Laboratory, The Salk Institute, La Jolla CA 92037.

Thalamocortical afferents promote the differentiation of the developing neocortex into functionally and architecturally distinct areas. We have studied the degree of precision in the matching of axons from the primary sensory thalamic nuclei to their appropriate target regions in developing rat cortex. In adult rats, neurons in the ventrobasal (VB), dorsal lateral geniculate (dLG), and medial geniculate (MG) thalamic nuclei project to S1, V1 and A1, respectively. The fluorescent tracers, FB, DY, DiI, were injected into presumptive somatosensory (S1), visual (V1) and auditory (A1) regions of rats aged from E17, when thalamic axons are growing to their appropriate regions, to P7, well after thalamic axons have arborized in the cortical plate.(E0 is day of insemination, P0 is the day of birth). At P7, the specificity of thalamocortical relationships is adult-like; injections into a primary sensory area did not label neurons in "inappropriate" sensory thalamic nuclei. However, at embryonic and neonatal ages, thalamic neurons can be retrogradely labeled from inappropriate cortical regions; although found in every case, these neurons are rare. This finding indicates that a very small proportion of VB, dLG and MG axons grow to inappropriate cortical regions. What is most striking, though, is the high degree of precision in the targeting of these primary sensory thalamic axons to appropriate cortical regions and their subsequent invasion of the cortical plate. The targeting specificity of thalamocortical axons suggests the operation of a highly effective marking system that matches thalamic axons with their appropriate cortical region, and is consistent with the crucial role of these axons in the differentiation of cortical areas. Support: NIH fellowship F05 TW04401 and grant EY07025.

34.7

AN ACTIVITY-DEPENDENT COMPONENT OF PLASTICITY IN THE DEVELOPING RAT NEOCORTEX. B.L. Schlaggar* and D.D.M. O'Leary. Molecular Neurobiology Lab., The Salk Institute. La Jolla, CA 92037.

The development of barrels in rat somatosensory cortex (S1) requires an intact periphery during the first postnatal week. Electrocautery of a row of vibrissae follicles during this critical period leads to fusion of the corresponding row of barrels, retraction of that row, and expansion of adjacent rows. These effects are most profound following neonatal cautery, attenuate thereafter, and are not evident after P5 (Woolsey & Wann 76; Belford & Killackey 80). Afferent activity is hypothesized to direct the normal formation of these patterns and to mediate developmental plasticity in S1, as has been demonstrated in visual cortex (V1) (Hubel & Wiesel 65; Stryker & Harris 86; Kleinschmidt et al 87). Yet, application at birth of the activity blocker, TTX, to either the infraorbital nerve (Henderson et al 92) or to S1 (Rhoades et al 92) does not disrupt normal barrel development. We have examined whether plasticity in S1 following peripheral manipulation is dependent upon cortical activity. In P0 rats, Elvax (a vehicle suited for prolonged, controlled release of drug - prepared by G. Prusky & M. Constantine-Paton) loaded with either TTX or AP5 (specific antagonist of NMDA receptor) was placed over the right S1. Follicles of the left C-row were destroyed by cautery on either P0, 2, or 3. Row area, visualized with AChE histochemistry in tangential sections, was measured at P7-8, normalized for total barrelfield size, and compared with drug treated, non-lesioned pups and control treated (Elvax with .1 M PBS or L-AP5), lesioned pups. Regardless of the age at cautery, 40-60% of the territorial change is blocked with TTX or AP5. Activity-blockade also interferes with the fusion of C-row in pups lesioned on P2 or 3. Neither AP5 nor TTX disrupted barrel development in non-lesioned animals as determined with AChE. Our results indicate that cortical activity, perhaps mediated through the NMDA receptor, plays a critical role in the developmental plasticity of S1, as in V1. (NIH PO1 NS17763 & RO1 EY07025)

34.8

NON-LINEAR CORTICAL FUNCTIONAL REPRESENTATION CHANGES FOLLOWING PARTIAL DEAFFERENTATION OF RAT FACIAL VIBRISSAE. D.M. Liu, D.A. Sirois, K.M. Gallo and P.J. Hand*, Dept. Animal Biology, Sch. Vet. Med., and Mahoney Institute of Neurological Sciences, Sch. Med., Univ. of Pennsylvania., Phila., PA. 19104.

Previous studies in this laboratory have shown the expansion of a deafferentation spared C3 vibrissa (SC3) representation in first somatosensory cortex (SI). Currently, an important issue in neocortical plasticity is whether cortical neuronal networks alone are sufficient for plasticity or whether subcortical components of a system contribute to the plasticity. In the present study, we investigate the interrelation of functional (metabolic) reorganization in the classical central vibrissa-trigeminal-SI cortical pathway using the 14C-2-deoxyglucose (2DG) method. Unilateral subtotal vibrissa deafferentation sparing C3 (SC3) was performed in Sprague-Dawley rats at postnatal day 2. Ninety days post-deafferentation, central functional representations and local glucose utilization rates (LCGU) produced by activation of SC3 and contralateral control C3 vibrissae (CC3) were compared. Preliminary results revealed that the % increases of the central representation of the SC3 (as compared to CC3) in the trigeminal nuclear complex, ventrobasal thalamus, and SI cortex were 39.13 ± 5.71, 193.26 ± 32.53, and 388.30 ± 35.84 %, respectively. Conversely, the LCGU was attenuated (12.72%) in SI, but showed no significant changes in the above subcortical structures when compared to CC3. The present results demonstrate that chronic vibrissa deafferentation, sparing C3 vibrissa, produced a different magnitude of SC3 representation expansion at the different levels of the central vibrissa-trigeminal pathway. This results in a non-linear increment in SC3 SI cortical functional representation changes that suggest both an autonomous intracortical and a subcortically-dependent plasticity. Supported by NIH grant NS-22283.

34.9

PATTERNED SEROTONERGIC PROJECTIONS TO S1 ARE NOT DEPENDENT UPON CUES IN THE NEONATAL CORTEX. C.A. Bennett-Clarke,*M.H. Hankin and R.W. Rhoades. Dept Anatomy, Medical College of Ohio, Toledo, OH.

In normal perinatal rats, serotonin-immunoreactive (5-HTIR) axons from the dorsal and median raphe nuclei form dense patterned terminal projections in sensory cortices that show a spatial correspondence with the terminal arbors of thalamocortical afferents (TCA). An important issue is how the patterned 5-HTIR projections are established. A previous study (*Soc Neurosci Abstr* 17:745) showed that there was not sufficient topography within the raphe nuclei to account for the establishment of patterned 5-HTIR. Alternatively, developing 5-HT axons may be influenced by cues residing within the developing cortex or by interactions between growing 5-HT axons and elements along the raphe-cortical pathway (e.g., TCAs or cues in the cortical subplate/developing white matter).

To address this issue, we transplanted fetal raphe tissue (E16-18 rat) to the superficial cortex adjacent and caudal to somatosensory cortices in P1 recipient rats. One day prior to transplantation, recipient pups were depleted of endogenous 5-HT by injections of the neurotoxin 5,7 DHT (either intracranial or subcutaneous), or by lesions of the ascending serotonergic pathway (medial forebrain bundle). Control recipient animals received no lesion. Pups were killed at P7-8, cortices and brainstems were processed for 5-HT immunohistochemistry. Animals with a complete loss of patterned 5-HTIR in the cortex contralateral to the transplant were considered for further analysis.

Transplanted tissue usually contained numerous 5-HTIR cells which formed projections that extended as much as 2mm beyond the boundaries of the graft. Transplant-derived 5-HT projections clearly invaded adjacent S1 cortex, but did not, in any case, form a patterned projection.

One explanation for these observations is that the cortex does not contain intrinsic cues that establish the formation of patterned projections of 5-HTIR cortical axons. Further studies will examine whether patterning cues for 5-HT axons are found at subcortical levels.

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34.10

TRIGEMINAL (V) PRIMARY AFFERENT STRUCTURE-FUNCTION RELATIONSHIPS AFTER DEAFFERENTATION OF RAT MOLAR PULP. B.J. Sessle*, J.W. Hu and M.F. Jacquin. Faculty of Dentistry, University of Toronto, Ont. M5G 1G6, Canada; Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Prior studies have documented changes in V brainstem neuron properties 1-2 wks after molar pulpotomy in the adult rat (Hu et al, Neurosci. Abst., 91) including increases in mechanoreceptive field size and the expression of inputs from more than one V division. To test the hypothesis that pulp deafferentation induces altered V primary afferent projections that account for these changes in V brainstem neurons, intra-axonal recording and HRP labeling methods were applied to 21 V primary afferents 10-14 days following pulpotomy of the 3 mandibular molars in adult rats and their collateral and terminal arborization patterns in subnucleus interpolaris were compared to functionally equivalent fibers in normal animals (Jacquin et al, J. Comp. Neurol., 88). All 21 fibers had A-beta conduction velocities and low-threshold mechanoreceptive fields restricted to a single whisker, or a small patch of hairy skin or guard hairs. They gave rise to arbors whose shapes were similar to those of comparable fibers in normal rats. Vibrissa afferents (N = 17) were subjected to quantitative analysis. Total numbers of their collaterals in deafferented rats (mean ± SD: 7.4 ± 1.3) did not differ from those in normals (8.2 ± 1.7). Transverse arbor areas (5945 ± 2215 μm^2) also did not differ from normals (6130 ± 2060 μm^2). Preliminary data also failed to reveal changes in the total numbers of boutons/collateral. Collaterals in, or bordering upon, mandibular regions also were indistinct from normal. These data suggest that spared A-beta primary afferents may maintain normal central morphologies in interpolaris 1-2 wks after mandibular pulp deafferentation. Supported by NIH Grant DE04786.

34.11

Mapping The Developing Rat Trigeminal Complex for Somatostatin and Calbindin D-28k. E.M.Mikota, C.Welt* and S.C.Feldman. Depts. of Orthodontics, Neuroscience and Anatomy,Cell Biology &Injury Sciences. UMDNJ- New Jersey Dental School and New Jersey Medical School, Newark, N.J. 07103.

The trigeminal nuclei contain the neuropeptide somatostatin (SS) and the calcium binding protein Calbindin D-28K. In this study we examined the temporal and spatial changes in the distribution of these proteins at representative ages from embryonic day 15 (E15), the stage at which the trigeminal nuclei are clearly identifiable, to adulthood. Both SS and Calbindin D-28k were demonstrated using previously characterized antisera. SS was not seen at E15, but small numbers of immunoreactive neurons and fibers were present in all three nuclei at birth (day 0). Scattered Calbindin D-28k neurons and fibers were demonstrable in the nuclei at E15 and showed a marked increase at birth. On day 7 the pattern and density of SS- and Calbindin-containing neurons and fibers was not significantly different from that seen in adulthood. The developmental pattern of SS differs from that in cerebral cortex where SS is expressed in neurons which are morphologically immature and are possibly still migrating. However, Calbindin levels in the neocortex and cerebellum peak after migration is complete. The possibility must be entertained that the relationship between expression and neuronal maturity differs for different parts of the CNS.

34.12

ORGANIZATION OF THE PROXIMAL SEGMENT OF THE INFRAORBITAL NERVE AT MULTIPLE INTERVALS AFTER AXOTOMY AT BIRTH: A QUANTITATIVE EM STUDY IN THE RAT. J.Z. Rana, J.P. Golden, J. Davis, W.E. Renehan*, D.S. Zahm & M.F. Jacquin. Anatomy & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Although much is known of the effects of infraorbital nerve (ION) section at birth, little is known about the short- and long-term effects of this lesion upon the ION itself. 19 newborn rats were subjected to left ION section and perfused 1, 2, 4, 7, 17 or 90 days later. Left IONs were removed proximal to the lesion and axon #, types and fasciculation patterns were assessed with EM methods. The axotomized ION contained 13,945 ± 10,335, 14,112 ± 3,501, 16,531 ± 1,904, 9,045 ± 1,465, 7,018 ± 4,212 and 8,672 ± 1,030 axons (mean ± SD) at these respective ages. These values are below the 33,059 reported for the normal adult ION and the 42,219 reported for the newborn ION. At the above-listed ages there were unusually low %s of myelinated axons in the axotomized ION: 8±2, 17±7, 8±5, 8±0, 18±5 and 27±6, respectively, vs. the 60±6% reported for the normal adult ION. The vast majority of the remaining fibers were unmyelinated, whereas degenerating myelinated axons never accounted for more than 2% of all axons. The # of fascicles in the axotomized ION ranged from 3-23 on day 1, 16-24 on day 7, and 11-20 on day 17. These values overlap with those from the newborn ION (5-15) and the normal adult ION (18-25). Thus, 1) extensive, though variable, axon loss occurs within 1 day of neonatal ION section, 2) the 74% fiber loss at 90 days is not achieved until 7 days after the lesion, 3) the preferential survival of unmyelinated axons may underly some CNS effects of this lesion, and 4) CNS pattern alterations induced by ION section do not reflect gross changes in ION fasciculation patterns. DE07734.

TRANSPLANTATION: PARKINSON'S—FETAL TISSUE

35.1

THE TIME COURSE OF FIBER OUTGROWTH IN GRAFTED HUMAN DOPAMINERGIC CELLS DOES NOT SEEM TO OBEY ESTABLISHED PRINCIPLES J.J. López-Lozano* and B. Brera Neurobiology Unit, Dept. of Neurology, and Exp. Surgery, Clínica Puerta de Hierro, 28035 Madrid, Spain

Parallel to our program of human ventral mesencephalon transplantation in parkinsonian patients begun in 1988, we undertook a series of experiments in which human fetal ventral mesencephalic tissue (HFVM) obtained on the same day and under the same conditions as that used for the clinical trials (Restor Neurol 1991;4) was implanted, in cellular suspension form (Brain Res 1989;86:351), into the denervated striatum of immunosuppressed rats (CyA, 10 mg/kg/ip). Our initial aim was to assess the tissue viability and analyze its in vivo behavior as an indirect way of determining the viability of the transplants done in humans. However, after histological and immunohistochemical processing of the tissue (TH and GFAP) and analysis of the results under an Olympus microscope interfaced to an IMCO-10 (Kontron)-MIP operating system (Micron), we observed that not only do the HFVM neurons survive transplantation (as had been reported by other authors), but they are capable of producing long processes (up to 300 µm in flat 40 µm sections) in less time than that reported to date (2-4 wk). This contradicts, in principle, earlier results showing that the time course of fiber outgrowth appeared to be slower for human fetal substantia nigra cells than for other similar animal cells, and leads to the hypothesis that, somehow, the HFVM neurons retain the capacity to govern their ontogenic development and maturation even when isolated from their natural environment. This finding suggests that the tissue management and/or the procedure for procuring the cellular suspension may be important factors in the behavior of the transplanted tissue, and that the environmental conditions (neural cultures or brain implants) may play a significant role in the morphological changes and in the time course of development observed in these cells. These findings merit further investigations.(Supported by CACYT 86/0461 and CO 29/91).

35.2

EFFECTS OF MPTP ON HUMAN NIGRAL TISSUE GRAFTED TO DOPAMINE DEPLETED RATS.
I. Strömberg[1]*, P. Almqvist[2], M. Bygdeman[3], E. Sundström[2]
Departments of [1]Histology & Neurobiology, [2]Geriatrics, and [3]Obstetrics & Gynecology, Karolinska Institute, Stockholm, Sweden.

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is known to cause Parkinson's disease in man. Human fetal tissue was examined for its sensitivity to MPTP. Human ventral mesencephalic tissue was grafted to the lateral ventricle of unilateral dopamine denervated immunosuppressed rats. Rotational behavior was monthly tested and when apomorphine induced rotations were reduced, subcutaneous injections of MPTP were performed. In two out of seven rats tested, the rotational pattern reappeared, and in the rest, apomorphine induced rotations stayed low. Histological evaluations of these two rats with increased turning behavior revealed surviving grafts using antibodies against human specific Thy-1. Few tyrosine hydroxylase-(TH) positive neurons were seen within the graft area, but no TH-positive nerve terminals could be detected in dorsal striatum. However, ventrally a TH-positive network was found. In rats that still had reduced rotational behavior after the MPTP-treatment, both TH-immunoreactive neurons within the grafts as well as a dense TH-positive network was found in dorsal striatum. When using antirat antibodies to detect extravasation of serum proteins, indicating a disturbance in the blood brain barrier, immunoreactivity was detected in parts of the grafts only in those animals with increased rotational behavior after MPTP-treatment. In conclusion, MPTP can affect human nigral tissue grafted to the immunosuppressed rats.

35.3

SHORT TERM CORTICOSTEROID TREATMENT IMPAIRS SURVIVAL OF FETAL DOPAMINE CELLS TRANSPLANTED INTO A RAT MODEL OF PARKINSON'S DISEASE. P. Patino*, EH Kriek, CJ Hutt, JX Qi, and CR Freed. Univ. of Colo. Sch. Med., Denver, CO.

In order to see if the antiinflammatory action of corticosteroids can help survival of fetal dopamine cells following transplant into dopamine depleted striatum, we have injected embryonic ventral mesencephalic tissue (ED 15) into male Sprague Dawley rats which had unilateral 6-OHDA lesions of the medial forebrain bundle. For 4 days after transplant, tapering doses of 30 to 15 mg/kg per day methylprednisolone were administered. Concurrent control animals were transplanted in the same fashion but did not receive steroid injections. Contrary to expectations, animals which received methylprednisolone had lower quality transplants, both as assessed behaviorally with methamphetamine 5 mg/kg i.p. three months after transplant and as measured by tyrosine hydroxylase immunocytochemical staining of the transplant tracks. Using a histologic scale we have developed in which 1 = 1-10 TH positive cells per section and 5 = > 150 cells per section, control animals had an average score of 4.17 ± 0.24 while treated animals had 2.55 ± 0.27. These data indicate that short term corticosteroid administration impairs transplant survival, possibly by reducing inflammation at the time of transplant.

35.4

EVIDENCE FOR HOST-DERIVED GLUTAMATE AFFERENT MODULATION OF INTRASTRIATALLY TRANSPLANTED DOPAMINE NEURONS. T. Kondoh* and W.C. Low. Departments of Neurosurgery and Physiology, and Program in Neuroscience, University of Minnesota Medical School, Minneapolis, MN 55455.

Grafted dopamine neurons have been shown to form functional graft-to-host fiber connections when transplanted into the host striatum. The present study was designed to determine whether fibers from the host brain, in turn, can modulate graft-derived dopamine release. In normal animals glutamatergic afferents to the striatum from the cortex have been shown to regulate nigrostriatal dopamine neurotransmission via axo-axonic connections. We have used methods of *in vivo* microdialysis and electrochemical detection to determine whether similar regulatory mechanisms are established between host glutamatergic afferents and fibers from grafted dopamine neurons. Three groups of animals were studied: rats with unilateral 6-OHDA lesions of the nigrostriatal pathway; rats with unilateral 6-OHDA lesions and grafts of ventral mesencephalic dopamine neurons; and rats with intact nigrostriatal fiber systems. Extracellular dopamine levels in the striatum were determined in each group under basal conditions and following the administration of dihydrokainic acid (DKA), a glutamate uptake blocker. DKA administration in the perfusate (5 mM) resulted in a substantial elevation in dopamine release in normal animals, a modest elevation in animals with 6-OHDA lesions, and intermediate levels in animals with dopamine grafts. Analysis of variance revealed significant group effects [$F(2,12) = 46.52$; $p < 0.05$]. Post-hoc pair-wise comparisons further revealed that dopamine levels in the intact animals were greater than that of 6-OHDA and transplant animals ($p < 0.05$). Moreover, striatal dopamine levels in transplant animals were significantly greater than that of 6-OHDA animals ($p < 0.05$). These results suggest that glutamatergic afferents from the host brain are capable of regulating dopamine release by grafted mesencephalic neurons. (Supported by NIH RO1-NS-24464).

35.5

MORPHOLOGICAL OBSERVATIONS OF FETAL DOPAMINERGIC CELLS TRANSPLANTED IN THE SINGLE DOPAMINERGIC OR COMBINED DOPAMINERGIC/SEROTONERGIC DENERVATED RAT STRIATUM. H.W.M. Steinbusch*, F.L. van Muiswinkel, J.G.J.M. Bol. and J. De Vente Dept. Pharmacology, Faculty of Medicine, Free University, Amsterdam, The Netherlands

Dopaminergic denervation of the caudate putamen complex with 6-OHDA is often associated with an animal model for Parkinson's disease. However, from clinical studies it is known that the disease is not only affecting the dopaminergic system, but that also the serotonergic system is implicated. Due to this discrepancy between the clinical observations and the animal model we investigated the effects of the denervation of both the dopaminergic as well as the serotonergic system on the survival and outgrowth of fetal dopaminergic cells. On the basis of this study we hope to develop a more elaborate model which better reflects the actual state of this disease. Rats were divided into three groups. One group receives only an unilateral 6-OHDA lesion both in the medial forebrain bundle as well as in the vta. The second group receives a single unilateral 6-OHDA lesion in the vta and a combined unilateral mixture of 5,7-DHT and 6-OHDA in the medial forebrain bundle. The third group serves as control. After two weeks group one and two received an injection of a fetal dopaminergic cell suspension (E 15) in the denervated caudate-putamen. After 4 months survival all rats were perfused with paraformaldehyde or glutaraldehyde and processsed for tyrosine hydroxylase-, dopamine- and serotonin-immunohistochemistry. For morphological analysis we used an IBAS 2000 system in which we counted the total number of surviving dopaminergic cells and the total area of newly formed dopaminergic fibres. In both groups we observed a good cell survival and outgrowth of the implanted dopaminergic cells. Possible differences between the two groups will be discussed.

35.6

EFFECT OF CHRONIC LEVODOPA TREATMENT ON THE DEVELOPING NIGROSTRIATAL DOPAMINE SYSTEM. David M. Yurek[1*] and John R. Sladek, Jr.[2] [1]Univ. of Kentucky College of Med., Div. of Neurosurgery and Dept. of Anatomy & Neurobiology, Lexington, Kentucky 40536, and [2]Neuroscience Institute, UHS/Chicago Med. Sch., Chicago, Illinois 60064.

Recent studies suggest that developing dopaminergic neurons may be adversely affected by chronic exposure to dopamine (DA) agonist treatment. For instance, neurite outgrowth of cultured embryonic DAergic neurons appears to be impaired when levodopa is added to culture media. A similar effect is also observed in the development of embryonic DAergic neurons that are grafted into the DA-denervated striatum of animals receiving chronic levodopa treatment. These findings suggest that levodopa treatment may be contraindicative in neural grafting studies that utilize embryonic DAergic neurons as a source of DA replacement therapy.

These studies were designed to examine what effect levodopa might exert on the development of immature DAergic neurons in the normally developing DAergic nigrostriatal pathway. At gestation day 10, pregnant female Sprague Dawley rats began either a chronic regimen of levodopa treatment or saline treatment and these treatments were continued until the day of parturition; tablets of Sinemet® (10:1 levodopa-carbidopa) were pulverized, suspended in 0.9% saline, and administered to the pregnant female rats at a dose of 50 mg/kg (s.c.) twice daily. Subsequently, rat pups exposed to levodopa treatment as fetuses continued to receive levodopa (50 mg/kg, i.p., twice daily) on day 2 and this treatment was maintained for a four week period. After four weeks of treatment these animals were sacrificed and brains were removed for morphological analysis. Both tyrosine hydroxylase immunocytochemical analysis and catecholamine histofluorescent analyses were performed to examine and compare if there were any qualitative and/or quantitative differences between animals exposed to chronic saline and those exposed to chronic levodopa. Preliminary TH ICC analysis indicates that exposure to chronic levodopa produces incongruent patterns of TH staining in the striatum and substantia nigra. These data will be presented with additional morphometric data and the results obtained from histoflourescent studies. This research was supported by the Univ. of Kentucky Med. College Research Fund.

35.7

INCREASED DYNORPHIN LEVEL IN SNR AFTER L-DOPA TREATMENT IS NORMALIZED WITH FETAL DOPAMINERGIC GRAFT. E. Roy, P.-Y. Côté, L. Grégoire, D.P. Gaudin, A. Parent* and P.J. Bédard Neurobiology Res. Center, Fac of Med., Laval Univ., Québec, CANADA

Recently, observations by Engber et al. (Brain Res. '91) show increased dynorphin levels in substantia nigra pars reticulata (SNR) on the lesioned side following chronic intermittent treatment with L-DOPA in 6-hydroxydopamine (6-OHDA) -lesioned rats. The purpose of this study was to confirm whether or not a dopaminergic graft is able to reverse the increase in dynorphin observed with L-DOPA treatment. Female rats were lesioned with 6-OHDA, selected with apomorphine and amphetamine circling tests and distributed in 2 groups: half received a striatal fetal nigral graft. Half of each group was treated chronically with L-DOPA and benserazide. The other group received benserazide and saline and served as control. All brains were processed immunohistochemically to visualize dynorphin (DYN-IR) in the SNR. The results were analysed by computerized image-analysis system using grey scale. The SNR on the lesioned side was compared to that on the unlesioned side. Among lesioned animals, only the levodopa-treated group showed increased DYN-IR on the lesioned side. This increase was abolished by the fetal nigral graft. These results confirm that a fetal nigral graft can correct not only the changes in neurotransmitters due to denervation but also those induced by L-DOPA treatment. [Supported by MRC and Canadian Network of Centres of Excellence]

35.8

FETAL DOPAMINERGIC MICROGRAFTS IMPLANTED INTO THE NEONATAL AND ADULT SUBSTANTIA NIGRA OF 6-OHDA LESIONED RATS. G. Nikkhah, M.G. Cunningham+, W.-M. Duan, K. Wictorin, A. Björklund*. Dept. of Medical Cell Research, University of Lund, S-223 62 Lund, Sweden; +Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.

Dopaminergic (DA) micrografts have been implanted homotopically into the substantia nigra in the developing and the mature CNS to investigate 1) the patterns of morphological integration; 2) spontaneous and drug induced behaviour; and 3) differences related to the state of development and plasticity of the host. Postnatal-day-one (P1) animals recieved bilateral intraventricular injections of 6-OHDA (2x55µg), whereas in the adult rats the right ascending mesostriatal pathway was lesioned stereotactically with 6-OHDA (16µg). Using a glass capillary (OD 50 µm), 200nl deposits of E14 ventral mesencephalic cell suspension were grafted into the right substantia nigra (40-60.000 cells/rat) in P3 pups and adult rats. Different behaviours were tested in the neonatal hosts including spontaneous, stress and drug induced rotation and paw reaching, 3 and 6 weeks post grafting. In the adult recipients drug induced rotation and paw reaching was tested between 6 and 16 weeks post grafting. The brains were then processed for TH-immunohistochemistry. Our results indicate that intranigral DA micrografts can exert functional effects on behavioral deficits both in neonatal and adult 6-OHDA lesioned rats.The grafts were more efficiently integrated into the neonatal than in the adult hosts, and induced a wider spectrum of behavioral recovery. This suggests, that the dynamic influence of the developing brain may promote the anatomical and functional capacities of intranigral DA grafts.

35.9

DOES DISCONTINUATION OF CHRONIC LEVODOPA ADMINISTRATION IN A RAT MODEL OF PARKINSONISM ALLOW RECOVERY OF FUNCTION OF GRAFTED EMBRYONIC DOPAMINE NEURONS? K. Steece-Collier*[1], T.J. Collier[1], F.S. Junn[1], D.M. Yurek[2], and J.R. Sladek, Jr.[3]; [1]Dept. of Neurobiol. & Anat., Univ. of Roch. Sch. of Med., Roch., NY 14642; [2]Dept. Neurosurg., Univ. of KY, Lexington, KY, 40536; [3]The Neurosci.Inst., Univ. Health Sci./Chgo Med. Sch., North Chicago, IL 60064.

We have been exploring the extent to which chronic levodopa therapy may interact with the beneficial effects of embryonic nerve cell grafts in rats with unilateral nigrostriatal lesions. We have previously shown that chronic levodopa (50 mg/kg, b.i.d.,i.p., for 6 weeks) impairs morphology (Steece-Collier et al., 1990, Exp. Neurol. 110:201), decreases effectiveness of grafted neurons to reverse dopamine (DA) agonist-induced rotational abnormalities which accompany unilateral lesions (Yurek et al., 1991, Exp. Br. Res. 86:97), and reduces the ability of grafts to reverse striatal DA receptor changes (Steece-Collier et al., 1990, Soc. Neurosci. Abst. 275.11). We are currently examining whether discontinuation of drug treatment after an initial 6 week administration results in recovery of the aforementioned parameters. Preliminary data from amphetamine-induced rotational studies, again, confirm that a graft in the presence of levodopa is less effective in reversing rotations (14.0% reduction from baseline, n=5) compared to grafts in saline injected controls (69.5% reduction) 6 weeks after transplantation. These same animals were then withdrawn from their respective injection paradigm for another 6 weeks and subsequently tested for response to amphetamine. Overall, there appeared to be functional recovery in the levodopa treated animals (76.4% reduction). However, it should be mentioned that 2 of the 5 animals in this group showed no sign of recovery. Control animals retained approximately the same level of behavioral improvement compared to 6 weeks after grafting. Definitive conclusions regarding the reversibility of the detrimental effects of levodopa subsequent to withdrawal of drug therapy is pending completion of behavioral analysis. We are also examining histological and receptor parameters of these animals. Supported by United Parkinson Foundation.

35.10

QUANTITATIVE ANALYSIS AND SERIAL RECONSTRUCTION OF DOPAMINE NERVE CELL GRAFTS FROM EARLY GESTATION FETAL MONKEY BRAIN. J.R. Sladek, Jr.,* S.J. Cooper, P.E. Hawkins, T.J. Collier[1], J.D. Elsworth[2], J. R. Taylor[2], R.H. Roth[2] and D.E. Redmond, Jr.[2] Neuroscience Institute and Department of Physiology & Biophysics, The Chicago Medical School, North Chicago, IL 60064; [1]Dept. of Neurobiology and Anatomy,University of Rochester, Rochester, NY 14642 and [2]Departments ofPsychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

We developed two approaches for more fully analyzing the geometry and 3-dimensional orientation of nerve cell grafts. We examined grafts from two early gestational stages placed into the head of the caudate nuclei in MPTP-treated monkeys. Animals were sacrificed three months after implantation of solid, ventral mesencephalic grafts. One animal received tissue from a 44 day donor; two others from a single 49 day donor. Tissues were stained for tyrosine hydroxylase and photographic impressions were scanned into a computer-operated microdensitometer where they were aligned, assembled, and edited for 3-dimensional reconstructions and rotations. The second approach allowed direct microscopic video input into a Macintosh Quadra interfaced with a Canon CLC color printer. Percentages were calculated from these digitized, outlined images of each graft and target. The animal that received the full complement of mesencephalic DA neurons contained large,well-circumscribed grafts with over 13,000 DA neurons in total. These six grafts respectively occupied on average 2.6, 7.3, 6.3, 6.4, 6.9, and 8.9 percent of the caudateas seen in two dimensions. At individual coronal levels, grafts occupied as much as 14.8 percent of the area of the caudate. An animal that received one-half of the mesencephalon from a 49 day old embryo had four grafts that could be measured. They averaged 2.5, 5.4, 5.0, and 2.4 percent of the caudate. DA cell numbers in this animal were on the order of 1,300 in total. The use of this technology aids correlations between graft size, number of surviving cells, and degree of improvement from MPTP-induced toxicity as a next step toward developing more optimal transplant procedures Supported by USPHS Grants PO1 NS 24032, BRSG RR-05366 and the Axion Research Foundation. DER is supported by RSA MH00643.

35.11

INFLUENCE OF DONOR AGE ON SURVIVAL OF HUMAN EMBRYONIC NIGRAL GRAFTS T. B. Freeman*, G. M. Nauert, P. R. Sanberg, D. W. Cahill, C. W. Olanow, J. H. Kordower. Div. Neurosurgery, Univ. of S. Florida, College of Medicine, Tampa, Fl. 33606

The age of embryonic donors is critical for graft survival in animal models of parkinsonism. The optimal period for rodent graft harvesting occurs when the substantia nigra is undergoing its early development, but before the nigrostriatal pathway has developed. Transplants harvested after this period are axotomized in the process of graft preparation leading to markedly diminished graft viability. Solid rodent grafts have a slightly prolonged "window" for harvesting when compared to suspension grafts.

We compared the ability of solid and suspension grafts of human embryonic dopaminergic neurons at different embryonic stages to survive grafting into 6-OHDA lesioned immunosuppressed rats. Suspension grafts survived best when donor age was between 34 and 56 postovulatory days. Implants displayed numerous healthy tyrosine hydroxylase immunoreactive (TH-IR) neurons which sent extensive neuritic processes into the host striatum. Suspension grafts survived poorly when donor age was greater than 65 days.

Solid implants of human ventral mesencephalic tissue displayed good-excellent survival of TH-IR neurons when donor age was between 43-65. No solid grafts survived when donor tissue was older than 72 days.

These results demonstrate that the upper limit for survival of human embryonic dopaminergic suspension grafts correlates well with the period of development of the human nigrostriatal pathway. The "window" for donor age of solid human embryonic dopaminergic grafts appears to be extended by about nine days in comparison to suspension grafts.

Many clinical protocols that use neural grafts in the treatment of Parkinson's disease use fetal donors that are older than 65 days. Our data suggests that these protocols are likely to result in transplantation of nonviable grafts. (Funded by a grant from UPF).

35.12

TYROSINE HYDROXYLASE AND C-FOS IMMUNOREACTIVITY IN FETAL TISSUE TRANSPLANTS FOLLOWING TREATMENT WITH D-AMPHETAMINE. Mantana K. Norman*, Sunny Y. Lu, Jennifer M. Klug, S. Murthy Ammisetti, Robert B. Norgren and Andrew B. Norman. Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

Transplanted fetal striatal tissue survives and grows within the adult host striatum and can be innervated by host dopaminergic afferents. It has been suggested that such afferent connections are functional in nature and may mediate the reversal of lesion-induced behavioral deficits. The expression of the immediate-early response gene c-fos in striatal neurons is stimulated by dopamine released from presynaptic terminals by psychomotor stimulants such as d-amphetamine. The expression of the fos protein can act as a neurochemical marker of functional postsynaptic activation. We investigated the relationship between tyrosine hydroxylase (T-OH) immunoreactive fibers and fos immunoreactive cells in fetal tissue transplants. Male Sprague-Dawley rats received unilateral or bilateral intrastriatal transplants of fetal (E 15-17) rat striatum or fetal rat cortex into unlesioned striatum. Five weeks after transplantation, rats were administered d-amphetamine (5 mg/kg i.p.) and perfused three hours later. Individual brain sections were double-labeled for fos and T-OH immunoreactivity using DAB and indophane blue, respectively as chromogens. There was no specific concordance between the fos immunoreactive cells and T-OH containing fibers in either the striatal or cortical transplants. These data may suggest a lack of functional connectivity between T-OH immunoreactive fibers and transplant neurons with respect to the expression of c-fos at five weeks post-transplant. (Supported by RO3 MH45253)

35.13

ALTERATIONS IN MORPHOLOGY AND ELECTROPHYSIOLOGY OF TRANSPLANTED NEOSTRIATAL NEURONS IN RATS: DOPAMINE AND EXCITATORY AMINO ACIDS. E.A. Nansen, R. Cohen, Z. Radisavljevic, M.A. Ariano[1], D.R. Sibley[2], R.S. Fisher and M.S. Levine*, MRRC UCLA L.A. CA 90024, Neurosci. Inst. Chicago Med. Sch. N. Chicago IL 60064[1], Mol. Pharmacol Unit ETB NINDS Bethesda MD 20892[2].

Experiments were designed to assess the presence of dopamine (DA) D_2 receptors on transplanted neostriatal neurons (TSNs) and to determine the functional responses of TSNs to iontophoretic application of DA and excitatory amino acids (EAAs). Standard methods were used to transplant fetal neostriatal cells into the host neostriatum. Some TSNs were first incubated in the fluorescent dye, fast blue to distinguish transplant from host cells. After 12 months, fast blue-containing neurons were found in the transplant. Sections were immunostained with rabbit antisera raised against a 54-peptide sequence of the D_2 DA receptor (PNAS, 1991, 88:1441-1445). Immunoreactivity was visualized in the neuropil and in cells of the transplant as well as the host. However, immunoreactivity was decreased in TSNs compared to the host neostriatum. Previous studies from our laboratory have demonstrated that TSNs become hyperresponsive to synaptic activation. In the present experiment standard brain slice techniques were used to assess the responses of TSNs to application of EAAs and DA. Both TSNs and host neostriatal cells were excited by application of glutamate (GLU) or N-methyl-d-aspartate (NMDA). Threshold currents to induce these responses were significantly lower in TSNs compared to host cells. DA attenuated responses evoked by GLU or NMDA in TSNs. However, while DA attenuated responses evoked by GLU it potentiated responses evoked by NMDA in host cells. Together, the morphology and electrophysiology provide evidence that TSNs display alterations in both DA and EAA systems. Supported by USPHS HD05958.

36.1

REDUCTION OF MOTOR IMPAIRMENT BY ADRENAL MEDULLARY TRANSPLANTS (AMT) IN AGED RATS. F. García-Hernández*, M.T. Pacheco-Cano and R. Drucker-Colín. Instituto de Fisiología Celular, UNAM. Apdo. Postal 70-600, México, D.F.

We have reported that L-DOPA and D-Amphetamine substantially improve the motor performance of *aged* rats in the inclined beam test (IBT). Here we report the effect of AMT upon the motor performance of aged rats in the IBT. Twenty male Wistar rats, mean age 25.6 ± 0.9 months were used. Rats were grafted with 300,000 cultured fetal chromaffin cells, placed into the head of the left caudate nucleus (Group A) or into both caudates (Group B). Group C was grafted with one fresh fetal adrenal medulla as a block into the lateral ventricle. Group D sustained sham grafting. Rats were evaluated for 3 months after grafting. Our results show that cultured AMT and sham grafts had no effect upon the motor deficits of aged rats. Only the block grafted group showed a significant recovery of motor impairment. Cultured cell grafts had poor survival rates with few chromaffine cells. Block AMT showed abundant chromaffine cells with round and neuron like shapes. Results suggest that fresh AMT reduce the motor impairment of aged rats for as long as 84 days. Cultured cell grafts may not induce recovery at least in the aging brain, maybe do to an unfavorable environment.

36.2

THE DIFFERENTIAL RESPONSIVENESS OF CULTURED VS. GRAFTED BOVINE CHROMAFFIN CELLS TO TROPHIC SUBSTANCES. J.D. Ortega*, J. Sagen, and G.D. Pappas. Dept. of Anat. and Cel Biol., Univ. of Il at Chicago, Chicago, Il 60612.

Previous work in our laboratory has demonstrated the long term survival of bovine chromaffin cells grafted into the CNS parenchyma of adult rats. Our studies reveal that bovine chromaffin cell xenograft survival is not compromised by the placement of these grafts into CNS regions lacking in NGF content. In contrast, studies in other laboratories suggest enhanced survival of chromaffin cells in adrenal medullary tissue transplanted in rat CNS by either the cografting of NGF-producing cells or by the continuous infusion of exogenous NGF. In addition, it has also been reported that these grafted chromaffin cells respond to trophic factor supplementation by sprouting processes into the host parenchyma. The purpose of this study was to determine whether exogenous trophic factors could enhance the survival and differentiation of isolated bovine chromaffin cells. Studies on cultured bovine chromaffin cells revealed that a complex combination of factors may lead to enhanced survival and process formation in vitro. For example, while cells maintained in serum-free media with no added trophic factors, or with NGF or bFGF alone did not survive well or sprout processes, the combined addition of NGF and bFGF, or pieces of sciatic nerve markedly increased both chromaffin cell survival and process formation. In contrast, chromaffin cell survival and sprouting did not appear to be enhanced in the CNS grafts. These results suggest that the survival of chromaffin cells in culture is dependent on one or more serum factors and that process outgrowth from cultured cells may be dependent on the combined effects of these serum factors with other growth factors. Furthermore, grafted cells survive transplantation without exogenous trophic factors suggesting that endogenous host factors exist that enhance graft survival. In contrast, it does not appear that the appropriate factors or combination of factors for the promotion of process outgrowth are present in the host CNS (Supported by N.I.H. grants NS28931 and NS25054).

36.3

EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX I AND II IN BOVINE CHROMAFFIN CELL GRAFTS INTO RAT MIDBRAIN. G.J. Rice, R.P. Becker, J. Sagen, and G.D. Pappas*. Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL 60612.

Rats received isolated bovine chromaffin cells into periaqueductal gray (PAG), with or without daily i.p. injections of cyclosporin A (CsA). Rat CNS and lymph nodes were fixed with 4% formaldehyde, cryosectioned at 0.5μm and treated with monoclonal antibodies (MoAb) against rat MHC I or MHC II. Donkey anti-mouse F(ab')$_2$ conjugated to Texas red was used as a secondary antibody for fluorescent light microscopy. For negative controls, the primary MoAb was replaced by non-specific mouse IgG. Lymph node, in which all nucleated cells express MHC I, and in which B cells, macrophages and dendritic cells express MHC II, was used for positive controls.

Normal rat PAG and sham-operated PAG with CsA showed neither MHC I, nor MHC II expression. Eight week transplants of chromaffin cells to PAG without CsA contained few chromaffin cells. In these grafts, MHC I was found associated with endothelial cells, while cells, presumed to be infiltrating leukocytes and/or resident glia, showed staining for both MHC I and MHC II. In contrast, eight week transplants to PAG in rats with CsA contained numerous chromaffin cells, with MHC I staining similar to that of grafts without CsA. In these grafts, however, no MHC II expression was found.

These results show that MHC expression in xenografts of bovine chromaffin cells to PAG, either with or without CsA, is induced by the donor tissue; sham injections do not produce expression of MHC. In CsA animals, MHC II is absent and transplants are robust. In animals without CsA, both classes of MHC are found in the grafts, and chromaffin cells are largely absent. These results suggest that xenograft survival depends, at least in part, on suppression of MHC II expression. (Supported in part by NS25045 and NS28931).

36.4

NEURONAL TRANSFORMATION OF CHROMAFFIN CELLS BY CO-CULTURE WITH PURIFIED SCHWANN CELLS. C. Paramore, S. Meadows*, R. D. Madison, and D. A. Turner. Neurosurgery and Neurobiology, Duke Univ. Med. Ctr., and VA Medical Center, Durham, NC 27710.

Transplantation of adrenal chromaffin cells into the striatum has been proposed as a treatment of Parkinson's disease. Recent experiments have focused on co-grafting chromaffin cells with peripheral nerve, thus providing trophic factors to support the graft. The co-culture of chromaffin cells with cultured, activated Schwann cells may generate phenotypically neuronal chromaffin cells which can survive transplantation and integrate better than standard peripheral nerve co-grafts.

We have tested the hypothesis that purified Schwann cells can rapidly induce chromaffin cells to assume a neuronal phenotype. Neonatal rat chromaffin cells were co-cultured with purified rat Schwann cells. Chromaffin cells were observed to accumulate in clumps on Schwann cells and to extend processes within one week. This effect was much more pronounced than that seen with chromaffin cells cultured solely with 100 ng/ml NGF. Schwann cell-conditioned medium was as effective as NGF in eliciting neurite outgrowth from chromaffin cells. However, these Schwann cell-mediated effects were not blocked by antibodies to NGF. Chromaffin cells co-cultured with dissociated peripheral nerve did not clump or extend neurites. We have demonstrated that purified Schwann cells are able to profoundly influence chromaffin cells to aggregate and differentiate into a neuronal phenotype *in vitro*. This effect is dependent on both soluble factors produced by Schwann cells and on direct contact between cells. We conclude that chromaffin cells may be easily induced to assume a neuronal phenotype in the presence of purified Schwann cells. Striatal grafts of these co-cultures may prove superior to currently utilized techniques of adrenal medulla transplantation in Parkinson's disease.

36.5

COMPARISON OF BOVINE ADRENAL MEDULLARY GRAFTS AND ISOLATED CHROMAFFIN CELL IMPLANTS IN RATS AND HEMIPARKINSONIAN MONKEYS. **S.B. Schueler*, J.D. Ortega, J. Sagen, and J.H. Kordower[1], Dept. Anat. & Cell Biol. Univ. of Illinois Sch. Med., I Department of Neurological Sciences, Rush Presbyterian Med. Ctr., Chicago, IL 60612 USA.**

Our present study compared the survival of intrastriatal implants of whole bovine adrenal medullary suspension with isolated adrenal chromaffin cell suspensions, and isolated adrenal chromaffin cells suspensions recombined with their supporting cells (endothelial cells and fibroblasts). Immunosuppressed rats receiving intrastriatal isolated chromaffin cell suspensions displayed robust grafts containing numerous healthy-appearing tyrosine hydroxylase (TH)- and dopamine-B-hydroxylase (DBH)-immunoreactive chomaffin cells. In contrast, smaller, more necrotic grafts with TH and DBH immunoreactivity were seen in animals receiving whole bovine adrenal medullary suspensions. Interestingly, no surviving chromaffin cells were seen in rats receiving isolated chromaffin cells recombined with adrenal medullary support cells (endothelial cells and fibroblasts). However, rats receiving these latter implants displayed dense TH-immunoreactive fibers in surrounding the graft. Isolated bovine chromaffin cell suspensions were also intrastriatally transplanted into immunosuppressed, hemiparkinsonian Rhesus monkeys. Robust survival of TH- and DBH-immunoreactive xenografts were observed which is in contrast to the poor survival seen previously with whole adrenal medullary autografts. This data suggests that adrenal medullary supporting cells may compromise the chromaffin cell survival. (Supported by the UPF)

36.6

RESTORATON OF STRIATAL PRE- AND POSTSYNAPTIC SYMMETRY FOLLOWING ADRENAL MEDULLA GRAFTS. E.J. Curran* and J.B. Becker, Neuroscience Program and Psychology Department, The University of Michigan, Ann Arbor MI 48109.

Intraventricular adrenal medulla grafts differentially effected the rotational behaviors induced by amphetamine and apomorphine in rats with unilateral lesions of the nigrostriatal dopamine pathway. Some animals showed a graft-induced decrease in the behavioral response to both amphetamine and apomorphine, whereas other animals had a graft-induced decrease only in the response to amphetamine or apomorphine. Bilateral *in vivo* microdialysis experiments revealed that animals with a graft-induced decrease in the behavioral response to amphetamine (AMPH RECOV) expressed an increased symmetry in presynaptic dopamine activity between the two striata. This enhanced presynaptic symmetry was not observed in animals that had a decrease exclusively in the behavioral response to apomorphine (APO RECOV). Examination of postsynaptic D1 and D2 dopamine receptor binding densities with *in vitro* autoradiography revealed a significant increase in D2 receptor binding within the dopamine denervated striatum whereas no lesion effect was observed in D1 receptor binding density. AMPH RECOV animals had a decrease in D2 receptor binding density within the denervated striatum; this effect was most prevalent within the dorsomedial quadrant of the denervated striatum. A slight but significantly smaller decrease in D2 receptor binding was observed in APO RECOV animals. It is concluded that more than one mechanism is promoting the behavioral effects of intraventricular adrenal medulla grafts. AMPH RECOV is associated with increased presynaptic dopamine function and a decrease in postsynaptic D2 dopamine receptor up-regulation. APO RECOV animals do not, however, exhibit increased symmetry in the pre or postsynaptic dopamine measures; indicating that different mechanism(s) may be involved in APO RECOV. (NS22157)

36.7

ENCAPSULATED CHROMAFFIN CELLS ISOLATED FROM BOTH MPTP-LESIONED AND UNLESIONED PRIMATES AMELIORATES A RAT EXPERIMENTAL PARKINSON MODEL.
S.A. Tan*, B.A. Zielinski, S.R. Winn, P.A. Tresco, P. Aebischer. Brown University,Providence,RI 02912

Adrenal chromaffin cell autographs implanted in patients afflicted with Parkinson's disease have been shown to only mildly ameliorate motor deficits. Low Dopamine (DA) content of the adrenals of parkinsonian patients may explain the paucity of improvement. Monkey adrenal chromaffin (MAC) cells isolated from animals rendered parkinsonian by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and from unlesioned primates were selected to study the potential harmful effect of MPTP on chromaffin tissue. Following isolation of the adrenal tissue, the chromaffin cells were encapsulated in an anionic and a cationic polyelectrolyte respectively. HPLC analysis of capsules maintained in vitro revealed release of epinephrine and norepinephrine in tissue isolated from both lesioned and unlesioned animals. Dopamine release, however, was undetectable from both types of isolations and encapsulations. Anionic polyelectrolyte-encapsulated MAC cells were evaluated in the unilateral 6-OHDA lesioned rat model and were shown to reduce apomorphine-induced rotational asymmetry by 40% as compared to empty capsules. DBH and PNMT immunopositive cells were found in animals exhibiting a rotation reduction. MAC cells isolated from MPTP-lesioned and unlesioned primates release comparable levels of catecholamines. Dopamine release, however, is undetectable. The improvement of rotation asymmetry suggests an alternative mechanism of action in the amelioration of rat experimental parkinsonism.

36.8

INTRASTRIATAL DELIVERY OF NERVE GROWTH FACTOR WITH BIODEGRADABLE MICROSPHERES.
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The survival and behavioral effect of adrenal medulla (AM) grafts in the striatum improve with the prolonged, local administration of NGF. We have developed biodegradable, biocompatible polymeric microspheres for the sustained delivery of NGF. Microspheres (3-25 μm diameter) were prepared with Poly(L-Lactide)-Co-Glycolide 70:30, according to Ogawa's method, with 0.025% (w/w) NGF (mouse 7S,Sigma). *In vitro* release was detected up to 5 weeks using enzyme-linked immunosorbent assay. NGF biological activity was preserved throughout this period as demonstrated by the chick embryo dorsal root ganglion assay, which was blocked with rabbit anti-2.5S NGF. The *in vivo* release was shown with immunohistochemical detection of NGF inside and surrounding microspheres implanted in the cat striatum. The effect of these microspheres on the behavioral recovery after stereotaxic AM grafts in 6-OH-dopamine lesioned male Sprague-Dawley rats is currently being studied. In our preliminary experiment three groups of 4 rats each received either 1) AM fragments, 2) AM with 5 mg of NGF microspheres or 3) 5 mg of microspheres solely. Followed for 5 months, significant ($p < 0.01$) reduction of apomorphine (0.1 mg/kg SQ) induced rotations compared to the preoperative performance was observed only in the first two groups, although NGF microspheres did not increase the magnitude of this reduction. Although this initial result is possibly due to the low concentration of NGF used, the results show that growth factors can be delivered with this approach. Supported by the United Parkinson Foundation and the American College of Surgeons.

36.9

FUNCTIONAL RECOVERY IN HEMIPARKINSONIAN PRIMATES TRANSPLANTED WITH POLYMER ENCAPSULATED CELLS
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Neural grafting using cellular xenografts which are protected from immune rejection by permselective membrane capsules provides an unlimited potential source of neuroactive substances for the treatment of degenerative neurologic diseases. The therapeutic potential of this approach was evaluated in a non-human primate model of Parkinson's disease. Eight *macaca fascicularis* monkeys exhibited a contralateral, Parkinson-like movement disorder following unilateral intra-carotid injection of MPTP. In order to allow quantitative behavioral assessment of the lesion before and after neural grafting, primates were chaired and trained to pick food rewards from multiwell plates prior to MPTP lesioning. The functional deficit was evaluated daily by measuring the time required for each upper limb to independently empty trays loaded with food treats. All animals showed significant unilateral impairment of their ability to pick food from the tray following MPTP injection. PC12 cells, a dopaminergic cell line, were encapsulated and transplanted into the striatum of 5 hemiparkinson monkeys. Four out of 5 of these animals showed a significant behavioral improvement. Tyrosine hydroxylase positive, encapsulated PC12 cells were observed in the lesioned striatum for up to 5 months post-implantation. The three remaining monkeys were implanted with either encapsulated bovine chromaffin cells or empty polymer envelopes and showed no amelioration of their behavioral deficit. These results suggest that the delivery of dopamine through cell encapsulation technology represents a promising avenue for the treatment of Parkinson's disease.

36.10

IMMUNOCYTOCHEMICAL ANALYSIS OF PC12 CELL IMPLANTS INTO RODENT AND MONKEY STRIATUM.
B.R.Frydel,* P.E. McDermott, M.J.Donegan, P.M.Krueger, F.A.Kaplan, M.Goddard, A.Signore, D.F.Emerich, CytoTherapeutics, Inc. Providence, RI 02906

PC12 cells encapsulated within polymeric membranes have been reported to survive in xenogeneic hosts for extended periods of time and alleviate the behavioral deficits in both rodent and primate models of Parkinson's disease (PD) (Aebischer et al,*Exp Neurol* 111:269, 1991). The safety of this approach in treating human disease depends on the rejection of xenogeneic PC12 cells in discordant hosts. The present study evaluated the survival of unencapsulated PC12 cells following transplantation in allo- and xenogenic recipients. Using methods described previously (Frydel et al, *Soc Neurosci Abst.* 17:569,1991) we injected PC12 cells at a concentration of 10^6 cells/4ul of PBS into rodent striata (Sprague-Dawley rats and Hartley guinea pigs), and 10^6 cells/50ul into 4 different sites of cynomolgus monkey putamen and caudate. Rodents were sacrificed at 1, 2, 4, 12 and 24 weeks, and monkeys at 12 and 24 weeks after injections. All animal brains were processed and stained for tyrosine hydroxylase, glial fibrillary acidic protein, a cell surface marker for PC12 cells (Thy1.1), Nissl substance, and hematoxylin and eosin. All rats showed surviving PC12 cells 1 week after injection and about half developed tumors by two weeks. Guinea pigs with PC12 cell implants showed surviving PC12 cells 1 week after the surgery, but beyond this time point only glial scars with scattered macrophages and no surviving PC12 cells were observed. In monkey brains, after 12 and 24 weeks, all four injection tracks were visible because of residual scarring. Histological analysis demonstrated macrophages, glial cells, and scattered to occasional eosinophils. No traces of PC12 cells were detected. These data indicate that PC12 cells do not survive unencapsulated in a xenogenic host, suggesting that the use of encapsulated PC12 cells may be a viable and safe approach for treating human diseases such as PD.

36.11

ENHANCED CATECHOLAMINE OUTPUT FROM ENCAPSULATED CHROMAFFIN CELLS IN LESIONED VS. NON-LESIONED RAT STRIATUM.
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Polymer-encapsulation provides an immunoisolatory retrieval system to evaluate the influence that site-specific and/or lesion effects may have on transplanted cells in the CNS. The present study analyzed the catecholamine release profile of encapsulated bovine adrenal chromaffin (BAC) cells prior to and following a 6 week implantation period in lesioned vs. non-lesioned rat striatum. Rat striatum was lesioned with either 20 nm of 6-OHDA in ascorbate, or 225 nm quinolinic acid in PBS. Controls were injected with vehicle alone. Three weeks after the striatal injections, the 6-OHDA (n=6) and quinolinic acid (n=6) animals were bilaterally implanted with 5 mm long tubular polymer capsules containing BAC cells. The capsules were pre-qualified and matched according to basal, 20 μM nicotine-evoked, and 56 mM potassium-evoked release of norepinephrine (NE) and epinephrine (EPI). Six weeks after implantation the capsules were retrieved, placed in PBS, and re-assayed. In contrast to pre-implant values, the retrieved capsules, irrespective of striatal treatment, released proportionally more NE and EPI than their *in vitro* counterparts. Furthermore, capsules retrieved from lesioned striatum revealed at least a 10 and 5 fold increase in the output of nicotine-evoked NE and EPI, respectively, when compared with their *in vivo* controls. These results suggest that the lesioned conditions provide an environment that enhances BAC cell survival and/or functionality as assessed by an apparent up-regulation of nicotinic receptors.

36.12

ENCAPSULATED PC12 CELLS PROMOTE RECOVERY OF MOTOR COORDINATION AND BALANCE IN AGED RATS.
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Aged rats exhibit disturbances in balance and coordinated movement together with a generalized decrease in locomotion. Previous studies have demonstrated that these age-related deficits may be partially corrected by the systemic administration of dopaminergic agonists or implantation of dopamine-rich nigral grafts into the striatum. The following studies evaluated the ability of encapsulated dopamine-rich PC12 cells to reduce the motor deficits in aged rats. Prior to surgery, aged (24 months) and young (6 months) rats were tested on a battery of tests to evaluate locomotion, strength, coordination and balance. Relative to young animals, the aged animals were: *(1) hypoactive; (2) remained suspended from a horizontal wire for less* time; (3) were unable to descend a wooden pole covered with wire mesh in a coordinated manner; (4) fell more rapidly from a rotating rod and (5) were unable to maintain their balance on wooden beams of varying widths. PC12 cell-loaded capsules were sectioned into 4 mm lengths and cultured in vitro to establish a basal level of dopamine release using HPLC. Following testing, the aged animals were randomly divided into one of three experimental groups (N=6-8/group) and received either no implant, empty capsule implants or PC12 cell-loaded implants. All implants were bilateral into the striatum and animals were retested three weeks following surgery. Following implantation of encapsulated PC12 cells, animals exhibited a significant ($p < 0.05$) amelioration of coordination and balance when tested on the rotating rod and wooden beams. No recovery was observed on the other behavioral tests and no effects were observed in any of the animals receiving empty capsule implants. Immunocytochemical analysis revealed the presence of numerous tyrosine hydroxylase-positive PC12 cells within the implanted capsules. This study further supports the notion that implantation of encapsulated neurotransmitter-secreting cells may provide a useful treatment for age-related human degenerative diseases.

37.1

EFFECT OF ARGININE ANALOGUES ON INDUCIBLE NITRIC OXIDE SYNTHASE ACTIVITY OF RAT ASTROCYTES. S.F. Roberts*, E. Galea, D.J. Reis and D.L. Feinstein. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Primary astrocyte cultures express a Ca^{2+}-independent NOS in response to treatment with lipopolysaccharide (LPS) (Galea et al., *Soc. Neurosci. Abstr.*, 1992). We examined the subcellular distribution and effects of inhibitors of the induced enzyme. Astrocyte cultures were prepared from neonatal rat cortex, exposed to LPS (500ng/ml for 24 hr), harvested, and NOS activity was determined by the conversion of ^{14}C-L-arginine to ^{14}C-L-citrulline. Both the cytosolic and membrane fractions contained NOS of similar specific activity, but the total activity recovered for the membrane-associated form was less than 50% of that recovered for the soluble enzyme. The apparent K_m and V_{max} values obtained for membrane astrocyte NOS were 15.3 μM and 93.2 pmol $min^{-1}mg^{-1}$, respectively, and were comparable to those of the soluble enzyme. The arginine analogs N^G-methyl-L-arginine (NMA), N^G-amino-L-arginine (NAA) and N^G-nitro-L-arginine (NNA) inhibited the soluble astrocyte NOS with comparable potencies, with NAA giving the lowest IC_{50} (11.7 μM in the presence of 20 μM L-arginine). The IC_{50}s obtained for NNA and NAA inhibition of the membrane astrocyte NOS were nearly identical to their respective values for soluble astrocyte NOS, but NMA exibited a lower IC_{50} with the membrane NOS (3.1 μM compared to 12.8 μM with the soluble enzyme). When assayed under the same conditions, NNA was a much more potent inhibitor of the constitutive, Ca^{2+}-dependent NOS from bovine cerebellum (IC_{50} 0.95 μM). We conclude that (a) inducible astrocyte NOS is distributed into both membrane and soluble fractions; and (b) the induced astrocyte NOS enzymes show an inhibition pattern that closely resembles that of the macrophage inducible NOS and is distinct from the brain constitutive type.

37.2

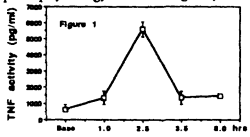
INDUCTION OF CALCIUM-INDEPENDENT NITRIC OXIDE SYNTHASE ACTIVITY IN PRIMARY RAT ASTROCYTE CULTURES. E. Galea*, D.L. Feinstein and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

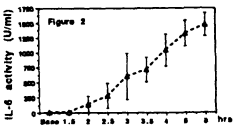
Nitric oxide synthase (NOS) is constitutively expressed as a Ca^{2+}-dependent form in brain. We sought to determine whether an inducible (Ca^{2+}-independent) form of NOS, characteristic of macrophages, can be expressed in cells of the CNS. Primary cultures of neonatal rat cortical astrocytes or neurons were exposed to bacterial lipopolysaccharide (LPS). Incubation of glia with LPS resulted in the appearance, within 8h, of NOS activity directly related to duration of exposure and dose of LPS, with exposure of 1h sufficient to induce enzyme. Induction was potentiated by interferon-γ and blocked by actinomycin-D indicating de novo transcription. Cytosol of LPS-treated cultures produced a Ca^{++}-independent conversion of L-arginine to L-citrulline completely blocked by N^G-monomethyl-L-arginine, indicating the presence of induced NOS. This enzyme had an apparent K_m of 16.7 μM for L-arginine, and was dependent on NADPH, FAD and tetrahydrobiopterin. A polyclonal antibody to inducible mouse macrophage NOS (courtesy C. Nathan) stained 20% of cells in culture in which the content of microglia/macrophages is < 5%. Thus, only a subpopulation of astrocytes express NOS protein. In C6 glioma cells, LPS alone had no effect but coincubation with TNF-α and/or IL-1β significantly increased NOS activity. LPS did not induce NOS in primary neuron cultures. We conclude that astrocytes can express an inducible form of NOS differing from the Ca^{++}-dependent constitutive form of neurons, but similar to inducible NOS of macrophages. The expression of the enzyme may be modulated by cytokines. Inducible NOS of glia may contribute to the neuronal damage associated with ischemic, degenerative and/or demyelinating diseases.

37.3

HUMAN ASTROCYTE CYTOKINE PRODUCTION IN RESPONSE TO ENDOTOXIN. S.Sharif*, I.B.G. Ghajar, R. Wang and R.J. Hariri. Aitken Neurosurgery Laboratory, Cornell University Medical College, New York, N.Y. 10021

Recent evidence indicates that astrocytes perform various functions essential to homeostasis in the CNS. A relatively new role ascribed to these cells involves immunocompentency, suggesting that astrocytes function in a similar fashion to macrophages. We recently reported that human astrocyte cultures generate leukotrienes in response to mechanical injury, and exposure to LPS (Shepard, etal 1991). We sought to determine whether the response of human astrocytes in culture to LPS included cytokine production characteristic of intracranial inflammation. Human astrocyte cultures were established from normal human brain surgical specimens (Hariri, etal, 1989). Primary through tertiary confluent cultures were exposed to 0.5μg/ml LPS (E. coli 0127:B9, Difco Labs, Detroit, Mich) and serial supernatant collections were made. Studies were performed in 4 cell lines run in duplicate. Levels of TNF-a were determined by a cytotoxicity assay (WEHI-164 clone fibrosarcoma cell line) (Espevik et al, 1986). IL-6 bioactivity was determined using IL-6 dependent B cell hybridoma B9 as previously described (Aardon et al,1987). Il-1b activity was measured by an enzyme linked immunoassay (Cayman Chemical, Mich.) We observed an increase in TNF-a as early as 1 hr after exposure which peaked by 2.5 hrs and declined by 8 hrs (figure 1). IL-6 activity was detectable 2 hrs after exposure to LPS and continuously rose thoughout the 8 hr experimental period.(Figure 2) IL-1b activity was detectable 4 to 24 hrs after LPS exposure. These data suggest that human astrocyte production of immunoregulatory molecules following exposure to LPS may play a role in the pathophysiology of neurologic dysfunction associated with the sepsis syndrome.





37.4

ARACHIDONIC ACID INDUCES INCREASE IN CYTOSOLIC CALCIUM CONCENTRATION IN RAT BRAIN ASTROCYTES (RBA-1). T.I. Peng*[1] T.C. Jou[2] P.D. Coleman[3] S-S. Sheu[4] [1]Neurosci. Prog. [3]Dept. Neurobiology & Anatomy. [4]Dept. Pharmacology, Univ. Rochester, Rochester, NY 14642. [2]Yang-Ming Medical College, Taipei,Taiwan

Arachidonic acid (AA) has been shown to be released from neurons upon neurotransmitter stimulation and been implicated to play important roles in synaptic plasticity. The effect of AA on cytosolic Ca^{++} concentration ($[Ca^{++}]i$) was studied in an astrocyte cell line (RBA-1) which was isolated and purified from neonatal rat cerebrum. $[Ca^{++}]i$ was measured with fluorescent Ca^{++} indicator Indo-1. A slow, progressive rise of $[Ca^{++}]i$ was observed when AA was applied exogenously. Latency of onset of rise of $[Ca^{++}]i$ was about 30 seconds to 1 minute, while time needed to reach to the plateau ranged from 3 to 5 minutes. ED_{50} of this response is about 20μM which elevated $[Ca^{++}]i$ into low micromolar range. With 35μM AA, the response started to get saturated. The action of AA was unaffected by inhibitors of cyclo-oxygenase (indomethacin), lipoxygenase (eicosatetraynoic acid) (ETYA), cytochrome P450 epoxygenase (proadifen). Other unsaturated fatty acids also exerted similar effect but with much less potency; whereas saturated fatty acids had little effect. Increase of $[Ca^{++}]i$ was mainly due to Ca^{++} influx across plasma membrane, which could be blocked by Ni^{++}, but not by Cd^{++} or nifedipine ``(voltage sensitive channel blockers). In conclusion, these data show that arachidonic acid induces a rise of $[Ca^{++}]i$ via influx of Ca^{++} across plasma membrane, probably through receptor operated calcium channel. The effect of arachidonic acid is not mediated via its metabolites, but by a direct action of arachidonic acid.

37.5

ROLE OF EXTRACELLULAR CARBONIC ANHYDRASE IN CNS. M. Chesler* & JCT Chen. Dept. of Neurosurg. & Dept. of Physiology & Biophys., NYU Med. Ctr., 550 First Ave., NY, NY 10016.

In brain, carbonic anhydrase (CA) is localized mainly within glia (1), yet, its inhibition with acetazolamide markedly increased *extracellular* pH transients associated with neural activity (2). These effects are difficult to reconcile with a role of intracellular CA, but can be readily explained by a decreased rate of buffering, due to inhibition of putative extracellular CA. We addressed this issue in rat hippocampal slices using a dextran-conjugated inhibitor of CA. Extracellular alkaline transients, detected with double-barreled pH microelectrodes, were elicited in three ways: (a) by repetitive stimulation of the Schaeffer collaterals, (b) by pressure ejection of glutamate or (c) by pressure ejection of NaOH.

Superfusion of slices with 10 μM acetazolamide enhanced extracellular alkalinizations evoked by all three methods. The enhancement of the alkaline shifts evoked by NaOH ejection (405 ± 74 % over control) suggested that this was mainly an extracellular effect. This was confirmed using a CA inhibitor (CL 13475) conjugated to a high molecular wt dextran (3) This compound, superfused at 1 mg/ml, enhanced stimulus and glutamate evoked alkaline shifts by 232 ± 29 % over control. Pressure ejection of the dextran conjugate caused a reversible, 4-5 fold enhancement of alkaline shifts evoked by glutamate or NaOH ejection.

We conclude that brain extracellular space has sufficient CA activity to significantly speed the rate of buffering by CO2 and bicarbonate. Our results suggest that extracellular CA is necessary to rapidly buffer alkaline shifts that accompany excitatory synaptic transmission. (1) Giacobini (1962) J. Neurochem. 9:169-177. (2) Kraig et al. (1983) J. Neurophysiol. 49:831-850. (3) Karlmark et al. (1979) Acta Phys. Scand. 106:145-150. Supported by NINDS grant NS-27011.

37.6

ANALYSIS OF THE QUANTITATIVE RELATIONSHIP BETWEEN RECEPTOR DENSITY AND CALCIUM REGULATION IN ASTROGLIA. Y. Shao and K.D. McCarthy*. Dept. of Pharmacology, The University of North Carolina, Chapel Hill, NC 27599-7365

Alpha$_1$-adrenergic receptor (α_1-AR) agonists elevate $[Ca_i^{++}]$ in 60-80% of astroglia *in vitro*. Likewise, 60-70% of astroglia exhibit specific binding sites for the α_1-AR selective antagonist, ^{125}I-HEAT. The density of α_1-AR binding sites varies markedly on individual astroglia, ranging from a few to over 2,000 binding sites per 1,000 um^2 surface area. In the present study, we examined the relationship between the density of α_1-AR binding sites on astroglia and their ability to respond to α_1-AR agonists with a rise in $[Ca_i^{++}]$, using a video-based imaging system combined with receptor autoradiography. The ability of a given concentration of phenylephrine (PE) to elicit a calcium response correlated well (r = 0.98) with α_1-AR density, the higher the receptor density the greater the probability that a given astroglial cell would respond to PE. However, the amplitude of the Ca^{++} response did not correlate with the α_1-AR density. Cells with a low α_1-AR density (< 10 binding sites/1,000 um^2) could generate a rapid Ca_i^{++} response with a similar amplitude to that of cells with a high α_1-AR density (> 1,000 binding sites/1,000 um^2). To evaluate the relationship between receptor occupancy and calcium response, we varied PE concentrations and α_1-AR density while monitoring the calcium response of individual cells. Interestingly, for a given cell the PE dose range required to move from zero to maximal increases in $[Ca_i^{++}]$ was very narrow (< 5x). Over this same dose range the latency between exposure to PE and the Ca_i^{++} response decreased. Irreversible inactivation of up to 90% of α_1-ARs by treatment with phenoxybenzamine reduced the potency of PE but not the maximal Ca_i^{++} response obtained. In summary, our results indicate that most of astroglial cells express a substantial level of 'spare' α_1-ARs that increase the sensitivity of these cells to α_1-AR agonists. Once activated, individual astroglial cells tend to generate a maximal calcium elevation which is independent of the total α_1-AR density.

37.7

SPREADING DEPRESSION, TO A GREATER EXTENT THAN SEIZURE ACTIVITY ALONE, UPREGULATES LEVELS OF mRNA FOR GLIAL FIBRILLARY ACIDIC PROTEIN. D.J. Bonthius*, J.L. Stringer, E.W. Lothman and O. Steward. Departments of Neuroscience, Pediatrics and Neurology, University of Virginia, Charlottesville, Virginia 22908.

It has been shown that spreading depression and seizure activity can each lead to upregulation of mRNA for glial fibrillary acidic protein (GFAP). The purpose of this study was to compare the relative strengths of spreading depression and seizure activity as signals for upregulation of mRNA for GFAP. Stimulating electrodes were placed bilaterally in the angular bundle, and glass microelectrodes were placed bilaterally in the dentate gyrus granule cell layer of urethane-anesthetized rats. One of the recording electrodes was a double barrel electrode with the second side filled with potassium exchanger for detection of the level of extracellular potassium. Stimulus trains of 20 Hz were delivered through the right angular bundle stimulating electrode. Stimuli were given every 5 minutes for a total of 18 stimuli. The duration of seizure activity was measured. Some of the animals (n=5) had only seizure activity, while others (n=3) had seizure activity accompanied by spreading depression, as documented by a large DC shift and an increase in extracellular potassium concentration to greater than 15 mM . The animals were killed 24 hours after the last stimulus, and levels of GFAP mRNA were assessed by *in situ* hybridization. Seizure activity in the absence of spreading depression induced a 4-fold increase in the level of GFAP mRNA in the dentate gyrus hilus, dentate gyrus molecular layer and hippocampal stratum lacunosum. Spreading depression induced a 10-fold increase in these same regions. The degree of upregulation correlated with the occurrence, but not the duration, of seizure activity and spreading depression. The results suggest that spreading depression is a substantially stronger signal than seizure activity for GFAP mRNA expression. Supported by NIH Grants NS12333 and NS28877.

37.8

GLIAL CELLS *IN SITU* IN HIPPOCAMPAL SLICES EXPRESS VOLTAGE-ACTIVATED Na^+ AND K^+ CHANNELS

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Glial cells in culture express a large range of ion channels. To assess whether such channels are also expressed *in vivo* we obtained whole-cell recordings from both thin (100-150 μm) and standard (400-500 μm) slices of 4-28 day old rat hippocampi. Recordings from both preparations showed voltage-activated ion channels to be expressed in astrocytes and oligodendrocytes. *Oligodendrocytes* typically had low input resistances and large K^+ conductances at rest. Resting potentials were between 60-70 mV. The predominant current recorded was an inwardly rectifying K^+ current (Kir) with little time-dependence. *Astrocytes* expressed up to 3 types of time- and voltage-activated K^+ currents: i. activating, non-inactivating outwardly rectifying K^+ currents (Kd), ii. transient, "A"-type K^+ currents, and iii. inwardly rectifying K^+ currents. Resting potentials in astrocytes were highly variable (-40 to -95 mV in 2.5 KCl), and appeared to differ within hippocampal subregions, being more negative in stratum moleculare than in radiatum or the cell layers of the CA regions. Different K^+ channel complements were also observed in astrocytes in these regions. Although most astrocytes only expressed K^+ currents, in a few instances, small transient inward Na^+ currents were recorded in addition to (Kd-type) K^+ currents. Na^+ current amplitudes in astrocytes (30-100pF, corresponding to current densities of 1-3 pA/pF) were much smaller than in hippocampal neurons which had Na^+ currents > 20nA, corresponding to current densities of up to 300 pA/pF. *(Supported by the VA, NIH, and NMSS)*

37.9

A PATCH-CLAMP STUDY OF ION CHANNEL EXPRESSION BY ASTROCYTES *IN SITU*. B.A. Clark* and P.Mobbs, Dept. Physiology, University College London, Gower St., London, WC1E 6BT, UK.

The expression of voltage-gated and receptor-operated ion channels by astrocytes has been extensively investigated in glial cultures obtained from different regions of the central nervous system. However, much less is known about the channels expressed by astrocytes *in vivo*. We have developed a whole-mount preparation of the intact isolated rabbit retina in which a population of astrocytes are readily accessible for whole-cell patch-clamping. This population of cells were identified as astrocytes by labelling with an antibody directed against glial fibrillary acidic protein and also by filling the cells with Lucifer Yellow during recordings. We have previously shown that these astrocytes have non-NMDA-type glutamate-gated channels and $GABA_A$-type chloride channels (J.Neurosci. Feb 1992). Since these agonists depolarise astrocytes *in vivo* we have investigated the expression of voltage-gated currents in these cells. Whole-cell patch-clamp experiments revealed the presence of a tetrodotoxin-sensitive fast sodium current, time dependent and time independent potassium currents and an L-type calcium current.

The functions of these channels are unknown, however, the combination of receptor-operated and voltage-gated channels found in these astrocytes provides a means by which neurons may influence potassium buffering and other proposed glial cell functions.

Supported by the MRC, Wellcome Trust and Wolfson Foundation

37.10

POST-SYNAPTIC POTENTIALS RECORDED FROM GLIAL CELLS OF THE PITUITARY PARS INTERMEDIA. L.A. Mudrick-Donnon, P.J. Williams, Q.J. Pittman, and B.A. MacVicar. Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Although glial cells have historically been considered passive elements, they have been shown recently to possess both voltage dependent and ligand gated ion channels. We have now observed that at least one type of glial cell, the stellate cell of the pituitary pars intermedia (PI), can, like neurons, respond to synaptic input with post-synaptic potentials. The stalk attched-whole pituitary preparation was used with a stimulating electrode placed on the pituitary stalk to activate afferent fibres. The PI is divided into discrete lobules and comprised primarily of melanocytes and stellate cells; intracellular recordings were obtained from both cell types. Whereas melanocytes demonstrated a neuron-like profile, the stellate cells possessed electrophysiological characteristics of macroglia. Stellate cells were identified by a resting potential more negative than -65mV, low input resistance (<50 MΩ), and no detectable voltage activated conductances. Stalk stimulation evoked a Ca^{2+}-dependent post-synaptic response in the stellate cells consisting of a short depolarization (<100 ms) and a long lasting hyperpolarization (45-75 s). The depolarization was mimicked by GABA application and blocked by the $GABA_A$ antagonist bicuculline (10 μM). The hyperpolarization was mimicked by dopamine and blocked by the D_2 antagonist sulpiride (2 μM). To confirm the glial nature of these cells, neurobiotin was injected into the impaled stellate cells and subsequently visualized. The tissue was then immunostained for GFAP. Positive double staining was observed in all stellate cells from which post-synaptic potentials had been recorded. These data provide the first demonstration of synaptically-evoked potentials in glial cells. Tonic synaptic modulation of glial K^+ and Cl^- permeability could regulate both extracellular homeostasis and melanocyte excitability.

This work was supported by the MRC of Canada.

37.11

DEHYDRATION-INDUCED PROLIFERATION OF SUPRAOPTIC NUCLEUS (SON) AND POSTERIOR PITUITARY ASTROCYTES REVISITED.

P. Murugaiyan*, P.S. Klinkhachorn and A.K. Salm, Dept. of Anatomy, West Virginia University, Morgantown, WV 26506.

Previous studies have shown a proliferation of astrocytes in the posterior pituitary and SON after a prolonged period of slight dehydration (Exp. Neurol, 1968, 20:460; JCN, 1977, 175 (4):373). We are examining this model system in order to gain insight as to the factors governing adult astrocyte mitogenesis in vivo. The proliferation marker bromodeoxyuridine (BrdU) was used to study mitogenesis in astrocytes and other cells in the SON and pituitary of dehydrated rats. Ten adult male rats were dehydrated by substitution of 2% NaCl for water for periods ranging from 1,3,6 and 9 days. During this time, these animals and their paired controls were injected with 20mg/kg of BrdU every day. Following fixation and histological processing, 6μm sections of SON and pituitary were labelled with fluorescent antibodies to BrdU and the astrocyte marker glial fibrillary acidic protein (GFAP). Observations of sections in each group revealed a dose-dependant increase in the number of BrdU+ cells in both the SON and posterior pituitary of dehydrated rats as compared to controls. Many of these cells were also GFAP+. The onset of proliferation was observed in posterior pituitary as early as 24 hours following saline substitution. In addition to the above findings, we also saw a substantial proliferation of cells in the anterior pituitary under all conditions as well as a decrease in the basal level of BrdU+ cells in the intermediate lobe of dehydrated subjects relative to their controls. These results suggest that astrocytes in the SON and posterior pituitary are very rapidly sensitive to mitogens accompanying dehydration and that this sensitivity continues as the stimulus persists. (Supported by BSRG# 9410(300) and NSF# BNS-9109827).

37.12

ASTROGLIAL "ENSHEATHMENT" OF INERT, NEURON-SIZED BEADS. A. K. Salm*, Dept. of Anatomy, West Virginia University, Morgantown, WV 26506.

Neurons in the central nervous system are extensively ensheathed by astrocytic processes, except in regions where synaptic, or other, contacts are made with adjacent cells. What factors influence where and how this ensheathment is accomplished? In these studies, astroglia were co-cultured with inert, 23 μm diameter, polystyrene beads to examine how these cells interact with beads in the presence or absence of neuron-derived biochemical cues. Beads added to astroglial cultures floated. However, some began to have contact with underlying astroglia within 4 hours. By 12 hours, numerous beads were firmly adherent to cells; even when cells were plated sparsely, suggesting that a dynamic interaction united them. After 4 days, many beads were almost entirely surrounded by astrocytic processes. Phase-contrast microscopy, 3-dimensional confocal microscopic reconstructions of fluorescently immunostained material, and scanning EM, shows that the "ensheathment" appears to be accomplished by a contortion of the astroglia, coupled with the extension of numerous processes around the beads. When doubly labelled with antibodies to the astrocyte marker GFAP and BODIPY-phalloidin, both GFAP+ and actin filaments were seen to be present in processes, although their distribution differed. In parallel studies, when beads were plated onto confluent rat meningeal fibroblasts or alveolar macrophages; only infrequent bead-cell complexes developed after 2 weeks in culture. In other experiments, beads were plated onto astroglia that had been previously co-cultured with neurons, and then the neurons removed. Such cells displayed an elongated morphology and failed to form any complexes with beads. Initial results with this model system suggest that astrocytes may constituatively act to ensheath neuron- like objects in their environments, and that this process may be modulated by neuron-derived cues. (Supported by BRSG 9410(300).

37.13

VIP AND FORSKOLIN INDUCES NUCLEAR TRANSLOCATION OF PKC ISOENZYMES IN RAT CORTICAL ASTROCYTES. Z. Oláh[1], Cs. Lehel[1], D.E. Brenneman[2], A. Buonanno[2]*, W.B.Anderson[1] and D.V. Agoston[2]; [1]Laboratory of Cellular Oncology, NCI, [2]Laboratory of Developmental Neurobiology, NICHD, National Institutes of Health, Bethesda, MD 20892,

Astrocytes synthesize and secrete trophic substances critical to development and function of the central nervous system (CNS); however, the extra-and intracellular signalling pathways are poorly understood. Here we demonstrate that treatment of neonatal cortical astrocytes with vasoactive intestinal peptide (VIP), which has been demonstrated to act as secretagogue in this system, and the adenylate cyclase activator, forskolin, caused the redistribution of protein kinase C isoforms (PKC) from the cytoplasm to the nucleus. Primary cortical astrocytes derived from rat pups were treated with various concentrations of VIP and forskolin, PKC activity, subcellular distribution and the presence of various PKC-isotypes were measured in Western-analysis using polyclonal antibodies specific to different isoforms of PKC. Western-blot analysis revealed that rat cortical astrocytes possess the classical PKC α, but PKC β or PKC γ isotypes were not detected. In addition, we identified two novel isotypes (δ and ζ) present in rat cortical astrocytes. While PKC α isoform was found to be predominantly cytosolic in localization, PKC δ was associated with the plasma membrane and nuclear fractions. Following exposure to either VIP (subnanomolar-to-nanomolar range) or forskolin (micromolar range) most of the PKC-activity become nuclear in a concentration-dependent manner. Exposure of astrocytes to 1 μM PMA for 24 h caused the down-regulation of both PKC α and PKC δ, while neither the level nor distribution of PKC ζ was altered. Treatment of astrocytes with both VIP and forskolin resulted in elevated PKC α and δ protein levels in the nuclear fraction, while the subcellular distribution of PKC ζ remained unaltered. The observed changes in the nuclear localization of PKC α and PKC δ in response to the neurotransmitter VIP and to forskolin may be part of the intracellular signalling pathway mediating various cellular responses including the release of trophic molecules.

37.14

CYTOKINE INDUCTION OF L-ARGININE-DEPENDENT cGMP PRODUCTION IN GLIAL CELLS. M.L. Simmons* and S. Murphy Dept. of Pharmacology, College of Medicine, Univ. of Iowa, Iowa City, IA 52242

Primary astrocyte cultures and the C-6 rat glioma cell line can both be induced by lipopolysaccharide (LPS) to express a calcium-independent nitric oxide synthase (NOS). Such nitric oxide production has been previously demonstrated in crude cell homogenates using a bioassay for cGMP production, as well as by L-arginine dependent cGMP production in the intact glial cells themselves. In support of these previous findings, NOS-induction can also be demonstrated by increases in L-[^{3}H]-citrulline production from LPS-treated cells loaded with L-[^{3}H]-arginine. The NOS can also be induced in both primary cultures and C-6 cells by 17-18 hour treatment with the cytokines interferon-γ (IFN-γ), interleukin-1β (IL-1β), or tumor necrosis factor-α (TNF-α), as demonstrated by L-arginine-dependent (Nω-nitro-L-arginine methyl ester inhibited) cGMP production in intact cultures. While dexamethasone (1μM) could strongly attenuate or prevent NOS induction by LPS in either cell type, it had little or no effect on induction by cytokines alone or in combination. Induction by maximal concentrations of a single cytokine could often be potentiated by concurrent treatment with an additional cytokine or LPS, implying that multiple mechanisms of induction may be involved. These findings suggest that astrocytic nitric oxide production may play a role in immunological responses within the CNS. Supported by NS 29226 and the Life and Heath Insurance Medical Research Fund.

37.15

LASER CYTOMETRY OF POTASSIUM STIMULATED CHLORIDE ION UPTAKE IN CCF-STTG1 HUMAN ASTROCYTOMA CELLS USING MQAE M. Patricia Leuschen* Joint Division of Newborn Medicine, Creighton University - University of Nebraska Medical Center, Omaha,NE. 68198

CCF-STTG1, a human astrocytoma cell line, responds in a dose dependent manner to increased extracellular potassium (HiK) with cell swelling as measured by ^{14}C-3-O-methyl-D-glucose studies and cell injury as monitored by LDH efflux and MTT cytotoxicity studies. When evaluated using ^{36}Cl, the K$^+$ stimulated cell swelling was accompanied by chloride uptake. MQAE, a chloride sensitive fluorescent indicator (Molecular Probes, Eugene, OR) was used with an ACAS 570 laser cytometer (Meridian, Okemos, MI) to monitor the Cl$^-$ exchange in response to HiK. CCF,STTG1 cells grown on #1 glass were loaded with 5mM MQAE in 1:1 buffer:distilled water. Single cells were identified, scanned continuously at 410nM and monitored for 15 min under control conditions then 60mM HiK was added and scans continued for 15 min; KSCN and valinomycin were added to quench MQAE fluorescence. Standard curves were constructed to quantitate [Cl$^-$]. Control cells lost <5% fluorescence in 15 min; autofluorescence was minimal. MQAE studies documented a rapid exchange of Cl$^-$ (millisec) followed by a slower (min) sustained influx in response to HiK. The MQAE probe and ACAS laser cytometer are extremely sensitive to changes in pH and temperature but allow very specific monitoring of single cell anion exchange. The system has dramatic potential as a tool for evaluating the role of ion exchange in regulating astrocyte swelling due to hypoxia, release of neurotransmitters such as glutamate or HiK.

37.16

INTRACELLULAR pH REGULATION IN PRIMARY RAT ASTROCYTES AND C6 GLIOMA CELLS. L.D. Shrode, R.W. Putnam and J.B. Dean*. Dept. of Physiol. & Biophys., Wright State Univ. School of Medicine, Dayton, OH 45435.

Intracellular pH (pH_i) was studied in cultures of neonate rat brain astrocytes and C6 glioma cells (at 80-90% confluency) using the pH-sensitive fluorescent dye BCECF. Steady state pH_i (pH_o=7.4, CO_2-free, 37°C) in astrocytes was 7.01 ± 0.03 (n=58) (mean ± SE) and in C6 cells was 7.28 ± 0.02 (n=83). Upon removal of 30 mM NH_4Cl, astrocytes acidified to a pH_i of 5.97 ± 0.04 (n=24) and then recovered at a rate of 0.21 ± 0.02 pH units/min (n=8), while C6 cells acidified to 6.08 ± 0.04 (n=28) and recovered at a rate of 0.31 ± 0.03 pH units/min. In both cell types pH_i recovery was abolished by 1 mM amiloride or by a Na-free solution (N-methyl-D-glucamine). Upon exposure to 5% CO_2 (24 mM HCO_3, pH_o=7.4), astrocytes acidified to 6.84 ± 0.04 (n=18) and subsequently recovered at a rate of 0.31 ± 0.03 pH units/min to a new steady state pH of 7.29 ± 0.04. C6 cells exposed to CO_2 alkalinized at a rate of 0.23 ± 0.04 pH units/min (n=9) to a new steady state pH of 7.56 ± 0.02. CO_2-induced alkalinization was abolished by Na-free solutions in both cell types. Prolonged exposure to Cl-free solutions inhibited CO_2-induced alkalinization in astrocytes by 62% and in C6 cells by 61%. The remaining Cl-independent recovery was nearly abolished by DIDS in both cell types. Preliminary experiments, with acute exposures of cells to Cl-free CO_2-containing solutions, showed that under such conditions pH_i alkalinized from 6.91 to 7.34 at a rate of 0.35 ± 0.02 pH/min in primary astrocytes and from 7.1 to 7.53 at a rate of 0.26 ± 0.06 pH/min in C6 cells. These are similar to the cell pH responses to CO_2 exposure in the presence of Cl. In C6 cells, this alkalinization is abolished by Na-free solutions (0.04 ± 0.01 pH/min). *In summary*, pH_i regulation is similar in C6 cells and primary astrocytes, and is mediated by Na-H exchange and by two types of Na/HCO_3-dependent transporters (Cl dependent and Cl independent). Na-independent Cl/HCO_3 exchange, if present in these cells, has very low activity.

37.17

Astrocyte-dependent inhibition of C6 glioma cell growth in cultures treated with ß-Xyloside. D. Lorton*, M. Dority, and K. Brunden. Gliatech, Inc., Cleveland, OH 44122.

Previous studies support a role for proteoglycans (PG) in tumor cell growth and metastasis. In this study, a culture paradigm was developed to determine the effects of a PG biosynthesis inhibitor on glioma growth and migration. C6 glioma cells were seeded in a defined area onto a confluent monolayer of neonatal rat astrocytes and grown in medium that contained 1 mM 4-methyl-umberiferyl ß-D-xyloside (ß-xyloside) or in medium without this agent. ß-xyloside treatment results in a decreased synthesis of PG and an accumulation of free glycosaminoglycan (GAGS) in the culture medium.

C6 cells grown in the absence of ß-xyloside developed into a mass of thick cell clumps which grew laterally over the astrocyte monolayer. The glioma "tumor" diameters in the untreated group were 14.7 mm, 30.8 mm, and 36.7 mm by days 9, 16, and 21, respectively. The C6 cells grown with ß-xyloside remained a monolayer throughout the study period and had slower lateral growth than the untreated cells. The diameter of treated tumors were 7.4 mm, 11.6 mm, 14.1 mm and 16.5 mm by days 9, 16, 21 and 28, respectively. The ß-xyloside effects were astrocyte-dependent since C6 cells seeded onto dishes in the absence of the astrocyte monolayer covered the plates by 28 days regardless of the presence of the PG inhibitor. A similar slowing of growth and migration were observed when U 87 MG human astrocytoma cells were treated with ß-xyloside. These data suggest that an alteration of PG biosynthesis or an increased release of GAGS can affect the growth and/or spread of glioma cells in vitro.

37.18

THE ROLE OF A GLYCOPEPTIDE INVOLVED IN HOMOPHILIC ADHESION OF THE TRANSFORMANT EXPRESSING P0. T.Yazaki [1,2], H.Asou*[1], K.Kitamura[3], M.Miura[1], S.Toya[2] and K.Uyemura[1] Depts. of [1]Physiology and [2]Neurosurgery, Keio University School of Medicine, Shinjuku-ku, Tokyo 160, Japan; [3]Dept. of Physiology, Saitama Medical School, Saitama 350-04, Japan.

Protein zero(P0), a major glycoprotein in mammalian peripheral myelin, is a member of the immunoglobulin superfamily and plays a role in the formation and maintenance of peripheral myelin by homophilic adhesion, and in the promotion of neurite outgrowth of dorsal root ganglion neurons [T.Yazaki *et al*, Biomed. Res. 1991]. To determine its functional domains, P0 protein was expressed in C6 glioma cells by transfection with rat P0 cDNA. Dissociated cells were resuspended in complete medium and incubated for various times, and the number of particles at each time point was measured. The total number of particles decreased to 24% in 60 min for transformant (C6P0) cells, in contrast to only 68% for control (C6P0') cells. To clarify the mechanism of adhesion, mixed-cell aggregation experiments with DiI-labeled C6P0' cells and DiO-labeled C6P0 cells were performed. P0-expressing C6P0 cells adhered predominantly to each other to form aggregates, with the exception of attachment to a few C6P0' cells at the periphery. The purified glycopeptide of P0 protein inhibited adhesion by 80%, while synthesized peptides (amino acid sequences 83-90 and 100-107) did not cause a significant inhibition. These results suggest that the glycopeptide portion of P0 protein is an important domain in homophilic adhesion. [Supported by grants-in-aid for Scientific Research from the Ministry of Education, Science and Culture in Japan.]

38.1

THE RESUMPTION OF RAPID AXONAL TRANSPORT FOLLOWING A REDUCTION IN TEMPERATURE. R.E. Snyder* and R.S. Smith. Department of Applied Sciences in Medicine, and Department of Anatomy and Cell Biology, University of Alberta, Edmonton, Canada T6G 2G3.

Although it has been determined that the rapid axonal transport of protein ceases at low temperatures, little is known regarding transport following a return to normal temperatures. In this study we report on the transport of both protein and organelles subsequent to a reduction in temperature. All studies were performed using an *in vitro* sciatic nerve preparation of *Xenopus laevis*. Nerves were cooled to 1.0-2.5 °C for 1.5-13 h followed by a return to room temperature (23 °C). Transport of ^{35}S-methionine-labeled protein was studied using a position-sensitive detector of ionizing radiation and liquid scintillation analysis, and transport of organelles was studied in isolated axons using computer-enhanced video DIC microscopy.

Upon return to room temperature protein was found to resume anterograde transport within <30 min, but at a velocity of only 25-50% of its pre-cooling value. Up to 90% of the protein observed moving prior to cooling did not resume transport upon warming, and that protein which did resume transport underwent deposition to a stationary phase at a rate 5-10 times greater than normal. Anterograde organelle transport resumed within <15 min following warming, but with a reduced flux that gradually increased with time. Fifteen minutes following warming organelles were observed to move at normal velocities, however with long pauses between movements. We conclude that a reduction in temperature affects both the axonal transport mechanism and the relationship that exists between the carrier organelles and transported protein. (This work was supported by the Medical Research Council of Canada and the University of Alberta Central Research Fund.)

38.2

TEMPERATURE DEPENDENCE OF RAPID ANTEROGRADE AND RETROGRADE AXONAL TRANSPORT IN AMPHIBIAN NERVE R.S. Smith*, R. Oates and R. E. Snyder. Department of Anatomy and Cell Biology and Department of Applied Sciences in Medicine, University of Alberta, Edmonton, Canada T6G 2H7

We have determined the temperature dependence of the velocity of rapid anterograde and retrograde organelle transport in sciatic axons of Xenopus laevis in order to compare the transport properties of organelles with those of radiolabeled proteins in the same animal. Organelle motion in isolated myelinated axons was detected using computer-enhanced video DIC microscopy. To control specimen temperature, the microscope was cooled in a cold room and heated with an infra-red lamp. Specimen temperature was measured with a thermocouple inside the glass nerve chamber in the vicinity of the isolated axons.

Organelles were classified on the basis of their direction of transport, their apparent size (sub or supraresolution), and shape (circular or rod-shaped). Anterogradely transported subresolution organelles (images ~ 0.2 μm dia.) showed an exponential dependence of velocity (V μm/s) on temperature (T °C) over the range 0-35 °C: $V = 0.23 \exp(0.0843\ T)$, with a Q_{10} of 2.3 and apparent activation energy, E, 14 kcal. Retrograde subresolution organelles had a similar exponential temperature dependence of velocity: $V = 0.157 \exp(0.0976\ T)$, with a Q_{10} of 2.7 and E, 16 kcal. Supraresolution circular organelles moved slightly slower than subresolution organelles, but otherwise had identical temperature dependencies. Velocities of these organelles had a coefficient of variation that was large (36 ± 2%) and independent of temperature. Rod-shaped organelles (presumptive mitochondria) had velocities that were poorly correlated with temperature. We conclude that subresolution organelle transport has characteristics similar to those described for rapid protein transport with two notable exceptions: the coefficient of variation of organelle transport is much larger than that of protein transport, and unlike protein transport organelle transport continues uninterrupted below 5°C. (Supported by MRC and NCE, Canada)

38.3

TRANSPORT KINETICS OF NEUROFILAMENTS IN MOUSE OPTIC AXONS. P. Paggi*[1,2], R. J. Lasek[1] and M. J. Katz[1]. [1]Bioarchitectonics Cntr. CWRU School of Medicine, Cleve. OH 44106. [2]Dip. Biologia Cellulare e dello Sviluppo, Univ. "La Sapienza" 00185 Roma, Italy.

In mouse optic axons, there are relatively few neurofilaments (NFs) and the NF proteins are more lightly labeled than other slowly transported SCb proteins, which, however, move faster than the NFs; thus the radio label of some of the faster-moving SCb proteins may confuse NF proteins analyses that use one dimensional SDS/polyacrylamide gel electrophoresis (1D-PAGE). To test this possibility, 35S- or 3H-labeled amino acids were injected into the right vitreous body of anesthetized mice. At 1-198 days after the injection, the mice were killed and 2 mm segment of the right optic nerve (at 3-5 mm from the posterior of the eye) was removed to compare the NF kinetics obtained by 1D-PAGE and by the higher resolution two dimensional isoelectric focusing/PAGE (2D-PAGE). We found that 1D-PAGE is insufficient for definitive NF kinetics in the mouse optic axons. By contrast, 2D-PAGE provides essentially pure NF kinetics. In mice, more than 97% of the labeled NFs were distributed in a unimodal wave that moved at a continuum rates, between 3.0 and 0.3 mm/day, and less than 0.1% of the NF population traveled at very slowest rates of less than 0.005 mm/day. These results are inconsistent with the proposal (Nixon & Logvinenko, J. Cell Biol. 102: 647, 1986) that 32% of the transported NFs remain within the optic axons in an entirely stationary state. As has been found in other axons, the axonal transport system of mouse optic axons moves NFs relentlessly from the cell body to the axon tip.

38.4

DENDRITIC TRANSPORT OF NEWLY SYNTHESIZED RNA IS DISRUPTED BY MICROTUBULE POISONS. R. Kleiman,* G. Banker, and O. Steward, Dept. of Neuroscience, Univ. of Virginia, Charlottesville, VA., 22908.

Neurons transport newly synthesized RNA into dendrites via an energy-dependent mechanism. Some newly synthesized RNA is retained in dendrites following detergent extraction, suggesting that RNA that is in transit is associated with elements of the cytoskeleton (Davis et al., 1987, Nature, 330:477-479). We were interested in determining whether microtubules or microfilaments play a role in the dendritic transport of RNA. Rat hippocampal neurons maintained in culture for 14 days were treated with nocodazole to disrupt microtubules, or cytochalasin D to disrupt actin microfilaments. Neurons were pulse-labeled with ^{3}H-uridine for one hour to label newly synthesized RNA, then transferred to medium containing an excess of unlabeled uridine for an additional six hours to allow for dendritic transport. Neurons maintained in cytochalasin D exhibited essentially normal RNA synthesis and dendritic transport. Neurons treated with nocodazole 3 hours before the start of labeling and maintained in nocodazole until the end of the experiment did not transport the recently synthesized RNA out of the cell body. To determine if the effects of nocodazole were reversible, neurons were treated with nocodazole for 9 hours and then returned to control medium to allow for microtubule re-assembly. Neurons were then pulse labeled with ^{3}H-uridine for one hour and maintained in unlabeled medium for 13 hours before being fixed and processed for autoradiography. Under these conditions there was a partial recovery of dendritic transport, although the distance of RNA transport was less than in control neurons. These findings are consistent with the hypothesis that dendritic transport of RNA depends upon microtubules. Supported by NIH grant NS23094. RK was the recipient of predoctoral fellowship HD07323.

38.5

MEMBERS OF THE *MYOSIN I* FAMILY IDENTIFIED IN EMBRYONIC RAT BRAIN TISSUE.
C. Ruppert*, J. Reinhard, J. Godel and M. Bähler.
Friedrich-Miescher Laboratorium of the Max-Planck Society, 7400 Tübingen; Germany.

Myosin I molecules are single headed motor-molecules which produce force along actin filaments. There is suggestive evidence that *myosin I*'s are involved in organelle movements and growth cone motility, processes playing an important role during neuronal development. Therefore, we have begun to investigate the presence and identity of *myosin I* motor molecules in the developing rat brain. Degenerate oligonucleotide primers matching to different conserved sequence regions of known myosin molecules were used to amplify several cDNA fragments by PCR from embryonic rat brain RNA. With these fragments we have screened cDNA libraries and isolated clones corresponding to three different *myosin I* molecules, **myr.1**, **myr.2** and **myr.3** (***myosin I* rat**). The expression pattern of these clones in various rat tissues was analysed by northern blotting. All the clones under investigation exhibited a mRNA size of 4-5kb. All three showed a complex nonidentical expression pattern with a noticeably high expression in lung tissue. The message of **myr.1** is highly expressed in embryonic brain tissue but downregulated in adult brain. Therefore, **myr.1** may play a role during neuronal development. Furthermore, our data provide evidence that there exists a family of *myosin I* molecules in mammalian brain.

38.6

AXOPLASMIC PROTEINS MICROINJECTED INTO AXONS ARE RETROGRADELY TRANSPORTED TO THE CELL SOMA AND IMPORTED INTO THE NUCLEUS. R. Schmied, C.-C. Huang and R.T. Ambron*. Dept. Anatomy & Cell Biol. and Otolaryngology, Columbia Univ. P&S, New York, NY 10032

An unresolved question is how the distal axon communicates with the cell body to cause the changes in gene transcription required for regeneration and plasticity. We recently discovered a novel transport pathway that conveys proteins retrogradely from the axon to the nucleus (J. Neurosci., in press). This was shown using human serum albumin (HSA) coupled to a nuclear import signal peptide (sp) and rhodamine (r). When microinjected into axons of Aplysia neurons *in vitro*, rHSA-sp was rapidly transported along the axon and then into the nucleus; transport was blocked by co-injection of r-HSA-sp with sp, indicating that this process is dependent upon a saturable sp-recognizing receptor. To see if this pathway has physiological relevance, Aplysia axoplasm was extruded from nerves and the soluble proteins were separated by size. When fractions were microinjected into axons *in vitro*, one contained protein that was conveyed to the nucleus. This fraction had an 83 kDa protein that can also be isolated from the nucleus and is recognized by an anti-sp IgG. This protein is likely to be a retrogradely transported component.

The axonal transport phenomenon was observed *in vivo* in two different preparations. rHSA-sp was retrogradely transported within axons of nerves after application to their cut ends and after microinjection into the varicosities of neurons innervating heart muscle. These findings suggest that neurons *in vivo* use this transport mechanism for certain axonal proteins to signal the nucleus of events acting at the periphery.

38.7

ENDOCYTOSIS AND TRANSPORT OF CON A-COATED LATEX BEADS BY GROWING NEURITES. Q. Zheng, B. Lu*+ and M.M. Poo. Dept. of Biol. Sci., Columbia Univ., N.Y., NY 10027; +Lab. Mol. Cell. Neurosci., Rockefellor Univ., N.Y., NY 10021.

Transport of a specific population of endocytic organelles was examined using Con A-coated latex beads as a probe. The latex beads (100-800 nm) were coated with rhodamine-concanavalin A (Con A). After a brief incubation with the cultured *Xenopus* spinal neuron, the Con A-beads were endocytosed by the neuron and transported within the neuritic processes, as monitored by high-resolution time-lapse video microscopy (DIC and fluorescence). We found that most beads were collected by the motile filopodia of the growth cone and were transported to the base of growth cones on the filopodia surface, as shown by the effective quenching of bead fluorescence with membrane-impermeant ions. Bead movement on the filopodia surface (0.15-0.2 μm/s) was independent of the bead size and was actin-based, since it was inhibited by cytochalasins. The beads became internalized at the growth cone base, which appears to be the primary site of endocytosis. Internalized beads initially moved in retrograde direction. At later times, both anterograde and retrograde motions along the neurite were observed. Bead transport along the neurite is microtubule-based, since colchicine inhibited the motion, and the transport rate reached a maximum of about 1-2μm/sec, a value similar to that of rapid axonal transport in other systems. With limited number of endocytosed beads in each neurite and unambiguous tracing of individual beads over prolonged periods, the present method offers an useful approach for studying the regulatory mechanisms for the transport of cytoplasmic organelles.

38.8

CHARACTERIZATION OF THE INTERMEDIATE FILAMENT PROTEIN PERIPHERIN IN THE RAT SCIATIC NERVE.
S. Chadan, K.L. Moya, M.M. Portier, L. DiGiamberardino* and G. Filliatreau. INSERM U334 and CNRS URA 1285, C.E.A., S.F.H.J., Orsay, and Cellular Biochemistry Lab, Collège de France, Paris, France.

Peripherin (56kD, pI 5.6), a neuron specific type III intermediate filament protein, is expressed in primary sensory, sympathetic and motor neurons. Intermediate filament proteins share a common molecular structure which is thought to contribute to their ability to form filamentous networks in a coil-coil complex stabilized by disulfide bonds. We used metabolic labeling and gel electrophoresis to study the axonal transport of peripherin in rat motor axons. The results show that peripherin is transported in two waves in the slow component a (SCa) of anterograde axonal transport. In the faster wave, peripherin is cotransported with NF-L.

In experiments using non reducing conditions, we observed by electrophoresis analysis, a high molecular weight protein (120kD, pI 5.6; P120) that was recognized by a peripherin-specific polyclonal antibody. In order to characterize P120 further, we used monoclonal antibodies that recognize epitopes common to intermediate filament proteins. Under non-reducing conditions, P-120 is recognized by all antibodies used, suggesting that P120 is also an intermediate filament protein. The prevention of *in vitro* disulfide bond formation by the use of iodoacetamide shows that P-120 is not an artifact of the sample preparation conditions. Furthermore, incubation of P-120 in the presence of ß-mercaptoethanol resulted in a single 56kD band recognized by all the antibodies.

In general, there is a diminution of peripherin after birth, while relative levels of the dimeric form diminish more rapidly than those of the monomeric form. It is noteworthy that the induction of sciatic nerve regeneration leads to a marked increase in peripherin levels in the dorsal root ganglia starting 7 days after the nerve crush. The increase in P120 is greater than for monomeric peripherin. Our results show that peripherin is co-transported with NF-L and that it can form homodimers, which may provide an important buidling block for the intermediate filament network.

38.9

FORWARD FLOW OF PLASMA MEMBRANE ALONG THE LENGTH OF GROWING NEURITES. S. Popov and M-m. Poo*. Dept. of Biol. Sci., Columbia Univ., N.Y., NY 10027.

It is generally accepted that neurite grows by membrane insertion at the growth cone. In this study we have examined the pattern of new membrane addition in growing *Xenopus* neurites. The bulk membrane flow on the neurite surface was studied by monitoring the distribution of fluorescent lipid molecules, which were locally inserted into a small segment of the neurite within 60-70 μm from the growth cone. Results of quantitative digital imaging showed that the center of diffusion profile of these lipids shifted forward with an average rate of 2.0 + 0.2 μm/min (s.e., n=12), a value close to the rate of neurite extension in these cultures, suggesting that forward flow of plasma membrane along the neurite may account for membrane extension at the growth cone. Further evidence of membrane flow was provided by the observation of yolk granules attached to the surface of neurites. These granules moved forward with the same speed as lipid flow, suggesting that they can be used as markers of bulk membrane movement. We found that the rate of forward movement of the granules increases linearly with the distance from the soma, resulting in increasing separation with time between yolk granules on the same neurite. This increase in the distance between granules cannot be accounted for simply by the thinning of neurite, and membrane insertion must occur along the neurite. The data also suggest that on the average 35% of membrane extension in the neurite can be attributed to membrane flow from soma to the neurite and little or no preferential addition of new membrane occurred at the growth cone.

38.10

ULTRASTRUCTURAL LOCALIZATIONS OF A NEURONAL GROWTH-ASSOCIATED PROTEIN, SCG10 IN THE ADULT RAT BRAIN
Y.SUGIURA, K.IKEDA# and N.MORI* Division of Neurogerontolgy, Andrus Gerontology Center, Univ. Southern California, Los Angeles, CA 90089-0191, #Neuroscience, Beckman Research Institute, City of Hope, Duarte, CA91010

Neuronal growth-associated proteins(nGAPs), such as GAP-43/B-50, MAP2 and synapsin, contributes to the development and maturation of the nervous system. SCG10 is one of the nGAPs, which was initially identified as a neuronal marker in the developing peripheral nervous system. The brain expression of SCG10 was maximum in mid to late gestation of embryo but dramatically reduced in the adult. However, the expression persisted at low but significant levels in many areas of adult brain(see the abstract by T.Himi). These areas included the hippcampus, the cerebellar Purkinje cells, and the mitral cells of the olfactory bulb. These data suggest that SCG10 might play a role in maintaining the plasticity of nervous system. On the other hand, amino acid sequences of SCG10 (see the abstract by T.Okazaki) showed a hydrophobic region and a region with interesting similalities, though segmentally, to cytoskeletal molecules, including myosin, keratin and laminin. The predicted structure suggests that SCG10 may be involved in neuronal vesicle-cytoskeletal interactions. In order to explore this possibility we examined the subcellular localization of SCG10 in adult rat cerebellum using specific antibodies by immunoelectron microscopy. In the cell body, immunoreactivity of SCG10 was observed around the endoplasmic reticulum. The Golgi apparatus, mitochondria, lysosomes and other membranous structure were devoid of immunoreactivity of SCG10. In the molecular layer of cerebellum, the immunoreactivity of SCG10 was restricted to a region near the neuronal terminals adjacent to the plasma membrane. A small vesicle-like structure in dendrites or axons also showed a positive reaction.

The existence of SCG10 protein in neuronal terminals raises the possibility that SCG10 is involved in synaptic interactions.

38.11

PROTEIN MAINTENANCE IN AXOTOMIZED CRAYFISH MEDIAL GIANT AXONS. S. L. Tanner, E. E. Storm, R. A. Sheller, and G. D. Bittner*. University of Texas, Austin, TX 78712.

Axotomy of the medial giant axon (MGA) of the crayfish, *Procambarus clarkii*, produces a distal anucleate segment of axon which remains intact and functional for at least 6 months. The total protein banding pattern (as assessed on 1D SDS-PAGE silver stained gels) is very similar in intact and anucleate MGAs until about 7 months post-severance. At this time, proteins of apparent molecular weights of 33, 55 (tubulin), and 71kd are decreased in anucleate MGAs while many other proteins are not decreased. The similarities in the protein banding pattern between intact and anucleate MGAs severed for less than 7 months suggest that protein maintenance in anucleate MGAs is the result of suppressed protein turnover and/or local uptake of protein from extra-axonal sources, e.g. glial cell synthesis of protein which is taken up by the MGA (Sheller and Bittner, Brain Res., In Press).

When fast axonal transport proteins from anucleate MGAs are examined by SDS-PAGE/fluorography, most protein bands are much less detectable after 28 days compared to 1 day, i.e., the half-life of most fast transported protein is less than 1 month. This result indicates that many proteins in anucleate MGAs are degraded long before the degeneration of anucleate MGAs. Therefore, local uptake of proteins may be an important mechanism for protein maintenance of anucleate MGAs. However, when proteins in slow transport are examined, the most heavily labeled bands are located at 33, 55, and 71kd, presumably the same species of proteins which are decreased in 7 month anucleate MGAs. Furthermore, the 55kd band (tubulin) is generally not taken up by the MGA from local sources. These data suggest that some proteins delivered by slow axonal transport may not be derived from local sources and may exhibit slow protein turnover (possibly as long as the survival of the anucleate MGA). Supported by ATP grant to G.D.B.

38.12

HIGH NEUROFILAMENT PACKING DENSITY IN LESIONED LAMPREY GIANT AXONS IS CORRELATED WITH NEUROFILAMENT SIDEARM PROTEOLYSIS. G.F. Hall*[1], L. Chen[2] and V.M-Y. Lee[2]. 1 Dept of Neurology, Harvard Med. School, Brigham and Women's Hosp., Boston, MA 02115 and 2 Dept. of Pathology, Univ. Penn. School of Med., Philadelphia, PA 19104.

Axotomy of giant Muller and Mauthner axons in the lamprey spinal cord results in the rapid formation of dense filamentous masses (DFMs) in the cut axons within 150 μm of the lesion site (McHale and Cohen 1983). We show that monoclonal antibodies (RMO14, RMO44, RMO48) specific for the neurofilament "core" domain strongly stain amorphous masses corresponding to DFMs while failing to recognize neurofilaments elsewhere in the axon. Antibodies specific for the neurofilament C terminal "sidearm" domain (RMO34, RMO270) fail to recognize DFMs, but stain other axonal neurofilaments. We prepared Western blots from SDS denatured total protein preparations of 3 mm segments of the spinal cord at the lesion site at 1 day post lesion and compared them to blots of unlesioned cord and cord taken from a site distant from the lesion. We found that the "core" antibodies RMO14 and RMO44 labelled several neurofilament breakdown products present in the lesion site preparation that were not present in the controls. Other low molecular weight bands were recognized by a C terminal antibody (RMO270). We conclude that axotomy leads to proteolysis of the "sidearm" region of some neurofilaments in the vicinity of the lesion, and suggest that the loss of the sidearm may be responsible for the high packing density reported for neurofilaments near the lesion site in this system. Supported by NIH grant NS29281.

38.13

ABERRANT NEUROFILAMENT-GENE EXPRESSION IN ACRYLAMIDE NEUROPATHY. S. Kittur[1]*, H. Endo[1], P.S. Spencer[2], M I. Sabri[2]. [1]Molecular Neurobiology, NIA/NIH, Baltimore, MD 21224, [2]Oregon Health Sciences University, Portland OR 97201. The molecular mechanisms by which acrylamide produces pathological changes in central and peripheral neurons are not understood. The most widely accepted hypothesis is that acrylamide progressively inactivates certain neuronal component(s) and thereby compromises neuronal integrity. Recent studies from our laboratory show that acute and subacute intoxication with acrylamide causes depletion of microtubule-associated proteins (MAP1 and MAP2) and activates immediate-early gene (c-*fos* and c-*jun*) expression in rodent brain. This study examined the expression of mRNAs for light (L), medium (M), and heavy (H) chains of 10-nm neurofilaments (NF) in the brains of acrylamide-treated rats. Adult male Sprague-Dawley rats were treated with a single (100 mg/kg, i.p.) dose or repeated (0.03% w/v in drinking water) oral doses of acrylamide. Animals were sacrificed by decapitation 6 hours after a single injection or 4 weeks after repeated oral dosing. Brains were excised, total mRNA isolated and Northern blot analysis performed by hybridization with ^{32}P-labeled cDNA probes for NF-L, NF-M and NF-H. Acute acrylamide treatment significantly increased (approximately 50%) the expression of NF-M mRNA with no apparent change in either NF-L or NF-H mRNA. In contrast, repeated doses of acrylamide caused a significant increase in NF-L mRNA (approximately 22%); no changes were observed in NF-M and NF-H mRNAs. The expression of mRNA for ß-actin was not altered significantly by acrylamide. These results show that acrylamide causes aberrant NF-gene expression in rat brain. Supported in part by NS 19611.

38.14

NO EFFECTS OF PROPOFOL ON THE ACTIN AND TUBULIN ORGANIZATION OF RAT NEURONS AND GLIAL CELLS. A. G. Jensen, M Lindroth, K Björnström and C Eintrei*. Departments of Anaesthesiology and Pathology II, Linköping University Hospital, S - 581 85 Linköping, Sweden.

Neurons and glial cells from 14 - 16 - day - old rat embryos (Sprague-Dawley) were cultured in modified Eagles Minimum Essential Medium (Hansson E, in "A Dissection and Tissue Culture Manual", Alan R Liss Inc., 1989, pg 92), supplemented with 10% fetal calf serum, glucose, insulin, amino acids and antibiotics. The cells were attached to Poly - L - Lysin coated coverglasses placed into a 24 multiwell tissue culture plate for ten days at 37° C in a humidified atmosphere of 5% carbon dioxide/95% air. Thereafter the cells were exposed to three different concentrations of propofol (0.3 µg/ml, 3µg/ml and 10µg/ml) for 30 min., extracted for 10 min. with 0.5% Triton X-100 in a microtubule stabilizing buffer, and fixed for 15 min. in 4% PFA. We then studied the organization of actin in the cells using rhodamin - conjugated phalloidin, which is specific for filamentous actin, and observed the cells using fluorescence microscopy. The organization of tubulin was studied using indirect immunofluorescence

No major changes could be observed, neither in the actin architecture nor in the arrangement of tubulin, after incubation with propofol. The increase in propofol concentration from 0.3 to 10 µg/ml had no effects on tubulin or actin organization. These preliminary results indicate that propofol has no effects on the neuron or glial cell cytosskeleton

38.15

DISTRIBUTION OF γ TUBULIN IN THE NEURONS: IMPLICATIONS FOR THE ORIGINS OF NEURITIC MICROTUBULES. H. C. Joshi* and P. W. Baas[1]. *Dept. of Anat. & Cell Biol., Emory Univ. Sch. of Med., Atlanta, GA 30322; [1]Dept. of Anat., The Univ. of Wisconsin Med. Sch., Madison, WI 53706.

Axons and dendrites contain dense microtubule (MT) arrays that are not attached to a traditional MT nucleating structure such as the centrosome. Nevertheless, the MTs within these neurites are highly organized with respect to their polarity, and consist of a regular 13-protofilament lattice, the two known characteristics of MTs nucleated at the centrosome. These observations suggest either that axonal and dendritic MTs arise at the centrosome, or that they are nucleated locally, following a redistribution of MT nucleating material from the centrosome during neuronal development. To begin distinguishing between these possibilities, we have determined the distribution of γ-tubulin within cultured sympathetic neurons. γ-tubulin, a newly discovered protein which is specifically localized to the pericentriolar region of non-neuronal cells (Zheng, Y., M.K. Jung, and B.R. Oakley, 1991, Cell 65:817-823; Stearns, T., L. Evans, and M. Kirschner, Cell 65:825-836), has been shown to play a critical role in MT nucleating *in vivo* (Joshi, H.C., M.J. Palacios, L. McNamara, and D.W. Cleveland, 1992, Nature 356:80-83). Because the γ-tubulin content of individual cells is extremely low, we relied principally on the high degree of resolution and sensitivity afforded by immunoelectron microscopy. Our studies reveal that, like the situation in nonneuronal cells, γ-tubulin is restricted to the pericentriolar region of the neuron. Furthermore, serial reconstruction analyses indicate that the minus ends of MTs in both axons and dendrites are free of γ-tubulin immunoreactivity. The absence of γ-tubulin from the axon was confirmed by immunoblot analyses of pure axonal fractions obtained from explant cultures. The observation that γ-tubulin is restricted to the pericentriolar region of the neuron suggests that MTs destined for axons and dendrites are nucleated at the centrosome, and subsequently released for translocation into these neurites.

38.16

IDENTIFICATION OF A 38 KD PROTEIN BINDING TO NEUROFILAMENT SUBUNITS *IN VITRO*. L.D. Errante* and G. Shaw. Department of Neuroscience, University of Florida College of Medicine, Gainesville, FL 32610.

To examine the interactions of neurofilaments (NFs) with cytosolic proteins, five different types of NF affinity columns were made with: (1) crude spinal cord intermediate filament preparation; (2) purified NF triplet proteins; (3) purified NF-H; (4) purified NF-M; and (5) purified NF-L. A cytosolic preparation from fresh pig spinal cord was cycled through each affinity column and bound proteins were eluted with a linear salt gradient (0-0.5M NaCl). Bound proteins were visualized using SDS polyacrylamide gel electrophoresis (SDS-PAGE) and relative mobilities were determined. The most abundant protein binding to all NF affinity columns was a 38 KD protein. Depending on the NF affinity column used, the 38 KD protein was eluted off at salt concentrations ranging from 0.1M to 0.2M NaCl. Recently, we have identified the 38 KD protein as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by: (1) virtual identity of amino acid composition with GAPDH; (2) co-migration on one dimensional SDS-PAGE with GAPDH; (3) production of cyanogen bromide cleaved fragments similar to those produced from GAPDH; and (4) immunoreactivity of the 38 KD protein with a mouse polyclonal antibody to GAPDH. GAPDH is a glycolytic enzyme which has been shown to associate with microtubules and actin *in vitro*. Current studies are focusing on determining the binding constants and *in vivo* interactions of GAPDH with the NF network.

38.17

IDENTIFICATION OF A NEURAL-SPECIFIC HOMOLOG OF CLATHRIN-COATED VESICLE-ASSOCIATED MEDIUM CHAINS. J. Pevsner*, W. Volknandt and R.H. Scheller. HHMI, Dept. Molec. & Cell. Physiol., Stanford U. Medical Center, Stanford CA 94305.

Neurotransmitters are packaged and stored in vesicles in the presynaptic nerve terminal. Following exocytosis synaptic vesicle proteins are believed to be selectively endocytosed by a pathway involving clathrin-coated pits and vesicles. We have used an antiserum directed against electric ray synaptic vesicles to isolate a cDNA clone encoding a 47 kDa protein, ray p47. We subsequently isolated rat homologs p47A, which is expressed in all tissues examined, and p47B, which is neural-specific. These three proteins share 85% amino acid identity among each other and 30% identity to the medium chains (AP50, AP47) of adaptor complexes associated with clathrin-coated vesicles. These adaptor complexes mediate functions such as the internalization of proteins from the cell surface. We propose that p47A and p47B are constituents of novel adaptor complexes. p47B may be present in an adaptor complex in the nerve terminal mediating the recycling of synaptic vesicles.

38.18

CHARACTERIZATION OF AN INOSITOL POLYPHOSPHATE RECEPTOR FROM BRAIN: SIMILARITY TO THE CLATHRIN ASSEMBLY PROTEIN AP-2. [1]S.M. Voglmaier, [2]J.H. Keen, [3]G.D. Prestwich, [1]S.H. Snyder, and [4]A.B. Theibert*. [1]Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205, [2]Jefferson Cancer Inst., Thomas Jefferson Univ. Sch. of Med., Philadelphia, PA 19107, [3]Dept. of Chemistry, SUNY, Stony Brook, NY 11794, [4]Neurobiol. Res. Ctr./Dept. of Cell Biology, Univ. of Alabama, Birmingham, AL 35294.

Highly phosphorylated inositols, including IP_4, IP_5, and IP_6 (IPs) are present in mammalian brain. Changes in the levels of several IPs are elicited by neurotransmitter action. IPs may regulate calcium levels, modulate ion channels, or regulate other activities in the cell. Using IP-affinity probes, several high affinity inositol polyphosphate receptors have been isolated from rat brain. One of these, designated the IP_6R, binds IP_6(K_d=12nM)>IP_5=IP_4(K_d=50-100nM)>IP_3(K_d>1uM). Several subunits comprise the IP_6R, with IP binding sites contained in the 105-115 kDa subunits. Partial amino acid sequence was obtained from two peptides generated by tryptic cleavage of the 105-115 kDa subunits. These peptides were identical to sequences 499-515 and 914-924 contained in the α_A subunit of the clathrin assembly protein AP-2 (α-adaptin). In immunoblot analysis, antibodies to both the AP-2 α and β subunits recognize proteins in the pure IP_6R fractions. These results indicate that the IP_6R is very similar and may be identical to the plasma membrane associated AP-2, and suggests that IPs may be involved in regulation of vesicle transport in neurons.

38.19

DIFFERENTIAL SUBCELLULAR LOCALIZATION OF SNAP-25 PROTEINS MAY BE DIRECTED BY ALTERNATIVE SPLICING.

C. Bark & M. C. Wilson, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.*

The presynaptic nerve terminal protein SNAP-25, (synaptosomal associated protein of 25 kD), is a 206 amino acid polypeptide that contains two domains which may play important roles for biological function and targeting of the mature protein. The amino terminus can form an amphipathic helix and at least one out of four clustered cysteine residues is substrate for posttranslational palmitoylation. SNAP-25 is a component of fast axonal transport, however, in rodents the cellular localization of the protein undergoes a developmental switch. In neonates SNAP-25 accumulates in axons and perikarya, whereas in adults it appears selectively in terminal fields. We recently reported the presence of two distinct transcripts encoding the SNAP-25 protein. The two mRNAs, SNAP-25a & b, are developmentally regulated and encode proteins that only differ in 9 amino acids *(Bark & Wilson, Neurosci. Abs. 211.9, 1991)*. The two isoforms are generated by alternative splicing between two exons which span the only cysteine residues in the polypeptide. While SNAP-25a is a minor earlier form, the expression of SNAP-25b appears to be coincident with neuronal maturation.

To investigate whether regulated subcellular localization and targeting of the protein to presynaptic terminals is determined within the 9 amino acid differencies between the two isoforms, we are examining their expression in the pheochromocytoma cell line PC12. We have shown that undifferentiated PC12 cells express small amounts of endogenous SNAP-25a mRNA and that dBcAMP and/or NGF treatment induces increased mRNA levels and a change in exon utilization which correlates with the translocation of the protein into neurite terminals. To define the minimal signal sequence encoded by the alternative exons required for differential cellular localization, the distribution of both isoforms and of mutated proteins are being examined after transfection into cultured cells. *Supported by Swedish NSRC B-PD9714-303 (C.B.) and BNS9121121 (M.C.W.).*

38.20

REGULATION OF PROTEIN SYNTHESIS WITHIN SYNAPTODENDROSOMES: EFFECT OF POTASSIUM-INDUCED DEPOLARIZATION ON THE SYNTHESIS OF SYNAPTIC PLASMA MEMBRANE PROTEINS.

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We have previously proposed that some synaptic proteins are locally synthesized by subsynaptic polyribosomes. This conclusion is based on the observation that some proteins synthesized by isolated "synaptodendrosomes" become associated with the synaptic junction (Rao and Steward, 1991, J. Neurosci., 11, 2881-2895). Other studies have revealed that protein synthesis in synaptosomes is increased by depolarization (Weiler and Greenough, 1991, Mol. Cell. Neurosci., 2, 305-314). This effect on protein synthesis could be a generalized increase in translation or a differential increase in the synthesis of particular protein species. In this study, we examined whether the synthesis of particular synaptic plasma membrane proteins was differentially affected by depolarization.

Synaptodendrosome fractions were pulse-labeled with ^{35}S methionine in the presence of 2, 25, or 40 mM extracellular potassium concentrations. A synaptic plasma membrane (SPM) subfraction was obtained from the synaptodendrosomes and labeled proteins within the SPM subfraction were analyzed by SDS-PAGE and fluorography. There was a monotonic increase in labeling of all bands with increasing potassium concentrations. This difference in labeling was greater comparing the 2 and 25 mM condition than between the 25 and 40 mM conditions. These results suggest that depolarization leads to a general increase in protein synthesis within synaptosomes of proteins that associate with the synaptic junction, although the synthesis of some proteins may be affected more than others. *Supported by NIH NS12333 .*

38.21

THE COMPLETE SEQUENCE FOR BRAIN ß-SPECTRIN INDICATES A RELATIONSHIP WITH HEME BINDING PROTEINS (GLOBINS).

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Brain spectrin (fodrin), a major ubiquitous cytoskeletal protein participates in cell morphogenesis, neurotransmitter release and regulation of the lateral mobility of N-CAM 180. It consists of α and ß subunits that form antiparallel 100 nm long fibrous heterodimers, and the heterodimers associate head-to-head to form 200 nm tetramers. The ß subunit of spectrin is functionally important because it interacts with other molecules including F-actin, ankyrin and synapsin I. Elucidating the details of the primary structure of ß spectrin will facilitate understanding its functional interactions and may predict new functions for this molecule. We have determined the first full length sequence of nonerythroid ß spectrin (ß SpIIa) from mouse brain cDNA libraries. The nucleotide sequence contains 7165 basepairs encoding 2363 amino acid residues, which comprise 17 typical repeat units which share 59% identity with ß SpIa (ß subunit of human rbc spectrin). The predicted molecular weight of brain ß spectrin from the derived amino acid sequence is 274,449 daltons. Combining the knowledge of previously reported functional studies with our ß SpIIa primary structure, it is possible to make a number of predictions regarding functional sites within this ß spectrin molecule. Interestingly, the analysis of the ß SpIIa sequence indicated striking homology and similar structural characteristics of brain ß spectrin repeat ß11 and ß12 to the heme-binding domain of globins. We have demonstrated that purified brain ß spectrin binds heme in vitro, suggesting possible new functions in electron transfer, oxygen binding, or heme scavenging. Supported by NIH grant RO1NS19357 to SRG.

38.22

NEUROFILAMENTS OCCUR IN NEURONS OF THE SEA ANEMONE CONDYLACTIS GIGANTEA (Cnidaria: Anthozoa)

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Neuronal processes in most organisms contain filamentous cytoskeletal structures. In vertebrates, annelids and molluscs, neurofilaments (NFs) (10 nm intermediate filament cytoskeletal elements) are a major component of the nervous system and play mechanical, structural and dynamic roles. Mammalian neurofilaments are composed of three polypeptides with molecular weights of 200kD, 150kD and 70kD. Non-mammalian NFs may also exist as a triplet, but in some only a single NF protein is seen. In these cases, the single protein is typically immunologically cross-reactive with the mammalian 150kD and 200kD subunits. Antisera to the 150kD and 200kD subunits of mammalian neurofilaments were used to stain the nervous system in the subtropical sea anemone C. gigantea. At the level of light and electron microscopy, staining of neural components was found in an ectodermal nerve net and plexus. A detergent-insoluble protein of 190kD was isolated. Antisera absorbed with this 190kD protein no longer stained neuronal structures. The data indicate that neurons of C. gigantea possess neurofilaments, and that this family of neuronal proteins is of great antiquity. (Supported by the NIH, ARCS Foundation Inc., and the Hedco Foundation.)

GENE STRUCTURE AND FUNCTION I

39.1

REGULATION OF THE 66 KILODALTON NEUROFILAMENT PROTEIN GENE EXPRESSION DURING DIFFERENTIATION OF PC12 AND EMBRYONAL CARCINOMA P19 CELLS.

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The 66 kDa neurofilament protein (NF-66) and 70 kDa neurofilament protein (NF-L) share homology and are co-expressed to varying extents within CNS neurons but are encoded by separate genes. NF-66 expression *in vivo* is correlated with periods of axonal growth, whereas NF-L expression becomes maximal after the period of axonal growth and synapse formation. To begin to explore the mechanisms underlying the regulation of NF-66 we have investigated the pattern of NF-66 transcript and gene product expression during differentiation in two model systems; NGF inducible PC12 cells and retinoic acid (RA) inducible embryonal carcinoma P19 cells. In undifferentiated PC12 cells NF-66 mRNA and protein levels were very low. However, upon NGF treatment NF-66 mRNA levels increased markedly by 6 hours and reached a plateau at 24 hours; thus induction kinetics of NF-66 is faster than that of GAP-43 mRNA, which encodes another axonal growth related protein. Western blotting for NF-66 confirmed this induction pattern. In P19 cells, RA differentiation for 3, 5 and 7 days induced the robust expression of NF-66 mRNA and protein. Indirect immunofluorescent staining of NF-66 in differentiated P19 cells detected the gene product in neuron-like cells with long processes that could be reliably distinguished from non-neuronal cells. These observations provide the basis for further studies on the mechanism of NF-66 gene expression during neuronal differentiation. Supported by USPHS grant NS 23705 and HD27226.

39.2

BASIC HELIX-LOOP-HELIX AND Id-LIKE TRANSCRIPTION FACTORS IN NEUROGENESIS.

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The helix-loop-helix (HLH) transcription factors play important roles in specifying cell fate. In the mouse CNS, different HLH transcription factors are expressed from the early stages of neurogenesis to adulthood. Class A basic-HLH (bHLH) transcription factors ME1 and ME2 are expressed at high levels in proliferating neuroblasts. The expression of ME1 decreases dramatically when the genesis of neurons is finished. ME2 continuous to be expressed at high levels in certain structures (hippocampus) during differentiation. At least two Id-like proteins, which lack the basic, DNA binding domain have high expression levels in specific cell types during neurogenesis. Id2 is expressed in large neurons of cortical structures from the moment of their genesis to adulthood. In the cerebral cortex, it is expressed at high levels in layers II,III and V, in the olfactory bulb in mitral cells, and in the cerebellum exclusively in Purkinje cells. BI1 (Brain Id 1) is expressed at high levels in white mater, most likely in oligodendrocytes beginning from postnatal days 7-10. BI1 gene has different transcripts in pre- and postnatal development. The presence of different bHLH and Id-like transcription factors in the CNS indicates that they have important regulatory roles in neurogenesis.

39.3

THE TYPE 1 NEUROFIBROMATOSIS GENE PRODUCT IS ENRICHED IN DENDRITES OF SUBPOPULATIONS OF CNS NEURONS. N. Ratner*, M. Shipley, and M. Nordlund. Dept. of Anatomy & Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0521.

Von Recklinghausen's neurofibromatosis (NF1) is an inherited human disease that has multiple manifestations in the CNS. Patients with NF1 exhibit certain types of CNS tumors, learning disabilities, MRI abnormalities, and headaches. To begin to understand the function of the NF1 gene product (neurofibromin) in the normal CNS, we have surveyed its distribution in the rat brain using polyclonal and monoclonal antibodies (Daston et al., Neuron 8:415-428). Neurofibromin expression is limited to oligodendrocytes and neurons in the brain. Staining of oligodendrocytes defines a caudo-rostral gradient; oligodendrocytes are stained in the spinal cord, less in the brain stem but not in the forebrain. Subsets of neurons possess neurofibromin immunoreactivity. Pyramidal neurons are the only cells that express neurofibromin in the cerebral cortex. Purkinje cells are the major cell type labelled in the cerebellum. Subpopulations (75-80%) of neurons in brain stem, thalamus, and deep cerebellar nuclei contain significantly elevated levels of the protein. Dendrites, especially proximal dendrites, are prominently labelled in immunoreactive neurons. These data are consistent with the hypothesis that neuronal abnormalities, resulting from dysfunction of a metabolic process localized primarily in dendrites of specific neurons, underlie CNS-associated disease manifestations in NF1. Supported by NS28840.

39.4

THE PATTERN OF EXPRESSION OF THE NEUROFIBROMATOSIS TYPE 1 GENE PRODUCT IN THE ADULT AND DURING DEVELOPMENT.
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Neurofibromatosis type 1 (NF1 or von Recklinghausen's disease) is a common inherited neurological disorder affecting 1 in 3500 people. The predominant manifestations of NF1 are benign tumors of peripheral nerves comprised mainly of Schwann cells (neurofibromas), tumors of the iris, learning disabilities and hyperpigmentation of melanocytes. In addition, NF1 patients have an increased risk for certain neural malignancies. These symptoms can arise throughout life, but their initial onset is usually during infancy or early childhood. Knowledge of the distribution of the normal NF1 gene product during development should improve our understanding of the pathogenesis of this disease.

We have raised antibodies, against peptides encoded by portions of the human *NF1* cDNA, which specifically recognize a 220 kDa protein (neurofibromin) in both human and rat spinal cord (Daston et al., 1992, Neuron (8):415). In adult animals very little neurofibromin is expressed outside the nervous system. In contrast, in embryonic rats neurofibromin is widely distributed.Unlike most cell types, in which neurofibromin expression decreases with maturation, neurons begin to express high levels neurofibromin around the time of terminal differentiation. Immunostaining in the embryonic central and peripheral nervous systems is most intense in growing fiber tracts.

The broad distribution of neurofibromin in embryos suggests that it may play a general role in cell growth or differentiation. The localization of neurofibromin to specific cell types of mature neural tissue suggests a separate role in the normal functioning of the nervous system, consistent with the predominance of neurological symptoms in NF1. Supported by NIH NS28840.

39.5

IDENTIFICATION OF THE POSSIBLE MOLECULAR BASIS FOR INHERITED DIFFERENCES IN HUMAN CATECHOL-O-METHYLTRANSFERASE. MH Grossman*[1], J B Littrell[1], R Weinstein[1], CL Szumlanski[2] and RM Weinshilboum[2]. 1-Dept. of Pediatrics, Temple Univ. Sch. Med., St. Christopher's Hosp. for Child., Phila., PA 19134 and 2-Dept. of Pharmacol., Mayo Clinic, Rochester, MN 55095.

Inherited variations of human catechol-O-methyltransferase (COMT) activity levels in humans are controlled at a two allele, autosomal locus. The "low" activity enzyme is also relatively thermolabile, compared to the "high" activity form. The cloning of the human COMT gene has enabled us to examine the molecular basis of these inherited variations. Liver specimens from three individuals with presumed *L/L* genotypes and three with presumed *H/H* genotypes were obtained, total mRNA isolated and cDNA prepared. Sequence analysis of PCR-amplified COMT coding region was performed on multiple M13 subclones. A single nucleotide change at position #472 has been identified, with all *L/L* individuals having an adenosine and all *H/H* individuals having a guanosine. A corresponding change occurs in amino acid #158, with *L/L* having a methionine and *H/H* having a valine. The nucleotide difference identified here may represent the molecular basis of normal variations that contribute to an individual's ability to metabolize endogenous as well as administered catechol-like substances. (Supported by NIH grant NS24066 to MHG).

39.6

ANALYSIS OF THE PROMOTER SEQUENCE AND THE TRANSCRIPTION INITIATION SITE OF THE MOUSE SEROTONIN 1C RECEPTOR GENE. L. J. Bloem*, J. Liu, Y. Chen, L. S. Bye and L. Yu. Dept. of Medical & Molecular Genetics, Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

The serotonin 1c (5-HT_{1C}) receptor is found in many brain regions, but is particularly enriched on the epithelial cells of the choroid plexus. A major goal of neurobiology is to define the molecular processes that regulate the regional expression pattern of neuronal genes. As an initial step towards identifying *cis*-acting DNA sequences that control the expression of the 5-HT_{1c} receptor, we have isolated the promoter sequence of the 5-HT_{1c} gene and sequenced a 1.8 kb fragment. Primer extension experiments using mouse brain poly A^+ RNA mapped the transcription initiation site in this region. There are a number of sequence elements upstream from the transcription initiation site that are homologous to enhancer elements found in other eucaryotic genes. A plasmid containing the promoter region and a reporter gene, when injected into the nucleus of *Xenopus* oocytes, resulted in functional expression of the reporter gene. Primer extension reactions using the RNA extracted from the injected oocytes indicated a single transcription initiation site of the reporter mRNA.

39.7

CHARACTERIZATION OF THE RAT AROMATIC L-AMINO ACID DECARBOXYLASE GENE. V. R. Albert*, M. R. Lee, R. C. Silverman, R. Wurzburger and A. Aguanno. Dept. of Neuroscience, Roche Institute of Molecular Biology, Nutley, N.J. 07110.

Aromatic L-amino acid decarboxylase (AADC) is the enzyme which catalyzes the decarboxylation of L-dopa to dopamine in catecholamine cells and 5-hydroxytryptophan to serotonin in serotonin-producing neurons. AADC is also expressed in relatively large quantities in non-neuronal tissues such as liver and kidney, where its function is still unknown. Although the enzyme itself appears to be identical in both neuronal and non-neuronal tissues, it has been shown that in rat neuronal and non-neuronal tissues express AADC messages with distinct 5' untranslated regions (Krieger et al., PNAS, **88**; 2161, 1991). In order to understand how this is accomplished at the genomic level, and to learn more about its regulation, we have isolated rat genomic DNA encoding AADC.

The neuron-specific form of rat AADC cDNA was used to screen an EMBL3-SP6/T7 rat genomic library. Eight overlapping clones of 12-16 kb were obtained. These clones encompass approximately 50 kilobases of the rat AADC gene. Included in this DNA is most of the AADC coding sequence.

The organization of the AADC gene suggests that there are two separate promoters specific for the transcription of neuronal and non-neuronal forms of the AADC message. A small exon containing 68 bases of the neuronal-specific 5'end is located 9.5 kb upstream of the translation start site, which is contained in the third exon. Approximately 6 kb upstream from the neuron-specific promoter is another small exon containing 71 bases of the 5' end of the non-neuronal AADC message. These data suggest that the expression of neuronal and non-neuronal forms of the AADC message is controlled by transcription initiating at distinct promoters, followed by alternative splicing to exon 3 which contains the common translation start site.

39.8

CHANGES IN GENE EXPRESSION DURING INCUBATION OF HIPPOCAMPAL SLICES. P.J. Voulalas[1], T.S. Nowak, Jr.[2], and J.M. Sarvey[1]*. [1]Dept. Pharm, USUHS Bethesda, MD 20814 and [2]Dept. Neurology, Univ. Tenn., Memphis, TN 38163.

Slices of brain tissue are subjected to mechanical and ischemic stresses in the course of preparation. Previous studies have noted that the heat shock protein hsp70 accumulates in the vascular elements of incubated slices from various tissues, while ischemia is well known to induce neuronal expression of c-*fos* and hsp70 in brain. In the present study 400μm thick hippocampal slices from male Sprague-Dawley rats were prepared on a tissue chopper and placed in a perfusion chamber (artificial cerebrospinal fluid/95% O_2, 5% CO_2, 34 C, 3 ml/min); slices were removed at 0,30,60,90 and 120 min, and frozen sections were prepared and analyzed by *in situ* hybridization, using anti-sense probes for c-*fos* and hsp70. Striking increases in c-*fos* were observed in dentate, CA1 and CA3 at 60 to 120 min. Hsp70 followed a similar pattern of increased expression, but was less strikingly induced. The distribution of altered gene expression in hippocampal slices is comparable to that observed following ischemia *in vivo*. The longer duration of c-*fos* mRNA expression in slices than in intact tissues, perhaps may reflect the slow metabolic recovery of slices *in vitro*. We suggest that changes in gene expression can be useful markers for assessing the status of slices used for electrophysiological work.

39.9

EFFECT OF NICOTINE ON TYROSINE HYDROXYLASE AND DOPAMINE ß-HYDROXYLASE GENE EXPRESSION IN PC12 CELLS. B.K. Hiremagalur*, J. Nitahara, A. McMahon, and E.L. Sabban. Dept. Biochem. and Mol. Biol. New York Med. Coll., Valhalla, NY 10595.

Nicotine, a cholinergic agonist, stimulates catecholamine secretion and raises tyrosine hydroxylase (TH) gene expression in adrenal medullary cells. The latter is associated with increased cAMP levels although nicotine is reported to induce c-fos in PC12 cells. This study investigated the effect of nicotine on expression of TH and dopamine ß-hydroxylase (DBH), two enzymes whose activity can be regulated by prolonged nicotine treatment in vivo.

PC12 cells were treated with nicotine (200 μM) for various times, and TH and DBH mRNA analyzed by Northern blots. Transcriptional regulation was studied using transfected PC12 cells in which expression of chloramphenicol acetyl transferase (CAT) was under control of the 5' region of the TH gene. Nicotine induced both TH and DBH mRNA about 3-4 -fold, with a maximum at 48 hrs. The effect on TH was transcriptional since PC12 cells transfected with pTH-5' (-773/+27) and (-272/+27) showed a 4 fold induction of CAT activity with nicotine. Nicotine mediated CAT activity was inhibited by H89, a protein kinase A inhibitor. Both the TH mRNA and CAT activity were not induced by nicotine in a mutant PC12 cell line deficient in protein kinase A activity. These results show that both TH and DBH are regulated in PC12 cells by prolonged periods of nicotine treatment. The effect on TH is transcriptional and appears to require a cAMP-dependent protein kinase.

39.10

Mechanisms of Enkephalin Regulation by Haloperidol. C.Konradi*, L.A.Kobierski, T.V.Nguyen, W.T. Dauer, and S.E. Hyman, *Molecular Neurobiology Department, Massachusetts General Hospital, Harvard Medical School.*

Chronic administration of D2 receptor antagonist anti-psychotic drugs, such as haloperidol, are known to increase mRNA and protein levels of proenkephalin in rat striatum. Using proenkephalin gene regulation as an endpoint, we have examined haloperidol-induced changes in intracellular signal transduction in striatal neurons. We find that proenkephalin mRNA is induced rapidly by haloperidol, with a time course suggesting that constitutively expressed transcription factors may play a critical role in the induction. Moreover, in long-term treatment paradigms, proenkephalin mRNA levels remain elevated at time points at which immediate early genes (IEG's), such as c-Fos, are no longer fully inducible. Gel shifts using crude nuclear extracts of rat striata treated chronically (12 daily injections of 2mg/kg haloperidol) or acutely (11 daily injections of saline, 1 injection of 2mg/kg haloperidol 2 hours before sacrificing), showed increased binding to consensus AP-1 sequences, but not to the enkephalin CRE-2 element which is known to be critically involved in gene activation. In contrast to levels of other IEG mRNAs, levels of junD (the protein product of which has been shown to bind to the CRE-2 element) do not change with chronic haloperidol treatment. We have therefore begun an analysis of the state of Jun D phosphorylation in response to acute and chronic haloperidol treatment.

39.11

DNASE I HYPERSENSITIVE SITES 5' OF THE RAT PROENKEPHALIN GENE. M. Holloway, E.F. La Gamma*. Dept. of Pediatrics, SUNY at Stony Brook, NY 11794.

Extracellular stimuli affecting mechanisms of transcriptional control of the proenkephalin gene link stimulus-secretion-synthesis coupling to transmitter phenotypic expression. In order to define distal and proximal genomic regions that might participate in transcriptionally active protein-DNA interactions, DNase I hypersensitivity assays were conducted. Four DNase I hypersensitive regions were identified at -3000, -2500, -1800 and -200 bp relative to the somatic start site. The appearance of a site over the proximal promoter/enhancer region (-200 bp) is of interest since we and others have published a detailed footprint and functional analysis of this region using rat tissues (*Mol. Cell. Neurosci.* 2:517, 1991 and 2:427, 1991). While liver (which does not express proenkephalin mRNA) displays essentially no hypersensitivity here, adrenal gland preparations show a strong signal and striatal tissue a significantly weaker signal. Cholinergic induction of proenkephalin mRNA was not associated with a change in hypersensitivity pattern. When analyzed, the -1800 bp region displays tissue-specific binding in gel shift assays. Cholinergic induction and the -3000 and -2500 bp regions have not yet been studied by gel shift. We are in the process of determining the extent that these regions are involved in the adrenal-specific regulation of proenkephalin expression and the choice of transmitter phenotype during development.

39.12

DOPAMINERGIC REGULATION OF PREPROENKEPHALIN GENE EXPRESSION IN STRIATAL ASTROCYTES J.DECRISTOFARO*, G.WEISINGER, E. LAGAMMA PEDS & NEUROBIOL, SUNY STONY BROOK, NY 11794.

Dopaminergic pathways regulate preproenkephalin (ppENK) gene expression in the rat striatum in vivo. Treatment with either a D1 antagonist SCH 23390 or a D2 agonist quinpirole increases striatal ppENK mRNA levels. Since both primary neurons and astrocytes express ppENK RNA, we sought to determine whether dopaminergic pathways regulate ppENK mRNA in non-neuronal cells. Striatal astrocytes isolated from neonatal rats and grown in culture for 2-3 weeks were then exposed to dopamine, 10-1000 uM. ppENK mRNA levels increased to a maximum of 3-fold at 100 uM. Astrocytes exposed to D1 agonist SKF38383 had a greater increase in ppENK mRNA than when exposed to D2 agonist quinpirole. The effects in culture were opposite to those seen in the intact striatum in vivo. D1 and D2 agonists given together were not synergistic. Using dopamine antagonists plus dopamine (100uM), neither D1 antagonist SCH 23390 nor D2 antagonist (sulpride or spiperone) alone or together completely blocked the effect of dopamine induced ppENK expression. These observations may be due to differences in dopamine receptor type binding specificity or may reflect activation of other dopamine receptor pathways (D3,D4,D5). Hence, ppENK mRNA is differential regulated by dopaminergic pathways in both neuronal and non-neuronal striatal cells. supported by NIH RR05736.

39.13

IS GAD_{67} EXPRESSION REGULATED BY TWO MECHANISMS? K. Rimvall and D. L. Martin*. Wadsworth Center for Labs and Research, New York State Health Dept., Albany, NY 12201.

Glutamate decarboxylase (GAD) activity is decreased by *ca.* 30% in cerebral cortex *in vivo* and 60-70% in cerebral cortical cultures following elevation of intracellular GABA levels by treatment with γ-vinylGABA (GVG) or GABA itself. In both systems the reduction in activity is due to a marked reduction of expression of GAD_{67} protein, as demonstrated by quantitative immunoblotting with selective antisera. Expression of GAD_{65} protein is unaffected. GAD_{67} mRNA was unchanged in rats treated with GVG despite a 75-80% reduction in GAD_{67} protein, suggesting that in this system the expression of GAD_{67} is regulated at the level of translation or protein degradation. GAD activity in cultures of striatal neurons is increased from 48 ± 7 nmol • h^{-1} • mg $prot^{-1}$ to 64 ± 7 by 10-μM quinpirole (a D_2 agonist), and 66 ± 7 by 10-μM SCH23390 (a D_1 antagonist). Others have demonstrated that the levels of GAD_{67} mRNA are altered in the striatum when rats are treated with quinpirole or SCH23390 or are lesioned with 6-hydroxydopamine. The contrast between the latter findings and the lack of change in GAD_{67} mRNA following GVG treatment suggest that the expression of GAD_{67} is controlled by at least two mechanisms. Supported by grant MH35664 from USPHS/DHHS.

40.1

TISSUE AND DEVELOPMENTAL EXPRESSION OF *SLO*, A CLONED DROSOPHILA CA^{2+}-ACTIVATED K^+ CHANNEL GENE. Robert Brenner, Yu-Yang Yu and Nigel Atkinson*. Dept. of Zoology, The University of Texas at Austin, Austin TX 78712-1064.

We are interested in how ion channel gene expression is regulated to generate the diverse electrical properties of excitable cells. Our focus is on the Drosophila Ca^{2+}-activated K^+ channel that conducts a current called ICF. This current has been characterized in adult and larval muscles and in larval neurons. The channel has a relatively high single channel conductance and is sensitive to block by charybdotoxin, is not blocked by apamin and plays a major role in shaping the action potential. The Drosophila slowpoke (*slo*) gene encodes a structural component of this Ca^{2+}-activated K^+ channel. Slowpoke was cloned by employing the well-developed genetics of Drosophila (Atkinson et al. 1991. Science 253: 551).

Alternative mRNA splicing of *slo* mRNA transcripts produce a number of messages that encode distinct but related polypeptides. We are characterizing the developmental and tissue specific expression pattern of these alternative products using *in situ* hybridization and RNA/PCR. A *slo* fusion protein has been expressed in bacteria and is being used to generate antibodies that recognize the Ca^{2+}-activated K^+ channel. Antibodies will be used to characterize the polypeptide and to determine the subcellular localization of this Ca^{2+}-activated K^+ channel.

40.2

CHARACTERISTICS AND MODULATION OF A CLONED CALCIUM-ACTIVATED POTASSIUM CHANNEL EXPRESSED IN XENOPUS OOCYTES. K.-Z. Shen,* A. Lagrutta, M.P. Kavanaugh, Y.-N. Wu, C.T. Bond, J.P. Adelman and R.A. North Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

A calcium-activated potassium channel was expressed in Xenopus oocytes by injection of RNA transcribed in vitro from a complementary DNA cloned from the slowpoke locus of Drosophila melanogaster. Unitary and macroscopic currents were studied in inside-out membrane patches. Channel opening required calcium ions on the cytoplasmic side, and activity increased both with depolarization and with increasing calcium concentration. The single channel conductance was 120 pS in symmetrical 120 mM potassium. This channel had a selectivity sequence of $K^+ > Rb^+ >> Na^+$, Li^+ and Cs+. It was apamin- and charybdotoxin-insensitive, but blocked by TEA, which was more sensitive when applied externally (K_D 0.15mM). The functional expression of calcium-activated potassium channels will allow the elucidation of structural features involved in the regulation and modulation of channel.

40.3

CA^{2+}-ACTIVATED K^+ CHANNELS FROM RAT BRAIN EXPRESSED IN MAMMALIAN CELL LINES AND *XENOPUS* OOCYTES . P.H. Reinhart* E. Shimony and F.M. Schmalz. Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710 and Dept. of Biochemistry Brandeis University, Waltham, MA 02254.

We have characterized two types of large-conductance Ca^{2+}-activated K^+ channels from rat brain cortex plasma membrane vesicles, and shown them to be modulated by protein kinases, protein phosphatases, and ATP (Chung et al., Science **253**:560; Reinhart et al., J. Neurosci. **11**:1627). Expression of potassium currents in *Xenopus* oocytes by injection of rat cortex mRNA has indicated that Ca^{2+}-activated K^+ channels only constitute a fraction of this current (approximately 5%). The expressed channels are blocked by iberiotoxin and charybdotoxin, high affinity blockers for some Ca^{2+} activated K^+ channels. Size selection of the mRNA demonstrated that the message coding for this current component was larger than 5.5 kb, but the enrichment of Ca^{2+}-activated K^+ channels in this fraction was less than 2-fold. To improve the signal to noise ratio we have developed an alternative screening protocol for Ca^{2+}-activated K^+-channels expressed in mammalian cell lines. The assay is based on the binding of fluorescent toxins to channels expressed in mammalian cell-lines. Site-directed mutagenesis was used to prepare iberiotoxin and charybdotoxin mutants suitable for labelling with rhodamine. These modified toxins blocked Ca^{2+} activated K^+ channels incorporated into planar lipid bilayers with affinities similar to wild-type toxins. Transformed COS cells, and oocytes injected with size-selected mRNA exhibited specific fluorescence signals. Cells transformed with a size-selected, uni-directional rat brain cDNA library are being screened for Ca^{2+}-activated K^+ channel expression.

40.4

REGULATION OF Ca^{2+}-ACTIVATED K^+ CHANNEL IN NERVE TERMINALS OF THE RAT NEUROHYPOPHYSIS. G. Wang* and J.R. Lemos. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

A slowly-gating, large conductance (type II) Ca^{2+}-activated K^+ channel was recorded from nerve terminals of the rat neurohypophysis using the patch clamp technique.

While it was not sensitive to charybdotoxin (at up to 360 nM), this channel could be blocked (IC_{50} = 0.2 μM) by externally applied tetrandrine, a bis-benzylisoquinoline alkaloid. Tetrandrine, however, appeared to have no effect on the Ca^{2+}-activated K^+ current of *pars intermedia* cells, which seems to be mediated by the type I channel.

Interestingly, addition of 200-400 μM ATP, but only in the presence of Mg^{2+}, to the cytoplasmic side of inside-out patches increased the open probability of the type II channel by an average of 729%. Thus, it is possible that phosphorylation of the channel results in its up-regulation.

In conclusion: (1) tetrandrine may be a specific blocker of the type II Ca^{2+}-activated K^+ channel; (2) up-regulation of the type II Ca^{2+}-activated K^+ channel is possibly catalyzed by a kinase closely-associated with the channel. (Supported by grants from NIH and NSF.)

40.5

CALCIUM- & PHOSPORYLATION-DEPENDENT K CHANNELS IN NERVE TERMINALS. K. Bielefeldt* & M.B. Jackson, Dept. of Physiology, University of Wisconsin, Madison, WI 53706.

Potassium channels can modulate hormone release from the neurohypophysis. We characterized a Ca-dependent K current in the rat posterior pituitary with patch clamp techniques. Prolonged depolarizations evoked a slowly activating outward current that could be blocked by Cd. In inside-out patches, a Ca-activated K channel (conductance: 161 $\pm$ 6.2 pS) could be identified. Channel openings depend on voltage and [Ca] on the cytosolic surface: The open probability increases e-fold for each 7 mV step; an increase in cytosolic [Ca] shifts the activation curve to more negative potentials (potentials for half-activation: 73 mV, 1.7 mV, and -25.7 mV for 100 nM, 250 nM, and 500 nM Ca, respectively). The channel exhibits complex gating behavior with two distinct open and close states; both voltage and [Ca] affect the open probability by decreasing the mean duration of long closures and increasing the frequency of prolonged openings. Channel activity disappears within 30 s to 5 min. This run down can be reversed by addition of MgATP to the cytosolic side. A non hydrolyzable ATP analogue (AMP-PCP) does not restore activity. Nerve endings in the posterior pituitary express a highly Ca-sensitive K channel which may be involved in the development of fatigue during high frequency stimulation. This channel is covalently modulated through phosphorylation which can be catalyzed by a closely associated kinase.

40.6

MAXI K^+ CHANNEL IN SINGLE ISOLATED EFFERENT NERVE TERMINALS FROM THE COCHLEA IS INHIBITED BY THE SCORPION VENOM CHARYBDOTOXIN AND THE AMINOGLYCOSIDE ANTIBIOTIC NEOMYCIN. Ph. Wangemann, S. Takeuchi, D.C. Marcus and D.L. Sinex*. Cell Physiology Lab and Biophysics Lab, Boys Town National Research Hospital, Omaha, NE 68131

Nerve terminals belonging to the medial efferent system of the gerbil were isolated *in vitro* from the organ of Corti under visual control. Patch clamp recordings were made from nerve terminal membranes. In 85% of seals, 2.0 $\pm$ 0.1 (average $\pm$ SEM; 33 seals) maxi K^+ channels (220 pS) were found and characterized in inside-out and outside-out patches. The open-probability (P_o) was close to zero when on the cytosolic side (CS) the free Ca^{++} concentration (Ca) was $\leq 10^{-7}$ M and when the voltage was negative on CS. There was no effect of Ca on P_o from the extracellular side (ES). Tetraethylammonium (20 mM) and charybdotoxin (10^{-7} M) reduced P_o from ES but not from CS. The ototoxic aminoglycoside antibiotic neomycin ($2.5*10^{-5}$ and $2.5*10^{-4}$ M in the presence of 0.7 mM Ca) inhibited the single channel current amplitude (i) at positive but not negative voltages from CS but not ES. Neomycin ($2.5*10^{-3}$ M in the presence of 0.7 Ca) inhibited i at both positive and negative voltages from CS but not ES. Neomycin had no effect on P_o at positive voltages on CS. The high incidence of this maxi K^+ channel suggests physiologic relevance which may include protection from noise-induced overstimulation. [Supported by NIH grants P01-DC00215-09, R29-DC01098, and R01-DC00212]

40.7

BIOTINYLATED CHARYBDOTOXIN BLOCKS CALCIUM ACTIVATED POTASSIUM CHANNELS IN FROG SACCULAR HAIR CELLS. M.M. Hagedorn* and W.M. Roberts, Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403

Most calcium channels and calcium-activated potassium [K(Ca)] channels in frog saccular hair cells occur in clusters that may correspond to presynaptic active zones. In search of anatomical evidence of this presynaptic localization, we have tested several biotinylated scorpion toxins for their ability to block K(Ca) currents. In low ionic strength extracellular saline (90% sucrose), addition of 100 nM biotinylated charybdotoxin (gift of Dr. K. Angelides) blocked nearly all of the K(Ca) current within 5 min. The channels remained blocked for >30 min after the toxin was removed. To rule out the possibility of blocking by an unbiotinylated contaminant, the toxin was analyzed by HPLC (C-18 column, 0.1% TFA, 5-30% acetylnitrile gradient over 50 min), which revealed two peptide fractions. Each reacted positively for biotin by an alkaline phosphatase dot-blot assay and each blocked the K(Ca) current. The entire biotinylated toxin was premixed with avidin-labeled latex microspheres (300 nM; Mol. Probes) for 8 hr, then pelleted at 13,000 x g. The supernatant and the pellet were tested separately for activity. The supernatant produced no blockage whereas the pellet partially blocked the channels. We conclude that biotinylated toxin is responsible for the observed channel blockade. To localize these channel areas at the level of the light and electron microscope, we are using 1.4 nm streptavidin gold particles (Nanoprobes). When complexed with the biotinylated charybdotoxin for 4 hr, this complex shows the same physiological characteristics as uncomplexed toxin. The anatomical visualization of these channels is in progress.

This work was supported by NIH grant NS27142 and grants from the Sloan and McKnight Foundations to WMR.

40.8

LOCALIZATION OF POTASSIUM CHANNELS LABELLED BY [^{125}I]-CHARYBDOTOXIN IN RAT BRAIN D. R. Gehlert*, S.L. Gackenheimer and D.W. Robertson Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285 and Ligand Pharmaceuticals, San Diego, CA.

The study of potassium (K) channel subtypes in neuronal function has been facilitated by the availability of specific peptide toxins. Charybdotoxin (ChTx), a 37 amino acid peptide isolated from scorpion venom, has been described as an inhibitor of both Ca^{++}-dependent and voltage-gated K channels. In rat brain homogenates, [^{125}I]-ChTx has been reported in bind only to a single population of voltage-gated K channels (Vázquez et al., J. Biol. Chem. 265: 15564-71, 1990). In order to map the distribution of [^{125}I]-ChTx binding sites in the rat brain, we performed a quantitative autoradiographic localization. We also compared the localization with ligands for the low conductance Ca^{++}-activated ([^{125}I]-apamin) and ATP-sensitive ([^{125}I]-iodoglyburide) K channels.

[^{125}I]-ChTx bound to sections of rat forebrain with a K_d of 1.1 nM and a B_{max} of 80 fmoles/mg protein. Routine binding studies were performed at a concentration of 300 pM at which greater than 90% of the binding was specific. The highest density of binding was observed in several white matter containing regions including fasciculus retroflexus and the lateral olfactory tract. High levels were seen in regions such as the paranigral nucleus, the deep layers of the superior colliculus and the ventral limb of the diagonal band. This distribution was distinctly different from that seen for other K channel ligands. In the hippocampus, a high level of [^{125}I]-apamin binding was seen in the CA1 region in stratum oriens and radiatum. [^{125}I]-ChTx binding was low in these regions with a moderate amount in stratum lacunosum moleculare. [^{125}I]-iodoglyburide binding was high in the pyramidal cell layer of CA2-3 and moderate in CA1.

These results indicate the distribution of the K channel labelled by [^{125}I]-ChTx in the brain is distinctly different from that seen with other K channel ligands and suggests differing effects of K channel modulators on brain function.

40.9

A PHYLOGENETIC COMPARISON OF THREE POTASSIUM CHANNEL BINDING SITES. S.E. Perschke, C.W. Bauer, K.C. Swick*, and P.M. Sweetnam. NovaScreen®, Baltimore, MD. 21224.

There are a variety of potassium channels which fulfill important functions in excitable tissues. The pharmacological value of these channels include protection from ischemia, modulation of secretory actions, smooth muscle relaxation, and antiarrhythemia. We have developed receptor binding assays for three functionally different potassium channel binding sites. The phylogenetic distributions of these potassium channel binding sites in neural tissue were determined in seven distinct vertebrates from shark to human. Binding characteristics for the radioligands, [^{3}H]Glibenclamide (ATP dependent), [^{125}I]Apamin (Ca^{++} activated, low conductance), and [^{125}I]Charybdotoxin (Ca^{++} activated, high conductance) were determined. Receptor densities of each channel increased with species complexity ([^{125}I]Charybdotoxin B_{max}: shark 0, rat 1.6 fmoles/mg tissue; [^{125}I]Apamin B_{max}: shark 0.49, rat 0.8 fmoles/mg tissue; [^{3}H]Glibenclamide B_{max}: shark 1.25, rat 4.9 fmoles/mg tissue). We were unable to detect any significant variation in binding affinity (K_d) across species.

40.10

CHARACTERIZATION OF APAMIN-SENSITIVE K-CHANNELS IN CULTURED RAT SKELETAL MUSCLE. S.R. Sampson* and S.V. Alboim. Otto Meyerhoff Center and Health Sciences Research Center, Bar-Ilan University, Ramat Gan 52900, Israel.

K-channels, of which there are several different types, play an important role in the regulation of electrical activity of excitable tissue. Little is known, however, of the factors that regulate their expression. Here, we have reported on some basic properties of apamin-sensitive Ca^{2+}-dependent K^+channels in cultured skeletal muscle obtained from the limbs of neo-natal rat pups. In studies performed with ^{125}I-apamin on whole-cell preparations, we found that the myotubes possess a single binding site for the ligand with a K_d of approximately 100 pM; the B_{max} was found to be of the order of 50 fmol/mg protein. These values differ from those previously reported for cultured mammalian myotubes (Hughes et al., EMBO Journal, 1:1039-1042, 1982). The number of ^{125}I-apamin binding sites increases with age to reach a plateau level at 4-5 days after plating. The largest increment occurs with fusion of myoblasts into multinucleated myotubes. The number of sites remains relatively constant after fusion, and prevention of fusion with 2.7 mM EGTA results in a steady decline in number of binding sites from pre-fusion to nearly undetectable levels. Apamin, in concentrations up to 100 nM had no effect on the rate of resting efflux of ^{86}Rb from mature myotubes. The results are consistent with the concept that apamin-sensitive Ca^{2+}-dependent K^+channels are of little importance in resting K-permeability in skeletal muscle but may be involved in generation of action potential-related changes in K-efflux. (Supported by the Krown Fund for Health Sciences Research, the Raber Fund for Neuroscience Research, and the Otto Meyerhoff Center).

40.11

ANTIARRHYTHMIC DRUG ACTIONS ON A CALCIUM-ACTIVATED POTASSIUM CHANNEL IN MOTONEURONS. J.G. McLarnon*, D. Sawyer, M. Michikawa and S.U. Kim. Dept. of Pharmacology & Therapeutics and Div. of Neurology, Dept. of Medicine, The University of British Columbia, Vancouver, B.C. V6T 1Z3.

Previous studies in this laboratory have shown that a number of drugs, generally classified as Class III antiarrhythmic compounds, block a calcium-dependent potassium channel K(Ca) in hippocampal neurons. We have recently studied the actions of one of these agents, tedisamil, on a K(Ca) channel in mouse motoneurons. Using inside-out patches with the bath solution containing 0.2 mM Ca^{2+} and 140 mM K^+ and the pipette solution containing 5 mM K^+, a K(Ca) channel with a conductance of 100 pS was isolated. The mean open time of the K(Ca) channel, with the patch held at 0 mV, was near 15 ms. Addition of tedisamil to the bath solution (0.1-10 μM) caused dose-dependent decreases in the channel mean open time. The mean open times were not significantly changed when the patch potential was varied. The drug action was consistent with open channel block of K(Ca) with the onward (blocking) rate constant in excess of 5×10^7 $M^{-1}s^{-1}$. These results would suggest that Class III antiarrhythmic drugs may have utility in the study and characterization of properties of K(Ca) channels in various tissue.

40.12

LOOSE COUPLING OF $(Ca)_i$ TO Ca-DEPENDENT POTASSIUM CURRENT DURING MUSCARINIC ACTIVATION. S. H. Thompson*, Stanford University, Hopkins Marine Station, Pacific Grove, CA 93950.

Muscarinic receptor agonists initiate a complex signaling cascade in N1E-115 cells that includes Ca influx, internal Ca release, and the activation of Ca-dependent potassium channels (19-24 pS). Potassium current and $(Ca)_i$ were monitored simultaneously by combining perforated-patch voltage clamp with fura-2 imaging. The Ca signal in response to carbachol peaks at about 400nM. 50% of the cells that express Ca-dependent potassium channels (determined by voltage clamp analysis of tail currents) fail to respond to the $(Ca)_i$ change with an increase in current. In cells that do respond with an increase in current, the current waveform is often substantially different than the waveform of the average intracellular Ca signal. Potassium channels respond to (Ca) changes in the immediate vicinity of the membrane which allows the (Ca) change in this compartment to be measured with much greater resolution than is possible with fluorescence imaging. We can draw two conclusions from our work; 1) in some cases, the near-membrane compartment is protected from the Ca release signal, 2) in other cases, processes localized to the near-membrane modify the time course of the Ca signal in that compartment.

40.13

PROPERTIES OF THE SLOW CALCIUM-ACTIVATED K^+ CURRENT IN RAT LOCUS COERULEUS NEURONS. S. S. Osmanović and S. A. Shefner*. Dept. of Physiol. & Biophys., University of Illinois, College of Medicine, Chicago, IL 60612.

Trains of action potentials in locus coeruleus (LC) neurons are followed by a Ca^{2+}-dependent post-stimulus hyperpolarization (PSH) which is composed of an early component (PSH_E) and a late component (PSH_L). The aim of the present study was to investigate the properties of the slow outward current underlying PSH_L (i_L). Intracellular recordings were made from rat LC neurons in completely submerged brain slices. The current underlying PSH was measured with single-electrode voltage clamp using the "hybrid clamp" protocol. Apamin (200 nM) or d-tubocurarine (200 μM) were added to block the early component of the outward current tail (i_E). i_L reversed at -111 ± 2 mV (n=5) and was reduced by decreasing external Ca^{2+}, addition of Cd^{2+}, or intracellular EGTA. The time course of i_L could be fitted with a single exponential (τ_L). Values of τ_L varied from 2.4 to 6.0 s with a mean of 4.3 s ± 0.4 s (n=12). The time course of decay of i_L showed a weak voltage dependency; depolarization decreased and hyperpolarization increased τ_L. If the number of spikes in the train was kept constant, bath application of 4-AP, Ba^{2+}, or TEA prolonged action potentials and increased the size and duration of i_L. The amplitude and duration of i_L was increased by caffeine (10 mM), and decreased by ryanodine (100 μM) and dantrolene (20 μM). i_L was not affected by noradrenaline (10 μM), 5-HT (100 μM), histamine (100 μM) or adenosine (100 μM). Superfusion of membrane permeable analogs of cAMP, 8-Br-cAMP and dibutyryl-cAMP (1 mM) did not affect i_L. Muscarine (1-10 μM) strongly enhanced i_L without affecting individual action potentials in the train. Enhancement of i_L by muscarine was blocked by pirenzepine (1 μM).

These results suggest that i_L is a Ca^{2+}-activated K^+ current with an unusually long duration, which could be partly due to the release of Ca^{2+} from intracellular stores. Its slow time course, voltage sensitivity and distinct pharmacological properties distinguish i_L from other types of Ca^{2+}-activated K^+ currents in central neurons.

Grant support: PHS AA05846-09 to S.A.S.

40.14

A Voltage-Dependent Calcium-Dependent Outward Current in Medial Pontine Reticular Formation Neurons of the Rat *In Vitro*. D.R. Stevens*, R.W McCarley and R.W.Greene. Dept of Psychiatry, Harvard Medical School and Neurosci. Lab. VAMC, Brockton, MA 02401 USA

The primary action of NE in most medial pontine reticular formation neurons is to reduce a voltage dependent potassium conductance. Using intracellular recording methods in a brainstem slice, this conductance was examined under voltage clamp in 71 neurons. Depolarizing step commands (-70 to -40 mV) activate a time-dependent non-inactivating outward current. This current is suppressed in low calcium or high magnesium medium. Cadmium (100 µM) and barium (100 µM) also block this current and the associated tail current, as does TEA. The tail current is voltage sensitive, with decreased conductance near -70 mV. When extracellular potassium is raised to 15 mM, the tail current reverses polarity near -50 mV, consistent with a K^+ conductance.

These results are consistent with the presence of a calcium-dependent potassium conductance which is voltage-dependent. This current resembles I_C of hippocampus but does not appear to contribute to action potential repolarization. This conductance contributes to the resting membrane potential and the interspike interval of medial pontine reticular formation neurons, resulting in an increased initial responsiveness to excitatory input as well as an increased non-adaptive repetitive firing rate.

This work supported by the Dept. of Veteran's Affairs and NIMH grant MH39683.

40.15

PROPERTIES AND ABUNDANCE OF Ca^{2+}-ACTIVATED K^+ CHANNELS IN HUMAN PERIPHERAL T LYMPHOCYTES. A. Nguyen, M. D. Cahalan*, and S. Grissmer. Dept. Physiol. & Biophys. UC Irvine, CA 92717.

Using excised patch and whole-cell recording we have characterized properties and abundance of Ca^{2+}-activated K^+ channels (K_{Ca}) in resting and activated human peripheral T lymphocytes. In contrast to Jurkat leukemic T cells, we found that the most common type of K_{Ca} channel is sensitive to block by CTX, but not by apamin. The expression of K_{Ca} channels increases dramatically as a function of activation. Freshly isolated resting $CD4^+CD8^-$ (helper T cells) and $CD4^-CD8^+$ (suppressor/cytotoxic T cells) peripheral blood T lymphocytes express on the order of ten K_{Ca} channels per cell, whereas T-cell blasts activated with the mitogenic lectin PHA for more than 1-2 days express hundreds. The calcium sensitivity of the channel in T-cell blasts was determined by simultaneous fura-2 measurement of Ca^{2+} and patch-clamp measurement of K_{Ca}-channel activation. The activation is highly sensitive to the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, suggesting that several Ca^{2+} must bind to the channel or an associated molecule in order to open the channels. The Ca^{2+} concentration at which half of the channels are activated is 500 nM. The K_{Ca} channels show little voltage dependence over a potential range from -100 mV to 0 mV and have a unitary conductance of ~30 pS in symmetrical 170 mM K^+. K_{Ca} channels are blocked by tetraethylammonium (TEA^+) and charybdotoxin (CTX) with half blocking doses of 40 mM and 3 nM, respectively. Both drugs also block voltage-gated type *n* channels with half blocking doses for TEA^+ and CTX of 10 mM and 3 nM, respectively. However, K_{Ca} channels are not sensitive to block by noxiustoxin (NTX), 4-aminopyridine (4-AP), or Ni^{2+} at concentrations that block type *n* channels in the same cells. We conclude that although K_{Ca} and type *n* channels share several similarities in ion permeation, the differences in gating mechanisms and pharmacological sensitivities indicate that the proteins are rather different.

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40.16

ETHANOL REDUCES MAXI-K^+CHANNEL ACTIVITY IN FROG SKIN GLANDS. B.J. Harvey, V. Urbach and J.J. McArdle*. Lab. Jean Maetz, CEA, BP 68, F-06230 Villefranche-sur-mer, FRANCE and *Dept. Pharmacol & Toxicol, New Jersey Medical School (UMDNJ), Newark, NJ 07103-2714, USA.

We have investigated the acute effects of a low concentration of ethanol (0.12% v/v) on the activity of charybdotoxin-sensitive, Ca^{2+}-dependent, outward-rectifier maxi-K^+ channels (200 pS) recorded from basolateral membranes of gland cells in intact epithelial sheets isolated by collagenase treatment from frog skin and mounted in Ussing chambers. Channel activity was defined as the product of the maximum number of open channels (N) and open probability (Po) calculated from $N.Po = \Sigma(n.t_n)$ where n represents the channel state, 0=closed, 1= 1 channel open etc, t_n is the duration of state n. Maxi-K^+ channels were activated by adding carbachol (10 μM) to the bath NaCl Ringer solution or by superfusing the gland cells with KCl Ringer. In cell-attached patches, ethanol reduced NPo within 5 min from 2.94 ± 0.18 to 1.83 ± 0.12 (n=11). The maximum inhibitory effect of alcohol was reached after 2 min. In membrane patches where n≤1 for periods of 5 min, the Po of control and alcohol states were 0.89±0.09 and 0.61±0.03 (n=8), respectively. Mean single channel open and closed times were, respectively, in control 108±12 ms and 4.9±0.3 ms, and in ethanol 13.2±1.5 ms and 50.1±6ms. Since ethanol reduced maxi-K^+ channel activity in intact gland cells but had no effect on NPo in excised inside/out patches our results are consistent with inhibition mediated by cytoplasmic factor(s).

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POTASSIUM CHANNELS: PHYSIOLOGY AND MOLECULAR BIOLOGY

41.1

CYCLICAL POTENTIAL CHANGES RECORDED FROM RAT CENTRAL AND PERIPHERAL AXONS: A MECHANISM FOR ECTOPIC IMPULSE GENERATION. R. Kapoor@, K.J. Smith#, P.A. Felts*#, and M. Davies#. @Inst. of Neurology, Queen Sq., London, WC1N 3BG and #U.M.D.S., Guy's Campus, London, SE1 9RT.

The internodal axolemma of myelinated fibres is not usually considered as a site for the generation of positive symptoms in neurological disorders. We have examined the possibility that the internodal axolemma may contribute to the production of ectopic impulses.

Using micropipettes filled with KCl (0.2-3M), recordings were obtained from the dorsal columns and spinal roots of normal, anaesthetized rats. In addition to intra-axonal recordings (n=31; V_{rest}. -56.7+/-11.6mV; action potential (AP) amp. 54.2+/-11.2mV) we also recorded APs (n=18, 42.3+/-10.9mV) in the absence of an appreciable DC potential (-4.1+/-12.7mV). In such recordings, interpreted as periaxonal, we observed cyclical negative shifts in potential (to -27.3+/-12.2mV, duration 0.1-10s) which were accompanied by a decrease in input resistance. These shifts either occurred spontaneously (0.1-5Hz), or in response to propagated APs or inward current commands. Notably, the shifts were sometimes associated with the generation of ectopic spikes. *In vitro*, similar potential shifts were inhibited by the application of TEA (2-10mM) or 4AP (1-5mM), and were absent using micropipettes filled with 3M NaCl.

The periaxonal recordings were probably made from internodal sites containing a high K^+ concentration, with the negative potential shifts arising from periodic increases in axolemmal K^+ conductance (David, *et al*, J. Physiol. 445:277-301, 1992): the resulting inward K^+ current would initiate the generation of ectopic impulses. We suggest that a high periaxonal K^+ concentration may similarly account for ectopic discharges in certain pathological conditions.

41.2

VARIATION IN I_H, I_{IR}, AND A LEAK CURRENT BETWEEN DIFFERENT DIAMETER ACUTELY ISOLATED ADULT RAT SENSORY NEURONS. R.S. Scroggs*♦, S.Todorovic▲, E.G. Anderson▲, and A.P. Fox♦. ♦Dept. of Pharm/Phys, University of Chicago, Chicago, IL 60637. ▲Dept. of Pharmacol., University of Illinois at Chicago, Chicago, IL 60612.

Inward rectifiers, leak currents and action potentials (APs) were studied in acutely isolated adult rat dorsal root ganglion (DRG) neurons. A slowly activating mixed Na^+/K^+ current, which was inwardly rectifying, was evoked by hyperpolarizing voltage commands in large (42-54μm), medium (32-37μm) and 46% of small (18-27μm) diameter neurons held. This current was selectively blocked by 1mM CsCl versus 1mM $BaCl_2$, and thus, resembled I_H. Neurons which expressed this slow current had short duration APs and exhibited time-dependent rectification in response to hyperpolarizing current commands, similar to A-type DRG neurons. 54% of small diameter neurons did not express the slowly activating current. These neurons had long duration APs and lacked time-dependent rectification, similar to C-type DRG neurons. The data suggest that time-dependent rectification results from activation of I_H.

In most medium diameter neurons, hyperpolarizing voltage commands evoked a rapidly activating current which became inwardly rectifying around the K^+ reversal potential (-94mV), was blocked by 100μM $BaCl_2$, and thus, resembled I_{IR}.

In most large diameter DRG neurons, superfusion with 100μM to 4mM $BaCl_2$, produced an increase in inward holding current. This indicated the presence of a standing outward "leak" current in these neurons which was sensitive to blockade by $BaCl_2$. Variation of ion current expression may be involved in shaping the electrical properties of different types of sensory neurons and the selective neuromodulation of assorted sensory modalities.

41.3

PROPERTIES OF SINGLE CHANNELS AND BACKGROUND CURRENT IN ACUTELY DEMYELINATED RAT SCIATIC NERVE. F.N. Quandt*, Multiple Sclerosis Center and Dept. of Physiology, Rush University, Chicago, IL 60612

Clinical signs and symptoms in multiple sclerosis are caused by block of propagating action potentials due to demyelination of nerve fibers in the central nervous system. In order to determine ways by which conduction can be restored following demyelination, more specfic information is needed regarding the electrical properties of the internodal axon membrane which is exposed during demyelination. Techniques developed by Jonas et al., 1989 (toad, Proc. Natl. Acad. Sci. USA 86: 7328); and Wilson & Chiu, 1990 (rat, J. Neurosci. 10: 3263) were used to expose the axon membrane in the internode for patch clamp recording. Rat sciatic nerve was incubated in collagenase followed by protease. Fibers were then placed in Kglutamate internal solution and dissociated using a "rolling pin" made from tygon tubing. Under this condition Schwann cells retract exposing the axon membrane, but only in a minority of fibers. Recordings have been made from intact membranes, as well as those in the inside-out configuration. The pipette solution contained normal physiological saline. Membrane current from one patch can exhibit both inward Na (50 pA peak) and outward K components. More than one type of K channel appears to contribute to the outward current. One type exhibited a conductance of 50 pS and a short open time (1 ms, 0 mV, 22° C), while the other had a 50% lower conductance and a prolonged open time. The background conductance which generates current not associated with gated channels was found to increase as the membrane was depolarized. Supported by the National Multiple Sclerosis Society.

41.4

ACTIVATION OF VOLTAGE-DEPENDENT K CHANNELS, REDUCES CYCLIC AMP FORMATION IN INTACT CELLS. M.Pizzi*, M.Memo, M.Belloni, PF. Spano. Div. Pharmacol., Toxicol., Exper. Therap.; Dept. Biom.Sci.Biotech.University of Brescia 25124 -I.

Dopamine D-2 receptor agonists, including BHT 920 and bromocriptine, and the K channel opener minoxidil share the property to hyperpolarize the plasma membrane by activating voltage-dependent K channels. We tested the capability of these drugs to inhibit the cAMP formation induced by forskolin either in intact or in broken pituitary cells. While bromocriptine was active in both experimental conditions, BHT920 and minoxidil inhibited cAMP formation in intact but not in broken cells. The concentration-dependent response of BHT 920 showed an IC-50 value of 0.7 µM and was specifically blocked by l-sulpiride. The effect of BHT 920 was not additive to that induced by minoxidil and less effective in the presence of K channels blockers. We suggest that BHT 920- and minoxidil-induced inhibition of cAMP formation is mediated by the activation of voltage-dependent K channels.

41.5

INWARD AND OUTWARD CURRENTS IN SLOW MUSCLE FIBERS OF THE CHICKEN. M. HUERTA*., X. TRUJILLO., C. VASQUEZ AND J. LOMELI. CENTRO UNIVERSITARIO DE INVESTIGACIONES BIOMEDICAS, UNIVERSIDAD DE COLIMA. APDO. POSTAL 199, 28000: COLIMA, COL., MEXICO.

The anterior latissimus dorsi muscle (ALD) of the chicken posses only slow fibers. The slow muscle fibers posses the ability to generate action potentials. Patch clamp of sarcolemmal spheres from stretched muscle fibers of the ALD muscle (Stein & Palade, Am. J. Physiol. 25:434-440, 1989) are a convenient preparation to study ionic currents in slow muscle of the chicken. In the present study, macroscopic inward and outward currents were recorded using the giant excised sarcolemmal patches (Hilgemann, Pflügers Arch. 415:247-249, 1989) variation of the patch electrode voltage clamp technique. The pipete solution contained in (mM): 172 Na(gluconate); 7 Mg(gluconate)2; 10 Dextrose and the bath solution contained (mM): 172 Cs(gluconate). The pH was adjusted with HEPES around to 7.4. In these conditions we found an inward current. It had a mean threshold of -60 mV, it follows to a peak in about 5 ms and it declines slowly to a new stable value in about 25 ms. This current is partially blocked by TTX (1X10-6M). If we use in the bath a solution in (mM): 172 K(CH3SO3) and the pipete solution in (mM): 172 TEA(CH3SO3); 7 Mg(CH3SO3)2; 10 Dextrose, we found and outward current who activates slowly with pulses of depolarization (25 ms) and its threshold is -40 mV. The current does not show inactivation. It suggest the presence of Na+ and K+ channels.

41.6

RAT AND GUINEA PIG VAGAL NEURONS HAVE DIFFERENT ELECTROPHYSIOLOGICAL PROPERTIES . P. Sah and E. M. McLachlan*. Dept of Physiology and Pharmacology, University of Queensland, Qld 4072. Australia.

We have compared the electrophysiological properties of neurons in the dorsal motor nucleus of the vagus(dmv) in rats and guinea pigs in transverse slices of brainstem maintained *in vitro* . Minor differences in the passive electrical properties of neurons between the species were consistent with the slightly larger dimensions of the guinea pig neurons revealed by intracellular injection of biocytin. However, action potentials in the guinea pig had a faster rate of rise, larger amplitudes and longer durations. In guinea pig neurons, two Ca-activated K currents were activated following calcium influx during the action potential (Neuron,7,257). These two currents accounted for the full duration of the afterhyperpolarization (AHP). In rat neurons an AHP of similar duration resulted in the absence of the second Ca-activated K current; instead the early apamin-sensitive current activated a transient voltage dependent K current (τ~400ms). These differences meant that in response to a step of depolarizing current, neurones in the guinea pig only discharged once or twice and then ceased firing. In rat neurones, this manoeuvre produced repetitive firing. In guinea pig neurons hyperpolarization of the cell beyond -60mV activated an inward current which could be blocked by Cs but not by Ba. This current was always much smaller in rat neurons. We conclude that, despite many similarities of size and electrical properties, DMV neurones in the two species differ in terms of several voltage and calcium dependent conductances which determine their active electrical behaviour.

41.7

NOVEL HYPERPOLARIZATION-ACTIVATED CURRENT MEDIATES INWARD RECTIFICATION IN CRAYFISH MUSCLE. W. Buño* and A. Araque. Instituto Cajal, Doctor Arce 37, Madrid, Spain.

The ionic current mediating inward rectification in opener muscle fibres was studied under two-electrode voltage-clamp. Hyperpolarizing pulses from -60 mV evoked an instantaneous voltage-independent current (I_L) followed by a time- and voltage-dependent inward current (I_{AB}). The mean reversal potential of I_{AB} (E_{AB}) at an extracellular K^+ concentration ($[K^+]_o$) of 5.4 mM was -61.8 mV. E_{AB} shifted towards positive potentials by 50.8 mV for a tenfold increase in $[K^+]_o$. The conductance underlying I_{AB} (G_{AB}) increased sigmoidally with hyperpolarization, starting close to the resting potential, saturating at a $G_{AB,max}$ of about -140 mV and showing a mean half activation at -94.4 mV. The activation curve of G_{AB} shifted 53.6 mV towards positive potentials with a tenfold increase of $[K^+]_o$. $G_{AB,max}$ did not increase in raised $[K^+]_o$. The activation and deactivation kinetics of I_{AB} were accurately described by single exponentials (time constants <100 ms).

I_{AB} was not modified in: (1) Na^+- and Ca^{2+}-free solutions; (2) intracellular EGTA; (3) extra or intracellular TEA; (4) extracellular Cs^+, Rb^+, Ba^{2+} or Mn^{2+}.

Low concentrations of Cd^{2+} or Zn^{2+} (<5 mM) strongly and reversibly reduced both I_L and I_{AB}.

Inward rectification in crayfish muscle is generated by a voltage- and time-dependent K^+ current I_{AB} which displayed many characteristics which distinguish it from all others.

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41.8

SINGLE CHANNELS IN STOMATOGASTRIC NEURONS IN CULTURE. W. D. Krenz and F. Nagy. LNPC, Univ. Bordeaux I, CNRS, 33120 Arcachon, France (SPON: European Neuroscience Association).

We have studied two membrane currents, a fast transient (I_A) and a Ca^{2+}-dependent ($I_{O,C}$) outward current in neurons from the stomatogastric ganglion (STG) of adult crabs (*Cancer pagurus*) in primary culture. Both have been shown previously to be present in STG neurons *in situ* and are supposed to be of importance in the generation and regulation of oscillatory discharge patterns. Single channel currents were measured in the cell-attached and inside-out patch configuration (Hamill et al., 1981: Pflügers Archiv 391) on cell bodies and growing neurites. In general the channel density was low. A voltage-dependent, transiently activated channel with voltage-dependent inactivation similar to that of I_A K^+ current was identified in few patches only. In cell-attached mode and with a physiological K^+ gradient its conductance was ~17 pS. Ca^{2+}-dependent channels were more abundant in membrane patches on the cell body as well as on neurites and growth cone veils. Their voltage dependence and sensitivity to Ca^{2+} at the intracellular membrane surface was similar to that of $I_{K,C}$ described in many other systems. In cell-attached mode and with physiological concentration gradients for K^+ and Ca^{2+} ions the single channel slope conductance of the linear region of the iV-plot was ~130 pS. During oxotremorine-induced membrane potential oscillations the single channel current changed in parallel with the membrane potential due to the cyclic changes in the driving force for the K^+ ion. In addition the open probability increased during the depolarized phase of the oscillation and in some cases additional channels were recruited, suggesting the influx of Ca^{2+} during the oscillations. In some patches small amplitude low conductance channels were found that may represent the channel of the Hodgkin-Huxley type delayed rectifier current. The functional implications of these results will be discussed.

41.9

VOLTAGE DEPENDENT GATING OF A SODIUM-ACTIVATED POTASSIUM CHANNEL FROM MAMMALIAN BRAIN. M. Esguerra* and I.B. Levitan, Graduate Dept. of Biochemistry & Center for Complex Systems, Brandeis University, Waltham, MA 02254.

Intracellular sodium activates a large-conductance (~170pS) potassium (K_{Na}) channel in neurons cultured from the rat brain (Egan et al., 1992, *J. Neurosci.*, in press). As part of ongoing studies of this channel's modulation, we recorded K_{Na} channel activity in inside-out patches excised from cultured rat olfactory bulb neurons and determined kinetic rate constants for channel openings and closures under varying levels of $[Na^+]_i$ (0-150mM) and transmembrane voltage (+60 to -100 mV, inside negative). As reported previously, the open probability (Np_o) of K_{Na} channels increased with depolarization at all sodium concentrations (n=6). In the hyperpolarized range of membrane voltages, further hyperpolarization by about 30 mV induced up to 5-fold increases in mean open time duration (n=7), particularly under conditions of low $[Na+]_i$. These changes were associated with a decrease in the frequency of short-duration closures within bursts of channel activity. These results suggest that membrane voltage modulates the sensitivity of K_{Na} channels to internal sodium concentration. A similar mechanism has been shown to confer voltage sensitivity on K_{Ca} channels; this functional similarity between K_{Ca} and K_{Na} channels supports the hypothesis that ion-gated potassium channels comprise a family of related membrane proteins.

41.10

SODIUM-ACTIVATED POTASSIUM CHANNEL OF AVIAN NEURONS: SUBCONDUCTANCE LEVELS AND GATING PROPERTIES STUDIED BY A NOVEL ANALYSIS PROCEDURE. R. Fesce and C. Haimann (Spon: European Neuroscience Association) "B. Ceccarelli" and CNR Cytopharmacol. Centers, Scient. Inst. San Raffaele, Dept. of Pharmacology, Univ. of Milano, 20129 Italy.

Sodium-activated potassium channels (K_{Na}) in neurons and heart are characterized by a main open channel conductance varying from 100 to 210 pS, in the different preparations, and by frequent transitions to other conductance levels or substates. This behaviour seems not to depend on Na^+ concentration near the plasmalemma (Na_i), and it has been suggested to arise from conformational changes of a single pore channel. K_{Na} openings occur in bursts, and previous data from open time distributions indicate that Na_i promotes the opening of the channel but has little influence on the durations of individual openings.

In order to investigate the mechanisms of the action of Na_i, and of other ions affecting neuronal K_{Na}, we developed a novel procedure to identify subconductance levels and to determine their gating properties. The procedure is based on the detection of minima in the signal variance for identification of conductance levels (Patlak, J. Gen. Physiol. 92, 413, 1988) and on automated analysis of the transitions between levels. The analysis gave consistent and reliable estimate of the number of substates (at least five), of the corresponding conductances, dwell times, and probability of exiting towards each other level.

Our data confirm that Na_i mainly affects the kinetics of closed states and help defining a kinetic model for neuronal K_{Na} channel to study the mode of action of blockers and modulators.

41.11

THE MINK POTASSIUM CHANNEL IS INWARDLY RECTIFYING. E. M. Blumenthal* and L. K. Kaczmarek, Interdepartmental Neuroscience Program, Yale Univ. Sch. of Med., New Haven, CT 06510.

The minK protein produces a very slowly activating voltage-dependent potassium current when expressed in *Xenopus laevis* oocytes. This protein is profoundly different from other cloned potassium channels, both in its small size (130 amino acids) and the slow kinetics of the expressed current. We used the oocyte expression system to study the current-voltage relationship of the minK channel.

Because single minK channels have not yet been seen, we studied the instantaneous changes in the active current in response to voltage jumps. This procedure should yield the single channel I-V relationship. We found that minK is strongly inwardly rectifying. In 2mM external potassium, steps from -60mV to between -20mV and 60mV showed the current saturating with increasing driving force. In addition, comparison of tail currents in 2mM and 113mM external potassium showed that the inward slope conductance is 6.3-fold higher than the outward slope conductance. No other cloned potassium channel is known to exhibit this property. MinK promises to be an excellent system for studying the structural basis for rectification.

41.12

EXPRESSION AND MODULATION OF THE Kv1.5 POTASSIUM CHANNEL IN A RAT ANTERIOR PITUITARY CELL LINE. D. Saal, S. Chung and L.K. Kaczmarek*, Interdepartmental Neuroscience Program and Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The rat anterior pituitary cell line, GH4, has been shown to have both A-type and delayed rectifier voltage-gated potassium channels. The cells express mRNA for the *Shaker* -like potassium channel Kv1.5. It has previously been shown that the steroid hormone dexamethesone increases the level of Kv1.5 mRNA, as well as the magnitude of the A-type current, in GH4 cells. We now have found that the delayed rectifier current is also increased by dexamethesone. In addition, we have used the PGEX system to generate a glutathione S transferase - fusion protein that contains the N-terminal 165 amino acids of Kv1.5. Using antibodies raised against this fusion protein, immunoflorescent staining has been detected in GH4 cells as well as Kv1.5 transfected fibroblasts. By using florescence activated cell sorting, we have demonstrated that the Kv1.5 protein level is also changed by dexamethasone. In ongoing experiments we are attempting to determine whether the Kv1.5 protein contributes to the A type or delayed rectifier current by applying antisense oligonucleotides to the cells to block translation of the Kv1.5 mRNA.

41.13

OVEREXPRESSION OF A NEW CLASS OF K⁺ CHANNEL ABOLISHES THE SPONTANEOUS BURSTING ACTIVITY IN *APLYSIA* NEURON R15. B. Zhao*, B.-K. Kaang, E. R. Kandel, and Tai Kubo. Ctr. Neurobiol. & Behav., Columbia P&S, and HHMI, NY, NY 10032.

Potassium channels make important contributions to the control of the membrane excitability and firing pattern in neurons. The ability to express various cloned *Aplysia* K⁺ channels in identified *Aplysia* neurons has allowed us to analyze the relative contributions of specific K⁺ channels to different aspects of the behavior of individual identified cells. By examining the H5 region, the putative pore-forming region, in several cloned *Aplysia* K⁺ channels, a remarkable conservation in codon usage was found at several potentially highly degenerate positions. Using this region-specific codon usage, we designed oligonucleotide probes based on the H5 region and scceeded in cloning two new K⁺ channels (AK04 and AK05). Each seems to represent a new class of K⁺ channel. We here describe AK05. When expressed in *Xenopus* oocytes this current showed no inactivation even with long depolarizing pulses. We therefore subcloned AK05 into pNEX, a neuronal expression vector and expressed AK05 in the bursting *Aplysia* neuron R15. Twenty-four hours after microinjection of AK05 into R15 a non-inactivating K⁺ current of about 2-3 μA developed. This current completely eliminated the spontaneous bursting activity of neuron R15, so that the cell became silent. This is in marked contrast to the effects we see in the same cell (R15) with AK01a overexpression. AK01a is a transient current that mainly affected the duration and afterhyperpolarization of action potentials. This suggests that by using overexpression of different K⁺ channels one can selectively redesign the different components of electrical excitability in neurons.

41.14

PROTEIN KINASE MODULATION OF *APLYSIA* POTASSIUM CHANNEL ACTIVITY EXPRESSED FROM A CLONED cDNA AND ITS PHOSPHORYLATION SITE MUTANTS. T. Kubo* and Y. Furukawa. Ctr. for Neurobiology & Behavior, Columbia P&S, New York, NY 10032; Hiroshima Univ. Integrated Arts & Sciences, Hiroshima, 730 Japan.

Ion channels are known targets for protein kinases, and the modulation of channel activities by phosphorylation is thought to be critical for controlling many aspects of neuronal activity. To explore the molecular basis of ion channel modulation, we have started to examine the effects of kinase activators on the current expressed by cloned *Aplysia* K⁺ channel (AK01a, a homologue of *Drosophila Shaker* channel). This channel produces a fast transient outward K⁺ current when expressed in *Xenopus* oocytes or in *Aplysia* neurons (B.-K. Kaang et al., *PNAS*, **89**:1133, 1992). Application to oocytes expressing AK01a of protein kinase C (PKC) activators, β-phorbol 12-myristate 13-acetate (PMA), phorbol-12, 13-dibutyrate (PDBU), or 1-oleoyl-2-acetyl-sn-glycerol (OAG) increases the inactivation time constant of the current, and the total charge movement through the channels. I_{Adepol}, which was identified as corresponding to the AK01 in native current in *Aplysia* neurons, was also modulated similarly by PMA. Inactive phorbol ester produced no change. The primary structure of AK01a contains three putative PKC phosphorylation sites and one putative cAMP-dependent protein kinase phosphorylation site. We now are examining mutants in these phosphorylation sites to determine how these residues contribute to the modulation.

41.15

EXPRESSION OF *SHAKER* FAMILY K^+ CHANNEL mRNA IN THE SPINY LOBSTER, *PANULIRUS INTERRUPTUS*. D.J. Baro*, C. Cole, M. Tapia, A. Zarrin, A. Chen, T.R. Podleski and R.M. Harris-Warrick. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

We are investigating the role of intrinsic neuronal properties in the generation of the pyloric motor pattern in the spiny lobster, *Panulirus interruptus*. The transient K^+ current (I_A) helps regulate the firing phases of the pyloric neurons and is expressed differentially in these neurons. We are extending the exploration of I_A to the molecular level in order to eventually quantitate the expression of the I_A channel gene(s) and manipulate the expression of I_A in the individual neurons of the pyloric central pattern generator. We used an RNA PCR-based approach to determine which members of the *Shaker* family of K^+ channels are expressed in the spiny lobster. Thus far we have isolated portions of the lobster *Shaker*, *Shab*, *Shaw* and *Shal* genes. Each gene shows a very high degree of homology to its *Drosophila* homologue. Limited regions within the conserved core of the proteins display between 82% and 100% amino acid identity to the *Drosophila* homologue, and 74-80% identity at the nucleotide level. Not surprisingly, the PCR data indicate the existence of only one gene in each of the *Shaker* subfamilies, as opposed to the multiple genes found in the subfamilies of higher vertebrates. We are now in the process of isolating complete open reading frames for each gene using a PCR-based approach as well as the more conventional screening of cDNA libraries. Supported by NIH grant NS25915.

41.16

ANALYSIS OF PRODUCTS OF TWO GENES ENCODING HIGH-VOLTAGE ACTIVATING, TEA-SENSITIVE, A-TYPE K^+ CURRENTS. E. Vega-Saenz de Miera, H. Moreno, K. Sen, D. Lau and B. Rudy, Dept. of Physiology and Biophysics and Dept. of Biochemistry, New York University Medical Center. 550 First Avenue. New York, N. Y. 10016

Recently we and others cloned several cDNAs encoding voltage-gated K^+ channels that are distantly related to the *Drosophila* Shaw gene: KShIIIA, NGK2-KV4, KShIIIC-Raw3 and KSHIIID. Products of the last two genes encode transcripts that express in *Xenopus* oocytes high-voltage activating, TEA-sensitive, transient K^+ currents. Both KShIIIC and KShIIID differ from KShIIIA and NGK2, which express non-inactivating currents, by the presence of an amino terminal insert which is responsible for channel inactivation. Both inserts have a similar cysteine-containing sequence near the amino end. In Raw3 this sequence has been suggested to be involved in the modulation of inactivation by oxidation (Ruppersberg et al, 1991, *Nature* 352,711). Both channels have the same voltage dependence of inactivation but the midpoint of the steady state inactivation curve in KShIIIC is 20-30 mV more negative than that of KShIIID. As a result of this difference KShIIID channels conduct in the steady-state over a much broader window of potentials. In addition, the inactivation of KShIIID channels proceeds with significantly slower kinetics than that of KShIIIC. Based on analysis of heteromultimers and chimeras we conclude that the different amino terminal inserts determine the observed differences in inactivation. Inactivation from the closed state appears more significant for these channels than for inactivating Shaker channels. In the case of KShIIIC and KShIIID there is a correlation between inactivation rate and the voltage-dependence of steady-state inactivation. ShIII proteins expressing noninactivating channels may be components of inactivating channels when forming heteromultimers with KShIIIC or KShIIID. In situ hybridization studies exploring whether these transcripts are expressed in the same cells will be shown.

41.17

DRK1 mRNA IS INDUCED BY NGF IN RAT PHEOCHROMOCYTOMA PC12 CELLS. B. Rudy*, D. Lau, J.W. Lin, J. Pollack, and C. Kentros; Dept. Physiology and Biophysics and Dept. of Biochemistry, NYU School of Medicine, NY NY 10016

The DRK1 gene encodes transcripts which express voltage-dependent delayed rectifier type K^+ currents which activate relatively slowly and are resistant to TEA and CTX. Rat pheochromocytoma PC12 cells express several Sh transcripts, including DRK1. Northern blot analysis of RNA isolated from PC12 cells at various time points following NGF stimulation reveals NGF-mediated induction of transcripts of the DRK1 gene. The levels of DRK1 transcripts have also been found to increase markedly following denervation of rat skeletal muscle (Trimmer et al, 1990 Soc. Neurosci. Abst. 16,3). Analysis of patch-clamp recordings reveals a current in PC12 cells ($I_{(TEA\text{-}CTX)r}$) which has electrophysiological and pharmacological properties quite similar to those of channels formed by injection of DRK1 cRNA into Xenopus oocytes. $I_{(TEA\text{-}CTX)r}$ is also induced by NGF in a manner that is temporally consistent with the hypothesis that proteins encoded by the DRK1 gene are a major component of this channel in situ. When hybridized to Northern blots of PC12 cell mRNA, DRK1-specific probes label three bands approximately 4.3kB, 9kB, and 10kB in length. Interestingly, only the 10kB band is significantly induced by treatment with NGF. Experiments investigating the possible nature of this seemingly transcript-specific induction will be discussed.

41.18

EXPRESSION AND PURIFICATION OF A RAT BRAIN DELAYED RECTIFIER POTASSIUM CHANNEL (Kv2.1) IN Sf9 INSECT CELLS. F.A. Sorond†, M. Ladner‡, A.E. Lacerda, A.M. Brown†*. Depts. of Molecular Physiology & Biophysics and †Neuroscience, Baylor College of Medicine, Houston, TX 77030, and ‡Chiron Corp., Emeryville, CA 94608.

The Kv2.1 channel has been expressed in Sf9 insect cells using a tagged construct subcloned into the baculovirus expression vector. The tag is an eight amino acid peptide (KT3) engineered on the carboxy terminus of the protein. Tagged and untagged constructs expressed similar currents. Whole cell recordings from the Sf9 cells have demonstrated that the tag does not alter the electrophysiological properties of the channel. The current densities were more than 60 pA/pF. Antibodies against the tag were used to purify the expressed protein from the transfected SF9 cell membranes using affinity chromatography. The purification of the channel protein has been confirmed using Western blots. Work is presently under way to analyze the channel properties of the expressed protein.

41.19

IDENTIFICATION OF A K^+ CHANNEL SUBFAMILY SPECIFIC OLIGOMERIZATION DOMAIN. N.V. Shen* and P.J. Pfaffinger. Div. of Neuroscience, BCM, Houston, TX 77030.

We are investigating how K^+ channel subunit proteins form the set of ion channels made by particular neurons. The great diversity of identified K^+ channel subunit proteins poses a critical problem in understanding how the electrical properties of neurons are determined, yet little is known about how specific K^+ channels are formed. Our work focuses on the analysis of K^+ channel subunit proteins synthesized in vitro from the Aplysia *Shaker* cDNA AK01a. In this system, K^+ channel subunit proteins are synthesized into membranes and form spontaneous oligomers with an apparent N of 4 (Pfaffinger and Chen, 1992). We have now analyzed nine C-terminal truncation constructs, covering the entire AK01a channel protein, in this expression system. All proteins extending past the S1 putative transmembrane domain were incorporated into the membranes. We have identified two glycosylated sites located between S1 and S2. We also tested if these constructs could associate into oligomers of wild-type K^+ channel subunit proteins. The nine truncation constructs were made with a novel epitope fused at the C-terminus (Tag 1). The Tag 1 truncation mutants were co-translated with the wild-type AK01a, which lacks a Tag 1 site, and with a *shaw* subfamily cDNA, AK20. To test for oligomerization, we attempted to co-immunoprecipitate the wild-type, non-tagged channel proteins, with the anti-Tag 1 antiserum after solubilization in CHAPS. Most of the truncation mutants co-immunoprecipitated wild-type AK01a protein with high efficiency; only the shortest protein, lacking a conserved N-terminal domain failed to do so. AK20 protein, however, does not co-immunoprecipitate in these assays. These results indicate that an important domain for K^+ channel subunit protein oligomerization is located in this conserved N-terminal domain and is subfamily-specific.

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41.20

N- AND C-TERMINAL CYTOPLASMIC DOMAINS ALTER A PARTICULARLY SLOW INACTIVATION OF THE NGK2 (Kv3.1) CHANNEL. T. Kawamura #, S. Yokoyama #, J. Yamashita # and H. Higashida*#Departments of Biophysics and #Neurosurgery, Kanazawa Univ. Sch. of Med., Kanazawa 920 , Japan.

In voltage-dependent potassium channels, N- and C-terminal domains have effects on various types of inactivaton. The *Shaw*-related voltage-dependent potassium channel, NGK2 (Kv3.1), exhibits a particularly slow inactivation during several seconds (Ito et al., Proc. R. Soc. B., in press, 1992). This type of inactivation has been also identified in NG108-15 cells, mouse neuroblastoma cells and rat hippocampal neurons. To determine whether N- and/or C-terminal cytoplasmic domains of the NGK2 channel alter this slow inactivation, we made deletion mutants in either or both of N- and C-terminal cytoplasmic domains. We also constructed the chimeric potassium channel formed by replacing C-teminal region of the NGK1 (Kv1.2) channel, which belongs to the *Shaker*-related subfamily, with that of the NGK2. These mutant channels were expressed stably in B82 mouse fibroblast cells, and analyzed using discontinuous whole-cell voltage clamp technique. We found that the slow inactivation was removed in all of the deletion mutant channels tested. Furthermore, the chimeric channel did not show the NGK2-specific inactivation. But the steady-state activation curves of the mutant channels were nearly same as that of intact NGK2 channels. These results suggest that both of the N- and C-terminal cytoplasmic domains may play an important role in the slow inactivation of the NGK2 channel.

41.21

REGULATION OF NEURONAL VOLTAGE-DEPENDENT K^+ CHANNELS BY cAMP-DEPENDENT PROTEIN KINASE: STUDIES WITH FIBROBLAST CELLS STABLY TRANSFECTED WITH Kv1.2 K^+ CHANNEL cDNA.

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In CL1023 fibroblast cells stably transfected with Kv1.2 cDNA, K^+ currents with delayed rectifying properties were recorded using the whole-cell recording technique. The expressed channel protein has a stretch of amino-acids (putatively located intracellularly) which could be a potential phosphorylation site (McKinnon, *J. Biol. Chem.* **264**: 8230, 1989). We therefore investigated whether one or more protein kinases are involved in the regulation of this K^+ channel. In recordings with the perforated patch method (Amphotericin B as pore forming agent), application of 1 mM dibutyryl-cAMP caused ~20% increase in the K^+ current. Smaller effects (< 10% enhancement) were observed when the K^+ currents were recorded with the normal whole-cell technique which allows the intracellular contents to be exchanged with the electrode solution. However, the larger potentiation was retained when cAMP-dependent protein kinase (PKA) was included in the intracellular perfusion solution. With both the whole-cell and perforated patch techniques no significant effects of dibutyryl-cGMP (1 mM) or the protein kinase C activator phorbol 12-myristate 13-acetate (TPA) (100 nM) were observed. In addition, dibutyryl-cGMP had no effect on the K^+ currents in cells intracellularly perfused with cGMP-dependent protein kinase. We conclude that PKA is a likely candidate as a regulator of the Kv1.2 channel protein.

41.22

HOST-DEPENDENT *SHAKER* cDNA EXPRESSION IN CULTURED *DROSOPHILA* "GIANT" NEURONS BY GERMLINE TRANSFORMATION.

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Alternative splicing of *Drosophila Shaker* gene transcripts generates a number of functionally distinct K^+ channel subunits that can interact to form both homo- and heteromeric K^+ channels in *Xenopus* oocytes. How distinct K^+ channels assemble in the fly is unknown. In neurons, voltage-dependent A-type K^+ channels may assemble from a single *Sh* subunit type, from multiple *Sh* subunits, or from the products of other loci, alone, or in combination with *Sh* subunits. Allele-specific differences, suggestive of heteromeric assembly, have been observed: Sh^{rK0120} and Sh^{M} mutations show similar hyperexcitability at the neuromuscular junction, but voltage-clamp studies reveal different K^+ channel defects in muscle (Sh^{M} eliminates I_A while Sh^{rK0120} reduces it). The Sh^{M} mutation results from an insertion in the constant region of the gene, while Sh^{rK0120} is apparently a point mutation at an undetermined site. We have investigated the functional expression of A-type K^+ channels in germline transformants of these *Sh* mutants in which expression of *Sh* 29-4 cDNA is driven by the *hsp70* promoter. K^+ currents expressed in cultured embryonic "giant" neurons from several, independently derived, transformed lines have been studied by whole-cell patch-clamp recordings at different times following heat shock. All transformed lines express a "heat-induced" current that is characterized by a higher sensitivity to 4-AP than the "native" A current in the same neuron, and a slower rate of recovery from inactivation than either the "native" *Sh* K^+ current or the current expressed from *Sh* 29-4 mRNA in *Xenopus* oocytes. This component shows similar inactivation kinetics in all lines, but is about 3 times greater in amplitude, and slightly more sensitive to 4-AP, in Sh^{rK0120} than in Sh^{M} hosts. Our results indicate that expression of *Sh* 29-4 cDNA in *Drosophila* neurons may be independent of the insertion site but is clearly dependent on the mutant background. The mechanism by which different host backgrounds influence the expression of individual *Sh* subunits is unknown, and the possibility that heteromeric K^+ channels may be formed is currently under investigation.

41.23

EVOLUTIONARY RELATIONSHIPS WITHIN THE POTASSIUM CHANNEL MULTIGENE FAMILY.

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Ion transport across the lipid membrane was probably of critical importance for primordial cells in volume regulation and control of cellular processes. We have previously analyzed the molecular evolution of members of the ion channel multigene superfamily including Na^+, Ca^{++}, K^+ and cyclic nucleotide gated channels (M. Strong, K.G. Chandy and G.A. Gutman, submitted). We have aligned sequences manually and with the help of algorithms such as Clustal and Macaw, and have used several phylogenetic tree reconstruction methods including maximum and weighted parsimony, maximum likelihood and neighbor joining, to more confidently estimate the correct tree. Here we report the addition of new gene sequences to the K^+ channel phylogenetic tree. A branching order was found using a tree computed from nucleotide sequences whereby the rat K13 gene branches off of the *Shab*/DRK1 node followed by the branching of the rat IK8 gene. This topology still supports our earlier finding of a branching order which makes neighbors of *Shab/Shaw* and *Shaker/Shal*. Furthermore, an amino acid alignment which compares widely diverged K^+ channel sequences (courtesy of R. Guy; *Science* 254: 730) shows that the cAMP and cGMP gated channels cluster strongly with *eag*, *slo* and *Shal*, and, more weakly with *Shaker*, *Shaw* and *Shab* which progressively branch off of this tree. This tree can not be confidently rooted at present due to the lack of gene sequences from more distantly related organisms. Thus we are left to speculate on the rooted branching order of the gene family using voltage clamp data from organisms such as jellyfish, Paramecium, Saccharomyces, plants, and *E. coli*.

41.24

MOLECULAR COMPONENTS OF LOW-VOLTAGE-ACTIVATING A CURRENTS.

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Transient K currents which activate and inactivate at voltages near the threshold for Na action potential generation [low-voltage activating A-currents or $I_{A(LV)}$] are widespread in mammalian neurons, where they play an important role in modulating spike frequency as well as delaying the initiation of bursts of action potentials. Several mammalian cDNA's have been identified that express transient A-type currents: RCK4, KShIIIC and KShIIID, and the mammalian homologues of the *Drosophila* Shal gene. We will present comparisons of the currents expressed by these cDNAs in *Xenopus* oocytes and neuronal $I_{A(LV)}$. KSIIIC and KShIIID are not likely to be components of $I_{A(LV)}$ because they express channels which activate at very positive potentials and are very sensitive to TEA. RCK4 and, in particular, mammalian Shals may be components of some $I_{A(LV)}$: they express currents with voltage-dependence, kinetics and pharmacology similar to some $I_{A(LV)}$. Furthermore, the time constants of inactivation of the currents expressed by known mammalian Shals (I_{shal}) are not voltage-dependent as is the case for many $I_{A(LV)}$. However, I_{shal} show a prolonged slow component of inactivation and recover from inactivation extremely slowly, unlike $I_{A(LV)}$ in many neurons. *Xenopus* oocytes injected with rat whole brain poly-A RNA, express a fast recovering $I_{A(LV)}$ which lacks this very slow phase of inactivation. This current is the result of a not yet understood combination of components some of which can be separated by sucrose-gradient fractionation. Antisense hybrid arrest experiments exploring the role of Shal proteins in the $I_{A(LV)}$ channels seen with whole rat brain mRNA, and experiments aimed at reconstituting this $I_{A(LV)}$ will be presented.

ACETYLCHOLINE: NEURONAL NICOTINIC RECEPTORS I

42.1

PHARMACOLOGICAL CHARACTERIZATION OF NICOTINIC ACETYLCHOLINE RECEPTORS IN THE MEDIAL HABENULA NUCLEUS OF THE RAT, *IN VITRO*.

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Previous studies have demonstrated nicotinic acetylcholine receptors in the rat medial habenula (MHb) nucleus. We have investigated the pharmacology of the nicotinic response in intact MHb neurones using extracellular recordings. 350µm slices of rat brain were cut and recordings were made from single neurones using conventional electrophysiological techniques.

Carbamyl choline (carbachol) excited all MHb neurones recorded from with an increase in firing rate that was rapid in onset and dose dependent. The cholinergic response was blocked by the nicotinic antagonists hexamethonium and mecamylamine while the muscarinic antagonists atropine and pirenzepine were ineffective. In addition, an excitatory response was evoked by low concentrations of the ganglionic agonists nicotine, cytisine and 1,1-Dimethyl-4-phenylpiperazinium iodide.

42.2

FUNCTIONAL PRESYNAPTIC NICOTINIC RECEPTORS ON SYNAPTIC TERMINALS ATTACHED TO ACUTELY ISOLATED CENTRAL NEURONS.

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Presynaptic nicotinic receptors (nAChR) are thought to play an important role in the central nervous system by regulating transmitter release, but the mechanism of action of these receptors is far from established. We show that the action of presynaptic nAChR can be studied on a preparation of acutely isolated neurons that have retained synaptic terminals attached to their cell body. Neurons of rat interpeduncular nucleus were dissociated by mild enzymatic treatment and gentle mechanical dissociation in a medium containing a low concentration of calcium (0.2 to 0.5mM) and used on the same day. The presence of synaptic boutons on these isolated neurons was assessed by the presence of clusters of synaptophysin immunoreactivity. Furthemore, in whole-cell patch-clamp experiments, we frequently observed spontaneous synaptic currents. In part of these neurons, nicotine, acetylcholine and cytisine at concentrations of 10-30µM not only activated a typical postsynaptic cationic current but also elicited a rapid and massive increase in the frequency of synaptic currents which were GABAergic since they were blocked by bicuculline (10µM). Due to rectification, the postsynaptic nicotinic current was not observed at positive membrane potentials while the presynaptic effect persisted. This effect could be blocked by d-tubocurarine (10µM) and mecamylamine (10µM). Presynaptic action of nicotine required the presence of calcium in the external medium. These experiments should help to better understand the mechanism of action of presynaptic nAChR.

42.3

RELATIVE SELECTIVITY OF AGONISTS FOR BRAIN PRE-SYNAPTIC AND GANGLIONIC NICOTINIC RECEPTORS. J. Gordon, J. Blosser*, S. McCreedy and R. Simmons. Fisons Pharmaceuticals, Rochester, NY 14623.

Multiple nicotinic receptors (nAChRs), composed of α and ß subunits, exist in the central nervous system (CNS). Since the biological function and pharmacology of these subtypes are poorly defined, the potencies of 7 nicotinic agonists for 2 neuronal nAChRs were compared. $^{86}Rb^{+}$ flux into nerve growth factor-treated PC12 cells was used to measure ganglionic nAChR agonist activity. [^{3}H]Dopamine (DA) release from superfused rat striatal slices was used as a measure of a presynaptic CNS nAChR function. Potencies: striatal assay [cytisine(CY)≈azetidine(AZ)>nicotine(N)≈methylcarbachol(M)>dimethylphenylpiperazinium(D)≥carbachol(C)≥acetylcholine(A)]; PC12 assay [CY≈A≈D>N≈AZ>M≈C]. The rank order of A_{50}(PC12)/A_{50}(striatal) ratios was AZ≥CY≥M>N≥C>D>A (range 1-100). The A_{50} values from striatal (r=.8), but not from PC12 assays (r=.3), correlated with K_i values for [^{3}H]nicotine binding to rat brain membranes. These results suggest that 1) the presynaptic striatal and ganglionic nAChRs have distinct pharmacological profiles and 2) the agonist profile at the latter receptor is consistent with that of the α4ß2 subtype.

42.4

A MECHANISTIC STUDY OF NICOTINE-EVOKED NEUROTRANSMITTER RELEASE IN RAT BRAIN SYNAPTOSOMES. K.G. Fernandes*[1], S. L. Yates[2], E.N. Fluhler[1], P.M. Lippiello[1]. R. J. REYNOLDS TOBACCO CO.[1], Research and Development, Winston-Salem, NC 27102 and Duke Univ. Med. Center [2], Integrated Tox. Prog., Durham, NC 27710

Nicotine acts presynaptically to induce a dose-dependent release of ^{3}H-dopamine (DA) from rat and mouse striatal synaptosomes. However, the steps involved in evoking DA release following binding of nicotine to the nicotinic acetylcholine receptor (nAChR) are not well characterized. In particular, the role of Ca^{2+} and other ions in the DA release process is poorly understood. The effects of nicotine on intrasynaptosomal calcium concentration ($[Ca^{2+}]_i$) were studied using fura-2. While K^+ evoked a dose-dependent increase in $[Ca^{2+}]_i$, nicotine had no measurable effect. To further investigate the role of Ca^{2+} in this process, the effects of Ca^{2+} removal and specific/nonspecific channel blockers on DA release from rat striatal synaptosomes were examined. This process is Ca^{2+}-dependent, since in the absence of Ca^{2+} (+ EGTA), K^+-induced DA release was greatly attenuated and nicotine-induced release was completely blocked. Cobalt, a nonspecific Ca^{2+} channel blocker, decreased DA release in response to nicotine (15-fold) and K^+ (8-fold), while the L-type Ca^{2+} channel blocker, nicardipine, reduced nicotine-stimulated DA release by 50%. Tetrodotoxin (2μM) did not alter nicotine-induced DA release, suggesting that the release process is not amplified through recruitment of voltage-gated Na^+ channels. These data show that Na^{2+} flux through the nAChR is sufficient to induce DA release in a Ca^{2+} -dependent fashion, and that this dependence is in part associated with the activation of the L-type Ca^{2+} channel.

42.5

TRIMETHAPHAN ACTS AS AN AGONIST AT THE INHIBITORY NICOTINIC RECEPTOR ON RAT DORSOLATERAL SEPTAL NUCLEUS (DLSN) NEURONS. E.M. Sorenson* and J.P. Gallagher. Dept. of Pharm. and Tox., Univ. of Texas Med. Br., Galveston, TX 77555.

This laboratory has previously shown that the nicotinic agonists dimethylphenylpiperazinium (DMPP), nicotine and acetylcholine produce a direct inhibitory response at DLSN neurons by activation of a Ca^{2+} dependent K^+ channel (Wong and Gallagher, Nature 341:439,1989 and J. Physiol. (Lond.) 436:325, 1991). We now report that trimethaphan camsylate (TMP), an antagonist at excitatory nicotinic receptors in ganglia, appears to be an agonist at the inhibitory nicotinic receptor on DLSN neurons.

Standard intracellular recording methods were used to record from DLSN neurons *in vitro*. DLSN neurons which hyperpolarized in response to DMPP also showed concentration dependent hyperpolarizations in response to superfusions of 0.1 to 1000 μM TMP. The EC_{50} for TMP from the dose response curve was 10 μM which produced a hyperpolarization of 3.5 mV (n=4) from a membrane potential of -60 to -67 mV. The hyperpolarization in response to TMP persisted in the presence of 0.1 μM TTX (n=2), indicating that it is a direct response of DLSN neurons. During continuous superfusion with TMP, the hyperpolarization of DLSN neurons gradually faded and the membrane potential returned to baseline. The fading of the response to TMP appears to be due to desensitization of the inhibitory nicotinic receptor also activated by DMPP, since the neurons were unresponsive to DMPP during this time. The response to DMPP recovered after washing off the TMP for 30-60 min. The responses to superfusion of 10 μM DMPP and 10 μM TMP were examined in the same neuron. The ratio of the hyperpolarization due to TMP over the hyperpolarization caused by DMPP in the same neuron was 1 (n=3) indicating that TMP has a similar efficacy for the inhibitory receptor as DMPP. Supported by DHHS 1F32 DA05447-01 and The Council for Tobacco Research-USA, Inc.

42.6

NEURONAL NICOTINIC RECEPTOR HETEROGENEITY AND SYNAPTIC TRANSMISSION IN THE AVIAN BRAIN. W.R. Weaver* and V.A. Chiappinelli. Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, St. Louis, MO 63104.

Neurons in the chick lateral spiriform nucleus (SPL) respond to nicotinic agonists with depolarization and increased excitability. Nicotinic receptors in the SPL have high affinity for ^{3}H-nicotine but are insensitive to blockade by either kappa-bungarotoxin or α-bungarotoxin (Sorenson and Chiappinelli, Neuron 5:307, 1990). Using intracellular recording in chick brain slices, we have shown that stimulation of a cholinergic fiber tract lateral to the SPL elicits excitatory postsynaptic potentials (EPSPs) in SPL neurons through nicotinic synapses (McMahon, et al., Soc. Neurosci. Abstr. 17:359,1991). In situ hybridization suggests that SPL receptors may consist of α2 and/or α4 subunits in combination with β2 subunits (Morris, et al., Mol. Brain Res. 7:305, 1990).

We now report that the nicotinic receptor antagonist trimethaphan appears to distinguish different receptor subtypes located on SPL neurons. Widely different concentrations (50, 200, or 500 μM) of trimethaphan are required to block nicotinic responses in different cells. Within a given SPL neuron, similar levels of this antagonist are needed to block responses to both exogenously applied nicotinic agonist and to endogenous transmitter released by stimulation of the cholinergic fiber tract. These results demonstrate that heterogeneity of nicotinic receptors exists in the SPL and that these receptor subtypes can be pharmacologically distinguished. Supported by NIH Grant NS17574 to V.A.C.

42.7

PHARMACOLOGICAL CHARACTERIZATION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS USING WHOLE-CELL PATCH CLAMP IN CHICK BRAIN SLICES L.L. McMahon* and V.A. Chiappinelli. Dept. of Pharmacological and Physiological Science, Saint Louis University School of Medicine, St. Louis MO 63104.

Neurons in the lateral spiriform nucleus (SPL) of chick mesencephalon contain functional nicotinic receptors which have high affinity for ^{3}H-nicotine and are likely to contain α2 and/or α4 along with β2 subunits (Morris et al., Mol. Brain Res. 7:305, 1990). In this nucleus, we have previously reported marked depolarizations in response to the nicotinic agonists carbachol and nicotine that were significantly attenuated by classical nicotinic receptor antagonists d-tubocurarine (50 μM) and dihydro-β-erythroidine (100 μM) but were insensitive to blockade by either kappa- or alpha-bungarotoxin (Sorenson and Chiappinelli, Neuron 5:307, 1990). To further characterize these receptors, we have used whole-cell patch clamp recording techniques to measure agonist-induced nicotinic currents. Reversal potentials were obtained using voltage ramps and ranged from -20 to 0 mV. The relative potency of several nicotinic receptor agonists was estimated by comparing the current amplitude evoked by bath perfusion of a particular agonist to that evoked by the same concentration (1 -100 μM) of carbachol. The relative rank order is 1,1-dimethyl-4-phenylpiperazinium > carbachol ≥ nicotine > cytisine. Current amplitudes range from less than 100 pA for cytisine to 200-300 pA for nicotine and carbachol. With cytisine, recovery of responses to agonists was prolonged or absent, suggesting that cytisine may have a complex interaction with these receptors. Muscarinic responses were blocked in all experiments with 1 μM atropine. Supported by NIH Grant NS17574 to VAC.

42.8

PERSISTENT BLOCKADE OF CNS NICOTINIC RECEPTORS AFTER IN VIVO OR IN VITRO ADMINISTRATION OF CHLORISONDAMINE. H. El-Bizri* and P.B.S. Clarke. Dept of Pharmacology and Therapeutics, McGill Univ., Montreal, Canada H3G 1Y6.

Chlorisondamine (CHL) blocks behavioural responses to CNS nicotinic receptor activation in a quasi-irreversible fashion. The mechanism of this blockade was investigated in vitro by examining nicotine-induced ^{3}H-dopamine release from rat brain striatal synaptosomes. Synaptosomes were prepared from the crude P2 pellet, incubated with ^{3}H-DA, and superfused; 1 min samples were collected. Following 25 min wash, a brief pulse of nicotine and then of high K^+ buffer were given 10 min apart. Nicotine (0.01 - 100 μM) induced ^{3}H-DA release in a concentration dependent and Ca^{++} dependent manner. Release evoked by nicotine (1 μM) was blocked in a graded fashion by *in vitro* administration of mecamylamine, dihydro-beta-erythroidine and CHL (0.01 - 100 μM); blockade by CHL was not surmounted by a high concentration of nicotine (100 μM). *In vivo* administration of CHL (10 mg/kg sc) 1, 7, 21 or 42 days before sacrifice completely blocked release induced by 1 μM nicotine administered in vitro. These results suggest that chlorisondamine's persistent in vivo blockade does not solely result from retention of this bisquaternary amine by the blood-brain barrier. Funded by NIDA.

42.9

CENTRAL NICOTINIC RECEPTOR BLOCKADE NEITHER BLOCKS NOR MIMICS CHRONIC NICOTINE-INDUCED ^{3}H-NICOTINE BINDING UPREGULATION IN RAT BRAIN. P.B.S. Clarke* and H. El-Bizri. Dept of Pharmacology and Therapeutics, McGill Univ., Montreal, Canada H3G 1Y6.

It has been proposed that the paradoxical upregulation of ^{3}H-nicotine binding induced by chronic in vivo nicotine reflects long term receptor blockade (through desensitization). The nicotinic antagonist chlorisondamine (CHL) blocks nicotine's CNS effects quasi-irreversibly after central or systemic administration. We tested whether chronic CNS nicotinic blockade by CHL alters ^{3}H-nicotine receptor binding or alters chronic nicotine-induced upregulation. Rats were randomly allocated to 4 groups (n=12) and were pretreated with CHL (10 mg/kg sc) or saline, and treated chronically with nicotine (0.6 mg/kg sc bid for 10 days) or saline. Persistent central blockade was shown in tests of nicotine-induced locomotion given just before and after chronic treatment. Central blockade exerted by CHL was not reversed by chronic nicotine. Forebrain ^{3}H-nicotine binding density (B_{max}) but not affinity (k_D) was increased by chronic nicotine (19% increase, $p < 0.001$). CHL neither altered ^{3}H-nicotine receptor binding density when given alone nor did it alter upregulation induced by nicotine. Supported by NIDA.

42.10

DIFFERENTIAL EFFECTS OF CHRONIC NICOTINE INFUSION AND CORTICOSTERONE TREATMENT ON BRAIN NICOTINIC RECEPTORS AND TOLERANCE TO NICOTINE. J.R. Pauly*, S.R. Robinson, E.U. Grun and A.C. Collins. Inst. for Behavioral Genetics and Dept. of Psychology, Univ. of Colorado, Boulder CO 80309.

Previous studies from our laboratory have shown that behavioral and physiological tolerance to nicotine following chronic agonist exposure is correlated with an increase in the number of brain nicotinic receptors (nAchRs). Mice that are chronically treated with corticosterone (CCS) are also tolerant to nicotine, although this treatment reduces the number of brain nAchRs. The present study examined tolerance to nicotine and changes in CNS nAchRs following chronic nicotine infusions that were combined with prolonged exposure to CCS. Female C57BL/6 mice were implanted with jugular catheters and subcutaneous hormone pellets comprised of 60% CCS and 40% cholesterol (controls received pure cholesterol) and then infused with saline or nicotine (6.0 mg/kg/hr) for a period of 7 days. Sensitivity to nicotine was determined using a battery of tests (Y-maze activity, heart rate and body temperature). Homogenate receptor binding assays using 3H-nicotine and alpha-125I-bungarotoxin (BTX) were performed in 8 grossly dissected brain regions. Mice that were infused with nicotine were tolerant to an acute drug challenge as indicated by shifts to the right in nicotine dose-response curves; CCS-treated animals developed similar tolerance. Combining these treatments however had an additive effect on tolerance to nicotine. CCS did not significantly alter nicotine-induced increases in 3H-nicotine binding in any brain region. CCS exposure decreased 125I-BTX binding in several brain regions but nicotine infusions reversed this effect. These data suggest that agonist treatment and glucocorticoid exposure produce tolerance to nicotine via independent mechanisms. Supported by DA-00116, DA-05131 and a grant from RJ Reynolds Tobacco Co.

42.11

TOLERANCE TO CHRONIC NICOTINE TREATMENT MEASURED BY DOPAMINE AND RUBIDIUM RELEASE. S.R.Grady, M.J.Marks*, D.A.Farnham, and A.C.Collins. Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

Chronic treatment of mice with nicotine elicits tolerance to the effects of the drug and results in increases in the number of nicotinic binding sites. However, the functional state of nicotinic receptors after chronic nicotine treatment has not been intensely studied. Therefore, C57BL/6 mice were continuously infused with nicotine (4 mg/kg/hr), withdrawn from treatment for various amounts of time, and assayed for nicotine-induced release of dopamine from striatum and of rubidium from cortex and midbrain. The binding of [^{3}H]nicotine was increased by chronic drug treatment and returned to normal levels during withdrawal. Nicotine-induced dopamine release from striatial synaptosomes from treated mice was 20-25% lower than that of control mice immediately after cessation of treatment and remained lower for at least four days. Nicotine-induced rubidium release from midbrain was 30-35% lower in treated animals than in controls and remained lower for several days after withdrawal. However, nicotine-stimulated rubidium release from cortical synaptosomes of treated mice did not differ from that of control animals. The results suggest that tolerance develops to at least two different biochemical measures of nicotinic receptor function.
Supported by DA-03194 and DA-00116.

42.12

ANALYSIS OF NICOTINIC RECEPTORS BY QUANTITATIVE AUTORADIOGRAPHY AND IN SITU HYBRIDIZATION FOLLOWING CHRONIC TREATMENT WITH NICOTINE AND MECAMYLAMINE. J.L.van de Kamp*, J.R.Pauly, S.R.Robinson, M.J.Marks, and A.C.Collins. Institute for Behavioral Genetics, University of Colorado, Boulder, CO. 80309.

Continuous infusion of DBA/2 mice with either the nicotinic agonist, nicotine, or the nicotinic antagonist, mecamylamine, results in increases in nicotinic receptors measured by high affinity [^{3}H]nicotine binding. Simultaneous treatment with these drugs causes larger increases in receptor number than treatment with either drug alone. DBA/2 mice were chronically treated with saline (control), 4.0 mg/kg/hr nicotine, 4.0 mg/kg/hr mecamylamine, and a combination of mecamylamine and nicotine. Subsequently, [^{3}H]nicotine and alpha-[^{125}I]bungarotoxin binding sites were measured by quantitative autoradiography, and the levels of mRNA encoding the alpha-4, alpha-7, and beta-2 nicotinic receptor subunits were measured by in situ hybridization. Chronic treatment with either drug evoked increases in [^{3}H]nicotine binding; even greater increases were observed after treatment with both drugs simultaneously. Chronic treatment had less effect on alpha-[^{125}I]bungarotoxin binding. Levels of mRNA encoding alpha-4, beta-2 or alpha-7 nicotinic receptor subunits were unchanged, suggesting that regulation of the number of nicotinic receptors by chronic treatment with nicotinic agonists or antagonists may occur posttranscriptionally. Supported by DA-03194 and DA-00116.

42.13

TOLERANCE AND RECEPTOR CHANGES AFTER CHRONIC TREATMENT WITH NICOTINE AND MECAMYLAMINE. Y.Luo, S.Selvaag, M.J.Marks, and A.C.Collins*. Institute for Behavioral Genetics, University of Colorado, Boulder, CO. 80309.

Mecamylamine blocks nicotine effects in vivo but response to chronic exposure to mecamylamine on behavioral or biochemical measures of nicotinic systems is not well understood. Therefore, DBA/2 mice were chronically infused with mecamylamine either alone (4 mg/kg/hr) or in combination with nicotine (4 mg/kg/hr) and compared to mice treated with saline or nicotine (4 mg/kg/hr). Mice treated with nicotine, mecamylamine, or both drugs were apparently tolerant to the effects of nicotine 4 hours after cessation of drug treatment. However, the apparant tolerance to mecamylamine probably resulted because the antagonist had not been completely metabolized. Mice that had been chronically treated with nicotine tested 2 days after cessation were still tolerant, while those chronically treated with mecamylamine were not. Treatment with nicotine or mecamylamine resulted in significant increases in nicotinic binding sites measured either 4 hr or 2 days after cessation of chronic treatment. Increases in ligand binding observed after treatment with both drugs were additive. These results suggest that chronic treatment with either nicotinic agonists or antagonists increases nicotinic receptors, but that the mechanisms underlying these increases differ. Supported by DA-03194 and DA-00116.

42.14

A HYBRID PBPK/PD MODEL FOR NICOTINIC RECEPTOR DYNAMICS IN THE RAT BRAIN. E.N. Fluhler*, D.R. Plowchalk, P.M. Lippiello and J.D. deBethizy. Duke University Medical Center, Durham, NC and RJ Reynolds Tobacco Co., Winston-Salem, NC.

We have examined the *in vivo* time-course of nicotine binding to rat brain receptors and the resulting effects on receptor dynamics by linking a physiologically-based pharmacokinetic model (PBPK) for nicotine to a two-state receptor binding model. The receptor model assumes that brain nicotinic receptors exist in either high (R') or low (R) affinity states and that binding of nicotine can shift the relative proportions of the receptor states via isomerization reactions. Rate constants for nicotine binding and conformational changes in the receptor ($k_1 \ldots k_8$) were estimated from kinetic measurements of S-(-)-[^{3}H]nicotine binding to rat brain homogenates. Temperature dependence of the rate constants was addressed by scaling each constant from 0 to 37 ^{0}C based on a subset of binding data obtained at 37 ^{0}C. The concentration of nicotine in the brain compartment of the PBPK model was used as the input for the receptor model. Simulations were conducted using an administration paradigm that mimics a single puff from a cigarette (1 puff, 0.1 mg nicotine). Results of simulations predict rapid nicotine binding (<30 sec) to both R and R' followed by rapid conversion (<60 sec) of nearly all receptors (97%) to the high affinity conformer (proposed to be a desensitized state). The $t_{1/2}$ for the return of receptors to their original distributions was 3-4 hours. These results are consistent with the prolonged desensitization of some physiological responses to nicotine (e.g. prolactin release) and indicate that this hybrid model provides a unique tool with which to study rat nicotinic receptor dynamics *in vivo*.

42.15

NICOTINE TOLERANCE IN ADRENAL CHROMAFFIN CELLS AFTER EXPOSURE TO SMOKING RELATED NICOTINE CONCENTRATIONS. A.E. Bullock and A.S. Schneider*. Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208

Nicotine tolerance and dependence have been demonstrated in animals by many investigators, however the cellular mechanisms underlying these processes are not well known. Repeated nicotine exposure has been previously shown to reduce the levels of evoked catecholamine release from cultured bovine adrenal chromaffin cells at concentrations well above that of the estimated tissue levels of nicotine in smokers. We have demonstrated the development of nicotine tolerance at concentrations related to those found in the tissues of smokers (0.1 - 1.0 μM). Cells are preincubated with varying concentrations of nicotine and subsequently challenged with a nicotinic agonist. Adrenal chromaffin cell function is measured as nicotine-stimulated ^{45}Ca uptake and catecholamine release. Decreased nicotine-evoked calcium uptake and catecholamine release is seen in minutes after preexposure with both 0.1 and 1.0 μM nicotine at 37°C but not at 20°C. Consistent with desensitization of the secretory response, this decrease in nicotine-evoked catecholamine release is dependent on extracellular calcium, nicotine dose, temperature and is cross tolerant to other nicotinic agonists. Chronic exposure to nicotine results in a decrease in the secretory response that is sustained over a seven day period. The above results are being considered in relation to mechanisms of chronic nicotine tolerance and dependence.

42.16

NICOTINE AND cAMP ENHANCE ^{3}H-NICOTINE BINDING TO PC 12 CELLS. T.C. Madhok* and B.M. Sharp, Endocrine-Neuroscience Laboratory and Departments of Medicine, Hennepin County Medical Center and Univ. of Minnesota, Minneapolis, MN 55404.

Recently, we reported that nerve growth factor (NGF) increased the number of ^{3}H-nicotine binding sites on PC 12 cells (*Endocrinol.* 1992; 130:825). To further delineate mechanisms regulating the expression of neuronal nicotinic cholinergic receptors (nChRs) in response to nicotine or cAMP, the following studies were conducted. PC 12 cells were grown in nicotine hemisulfate (0.001-1.0 mM) or dibutyryl-cAMP (0.01-1.0 mM) or vehicle for 7 days, with daily replenishment of these agents. Cells were then harvested and specific ^{3}H-nicotine binding was measured. Specific binding was enhanced by nicotine (up to 2.5-fold with 1.0 mM) or dbcAMP (up to 5-fold with 1.0mM); this was dose and time-dependent. Maximal stimulation by dbcAMP occurred earlier (by 3 days) than that achieved by nicotine (5-7 days). Concurrent treatment with mecamylamine (0.01mM) prevented up-regulation by nicotine. Forskolin (10-100 μM), in combination with IBMX (1 mM), enhanced ^{3}H-nicotine binding; forskolin alone was ineffective. Specific ^{3}H-nicotine binding on PC 12 cell mutants (A126.1B2), lacking a cAMP-responsive system, was unaffected by dbcAMP. Finally, enhanced ^{3}H-nicotine binding did not appear to be related to cell differentiation, since nicotine only induced receptor up-regulation, whereas dbcAMP or NGF also stimulated neurite outgrowth. These studies provide an *in vitro* model for elucidating the mechanism of nChR up-regulation by nicotine and suggest that this may involve, in part, activation of protein kinase A. (Supported by DA 04446)

42.17

EFFECTS OF NICOTINIC AGONIST PRETREATMENT ON NICOTINE-INDUCED HYPOTHERMIA AND HYPOLOCOMOTION. M.J. Buckley*, M.W. Decker, and S.P. Arneric. Neuroscience Research, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064.

In mice, acute treatment with nicotine produces substantial reductions in body temperature and locomotor activity. In these experiments, we investigated the effects of pretreatment with nicotinic agonists and antagonists on this nicotine-induced reduction of body temperature and locomotor activity in male, CD-1 mice. Each mouse was given an injection of a nicotinic agent 3-4 min. prior to an injection of saline or (-)nicotine (6.2 µmol/kg IP). Four min. after this second injection, the mouse was placed in an activity cage for 10 min. to measure horizontal activity. After the activity session, temperature was measured using a rectal probe. Nicotinic agonists used for pretreatment included (-)lobeline, (-)cytisine, and (-)nicotine (0, 0.062, 0.62, and 6.2 µmol/kg IP). Antagonists used were mecamylamine (1.5, 5, and 15 µmol/kg) and hexamethonium (3 and 30 µmol/kg).

As expected, nicotine by itself dramatically reduced locomotor activity and body temperature. Mecamylamine attenuated these effects, but hexamethonium did not. Similar but slightly less pronounced reductions were found with cytisine, whereas lobeline had no effect by itself. Cytisine effects did not appear to be additive with those produced by nicotine, as pretreatment with cytisine did not significantly alter the effects of nicotine. Pretreatment with lobeline, however, dose dependently potentiated nicotine-induced hypothermia without affecting nicotine's reduction of activity. Interestingly, pretreatment with 0.62 µmol/kg nicotine significantly attenuated the hypothermic effect of a subsequent injection of 6.2 µmol/kg of nicotine.

These results suggest that nicotinic agonist effects on temperature and locomotor activity differ and are not simply additive. The different patterns of behavioral and physiological effects elicited by these agonists may be related to preferential activity at one or more nicotinic receptor subtype(s).

EXCITATORY AMINO ACIDS: EXCITOTOXICITY I

43.1

CYCLOHEXIMIDE TREATMENT PREVENTS NEUROTOXICITY AND C-*FOS* EXPRESSION IN ADULT RAT BRAIN FOLLOWING KAINIC ACID TREATMENT. S.S. Schreiber*, I. Najm, G. Tocco and M. Baudry. Dept. of Neurology and Neurosciences Program, Univ. So. Calif., Los Angeles, CA 90033.

Recent evidence has suggested a link between cell death mechanisms and macromolecular synthesis. Kainic acid (KA) causes a distinct seizure syndrome in adult rats and is selectively toxic to neurons in the pyramidal cell layers of the hippocampus and the piriform cortex. Systemic administration of KA also results in a rapid elevation of c-*fos* messenger RNA (mRNA) in multiple brain regions. In regions not damaged by KA the time course of c-*fos* induction is relatively brief, whereas elevated levels of c-*fos* mRNA are maintained for at least 16 hours in regions selectively vulnerable to KA, suggesting a role for Fos protein in KA-induced cellular damage. Therefore, we studied the effects of cycloheximide (CHX), a protein synthesis inhibitor, on KA-induced cell damage and c-*fos* expression. Adult male Sprague-Dawley rats were given CHX (2 mg/kg) 1 hour prior to KA (10 mg/kg), both by subcutaneous injection, and were sacrificed 1 and 16 hours, or 5 days following the onset of behavioral seizure activity. Either CHX or KA alone were given to control animals. Frozen brain sections were prepared for *in situ* hybridization using a ^{35}S-labeled c-*fos* cRNA probe. In animals that did not receive CHX pretreatment, increased c-*fos* mRNA was consistently observed in CA3 and CA1 pyramidal cells, as well as the piriform cortex 16 hours following seizure onset. In contrast, CHX pretreatment resulted in minimal or no increase in c-*fos* mRNA at 16 hours, but did not prevent widespread c-*fos* induction 1 hour following seizure onset. Furthermore, histological examination revealed no hippocampal cell loss in CHX-pretreated animals at 16 hours or 5 days after seizure onset. These results suggest that protein synthesis is required for KA-induced neurotoxicity, and that prolonged expression of c-*fos* mRNA in KA-vulnerable regions may be a marker for subsequent cell death.

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43.2

KAINATE-ACTIVATED COBALT UPTAKE IDENTIFIES A SUBPOPULATION OF CULTURED CORTICAL CELLS THAT ARE PREFERENTIALLY VULNERABLE TO KAINATE-INDUCED DAMAGE. D.M. Turetsky*, M.P. Goldberg and D.W. Choi. Dept. of Neurology, Washington Univ. School of Med., St. Louis, MO 63110.

Pruss et al. found that cerebellar granule or hippocampal neurons exposed to kainate for 20 min in the presence of 5 mM $CoCl_2$, take up Co^{2+}, visualizable by precipitation with $(NH_4)_2S$ and silver enhancement (Neuron 7:509, 1991). Murine cortical cell cultures exposed to 100-500 μM concentrations of kainate for 20 min exhibited Co^{2+} uptake in only a subpopulation of neurons (about 15% of the total). The idea that such Co^{2+} uptake is a marker for AMPA/kainate receptors linked to Ca^{2+}-permeable channels suggests the prediction that Co^{2+}-positive cortical neurons should have heightened vulnerability to kainate toxicity. Indeed, cortical cultures exposed to 100 μM kainate plus 10 μM MK-801 for 10 min developed minimal overall neuronal death 24 hrs later, as assayed by LDH release, whereas the Co^{2+}-positive subpopulation in the same cultures was decreased by 60-70%. Removing Ca^{2+} from the bathing medium during kainate exposure resulted in an almost complete preservation of the Co^{2+}-positive subpopulation. No such selective loss of Co^{2+}-positive cells was seen after NMDA exposure. These observations suggest that a distinct subpopulation of cortical neurons has AMPA/kainate receptors linked to Ca^{2+} permeable channels, and that this characteristic does convey vulnerability to kainate-induced, Ca^{2+}-mediated, damage.

43.3

THE INTERACTION BETWEEN BMAA AND BICARBONATE CREATES A NEW AGONIST SPECIES. C.N. Allen*[1], P.S. Spencer[1] and D.O. Carpenter[2]. [1]Cntr for Res. on Occup. & Environ. Toxicol., OR Hlth. Sci. Univ., Portland, OR; [2]Wadsworth Cntr. for Lab. & Res., New York State Depart. Hlth. & Sch. of Pub. Hlth., Albany, NY.

BMAA (N-methylamino-L-alanine), a component of the neurotoxic *Cycas circinalis* plant, requires the presence of $NaHCO_3$ for excitotoxicity. BMAA neurotoxicity has been linked to activation of NMDA, quisqualate (QA) and kainate (KA) receptors. We used cultured hippocampal neurons and whole cell patch clamp recording to identify the receptors activated by BMAA in the presence of $NaHCO_3$. BMAA activates ionic currents in 0.1 mM calcium which are reduced 50% in 0.5 mM extracellular calcium and 92% in 3.0 mM calcium. The BMAA-activated currents were not antagonized by APV (10-100 μM) nor by CNQX (1-10 μM). Addition of $NaHCO_3$ to BMAA (500 μM) potentiated the BMAA currents 224% for 10 mM NaHCO and 578% for 20 mM. $NaHCO_3$ had no effect on QA-, NMDA- or KA-activated ionic currents. The addition of $NaHCO_3$ alkalinizes the extracellular Ringer's solution which did not alter the $NaHCO_3$ potentiation of BMAA-activated currents. The BMAA+$NaHCO_3$ activated currents were unaffected by APV (10-100 μM) but were reduced 49% by CNQX (1 μM) and 80% by CNQX (10 μM). These data indicate that the interaction between BMAA and bicarbonate produced a new agonist species, possibly an α-carbamate which activates QA/KA type of glutamate receptors and may be responsible for the neurotoxicity of BMAA in $NaHCO_3$ solutions. This work is supported by NIH grants NS-23807 and NS19611.

43.4

NON-NMDA RECEPTOR-MEDIATED DAMAGE TO NADPH-D(+) NEURONS IS CA^{2+} DEPENDENT. J.H. Weiss*[,1] and D.W. Choi[2]. Dept. Neurology, U.C. Irvine, Irvine, CA 92717[1] and Dept. Neurology, Washington Univ. Med. Sch., St. Louis, MO 63110.

Studies in cortical culture have revealed that the neurodegeneration that occurs after brief periods of intense NMDA receptor activation is Ca^{2+} dependent. Similar experiments aiming to clarify the role of Ca^{2+} entry in non-NMDA receptor-mediated damage to cortical neurons are more difficult, as Ca^{2+} removal for the several hours needed for AMPA or kainate to produce widespread neurodegeneration is in itself damaging.

We thus examined the Ca^{2+} dependence of non-NMDA receptor-mediated damage to a small subpopulation of cortical neurons with unusual vulnerability to non-NMDA receptor-mediated injury, those containing high concentrations of the enzyme, NADPH-diaphorase (NADPH-d(+) neurons). Brief (30 - 40 min) exposures of cortical cultures to either 50 μM AMPA or to 100 μM kainate caused little overall neuronal loss, but destroyed over 70% of the NADPH-d(+) neurons. Ca^{2+} ion removal during the toxic exposure decreased NADPH-d(+) neuronal loss by over 50% ($p < 0.05$ by 2-tailed t test).

43.5

BLOCKING NON-NMDA RECEPTORS REDUCES TISSUE LOSS AFTER EXPERIMENTAL SPINAL CORD TRAUMA . J.R. Wrathall*, D. Choiniere and Y. D. Teng. Dept. of Anatomy and Cell Biology, Georgetown Univ., Washington, DC 20007.

We have recently found that administration of the potent non-NMDA antagonist NBQX (Sheardown et al., Science *247*:571, 1990) results in reduced hindlimb functional deficits after a standardized contusive injury of the thoracic cord. We have now analyzed spinal cord tissue from these experiments with quantitative morphological and immunocytochemical techniques. Cross-sections of the cord representing the epicenter (region of maximal damage) and tissue at specified distances rostral and caudal to the epicenter were stained with luxol fast blue-hematoxylin and eosin and used for morphometric analysis as previously described (Noble and Wrathall, Exp. Neurol., *88*: 134, 1985). In addition, sections 5 mm rostral and caudal to the epicenter were processed for 5-HT immunoreactivity and quantitatively assessed with an image analysis system. The results showed that 4 weeks after injury there were significantly larger areas of residual gray matter in spinal cords of the NBQX-treated group in sections 1, 2, and 3 mm caudal to the epicenter. The effect on total white matter showed a similar trend with a significant difference 2 mm caudal to the epicenter. Further, 5-HT immunoreactivity, a marker of descending pathways from the brainstem, below the lesion was almost twice as great in the NBQX-treated group as compared to vehicle controls. These results suggest that the reduction in functional deficits by NBQX may be mediated by sparing of tissue loss resulting from traumatic injury. (Supported by NIH-P O1-NS28130).

43.6

BRAIN METABOLISM OF β-*N*-METHYLAMINO-L-ALANINE (BMAA) AND PROTECTION OF EXCITOTOXICITY BY GABA-UPTAKE INHIBITORS. G.E. Kisby, V. Nottingham, R. Kayton, D.N. Roy *, P.S. Spencer. Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, Portland, OR 97201.

β-*N*-Methylamino-L-alanine (BMAA), a weak excitotoxic amino acid, is one of the many potentially toxic agents in cycad seed which is under study as a possible etiological factor for western Pacific amyotrophic lateral sclerosis (ALS). BMAA uptake into neural tissue is sodium-dependent, sensitive to temperature and pH, dependent on protein concentration, and saturates with increasing concentration of neurotoxin (Kisby *et al.*, 1992). Pretreatment of mouse cortical explants (2-3 weeks *in vitro*) with the non-selective GABA-uptake inhibitor nipecotic acid (100 μM and 1.0 mM) blocked BMAA uptake and BMAA-induced excitotoxicity (*respectively*). Metabolism of BMAA and aminopyrine by purified brain microsomes and mitochondria was inhibited by the P450 inhibitors SKF 525A and piperonyl butoxide. Brain microsomal metabolism of aminopyrine, BMAA, and MPTP (all at 1.6 mM) was inhibited by deprenyl (0.1-0.5 μM). Rat brain DNA was radiolabeled when incubated (0.5 h) with purified brain mitochondria and 1.6 mM L-[-$N^{14}CH_3$]-BMAA (0.2 μCi). These studies demonstrate that (a) BMAA is metabolized by brain tissue, (b) the metabolite(s) can interact with brain tissue macromolecules and (c) BMAA excitotoxicity is blocked by inhibitors of GABA uptake. Further study is needed before conclusions can be drawn about the potential role of BMAA in western Pacific ALS. [Supported by a grant from AHAF and NS19611]

43.7

L-PROLINE-LIKE EXCITOTOXICITY OF THE CYANIDE METABOLITE, 2-ICA (2-IMINOTHIAZOLIDINE-4-CARBOXYLIC ACID). R.S. Bitner, G.E. Isom and G.K.W. Yim*. Dept. Pharm. Tox., Sch. Pharm. & Pharmacal Sci., Purdue Univ., W. Lafayette, In 47907.

The amino acid L-proline, despite its monoacidic structure, produces neurotoxic damage to CA1 pyramidal neurons following intrahippocampal administration (Nadler et al. 1988). Our earlier study indicated that 2-ICA, a cyanide metabolite structurally similar to L-proline, induces EAA-like seizures in mice. To determine if 2-ICA could also induce the neurotoxicity seen with L-proline, the cyanide metabolite was infused i.c.v. in rats for seven days at a sub-convulsant dose (50 ug/hr). Histological examination revealed extensive loss of CA1 pyramidal neurons of the hippocampus in 2-ICA treated rats. In addition, icv injections of L-proline in mice produced EEA-like seizures at doses comparable to those required for 2-ICA. These findings emphasize the possibility of excitotoxicity with compounds possessing only one acidic group, and may constitute another excitotoxic mechanism involved in chronic cyanide neurotoxicty. (Supported in part by NIH grants ES4140 & RR0586.)

43.8

NMDA ANTAGONISTS HAVE LIMITED PROTECTION AGAINST AMPA-INDUCED INJURY IN THE IMMATURE BRAIN. W.H. Trescher* and M.V. Johnston, Kennedy Krieger Research Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Sensitivity to *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) mediated injury is enhanced in the immature brain, and the age of peak neurotoxicity is distinct but overlapping for the two agonists. It is unclear to what extent *in-vivo* AMPA-induced injury is mediated through NMDA receptor activation in the immature brain. Therefore, we investigated the activity of NMDA antagonists in AMPA-induced brain injury. On postnatal day (PND) 7, rat pups under deep anesthesia received a unilateral, intrastriatal injection of 10 nmol/0.5 μl of NMDA or AMPA. Antagonists were administered i.p. 15 min. after the intracerebral injections. The brains were removed on PND 14 and the severity of damage was determined by comparing the wet weights of the cerebral hemispheres ipsilateral (I) and contralateral (C) to the injection site according to the formula 100 X (C-I)/C (% damage). Neuroprotection was calculated as %protection, a term that reflects the degree of damage in drug treated vs saline treated groups. Data are presented as mean ± SEM. Intracerebral injection of 10 nmol NMDA produced 25.4 ± 1.6% damage compared to 16.4 ± 2.1% damage after intracerebral injection of 10 nmol AMPA ($p = 0.003$). Administration of three doses of MK-801 (0.01-1.0 mg/kg) produced dose related protection against NMDA induced injury (ANOVA, $p = 0.0001$) with maximal protection of 86.5 ± 3.6%. 1 mg/kg of MK-801 was only partially protective against AMPA-induced injury (24.1 ± 5.5%). The competitive NMDA receptor antagonist, CGS-19755 (10 mg/kg) was protective against NMDA-induced injury (82.2 ± 8.8% protection, $p = 0.0034$, vs control), but only partially protective against AMPA-induced injury (31.6 ± 12.2% protection, $p = 0.24$ vs control). The glycine receptor antagonist HA-966 (50 mg/kg) was partially protective against NMDA-induced injury (43.5 ± 12.5% protection, $p = 0.059$) and AMPA-induced injury (25.6 ± 7.6%, $p = 0.3$). The data indicate that a major portion of AMPA-induced injury in the immature brain is independent of NMDA mediated mechanisms. Supported by NIH grants NS01482 (WHT) and NS28208 (MVJ).

43.9

DETECTION OF TOPA (6-OH DOPA) AND TOPA QUINONE BY HPLC REVEALS A SPONTANEOUS DOPA TO TOPA CONVERSION IN AQUEOUS SOLUTIONS

TA Newcomer*, AM Palmer, PA Rosenberg & E Aizenman, Depts of Physiology and Psychiatry, University of Pittsburgh Sch Med, Pittsburgh, PA 15261, and Dept of Neurology, Children's Hospital and Harvard Med Sch, Boston, MA 02115.

2,4,5-Trihydroxyphenylalanine (TOPA) oxidizes in solution to form a quinone derivative that is a non-NMDA agonist and excitotoxin (Rosenberg et al., *P.N.A.S.* 88:4865; 1991). A HPLC analysis of TOPA quinone-containing solutions was performed in an effort to characterize its behavior under physiological conditions. A dual electrode coulometric detector was utilized. Factors such as potential (-400 to 400 mV), pH (3-8), methanol concentration (8-15%) and buffer (HEPES, phosphate) were varied. We have successfully defined optimal conditions to detect TOPA quinone and clearly distinguish it from closely related catechols. Under physiological pH, DOPA-containing solutions showed a partial conversion (0.1%) to TOPA and TOPA quinone. This conversion was greatly delayed by acidic conditions (pH 3). We are currently examining circumstances that may increase this conversion and which may explain some of the neurotoxic properties that other investigators have attributed to DOPA (Olney et al., *Exp. Neurol.* 108:269; 1990).

43.10

THE INTERPLAY BETWEEN IONOTROPIC AND METABOTROPIC GLUTAMATE RECEPTORS AFFECTS N-METHYL-D-ASPARTATE (NMDA)-INDUCED NEUROTOXICITY IN THE ADULT RAT RETINA.

R. Siliprandi*, J. Sautter, E. Fadda, M. Lipartiti, N. Schiavo and H. Manev. Fidia Research Laboratories, 35031 Abano Terme, Italy.

We have recently shown (Siliprandi et al., Visual Neuroscience, in press) that a single intraocular (i.o.) injection of NMDA in the adult rat resulted in a dose-dependent loss of retinal ganglion cells and a decrease of retinal choline acetyltransferase activity (ChAT). These effects were inhibited in a dose dependent manner by a single i.o. injection of MK-801, a noncompetitive NMDA receptor antagonist. Now we report that a complete protection can also be achieved by an intravenous injection of MK-801 (0.8 mg/Kg) given either 5 min before or within 15 min after i.o. administration of 12 nmoles of NMDA, suggesting a narrow time-window for rescuing treatments. (1S,3R)-1-Aminocyclopentane-1,3-dycarboxilc acid [(1S,3R)-ACPD)], a selective agonist for the metabotropic glutamate receptors, injected i.o. 30 min before NMDA (12 nmoles) did not cause any histological retinal damage or decrease in retinal ChAT activity, but reduced by 30% the NMDA-induced loss of ChAT activity. These results suggest that the interplay between metabotropic and NMDA-sensitive glutamate receptors is operative in the 'in vivo' retina, and that it may play a crucial role in the outcome of the pathological NMDA receptor over-activation.

43.11

CHARACTERIZATION OF GLUTAMATE EXCITOTOXIC ACTIVITY USING WHOLE BRAIN TISSUE CULTURE.

David Deupree*, Mina Yarom, Yi-Hsuan Lee and Jang-Yen Wu. Physiology and Cell Biology, University of Kansas, Lawrence, KS 66045.

Cultured neurons obtained from fetal rat brain homogenates were exposed to various glutamate agonists for prolonged periods (24 hrs). So far, non-NMDA agonists have produced the greatest degree of neurotoxicity, as measured by increased lactate dehydrogenase (LDH) activity following the exposures. This neurotoxic effect does not appear to be mediated via glutamate metabotropic receptors, as trans-ACPD produced no effect on LDH activity. Preincubation with trans-ACPD was ineffective in modulating quisqualate induced neurotoxicity. Thus the metabotropic modulation of NMDA induced neurotoxicity reported in the literature was not seen on quisqualate neurotoxicity in the present experiments. Further characterization and testing will continue and reported at the meeting.

Research supported by NIH grant #NS-20978 and the Marion Merrell Dow Foundation.

43.12

NEUROTOXICITY LINKED TO THE GLUTAMATE METABOTROPIC RECEPTOR.

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Activation of the glutamate (Glu) metabotropic receptor by Glu, quisqualate or the more selective agonist, *trans*-ACPD, triggers phosphoinositide hydrolysis, intracellular Ca^{++} mobilization and protein kinase C activation. These 2nd messenger functions can be blocked by AP3 (2-amino-3-phosphonopropionic acid). Subcutaneous treatment of infant rodents with AP3 for several consecutive days causes degeneration of virtually all nerve cells in the retina and some neurons in the brain (Tizzano et al, 1991). Moreover, when AP3 is injected directly into the adult rodent brain it results in degeneration of many neurons at the site of injection (Schoepp and Olney, unpublished). These findings suggest that under normal physiological circumstances, the Glu metabotropic receptor through its 2nd messenger functions may provide vitally important support for neurons either during development or in adulthood, and that disruption of this support can have lethal consequences for CNS neurons.

Here we report that *trans*-ACPD, when injected directly into the lateral ventricle of either the immature or adult rat brain, causes acute degeneration of specific neurons in the lateral septal nucleus. The 1S, 3R isomer of *trans*-ACPD, which acts as a pure agonist in driving the Glu metabotropic receptor and stimulating its 2nd messenger functions (Schoepp et al., 1991), potently reproduces this toxic action. When organotypic cultures containing the hippocampus and lateral septal nucleus are exposed to *trans*-ACPD, it causes an acute neurotoxic reaction localized to the lateral septum. Excessive activation of the 2nd messenger functions of the Glu metabotropic receptor is apparently responsible for the toxic effects on septal neurons in that AP3, which prevents excessive activation of these functions, prevents these toxic effects. It appears that the Glu metabotropic receptor harbors neurotoxic potential that can be unleashed either by suppression or hyperstimulation of this receptor.

Supported by HD 24237, AG 05681 and RSA MH 38894 (JWO).

43.13

Similarities between ischemia and metabotropic receptor-induced injury in organotypic hippocampal cultures.

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We have descrbed a model of ischemia in organotypic cultures of hippocampal slices that reproduces the characteristic regional vulnerability to ischemic injury. Ischemic conditions are created pharmacologically with 2-deoxyglucose and potassium cyanide. As *in vivo*, ischemic injury can be reversed if NMDA receptors are blocked during recovery . The distribution of injury caused by glutamate or simulated ischemia is distinct from NMDA or hypoglycemic injury.

Trans-ACPD,the selective agonist of the glutamate metabtropic receptor, reproduces the anatomic distribution of ischemic injury in the cultures and shares pharmacologic properites with ischemia and glutamate induced injury. Propidium iodide fluorescence was used to observe early membrane injury as it occured in the living culture. Several hours following a 30 min exposure to trans-ACPD (100 μM), injury was observed in the CA1 and hilar regions of the cultures, the regions most vulnerable to ischemic injury. Most of CA3 and the dentate gyrus were spared. We hypothesize that glutamate, released during and after ischemia, triggers injury at metabotropic receptors. The distribution of these receptors may determine the distribution of ischemic injury. TTX or MK-801 blocked ischemic, trans-ACPD or glutamate-induced injury. The toxicity of NMDA itself was not blocked by TTX. Thus, the injury appears indirect, mediated through secondary NMDA receptor activation. The secondary toxic effect may be synaptically mediated as it is blocked by TTX.

43.14

OCULAR AND OPTIC NERVE PATHOLOGY IN ADULT RATS ASSOCIATED WITH NEONATAL EXPOSURE TO D,L-2-AMINO-3-PHOSPHONOPROPIONIC ACID (D,L-AP3) AND 1-AMINOCYCLOPENTANE-TRANS-1,3-DICARBOXYLIC ACID (TRANS-ACPD).

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Metabotropic glutamate receptors are considered important in synaptic plasticity and nervous system development. D,L-AP3, a metabotropic receptor antagonist, produces developmental behavioral deficits and widespread acute neurodegenerative changes when administered to neonatal rats (Tizzano et al., NS Abstr., 1990 and 1991). Included in these changes are degeneration of retina and optic nerve. The present study describes the ocular lesions in adult rats associated with neonatal D,L-AP3 exposure and evaluates the effects of trans-ACPD, a metabotropic receptor agonist, on this D,L-AP3-induced developmental abnormality. Neonatal rats were dosed intraperitoneally on postnatal days 3-10 with saline (n = 5), 25 mg/kg/day trans-ACPD (n = 3), 400 mg/kg/day D,L-AP3 (n = 4), or a combination of 25 mg/kg/day trans-ACPD followed 15 minutes later by 400 mg/kg/day D,L-AP3. At 60 days of age, morphologic examinations were made of eyes and optic nerves. All D,L-AP3-treated rats had total atrophy of optic nerve and chiasm. Microscopically, these rats had severe retinal atrophy, retinal detachment, and cataract formation. Similar lesions occurred in all rats treated with a combination of trans-ACPD and D,L-AP3; however, the optic nerve atrophy was less pronounced in these rats than in those treated with D,L-AP3 alone. There were no differences between saline-treated control rats and those treated with trans-ACPD alone. These data suggest that a metabotropic receptor agonist, trans-ACPD, can reduce the severity of D,L-AP3-induced neonatal optic nerve atrophy. Early postnatal exposure to metabotropic receptor agonists/antagonists clearly alters normal development of the rat eye and optic nerve.

43.15

SEIZURE ACTIVITY STIMULATES THE POLYAMINE INTERCONVERSION PATHWAY IN RAT LIMBIC SYSTEM.

A. Bruce*, I. Najm, S. Hauge, S.S. Schreiber, G. Tocco, and M. Baudry. Neuroscience Program, USC, Los Angeles, CA 90089-2520.

The roles of the polyamines putrescine, spermidine, and spermine following injury in the adult mammalian CNS is still a matter of controversy. Although it has been repeatedly demonstrated that a variety of manipulations can produce a rapid induction of ornithine decarboxylase (ODC), the rate limiting enzyme in the polyamine synthesis pathway, little information is available concerning the induction of the interconversion pathway, which involves the activities of the enzymes spermine/spermidine acetyl transferase (SAT) and polyamine oxidase. We report here that the interconversion pathway is rapidly activated in the rat limbic system following kainic acid (KA)-induced seizure activity.

Groups of rats were sacrificed at various time intervals following systemic KA administration, and the brains were either dissected for biochemical analysis or frozen and used for *in situ* hybridization studies. SAT activity was increased several fold and up to 16 hours following KA administration in the hippocampus and piriform cortex. *In situ* hybridization using a ^{35}S-labelled SAT cRNA probe showed a rapid increase in SAT mRNA throughout the rat limbic system. By 16 hrs after KA treatment the increase in SAT mRNA was still present in CA3, CA1 and piriform cortex. Pretreatment of the animals with the polyamine oxidase inhibitor MDL 72527 resulted in larger accumulations of N-acetylspermine and N-acetylspermidine in KA treated animals than in control animals. The results indicate that seizure activity in limbic structures is accompanied by a rapid induction of SAT, an increased activity of the enzyme and therefore an increased polyamine interconversion.

(Supported by NIH grants NS 01337 to SSS and NS 18427 to MB).

43.16

EXTRACELLULAR POLYAMINES IN QUINOLINIC ACID-INJECTED STRIATUM.

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Disposition of endogenous polyamines (PA) is relevant for studying their modulatory role on the N-methyl-D-aspartate receptors. Recently, we showed the extracellular presence of striatal putrescine (PUT), spermidine (SPD), and spermine (SPM) by using the microdialysis in awake rats (Speciale et al., Soc. Neurosci. 17:106.11). We now report PA extracellular content during nerve cell loss. Wistar rats (200-225 g) were used in all experiments. Animals were injected intrastriatally with quinolinate (QUIN, 120 nmoles/2 μl) or saline (SAL, 2 μl). Seven days later, dialysis was performed by delivering Krebs buffer in the injected striatum for 320 min at the speed of 2 μl/min. At the beginning of perfusion (but not later on), lesioned rats (n=11) dialysate levels were significantly higher than controls (n=12) for PUT and SPD, and lower for SPM. A depolarizing stimulus was given by perfusing with isotonic Krebs buffer containing 75 mM KCl. In SAL-injected rats (n=6), PUT did not change; SPD rose from 1.41 ± 0.25 to 3.61 ± 0.58 pmoles/40 μl dialysate, and SPM from 0.19 ± 0.05 to 2.54 ± 0.79 pmoles/40 μl dialysate. In QUIN-injected rats (n=5), depolarizing stimuli did not affect PUT content, but, unlike controls, the rise of SPD and SPM was not observed. Data show changes of extracellular PA content consequent to excitotoxic nerve cell death and suggest the involvement of neurons in the liberation of SPD and SPM upon depolarization.

43.17

ATP DEPLETION RELEASES GLUTAMATE PRIMARILY FROM AXONAL TERMINALS OF HIPPOCAMPAL SLICES.

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Glutamate (Glu) may be released from synaptosomes during ATP depletion by reversal of normal uptake systems. However, immunocytochemical studies suggest much of the Glu in the CNS is found in neuronal cell bodies and dendrites. Whether both of these pools contribute to release induced by ATP depletion remains unknown. Release of Glu from rat hippocampal slices during ATP depletion was measured by HPLC and compared with loss of Glu-like immunoreactivity (GLIR) from neuronal cell bodies and axonal terminals. Slices incubated in oxygenated Hank's balanced salt solution (with 10 mM HEPES and 10 mM glucose; HBSS) for 1 hr showed GLIR primarily in terminal-like structures with little staining of cell bodies or dendrites. Addition of Glu (1 mM) with Glu antagonists to the HBSS produced greater retention of GLIR in cell bodies and dendrites. ATP depletion induced by dinitrophenol, azide, or other inhibitors produced dose-dependent releases of Glu. In slices preloaded with the transportable Glu analog D, L-threo-3-Hydroxyaspartate (OH-Asp), Glu and OH-Asp were released in a similar, Ca-independent manner during ATP depletion (R^2>0.90), suggesting releases occurred by reversal of Na-Glu cotransporters. Immunocytochemical localization of Glu in ATP-depleted slices suggests release was primarily from axonal terminals. ATP depletion reduced GLIR in structures with morphology consistent with axonal terminals while GLIR was retained in neuronal cell bodies and dendrites. ATP depletion also resulted in increased GLIR in some glia. These results suggest Glu is released primarily from axonal terminals and not neuronal cell bodies and dendrites during ATP depletion.

43.18

KYNURENIC ACID AND DIZOCILPINE DIFFERENTLY MODULATE GLUTAMATE-INDUCED ENERGY FAILURE IN LIVE NEONATAL BRAIN SLICES.

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Acute energy failure and recovery were compared in live 350μ brain slices perfused with different concentrations of glutamate (GLU) and GLU-receptor antagonists. Slices were perfused for sixty minutes with ACSF containing GLU in the range 0.5 mM to 10 mM and then immediately reperfused for sixty minutes with GLU-free ACSF. Interleaved ^{31}P/^{1}H NMR spectra were determined every five minutes. Perfusion with 0.5 mM GLU in the absence of Mg^{+2} decreased PCr to ≈75% of control but did not affect ATP. Inclusion of 1.2 mM Mg^{+2} prevented detectable NMR changes. Perfusion with 2.0 mM GLU (no Mg^{+2}) decreased PCr (≈60%) and ATP (≈60%); perfusion with 2.0 and 3.0 mM GLU in the presence of 1.2 mM Mg^{+2} caused a similar but partially recoverable decrease in PCr and ATP. Perfusion with 5.0-10.0 mM GLU caused larger nonrecoverable decreases. Two cerebroprotective agents, kynurenate (1.0 mM) and dizocilpine (150μM MK801), prevented the immediate energy failure caused by 2.0 mM GLU. Additionally, kynurenate and dizocilpine each ameliorated GLU-induced depletion of PCr and ATP, while accelerating recovery after reperfusion. During the first 20 minutes of GLU administration kynurenic acid improved metabolic response while dizocilpine did not. After 30 minutes of GLU administration dizocilpine-treated slices showed almost complete recovery, while kynurenate-treated slices did not. Acute energy failure thus appears linked to activation of more than one type of GLU receptor.

43.19

STRIATAL DAMAGE CAUSED BY 3NP INDUCED ENERGY COMPROMISE IS POTENTIATED BY AGING.

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3-Nitropropionic acid (3NP) produces excitotoxic lesions in the striatum by impairing energy metabolism (Beal et al., this volume). Since many neurodegenerative diseases, like Huntington's disease, are age dependent we hypothesized that striatal damage induced by 3NP may increase with age. We treated male rats systemically (30 mg/kg/i.p.) or intrastriatally (500 nmol) with 3NP. One week after treatment, rats were evaluated for striatal damage by histological and neurochemical methods. Systemic administration of 3NP rarely caused striatal damage in rats younger than 3 months of age (12% of cases) while rats above that age either died or showed severe striatal damage (87% cases). Lactate increases and ATP depletions at 3 hours following intrastriatal injections of 3NP were significantly greater in 4 month old than in 1 month old animals. There was a significantly greater depletion of striatal neurochemical markers in older than in young rats. Mild lesions showed sparing of NADPH-diaphorase neurons consistent with an NMDA-like excitotoxic lesions. Our results demonstrate an age dependent susceptibility of rats to striatal neuronal damage induced by energy compromise.

43.20

LOCAL AND SYSTEMIC ADMINISTRATION OF 3-NITROPROPIONIC ACID RESULTS IN EXCITOTOXIC STRIATAL LESIONS.

M. Flint Beal*, Emmanuel Brouillet, Bruce Jenkins, Rachana Srivastava, Deborah Samanta Roy, Bruce R. Rosen and Bradley T. Hyman. Neurology Service, Mass. General Hospital, Boston, MA 02114.

3-Nitropropionic acid (3NP) is an irreversible inhibitor of succinate dehydrogenase which results in striatal lesions and dystonia in man. In the present study we characterized the neurochemical effects of lesions produced by either intrastriatal or systemic administration of 3NP. Intrastriatal injections resulted in dose-dependent depletions of GABA, substance P, somatostatin, neuropeptide Y and dopamine. At 3 hours there were significant decreases in ATP and increases in lactate concentrations. Following systemic administration of 3NP striatal concentrations of dopamine and serotonin were spared. Systemic and local administration of 3NP resulted in selective striatal lesions with marked depletion of both Nissl stained and NADPH-diaphorase neurons. Chemical shift magnetic resonance imaging, showed focal increases in lactate in the striatum following systemic administration of 3NP. Prior decortication attenuated both intrastriatal and systemically induced 3NP lesions. These results show that both local and systemic administration of 3NP results in excitotoxic striatal lesions as a consequence of impairment of oxidative phosphorylation.

44.1

MODULATION OF KAINATE-INDUCED IMMEDIATE EARLY GENE EXPRESSION IN RAT BRAIN. X. Li*, L. Song, K. Kolasa and R.S. Jope. Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama, Birmingham, AL 35294.

Immediate early genes (IEG) have a critical role in the stimulus-response process of many cells by virtue of their protein products regulating gene transcription. Because of the importance of IEG in neuronal function, endogenous agents which modulate stimulation of IEG transcription, if they exist, could have profound effects. We used as model stimuli relatively low doses of kainate and pentylenetetrazole (PTZ). The dose (4 mg/kg) of kainate used does not cause convulsions and induces relatively slight increases in the IEG mRNA levels. Therefore, the results obtained should be relevant to other stimuli that only mildly induce IEG expression.

Kainate or PTZ increased c-fos, c-jun, jun B and jun D mRNA levels in rat brain dose-dependently. Kainate increased these mRNA levels predominantly in the hippocampus and PTZ was more effective in the cortex. Adrenalectomy (3 days) was used to eliminate endogenous glucocorticoid hormones. Compared with increases caused by kainate (4 mg/kg) alone, adrenalectomy significantly potentiated kainate-induced increases in the hippocampal mRNA levels of c-fos and jun B by 6.5-fold, of jun D by 2-fold, and tended to augment c-jun. Corticosterone administration blocked the potentiated stimulation of these mRNA levels caused by adrenalectomy. Adrenalectomy also significantly increased PTZ-induced c-fos mRNA in the cortex. These results demonstrate that glucocorticoids modulate IEG expression in the brain, raising the possibility that this interaction contributes to interneuronal and interindividual differences in responses to stimuli and to the effects of stress- or disease-induced changes in glucocorticoid concentrations.

These observations may be relevant in psychiatric disorders in which altered responses to external stimuli and/or abnormal glucocorticoid function have been implicated as well as to age-related neurodegeneration which is adversely influenced by glucocorticoids.

44.2

γ-D-GLUTAMYLAMINOMETHYL SULFONIC ACID (GAMS) DISTINGUISHES AMPA- FROM KAINIC ACID-INDUCED RESPONSES IN *XENOPUS* OOCYTES EXPRESSING CHICK BRAIN GLUTAMATE RECEPTORS N. Zhou*, L. G. Hammerland and T. N. Parks. Depts. of Anatomy and Biology, Univ. of Utah, Salt Lake City, UT 84132.

In the chick cochlear nucleus, we recently found both GAMS-sensitive and GAMS-insensitive glutamate receptor-mediated responses, with the latter mediating synaptic transmission (*Hear. Res.*, in press 1992). To further characterize the actions of GAMS, its effects on AMPA- and KA-induced responses were studied in 49 *Xenopus* oocytes injected 3-5 days previously with mRNA from the brains of E16-17 chick embryos and voltage-clamped at -60 mV (cf. *Mol. Pharm. 34*: 298, 1988). Both AMPA and KA induced smooth inward currents with Hill coefficients of 1.46 (AMPA) and 1.90 (KA). GAMS, at concentrations up to 1 mM, had no reliable effect on AMPA-induced currents but showed a consistent, dose-dependent and reversible effect on KA-induced responses; the slope of the Schild plot was 0.76 and the pA_2 value (± S.E.M.) -4.3 ± 0.2. We then compared the selectivity of quinoxalinedione antagonists with that shown by GAMS. DNQX, CNQX and NBQX all blocked the effects of both agonists completely, competitively, reversibly and dose-dependently, with Schild-plot slopes very close to 1.0. Against AMPA, observed pA_2 values were -6.6 ±0.1 for DNQX, -6.43±0.07 for CNQX and -6.8±0.1 for NBQX. Against KA, pA_2 values were -6.4 ±0.1 for DNQX, -6.6± 0.2 for CNQX and -7.2 ± 0.3 for NBQX. Although these data show that NBQX and CNQX are significantly more potent antagonists of KA- than of AMPA-induced effects, GAMS shows much higher selectivity for KA-induced responses and may therefore be a useful tool in studying the diverse types of glutamate receptors in the CNS.

Supported by USPHS grant DC00144 to T.N.P. We thank Prof. D. Yoshikami for generous help and Dr. T. Honore for the gift of NBQX.

44.3

EFFECT OF THE NON-NMDA ANTAGONIST, GYKI 52466, ON ISCHEMICALLY-INDUCED INTRACELLULAR AND EXTRA-CELLULAR ASPARTATE AND GLUTAMATE CHANGES IN RAT HIPPOCAMPUS AND STRIATUM. A.G. Chapman*, J.L. Graham, C. Moncada, B. Arvin and B.S. Meldrum. Dept. of Neurol., Inst. of Psych., De Crespigny Park, Denmark Hill, London SE5 8AF, England.

GYKI 52466 protects against striatal, but not significantly against hippocampal cell loss when assessed 7 days after 20 min of transient global (4VO) ischemia (1). GYKI also selectively inhibits 4VO-induced glu (but not asp) release (2). During 20 min of 4VO ischemia the striatal microdialysate levels of asp and glu increase 4-6 fold, and there are significant increases in the intracellular striatal levels of glu (25%) and asp (50%) following ischemia (at 0hr), with return to control levels by 72 hrs postischemia. GYKI (20 mg/kg) pretreatment blocks the ischemic increases in striatal intracellular glu and asp, and the increased striatal glu release. 4VO ischemia produces prolonged (0-72hrs) decreases in hippocampal intracellular glu (25-30%) and asp (30-45%), and increased hippocampal glu and asp release. GYKI does not reverse the hippocampal intra- and extra-cellular asp and glu changes.

1.Le Peillet et al.(1992) Brain Res. 571, 115-120
2.Arvin et al.(1992) NeuroReport 3, 235-238

44.4

ANIRACETAM POTENTIATES KAINATE- AND AMPA-INDUCED CURRENTS IN OOCYTES EXPRESSING CLONED GLUTAMATE RECEPTORS. J.F. McGurk*, R.S. Zukin and M.V.L. Bennett. Dept. Neurosci., A. Einstein Coll. Med., Bronx, NY 10461.

Kainate/AMPA receptors encoded by "flip" and "flop" splice variants of rat GluR1 and GluR3 RNAs show little desensitization to the agonist kainate and marked desensitization to the agonist AMPA, with "flop" variants desensitizing more than "flip". The nootropic drug aniracetam potentiated both kainate- and AMPA-induced currents in *Xenopus* oocytes injected with GluR1 "flip", GluR1 "flop" or GluR3 "flop" cRNA. For all receptor subtypes aniracetam (2mM) increased kainate (300μM) responses to ~140% of control. For GluR1 "flip" receptors aniracetam (2mM) increased AMPA (30μM) responses to 140% of control, while for GluR1 and 3 "flop" receptors potentiation by aniracetam was to ~200% of control. Aniracetam may reduce desensitization of AMPA responses, which could account for the greater potentiation observed for the "flop" variants. Onset and wash-off time of aniracetam were sufficiently fast (<1s) to indicate an extracellular site of action. Potentiation did not depend on extracellular Ca^{2+} concentration or transmembrane voltage. In oocytes injected with rat brain mRNA, aniracetam generally did not potentiate kainate responses, although potentiation to ~120% of control was sometimes seen. In the same oocytes, aniracetam increased AMPA responses to ~280% of control regardless of its effect on kainate responses. The difference in effects of aniracetam on homomeric GluR receptors and receptors encoded by brain mRNA may be due to heteromeric composition of the latter receptors or the presence of additional subunits.

44.5

PENTOBARBITAL BLOCK OF KAINATE- AND QUISQUALATE-INDUCED CURRENTS. W. Marszalec and T. Narahashi* Dept of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Mechanisms underlying the inhibition of non-NMDA currents by Na pentobarbital (PB) were studied in cultured rat cortical neurons. The block of whole-cell kainate-induced currents by PB (ED_{50}=75μM) was facilitated by receptor activation (use dependent). Currents evoked by 40 μM kainate were reduced 50±3% when 100 μM PB was co-applied for 5 sec. In contrast, kainate currents following a 10 min perfusion with PB alone were inhibited by <10%. Recovery from PB inhibition was use dependent, reversing within 5 sec of kainate application, but persisting longer than 10 min in its absence. Currents evoked by 10 μM quisqualate were rapidly desensitized to a steady state. Repeated applications of 100 μM PB reduced peak quisqualate currents 19±5% while inhibiting the smaller sustaining phase 49±5%. The onset of and recovery from PB's inhibition of kainate currents were use dependent, suggesting a PB binding site within the channel. The smaller inhibition of quisqualate currents may reflect a decreased access to the channel due to receptor desensitization. Supported by NIH grant NS14144.

44.6

ANTAGONISM OF ATPA AND AMPA-INDUCED DEPOLARIZATIONS IN THE RAT CORTICAL WEDGE BY NBQX, CNQX, AND PD 140532. L.J. Robichaud*, S.J. Hays, R.J Chang and P.A. Boxer. Neuroscience Pharmacology, Parke-Davis Research, Warner-Lambert Co. Ann Arbor, MI 48105

Selective affinity of NBQX and CNQX for AMPA versus kainate receptors has been reported in cortical membranes using specific radiolabeled ligands. In similar studies (RS)-α-amino-3-*tert*-butyl-4-isoxazolepropionic acid (ATPA) has been identified as a new agonist of the AMPA subclass of glutamate receptors. In the rat cortical wedge model, we obtained depolarizations to cumulative concentrations of ATPA, AMPA, and kainate. ATPA was the least potent agonist and produced depolarizations with a slow onset and offset that were qualitatively different from AMPA or kainate. Repeated depolarizations to ATPA, AMPA, or kainate (at their respective EC_{70}s) were obtained before and after thirty min treatment with varying concentrations of antagonist. NBQX, CNQX and the novel compound 6,7-dichloro-3,4-dihydro-3-oxo-N-(phenylsulfonyl)-2-quinoxalinecarboxamide (PD 140532) were potent and effective inhibitors of ATPA (IC_{50}s=0.12, 1.6, and 0.6 μM respectively) and AMPA (IC_{50}s=0.17, 0.4, and 4.3 μM respectively), but less potent against kainate. These data indicate that ATPA is a functional agonist in the cortical wedge, with responses antagonized by NBQX, CNQX and PD 140532 at concentrations similar to those inhibiting responses to AMPA but not kainate. The results are consistent with the assumption that ATPA is an agonist of the AMPA subtype of glutamate receptors.

44.7

GUANOSINE COMPOUNDS DECREASE ^{3}H-KAINATE BINDING AND INHIBIT KAINATE RESPONSES IN MAMMALIAN CEREBELLUM. C. Poulopoulou* and L.M. Nowak, Department of Pharmacology, Cornell University, Ithaca, NY 14853.

Kainate is known to dramatically increase intracellular levels of cGMP in cerebellum. For this study we examined some possible effects of cGMP on kainate receptors using radioligand binding and patch clamp recording methods. Crude synaptosomal membranes from rabbit cerebellum were used in binding assays and modulation of kainate responses was examined in cerebellar neurons cultured from 1-8 day old mice. Direct and Scatchard plots of ^{3}H-kainate binding revealed that kainate binds to two distinct sites with a 10 fold difference in affinity (K_{D_1} = 17nM and K_{D_2} =1.6nM) and a ratio of high to low affinity sites of 1: 2. This binding profile was altered by the inclucion of 2.5 mM cGMP in the incubation mixture; direct plots were now best fitted by one binding site with a K_D of 17nM. This may mean that either cGMP preferentialy competes for binding to the high affinity kainate binding site, or possibly the high affinity site is converted to a lower affinity. The pharmacological specificity of this effect was tested with guanosine and adenosine nucleotides (0.75-5mM) in displacement assays. All guanosine nucleotides (GMP, cGMP, GDP, GTP), were nearly equipotent in displacing ^{3}H-kainate binding while adenosine analogs apparently had no effect. Preliminary whole cell recording experiments on cerebellar neurons indicate that coapplication of cGMP with kainate in the bath has inhibitory effects on whole cell kainate currents, suggesting that cGMP exerts this inhitory action at a site on the extracellular surface of the receptors. Supported by NS24467.

44.8

INHIBITION OF SELECTED POPULATIONS OF KAINATE-ACTIVATED RECEPTOR-CHANNELS BY nonNMDA AGONISTS: AMPA, IBOTENATE AND L-GLUTAMATE. L.M. Nowak* and C. Poulopoulou, Department of Pharmacology, Cornell University, Ithaca, NY 14853.

Inhibition of kainate responses by L-glutamate, quisqualate and AMPA has been observed in different neuronal cell preparations. Several mechanisms of inhibition have been proposed, with one hypthesis being that some nonNMDA agonists decrease kainate responses by increasing desensitization of a common receptor. Having observed that kainate activates a variety of ion channels, we investigated the inhibition of kainate-activated channels in outside-out patches and asked: 1) are one or more populations of kainate-activated channels affected by these agonists, and 2) do all of the agonists affect the same channels. Patches were taken from cortical and cerebellar neurons grown in primary dissociated cell culture. Pipettes (3-8MΩ) typically contained (in mM): 145 CsCl, 10 Hepes-K (pH 7.2), 10 EGTA/1.0 CaCl2, and 2-4 ATP-Mg. Extracellular solutions contained (in mM): 150 NaCl, 2.8 KCl, 1.0 CaCl2, 10 Hepes-Na (pH 7.2) and 300 nM TTX. Kainate, AMPA, L-glutamate and ibotenate were applied alone, or in combination by slow or fast perfusion. Kainate opened channels with conductances of ~1, 2-4, 13, 25, and 36 pS, which where observed in different patches, and which had different kinetics, suggesting they represent different receptor-channels. AMPA, applied to some of these patches, activated the 13, 25 and 36 pS channels, and ibotenate activated the 25 and 36 pS channels. Coapplication of AMPA or L-glutamate with kainate primarily inhibited low conductance kainate channels, whereas ibotenate primarily inhibited the 13 pS channels activated by AMPA and kainate. Supported by NS24467.

44.9

ATPA DRUG DISCRIMINATION: A MODEL OF AMPA AGONISTIC EFFECTS IN VIVO? M.D.B. Swedberg* and P. Jacobsen. Novo Nordisk, CNS Division, Novo Nordisk Park, DK-2760 Malov, Denmark.

ATPA (2-amino-3-(3-hydroxy-5-tert-butylisoxazol-4-yl)propionate) is an agonist at AMPA (2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionate) receptors. To determine whether ATPA could serve as an in vivo model of AMPA agonism, rats were trained to discriminate ATPA at 10 mg/kg, i.p., 15 min after injection from no drug. The ATPA discrimination was easily acquired, and yielded a dose dependent generalization curve. The excitatory amino acid agonists NMDA (10-40 mg/kg), kainic acid (0.3 and 1 mg/kg) and AMPA (3 and 10 mg/kg), did not produce ATPA-like discriminative effects. These results indicate that the discriminative effects of ATPA are not mediated by NMDA or kainiate receptors. The failure of AMPA to generalize to ATPA may be attributable to AMPA's poor ability to penetrate the blood brain barrier. Excitatory amino acid antagonists are currently being investigated for their ability to reverse the discriminative effects of ATPA.

44.10

SELECTIVE DISPLACEMENT OF LOW AFFINITY ^{3}H-KAINATE BINDING CORRELATES WITH ANTAGONISM OF DOMOIC ACID TOXICITY. E.Ø. Nielsen*[1], T.H. Johansen[1], R.A.R. Tasker[2], S.M. Strain[2], L.H. Jensen[1], F. Wätjen[1] and J. Drejer[1]. [1]NeuroSearch A/S, Smedeland 26, 2600 Glostrup, Denmark; [2]Atlantic Veterinary College, Charlottetown, Prince Edward Island, Canada, C1A 4P3.

Binding sites for non-NMDA excitatory amino acid receptors have generally been studied using ^{3}H-AMPA and ^{3}H-kainate (^{3}H-KA). ^{3}H-KA binding sites can be separated into high- and low affinity sites, respectively, and be studied on the basis of the sensitivity of these two sites to Ca^{2+}. High affinity ^{3}H-KA binding sites may represent distinct KA receptors whereas low affinity ^{3}H-KA binding sites probably represent binding of KA to AMPA receptors. Most non-NMDA agonists and antagonists studied so far in ^{3}H-KA binding have shown little or no selectivity between high- and low affinity sites. One exception is domoic acid (DOM) which relative to KA has a higher affinity for low affinity ^{3}H-KA binding sites. From a series of novel isatinoximes developed as non-NMDA antagonists a few compounds have been characterized as potent and selective displacers of low affinity ^{3}H-KA binding. High affinity ^{3}H-KA binding was studied at 2 nM ^{3}H-KA ($-Ca^{2+}$) whereas low affinity binding was performed at 20 nM (+20 mM Ca^{2+}). NS102 displaced low affinity ^{3}H-KA binding with an IC_{50} of 0.7 μM with only partial inhibition of high affinity binding at 10 μM. NS102 showed non-NMDA antagonism in several *in vitro* functional tests. *In vivo* NS102 (10 mg/kg ip) antagonized behavioral effects including seizures induced by systemic DOM administration and blocked DOM induced neurodegeneration. However, NS102 only offered partial protection of behavioral responses to KA and could not block KA induced neurodegeneration. These findings indicate that DOM activation of non-NMDA receptors differs from activation by KA. This difference might be explained by an apparent selectivity of DOM for low affinity ^{3}H-KA binding sites. The identification of new non-NMDA antagonists such as NS102 with high selectivity for low affinity ^{3}H-KA binding sites may be valuable tools in the characterization of the functional importance of these binding sites.

44.11

CHARACTERIZATION OF KAINATE RECEPTORS IN RAT DORSAL ROOT GANGLION(DRG) NEURONS USING NOVEL S-WILLARDIINE ANALOGS. L.A. Wong*, M.L. Mayer, D.E. Jane† and J.C. Watkins†. Lab Cell & Molec Neurophysiol, NICHD, NIH and †Dept of Pharmacology, School of Medical Sciences, Univ of Bristol, UK.

Glutamate receptors in DRG neurons differ from those in the CNS in their desensitizing response to kainate and greater affinity for agonists *(Huettner, Neuron 1990, 5:255)*. The present study further investigates the pharmacology of these receptors by testing the action of a series of S-willardiine analogs. Whole cell patch clamp recordings using fast perfusion techniques were performed on acutely dissociated neonatal (P2-P10) rat DRG neurons. Dose-response analysis revealed the following potency relationship: S-Iodo-Willardiine(I-W)>S-Bromo-Willardiine(Br-W)>S-Chloro-Willardiine(Cl-W)>S-Nitro-Willardiine(N-W)>Kainate(Kai)>S-Fluoro-Willardiine(F-W). Application of saturating concentrations of these agonists (100 x EC50) resulted in peak responses of similar amplitude for all willardiine analogs but were smaller than those to Kai. With 2s agonist applications, all willardiine analogs evoked ~90% desensitization; in contrast, there was only a 50-70% reduction in peak amplitude for Kai. The rate of onset of desensitization in response to willardiines could be fitted by the sum of two exponentials, τ_1~100 ms and τ_2~900 ms. The potency sequence in our experiments is consistent with the rank order of agonist potency for depolarizing rat dorsal root C fibers *(Blake et al., Br. J. Pharm. Suppl. 1991, 104:334P)* but is the reverse of that observed in cultured hippocampal neurons: F-W>N-W>Cl-W~Br-W>I-W>Kai *(Patneau et al., J. Neurosci. 1992, 12: 595)*. Willardiines exhibit strikingly different pharmacology in hippocampal and DRG neurons and may be useful as tools for the differentiation of glutamate receptor subtypes in other parts of the CNS.

44.12

DIAZOXIDE POTENTIATES AMPA/KAINATE RECEPTOR CURRENTS ALLOSTERICALLY. J.C.R. Randle* and J.M. Lepagnol, Institut de Recherches Servier, 92150 Suresnes, FRANCE.

Diazoxide (DZX) was recently reported to enhance and prolong excitatory synaptic currents and L-glutamate or quisqualate-induced (but not kainate-induced) current in hippocampal cell cultures (Yamada and Rothman, *Soc. Neurosci. Abstr.* 17:896 (1991), independent of its actions on ATP-sensitive K^+ channels. We have examined this phenomenon in two-electrode voltage-clamp experiments on rat cortex mRNA-injected *Xenopus* oocytes. DZX (10-560 *u*M) had no effect on membrane current when applied alone, and only weakly inhibited responses to NMDA (30 *u*M) plus glycine (3 *u*M) or GABA (100 *u*M). However, DZX induced a rapidly appearing and reversible 2 to 3-fold increase in current responses evoked by all concentrations of AMPA (1-1000 *u*M); the EC50 of AMPA (8 *u*M) was unchanged. Curiously, DZX induced a leftward shift of the kainate concentration-response curve (EC50: control, 100 *u*M; +DZX, 30 *u*M). DZX increased responses to 3-30 *u*M kainate 3 to 5-fold but those to high (1-3 mM) concentrations only 10-15% (accounting for the lack of effect reported by Yamada and Rothman). DZX had little effect on the inhibition of kainate/AMPA currents by NBQX. Furthermore, DZX (1 nM - 1 mM) did not displace [3H]AMPA or [3H]kainate binding. Our data indicate that DZX acts allosterically to induce kinetically distinct potentiation of activation by AMPA and kainate of the AMPA/kainate receptor/channel.

44.13

PENTOBARBITAL ANTAGONIZES AMPA AND KAINIC ACID BUT NOT DNQX-SENSITIVE SYNAPTIC RESPONSES. E.W. Harris*, Biology Dept., Fisons Pharmaceuticals, Rochester, NY 14603

Glutamate appears to be an excitatory neurotransmitter in the mammalian CNS, acting at receptors denoted by selective agonists, for example, NMDA, kainic acid (KA), and AMPA or quisqualate. Many excitatory synaptic responses appear to have a large AMPA and/or KA receptor-mediated component because they are reduced by AMPA/KA competitive antagonists (e.g., DNQX). Pentobarbital has also been reported to antagonize quisqualate and KA more than NMDA. Therefore, its effects on responses to AMPA, KA, and excitatory synaptic responses have been examined.

In area CA1 of rat hippocampal slices, pentobarbital (10-100μM) antagonized the effects of bath-applied AMPA and KA, but not NMDA, similar to the effects of 10μM DNQX. Antagonism by pentobarbital was unaffected by bicuculline (30μM), or reducing Ca^{++} (0.2mM Ca^{++} & 5.8mM Mg). Therefore, pentobarbital appears to be acting as a direct AMPA/KA antagonist. However, 1000μM pentobarbital had no effect on excitatory synaptic field potentials, which were almost abolished by 10μM DNQX. In contrast, the NMDA antagonists AP5 and MK-801 did antagonize responses to bath-applied NMDA as well as NMDA-receptor-mediated synaptic responses (recorded in low-Mg^{++}/DNQX-containing medium).

The effects of pentobarbital seem inconsistent with competitive antagonism or channel blockade.

44.14

THE NEUROPROTECTIVE ACTIONS OF YM900, A NOVEL AND POTENT α-AMINO-3-HYDROXY-5-METHYLISOXAZOLE-4-PROPIONATE (AMPA) RECEPTOR ANTAGONIST, IN A GERBIL GLOBAL ISCHEMIA MODEL AND A RAT FOCAL ISCHEMIA MODEL. M. Shimizu-Sasamata*, S. Kawasaki, S. Yatsugi, J. Ohmori, S. Sakamoto, K. Koshiya, S. Usuda and K. Murase. Central Research Laboratories, Yamanouchi Pharmaceutical CO. LTD., 21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan.

Glutamate is thought to mediate the neurodegeneration following cerebral ischemia by acting on N-methyl-D-aspartate(NMDA) and non-NMDA subtypes of excitatory amino acid receptors. We have investigated the neuroprotective actions of YM900 [6-(1-imidazolyl)-7-nitroquinoxaline-2,3(1H,4H)-dione], a novel and potent AMPA receptor antagonist (Okada et al., this meeting), in a gerbil global ischemia model and a rat focal ischemia model.

Male Mongolian gerbils were subjected to 5 min of transient cerebral ischemia induced by bilateral common carotid occlusion. After 4 days, the CA1 subfield of the hippocampus was assessed for neuronal loss using a 4 point rating scale (from 0 = no damage to 3 = almost total damage). The mean score of the control animals was 3.0 ± 0.0. When administered at 60, 70, and 85 min or 360, 370, and 385 min after recirculation, YM900 (30 mg/kg i.p.) significantly prevented delayed neuronal death in the CA1 area (the scores were 0.7 ± 0.2 and 1.8 ± 0.4, respectively).

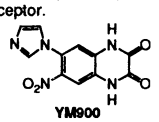
Male Fischer rats were subjected to permanent occlusion of the left middle cerebral artery. After 24 hr, the volume of ischemic brain damage was assessed quantitatively using an image analyzer. When administered by an continuous i.v. infusion of 20 mg/kg/h for 4 hr starting at post-ischemia, YM900 significantly reduced the volume of ischemic damage in the cerebral hemisphere by 31%.

Our data demonstrate that blockade of non-NMDA receptor decreases neuronal damage following both global and focal ischemia models and suggest that YM900 may be useful in the treatment of human stroke.

44.15

α-AMINO-3-HYDROXY-5-METHYLISOXAZOLE-4-PROPIONATE(AMPA) EXCITATORY AMINO ACID RECEPTOR ANTAGONISTIC PROPERTIES OF YM900 (6-(1-imidazolyl)-7-nitro-quinoxaline-2,3(1H,4H)-dione). M. Okada, K. Hidaka, J. Togami, K. Ohno, S. Tada, J. Ohmori, S. Sakamoto, and T. Yamaguchi*. Central Research Laboratories, Yamanouchi Pharmaceutical Co. LTD., 21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan.

We have synthesized a series of imidazolylquinoxaline-2,3-diones to develop a potent and selective AMPA excitatory amino acid receptor antagonist. Among of these compounds, we discovered YM900 which had high affinity for AMPA binding sites(S. Sakamoto et.al., XIIth International Symposium on Medicinal Chemistry, 1992.). Here we report YM900 is an antagonist at AMPA-type receptor.



YM900

YM900 with a Ki for the displacement of ^{3}H-AMPA=0.058μM, ^{3}H-Kainate=2.2μM, L-^{3}H-Glutamate(NMDA-specific site)>100μM and ^{3}H-Glycine(strychnine-insensitive site)=19μM had the highest affinity for AMPA binding sites. AMPA elicited a concentration-dependent increase in intracellular Ca^{2+} in cultured hippocampal neurons, and YM900 inhibited AMPA-induced Ca^{2+} increase. Injection of AMPA into the rat striatum resulted in a loss of enzyme marker for cholinergic neurons 7 days later, and YM900 co-injected with AMPA protected cholinergic neurons from AMPA-neurotoxicity. All of these data shows that YM900 is a novel and potent antagonist at AMPA-type receptor.

EXCITATORY AMINO ACIDS: RECEPTORS I

45.1

STRUCTURE AND SUBUNIT COMPOSITION OF AMPA RECEPTORS IN THE RAT BRAIN. C.D. Blackstone*, L.J. Martin, D.L. Price and R.L. Huganir, Dept. of Neuroscience, Howard Hughes Med. Inst., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

The oligomeric structure and regional distribution of AMPA-preferring glutamate receptors were studied using antipeptide antibodies to the receptor subunits GluR1-GluR4. These antibodies, which were generated against divergent regions of GluR1-GluR4 as well as a domain common to GluR2 and GluR3 (GluR2/3), detected distinct proteins ranging from 102-108 kDa on SDS-PAGE of rat CNS membranes, but not in peripheral tissues. Chemical cross-linking studies in cerebellar membranes revealed a fully cross-linked product of M_r=520,000 as detected by immunoblotting with the the GluR1 antibodies. This finding is consistent with the previously reported native CHAPS-solubilized receptor M_r=610,000 [*J. Neurochem.* **58**, 1118, (1992)] and suggests that AMPA receptors are pentameric complexes of these subunits. Immunocytochemical localization in the rat CNS using the GluR1, GluR2/3, and GluR4 antibodies showed a partially overlapping distribution of these subunits, with enrichment in hippocampus, striatum, cerebellar cortex, and cerebral cortex. Immuno-electron microscopy demonstrated that these receptors are post-synaptic, with enrichment in the post-synaptic densities of dendritic spines. Interestingly, the GluR4 subunit was localized primarily to astrocytes in hippocampus, olfactory bulb, and cerebral cortex, and was most prominant in the cerebellar Bergmann glia. These results indicate that AMPA receptors may exist as both hetero- and homo-oligomers on neurons and glia within the CNS. *Supported by the NIH and HHMI.*

45.2

CHARACTERIZATION OF THE GLUTAMATE RECEPTOR SUBUNIT GluR1 IN HUMAN POSTMORTEM TISSUE. C.R. Breese*, T.M. Bartel, S.W. Rogers, M.D. Browning, S. Leonard, Denver Veterans Administration Medical Center; and Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80220.

Antibodies to the functional GluR1 glutamate receptor subunit were utilized as probes to characterize glutamatergic receptors in human post-mortem brain tissue. Antibodies were raised in rabbits to the extracellular (residues 185-449) and putative intracellular (residues 658-889) domains of the rat GluR1 receptor protein (Rogers, *et al.*, J. Neurosci. 11:2713). Crude membranes from rat and human hippocampus were fractionated by SDS-PAGE and electrotransferred to Immobilon-P membranes. Using these antisera raised against rat antigen, we have been able to detect specific bands on western blots in both human and rat hippocampal membranes. These antisera recognized previously described bands at 105kDa and 97kDa in both humans and rats. Additional bands were observed at 180kDa from the antibody to the intracellular domain and at 145kDa from the antibody to the extracellular domain. Our studies demonstrate that glutamatergic receptors may be examined in human postmortem tissue by western blotting techniques utilizing antibodies raised to the rat protein, suggesting a high degree of homology in these receptors. These antibodies can thus be utilized in human postmortem tissue to examine receptor protein changes in various human disease states. [Supported by USPHS grant MH14212 and Veterans Affairs Medical Research Service.]

45.3

ANTIBODIES TO THE GluR-A AMPA RECEPTOR SUBUNIT LABEL 98 AND 57 KDa PROTEINS CONCENTRATED IN PSDs. D.T. McQuade, B.A. Bahr, M. Kessler, E.T. Esteban, B.L. Bakus, R.A. Hall, P.W. Vanderklish, G.L. Shaw*, K. Sumikawa, and G. Lynch. Center for the Neurobiology of Learning and Memory and Dept. of Psychobiology, University of California, Irvine, CA 92717-3800

The AMPA-selective glutamate receptor, a member of the ligand-gated channel family, is comprised of several subunits. Polyclonal antibodies (anti-GluR) against an extracellular domain (amino acids 163-188) of the GluR-A subunit was used to screen subcellular fractions from rat brain. Postsynaptic densities (PSDs) and synaptosomes were prepared in the presence of broad spectrum protease inhibitors and subsequently analysed by immunoblot. Affinity purified anti-GluR labeled two proteins of 98±2 and 57±2 kDa (S.D., $n=8$) that were highly concentrated in PSDs as compared to brain homogenates. The molecular mass of the larger antigen (98 kDa) is similar to that expected for the GluR-A subunit on the basis of its amino acid sequence. This protein was most concentrated in PSDs from the hippocampus, with lower amounts in neocortex > mesencephalon > cerebellum > brain stem. The density of the 57 kDa antigen had a partially reversed order with mesencephalon > cerebellum > brain stem > hippocampus > neocortex. Crude synaptosomes from forebrain were fractionated by equilibrium density centrifugation and assayed by immunoblot and [^{3}H]AMPA binding. The two antigens showed distinct profiles across the gradient, the smaller one being more concentrated in the denser fractions. [^{3}H]AMPA binding had two maxima which appeared to coincide with the immunoreactivity peaks. This suggests the possibility that a 57 kDa AMPA-binding protein exists with a size and brain distribution similar to that of a glutamate-binding protein in chick. It is noteworthy that two similar antigens (99 and 61 kDa) have also been identified in the mouse telencephalon and that their density decreased equally with age (3-25 months). A comparable reduction was also found for the B_{MAX} of [^{3}H]AMPA binding (Bahr *et al., Brain Res.*, in press). This further suggests that the smaller antigen is not a proteolytic fragment but represents a component of some AMPA receptor complexes. (This work was supported by grant AFOSR 89-0383 and the HFSP).

45.4

ANTIBODIES TO THE GluR-A, GluR-B/C, GluR-D, AND NMDA-R1 GLUTAMATE RECEPTOR SUBUNITS: WESTERN BLOT STUDIES. M. Hennegriff*[1], B.A. Bahr[1], R.A. Hall[1], K.M. Guthrie[3], R.S. Yamamoto[2], M. Kessler[1], C.M. Gall[3], and G. Lynch[1]. Center for Neurobiology of Learning & Memory[1], Department of Molecular Biology and Biochemistry[2], Department of Anatomy and Neurobiology[3], University of California, Irvine, CA 92717.

The recently reported cloning of NMDA receptors has expanded the list of excitatory amino acid receptors already characterized. In this study, polyclonal antibodies were raised against synthetic peptides corresponding to carboxyterminal regions of the AMPA receptor subunits GluR-A, GluR-B/C, GluR-D, as described by Petralia & Wenthold (*J. Comp. Neurol.* 318:329, 1992), and against the corresponding carboxyterminal region of the putative NMDA receptor cloned by Nakanishi et al. (*Nature* 354:31, 1991). As in previous studies, affinity purified antibodies to AMPA receptor subunits labeled antigens of about 100 kDa in rat brain homogenates samples. This molecular mass closely agrees with that deduced from the corresponding amino acid sequences. In contrast, antibodies to the NMDA receptor labeled 75±2 and 81±1 kDa (SD, n=7) doublet proteins which are much smaller than expected from sequence data (105.5 kDa). Of these, the lower molecular weight band was labeled more intensely. Prolonged staining led to the appearance of additional faint bands of 42, 48, and 62 kDa. Pre-immune serum subjected to affinity purification steps did not label any bands. In order to determine the regional distribution, the 75/81 kDa doublet was quantitated by laser densitometry in homogenate samples prepared from seven brain regions. The doublet was most abundant in neocortex, followed by hippocampus > striatum >> thalamus > olfactory bulb > cerebellum >> brain stem. The same regional distribution was found in synaptosomal membranes and resembles that seen in autoradiographic studies measuring NMDA-sensitive [^{3}H]glutamate binding (Monaghan & Cotman, *J. Neurosci.* 5:2909, 1985).

45.5

AMPA-SELECTIVE GLUTAMATE RECEPTOR SUBUNITS IN HIPPOCAMPAL NEURONAL CELLS. N. Eshhar[1], R.S.Petralia[1], C.A. Winters[2] and R.J. Wenthold[1]*. [1]Lab. of Neurochemistry, NIDCD and 2Lab. of Cell. and Molec. Neurophysiology, NICHD, NIH, Bethesda, MD 20892.

Recent immunoelectron microscope studies on rat brain sections using antibodies directed against the C-termini of AMPA-selective glutamate receptor (GluR) subunits revealed their localization to the postsynaptic membrane and density, dendritoplasm, and cell body, but not within the synaptic cleft. Tissue culture systems may provide further information concerning topology and distribution of GluR molecules in neuronal cells. In this study, a variety of methodologies such as immunohistochemical analysis utilizing immunofluorescence and enzyme-linked immunoassays, electron microscopy, and *in-situ* hybridization were used. The distribution of GluR immunoreactivity on fixed hippocampal neuronal cells (derived from 22 day old rat embryos) grown on hippocampal glial cells was observed in the cell body and dendrites but not in the axon. Staining was dense with antibodies to GluR1 and GluR2/3 (antibody recognizing GluR2 and 3) and light with antibody to GluR4. Visualization of receptor sites on live cells under light microscopy was not observed. Staining with antibodies to GluR1, 2/3 and 4 was detected in both cell bodies and dendrites 2 hours following their plating. In comparison, microtubule-associated protein (MAP-2) has been visualized in cells only 6 hours following their plating indicating that MAP-2 is not required for the initial distribution of GluR subunits into neurites. At one week in culture, several morphological types of neurons were present, although not all types were labeled consistently. The number of cells labeled with antibodies to GluRs increased with time in culture.

The relationship between where the protein is localized and where it is synthesized was revealed by preliminary in-situ hybridization analysis. We observed significant grain density in cell bodies only, indicating that synthesis of GluR subunits is limited to the neuronal cell body and segregation of GluR subunits in dendrites may be determined after translation.

45.6

ULTRASTRUCTURAL LOCALIZATION OF AMPA-SELECTIVE GLUTAMATE RECEPTOR SUBUNITS. R.S. Petralia* and R.J. Wenthold, Lab. of Neurochemistry, NIDCD, NIH, Bethesda, MD 20892.

Previous ultrastructural studies using polyclonal antibodies made against different glutamate receptor (GluR) subunits have indicated that these subunits are localized in postsynaptic densities as well as in the dendritoplasm and cell bodies (Petralia and Wenthold, J. Comp. Neurol. 318:329, 1992). These studies which utilized a preembedding avidin-biotin-peroxidase immunocytochemical method, indicated that the antigenic site might be intracellular since the synaptic clefts were unstained. However, it was not possible to distinguish between receptor molecules incorporated in the membrane and those of the cytoplasmic pool. Further, the antibodies were made against peptides representing the C-terminus, which is believed to be extracellular.

Consequently, we have begun studying ultrastructural localization of GluR subunits with postembedding immunocytochemistry. In this study, we compared the localization of GluR subunits with preembedding and postembedding immunocytochemistry in a number of brain structures including the cerebral cortex and cochlear nuclei of the rat.

A number of postembedding techniques were used to show localization of antibodies to GluR1 and GluR4, and a third antibody which recognizes both GluR2 and 3 (GluR2/3) and which gave the most distinctive labeling. When 75 nanometer sections were incubated with antibody to GluR2/3 using aldehyde fixation and LR White embedment, 10 nanometer gold particles were localized most commonly along the center of the synaptic cleft, and less commonly adjacent to the post- and presynaptic membranes or within the postsynaptic density. Cytoplasmic labeling was low to moderate, and showed a preference for a subset of neurites.

45.7

IMMUNOSTAINING FOR AMPA-SELECTIVE GLUTAMATE RECEPTORS IN THE SPINAL CORD OF RATS

A. Rustioni*[1], C.D. Blackstone[2], R.J.Wenthold[3] and R.J. Weinberg[1] [1]Dept. Cell Biology & Anatomy, U North Carolina, Chapel Hill; NC 27599; [2]Dept. Neuroscience, The Johns Hopkins U., Baltimore, MD 21205; [3]Laboratory of Neurochemistry, NIH, Bethesda, MD 20892.

We have employed recently characterized antisera that recognize four receptors subunits, GluR1-4, that respond to AMPA, kainate and glutamate when expressed in oocytes and cultured cells (Blackstone et al., 1992; Wenthold et al., 1992). Nembutal-anesthesized Sprague-Dawley rats were perfused with 4% paraformaldehyde and 0.1% picric acid with or without 0.2% glutaraldehyde. The addition of glutaraldehyde was required for good tissue preservation for electron microscopy. Fifty µm thick Vibratome sections were processed for immunocytochemistry. Best results were with primary antibody concentrations in the range of 1:1,000 to 1:10,000. Observations to be reported deal primarily with the results in the dorsal horn. In this part of the spinal cord stained cells bodies were small and concentrated in lamina II (substantia gelatinosa). Within this lamina, GluR1-positive cells were located more superficially than GluR2-3. Electron microscopy confirmed the staining of cell bodies and revealed immunostaining especially of dendrites contacted by axon terminals of various morphology. Less selective staining for neuronal somata was obtained with the use of GluR4. The distribution of immunostaining suggests a relation between the receptors expressed by neurons in different sublaminae of substantia gelatinosa and their anatomical and functional features.

45.8

DETECTION OF NMDA RECEPTOR mRNA BY *IN SITU* HYBRIDIZATION USING RADIOACTIVE AND NON-RADIOACTIVE LABELED SYNTHETIC OLIGONUCLEOTIDE PROBES.

W.M. Al-Ghoul*[1], D.E. Millhorn[2] and A. Rustioni[1]. [1]Dept. Cell Biol. and Anat., and [2]Dept. Physiol., University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

The nucleotide sequence of a rat brain cDNA that encodes a protein (NMDAR1) with NMDA receptor functional characteristics has been published recently by Moriyoshi et al. (Nature 354:31-37, 1991). Based on this sequence, we developed three synthetic oligodeoxyribonucleotide probes against highly conserved regions of NMDA receptor mRNA. The 29-, 32- and 48-nucleotide probes did not recognize any other mammalian cDNA or gene sequence registered in GCG database. Probes were 3'-labeled with α-thio-[^{35}S]dATP or digoxigenin-11-dUTP/dATP. ^{35}S- or digoxigenin-labeled probes were used in parallel *in situ* hybridization experiments performed on cryostat sections of quick-frozen rat CNS tissue. Hybridization was performed overnight at 37° C using a mixture of the probes. The hybridization signal was detected with autoradiographic (using NTB3 emulsion; Kodak) or immunohistochemical (using anti-digoxigenin antibody; Boehringer Mannheim) methods. High levels of hybridization were detected in selected neuronal populations in the hippocampus, dentate gyrus, cerebellum and cerebral cortex. The distribution of hybridization with our synthetic oligonucleotide probes was similar to the distribution described by Moriyoshi et al. using a 1,431-nucleotide ^{35}S-labeled RNA probe. Further experiments are underway in our laboratory using this method to detect changes in NMDA receptor mRNA levels under various experimental conditions.

45.9

WITHDRAWN

45.10

EXPRESSION AND DISTRIBUTION OF AMPA-SELECTIVE GLUTAMATE RECEPTORS IN CULTURED RAT HIPPOCAMPAL NEURONS. A.M. Craig[1], C.D. Blackstone[2], R.L. Huganir[2] and G. Banker[1]*, [1]Dept. of Neuroscience, University of Virginia, Charlottesville, VA 22908 and [2]Dept. of Neuroscience, Howard Hughes Medical Institute, The Johns Hopkins University, Baltimore, MD 21205.

Anti-peptide antibodies and oligonucleotide probes were used to study the expression and localization of some of the non-NMDA ionotropic glutamate receptor subunits in hippocampal neurons cultured from E18 rats. *In situ* hybridization was performed with oligonucleotides directed against the immediate 3' untranslated region of GluR 1, and against the divergent loop between TM1 and TM2 for GluR 2, 3, and 4. GluR 1 and 2 mRNAs were abundant in over 80% of the neurons, whereas the expression of GluR 3 and 4 was more restricted. The level of expression of all subtypes increased between 2 days and 2 weeks in culture. At all ages, the mRNAs were largely restricted to the cell body. Immunolocalization with an affinity-purified subtype-specific antibody against GluR 1 yielded selective staining of cell bodies and dendrites, as defined by morphology and co-staining for MAP2, with little or no staining of axons. The staining was blocked by pre-incubation of the antibodies with the specific peptide. Within the soma and dendrites, GluR 1 staining was not homogeneous. There were numerous small, densely stained spots superimposed on a more diffuse staining. By double label immunofluorescence, these spots co-localized with a subset of synaptophysin-rich puncta that have previously been identified by electron microscopy as presynaptic specializations. Thus, GluR 1 appears to be most concentrated at synaptic sites. An antibody which recognizes GluR 2/3/4c gave a staining pattern similar to that for GluR 1. We are currently studying localization of these glutamate receptors as neurons develop and form synaptic contacts in culture. *Supported by NIH grant NS17112, the Howard Hughes Medical Institute, and an MRC of Canada fellowship.*

45.11

SWITCH IN KAINATE/AMPA RECEPTOR SUBUNIT GENE EXPRESSION FOLLOWING GLOBAL ISCHEMIA IN RATS. D.E. Pellegrini-Giampietro*, R.S. Zukin, S. Cho[1] and W.A. Pulsinelli[1]. Dept. of Neuroscience, A. Einstein Coll. Med., Bronx, NY 10461, and [1]Dept. of Neurology, Cornell Univ. Med. Ctr., New York, NY 10021.

Severe, transient forebrain ischemia induces delayed damage to specific neuronal populations. Sustained Ca^{2+} influx through EAA receptors is thought to be responsible for this damage. AMPA receptors, in particular, are implicated in the pathogenesis of CA1 pyramidal cell postischemic death: whereas NMDA receptor blockers are ineffective, the AMPA receptor antagonist NBQX attenuates CA1 ischemic injury. It has been demonstrated that kainate/AMPA channels expressed from GluR1 and/or GluR3 cRNAs are permeable to Ca^{2+}; the GluR2 subunit acts as a switch to shutoff Ca^{2+} permeability. To investigate the molecular mechanisms underlying delayed postischemic cell death, kainate/AMPA receptor subunit gene expression was examined by *in situ* hybridization in the hippocampus of rats subjected to 10 min of global ischemia. Our results show that GluR2 expression postischemia is reduced in CA1 neurons at a time point (24 hrs) which precedes their degeneration, whereas GluR1 and GluR3 are not significantly changed. Immunocytochemistry indicates that the GluR2 protein is preferentially reduced in CA1 at 48 hrs. The switch in expression of kainate/AMPA receptor subunits precedes an increase in Ca^{2+} influx into CA1 cells. Timing of the switch suggests that it might play a causal role in postischemic cell death.

45.12

GLUTAMATE RECEPTOR GENE EXPRESSION IN SPINAL CORD OF ARTHRITIC RATS. S.G. Fan, R.S. Zukin*, D. Pellegrini-Giampietro, and B. Ault[1]. Dept. Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461 and [1]Sterling Winthrop Pharmaceuticals Research Division, Rensselaer, NY 12144.

Injury to peripheral tissue leads to inflammation and increased sensitivity to noxious stimuli. Hyperalgesia following peripheral injury is thought to be mediated in part by spinal cord plasticity. We used *in situ* hybridization to examine glutamate receptor gene expression following induction of inflammation by a unilateral intra-articular injection of lipopolysaccharide (10 µg) into the ankle. In control rats GluR1 expression was prominent throughout the layers of the gray matter of the spinal cord. Microscopic examination revealed labeling of neuronal cell somata in all major nuclei, with highest intensity over motoneurons. GluR2 was abundant in substantia gelatinosa and motor nuclei; emulsion dipped sections exhibited intense labeling over densely packed neurons in the superficial laminae of dorsal horn and individual motoneurons of ventral horn. GluR3 was expressed at low levels in layers IV - VI of dorsal horn and (most prominently) in the motor nuclei. GluR5 expression was of low abundance and selectively localized to the motor nuclei. One day after injection GluR1 expression was decreased by ca. 30% in the substantia gelatinosa (n=4). In contrast, no significant change was apparent in GluR2, GluR3, or GluR5 expression in any nucleus of the cord. At 72 hrs after injection, all four transcripts were expressed at near control levels. These findings provide evidence for a specific decrease in GluR1 expression in the cord in response to inflammation.

45.13

GLUTAMATE RECEPTOR SUBTYPES ARE DIFFERENTIALLY EXPRESSED IN CULTURED HIPPOCAMPAL ASTROCYTES. D. Fan, A.B. Johnson*, J.A. Kessler and R.S. Zukin. A. Einstein Coll. Med., Bronx, NY 10461.

The cloning of cDNAs encoding kainate/AMPA and NMDA receptors makes it possible to use techniques of molecular biology to examine the expression patterns of these receptors in cultured cells. Electrophysiological and pharmacological studies indicate that non-NMDA glutamate receptors occur in astrocytes, whereas NMDA receptors do not. However, glutamate-induced swelling in primary astrocyte cultures can be blocked by the NMDA receptor antagonist MK-801. We used *in situ* hybridization to examine the pattern of kainate/AMPA and NMDA receptor expression in astrocytes. Astrocytes from 1-2 day old neonatal rat hippocampi were maintained in culture for eight days. Monolayer cultures were hybridized under conditions of high stringency with riboprobes directed against GluR1, GluR2 and GluR3 (Kainate/AMPA) and NMDAR1 receptor mRNAs. Under these conditions, there is no detectable cross-hybridization among these probes. In cells hybridized to GluR1, labelling was prominent in the nuclear region and at a low level in the cytoplasm. In contrast, GluR2 and GluR3 expression was not detectable. The cells exhibited NMDAR1 mRNA labelling at a very low level, suggesting that NMDA receptors may be expressed in rat hippocampal astrocytes. These results indicate that GluR1 is the predominant glutamate receptor subunit mRNA expressed in rat hippocampal astrocytes.

45.14

EXPRESSION OF GLUTAMATE RECEPTOR SUBUNITS IN CHICK α-MOTONEURONS. D. Lowe*, P. Leach, R. Temkin, D.O. Smith. Neuroscience Training Program, Univ. WI, Madison, WI 53706.

Whole cell recordings from 6.5 day embryonic chick α-motoneurons indicate the presence of AMPA, kainate and NMDA glutamate receptor subtypes within each motoneuron tested. However, in excised membrane patches, no more than two subtypes were ever detected. AMPA and NMDA or kainate and NMDA were found together. AMPA and kainate were never in the same patch. Recent cloning of the glutamate receptor has led to the molecular cloning and electrophysiologic characterization of eight ionotropic subunits: GluR 1-4 (AMPA), GluR 5-7 (kainate) and NMDAR1. Using PCR based gene amplification, we have found GluR 1, 2, 4 and 6, and NMDAR1 in chick α-motoneurons. This well defined cell population appears to contain most if not all of the glutamate receptor subunits. If all subunits are present, functional specificity may not be determined by subunit expression. Specificity may arise from differential post-translational or post-transcriptional modifications, or subunit insertion may occur in specific configurations. Supported by NIH grant NS13600.

45.15

EXPRESSION OF GLUTAMATE RECEPTOR SUBTYPES IN MAMMALIAN SPINAL CORD. R. Temkin*, P. Leach, D. Lowe, D.O. Smith. Neuroscience Training Program, University of Wisconsin, Madison, WI 53706.

Ionotropic receptors for glutamate are classified into three groups: NMDA, kainate, and AMPA channels. In patch clamp electrophysiologic recordings of rat ventral spinal cord, each individual cell responds to all of these agonist types. Receptors of all three types have been cloned from rat brain, but some of the receptor subtypes have not been shown definitively in spinal cord using hybridization detection techniques. Using the more sensitive polymerase chain reaction to amplify a portion of cDNA of each receptor sequence, we have shown the presence of mRNA for all three classes of glutamate receptor, including some subtypes not previously shown in spinal cord. We have amplified AMPA selective channels GluR 1-4, kainate selective channels GluR 5 and 6, and the NMDA receptor, as well as the quisqualate sensitive metabotropic receptor, thus providing a molecular basis for the electrophysiologic responses. We are now looking for similar expression of glutamate receptor subtypes in human spinal cord of normal and pathological states. Supported by NIH grant NS13600.

45.16

GLUTAMATE-RECEPTOR GENE EXPRESSION IN HYPOTHALAMUS: IN SITU HYBRIDIZATION WITH 7 RECEPTOR SUBTYPES. A.N.van den Pol*, I.Hermans-Borgmeyer, S.Heinemann. Sect. Neurosurgery, Yale Med.Sch.,New Haven,CT 06510, Lab Molec. Neurobiol., Salk Inst., La Jolla,CA 92186.

Glutamate has received relatively little attention for its contribution to hypothalamic regulation. To examine the expression of glutamate receptor subtypes, we used in situ hybridization with ^{35}S-labeled RNA probes complementary to mRNA coded by seven of the glutamate receptor genes. GluR1 and -R2 were expressed in many hypothalamic areas including, but not restricted to, the ventromedial, arcuate, and dorsomedial nuclei, and in the preoptic area and mammillary region. GluR4 was weakly expressed in the supraoptic, paraventricular, and arcuate nuclei. GluR5 and -R6 labeling was detected in the suprachiasmatic nucleus, with a greater label density over the ventrolateral part which receives retinal input. Although detectable in other CNS regions, we found little GluR3 or -R7 label in hypothalamus. Controls with unrelated probes not expected in the hypothalamus were negative. Since the receptors may manifest different physiological properties, these data suggest a variety of cellular responses to glutamate may exist in the hypothalamus, and together with other data, underline the widespread role of glutamate in hypothalamic and neuroendocrine regulation.

45.17

INCREASED EXPRESSION OF GLUTAMATE RECEPTOR mRNAs IN HUMAN EPILEPTIC HIPPOCAMPUS. N. W. Kleckner*, Z. Cao, C. Moll, B. Bettler, S. Heinemann, and J. O. McNamara. Duke University and VA Medical Centers, Durham NC, 27710, and The Salk Institute, LaJolla, CA, 92186.

A 2-fold increase in radioligand binding to the AMPA subtype of glutamate receptor has been identified in the dentate region of hippocampi surgically removed from patients with medically refractory complex partial epilepsy (Hosford, et al., 1991). We hypothesized, therefore, that the mRNAs encoding the non-NMDA glutamate receptors would also increase in epileptic tissue. Epileptic hippocampi (n=10) were surgically removed from patients with medically refractory complex partial epilepsy, and control hippocampi (n=5) were obtained from rapid autopsy of patients with non-neurological causes of death. ^{35}S-labelled antisense and sense RNA probes were synthesized from plasmids containing a portion of the coding region of glutamate receptor cDNAs isolated from rat or human brain. The probes were hybridized to 10 µm sections of human hippocampus. Specific binding [total - nonspecific (sense probe) in attomoles/neuron] of RNA probes synthesized from rat and human GluR1 and GluR2 cDNAs was increased 2 or 3-fold in the dentate granule cell layer of epileptic hippocampi compared to rapid autopsy controls. The increase was specific in that no significant differences were observed in the CA1 and CA3 regions using either human or rat GluR1 and GluR2 RNA probes, and no significant change in the level of GluR3 mRNA was observed in any region of the hippocampus. Our results indicate that expression of mRNAs encoding the AMPA subtype of glutamate receptor is selectively altered in the hippocampus in human complex partial epilepsy. Increases in the GluR1 and GluR2 subunits, and not the GluR3 subunit, may underlie the previously observed increase in [^{3}H]AMPA binding (supported by NS08992, NS17771, NS24448).

45.18

Glutamate Receptor Expression Changes in Rat Hippocampus after Entorhinal Cortex Lesion. M.J. Velardo, G.M. Rose, E.S. Deneris, M.J. Marks, and S.W. Rogers*. Dept. Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262, Denver VAMC, Case Western Reserve Medical School, and Institute for Behavioral Genetics, University of Colorado.

Glutamate serves as a major excitatory neurotransmitter in the mammalian brain, and dysfunction of this system has been related to a variety of neurodegenerative diseases. cDNAs encoding subunits of the AMPA/kainate-type (GluR 1-4) and kainate-type (GluR 5-7) glutamate receptors have been identified. Each subunit is expressed in distinct but overlapping brain regions, and different subunit combinations confer specific physiological properties to the receptor. We have used *in situ* hybridization histochemistry to examine the expression of GluR1, GluR2, GluR3, and GluR5 RNA in the rat hippocampus for up to 14 days after partial deafferentation by unilateral entorhinal cortex lesion. We observed an increase in GluR1 but not GluR3 or GluR5 in the hippocampus of lesioned animals. The increase in GluR1 expression was predominantly in the molecular layer, and it occurred both ipsilateral and contralateral to the lesion. Surrounding the lesion site itself, GluR2 and GluR3 are expressed but not GluR1 or GluR5. We will report our findings in terms of glutamate receptor gene expression by neuronal cells and by glia in the partially deafferented hippocampus and in regions of glial scarring. Supported by a grant to SWR from the Pharmaceutical Manufacturers Association and NIDA grant DA03194 to MJM.

SEROTONIN RECEPTORS: 5HT$_{1A}$ SUBTYPES

46.1

STEREOSPECIFIC EFFECTS OF THE SELECTIVE AND SILENT 5-HT$_{1A}$ RECEPTOR ANTAGONIST, WAY100135, ON RAT HIPPOCAMPAL 5-HT LEVELS: A MICRODIALYSIS STUDY. C. T. Dourish*, J. Gurling, I. Wright, C. Routledge. Wyeth Research (U.K.) Ltd., Huntercombe Lane South, Taplow, Maidenhead, Berkshire SL6 0PH, U.K

WAY100135 is the first example of a selective, silent, 5-HT$_{1A}$ receptor antagonist (Fletcher et al., 1991, Soc. Neurosci. Abstr., Vol 17, P92). Thus, the compound has high affinity and selectivity for 5-HT$_{1A}$ receptors in vitro and is also a potent inhibitor of the behavioural effects of the 5-HT$_{1A}$ agonist 8-OH-DPAT. The action of WAY100135 at the 5-HT$_{1A}$ receptor is stereospecific, the activity residing in the (+) isomer; in the present study we examined the effects of WAY100135 and its enantiomers on the in vivo release of 5-HT in the rat hippocampus using microdialysis. Dialysis probes were implanted into the ventral hippocampus of male rats, and 24 h later were perfused with artificial CSF (1 ul/min). Following a 2.5 h stabilisation period 20 min samples were collected; 3 control samples were taken prior to s.c. administration of antagonist or vehicle. (±)-WAY100135, (+)-WAY100135, and (-)-WAY100135, (all at 10 mg/kg) had no significant effect on extracellular levels of 5-HT in the rat hippocampus. Increases in extracellular levels of 5-HT were observed in a number of animals following administration of (±)-WAY100135 and (+)-WAY100135 but this response did not reach statistical significance. In contrast, the 5-HT$_{1A}$ receptor partial agonists BMY7378 (5 mg/kg) and buspirone (1 mg/kg), and the potent agonist 8-OH-DPAT (100 ug/kg) all significantly decreased extracellular 5-HT. Pre-treatment with (±)-WAY100135 and (+)-WAY100135 (both 10 mg/kg, 30 min) completely blocked the inhibitory effect of 8-OH-DPAT (100 ug/kg) on 5-HT release. (-)-WAY100135 (10 mg/kg) had no effect on the 8-OH-DPAT response. These data demonstrate that (±)-WAY100135 and (+)-WAY100135 are selective and silent 5-HT$_{1A}$ receptor antagonists, at presynaptic 5-HT$_{1A}$ receptors in vivo, as they did not decrease 5-HT release. On the contrary, there was evidence of an increased 5-HT release, indicating that (±)-WAY100135 and (+)-WAY100135 may block a tonic inhibition of 5-HT neurones mediated by somatodendritic 5-HT$_{1A}$ receptors.

46.2

ELECTROPHYSIOLOGICAL STUDIES ON THE EFFECTS OF THE 5-HT$_{1A}$ RECEPTOR AGONIST / 5-HT REUPTAKE BLOCKER BMY 42568 ON THE 5-HT SYSTEM. C. de Montigny*, P. Blier and A. Lista. Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada H3A 1A1.

BMY 42568 is a buspirone derivative endowed with nanomolar affinity for 5-HT$_{1A}$ receptors as well as for the 5-HT reuptake carrier. In anesthetized rats, microiontophoretic applications of BMY 42568 produced a current-dependent suppression of the firing activity of dorsal hippocampus CA$_3$ pyramidal neurons, which was blocked by the 5-HT$_{1A}$ antagonist BMY 7378. The suppression of firing of these neurons by 5-HT applications was not prevented by the concurrent ejection of BMY 42568, indicating that this drug is a full agonist of these postsynaptic 5-HT$_{1A}$ receptors. A two-day treatment with BMY 42568 (5 mg/kg/day, s.c.) reduced the firing activity of dorsal raphe 5-HT neurons. There was a partial recovery after 7 days, and a complete one after 14 days of treatment. At this point in time, there was a marked shift to the right of the dose-response curve of the effect of i.v. LSD on 5-HT neuron firing activity, indicating a desensitization of their somatodendritic autoreceptors. The same 14-day treatment left unaltered the responsiveness of CA$_3$ pyramidal neurons to 5-HT, but enhanced the effectiveness of the electrical stimulation of their afferent 5-HT pathway. This enhancement was attributable to a desensitization of the terminal 5-HT autoreceptor, as indicated by the lack of an attenuated effectiveness of the stimulations when increasing the frequency from 1 Hz to 5 Hz. Interestingly, BMY 42568 is the first drug which displays a full agonistic activity electrophysiologically at postsynaptic 5-HT$_{1A}$ receptors. Since its long-term administration induces a desensitization of somatodendritic and terminal 5-HT autoreceptors, but not of postsynaptic 5-HT$_{1A}$ receptors, and since it exerts full agonistic activity at the latter, BMY 42568 is thus expected to be a potent antidepressant drug.

46.3

AGONIST OCCUPATION OF $5\text{-}HT_{1A}$ RECEPTORS PREVENTS THEIR INACTIVATION BY PERTUSSIS TOXIN. V. Hadrava*, P. Blier and C. de Montigny. Neurobiological Psychiatry Unit, McGill University, Montréal, Québec, Canada H3A 1A1.

Pertussis toxin (PT) drastically reduces the responsiveness of extrasynaptic $5\text{-}HT_{1A}$ receptors of dorsal hippocampus CA_3 neurons, but has no effect on the responsiveness of intrasynaptic $5\text{-}HT_{1A}$ receptors (*P. Blier et al., Neurosci. Abst. 16:462.5, 1990*). Since activation of G protein-linked receptors renders the Gi/o α subunit refractory to ADP ribosylation by PT (*M. Diversé-Pierluissi et al., Neurosci. Abst. 17:232.14, 1991*), the tonic activation of intrasynaptic $5\text{-}HT_{1A}$ receptors by endogenous 5-HT might protect them from PT inactivation.

To test this hypothesis, we studied the effect of PT on the extrasynaptic $5\text{-}HT_{1A}$ receptors in Sprague-Dawley rats treated with the potent $5\text{-}HT_{1A}$ agonist flesinoxan (5 mg/kg/day, s.c.) for 14 days by osmotic minipumps, starting 3 days prior to a unilateral intrahippocampal injection of PT (1 µg). The responsiveness of ipsilateral CA_3 dorsal hippocampus pyramidal neurons to microiontophoretic applications of 5-HT and of the selective $5\text{-}HT_{1A}$ agonist 8-OH-DPAT was nearly abolished by PT in control rats. In flesinoxan-treated rats, the reduction of these responses by PT was significantly attenuated. This suggests that the occupation of $5\text{-}HT_{1A}$ receptors prevented the inactivation of their G proteins by PT. We then investigated whether such protection may be extended to $GABA_B$ receptors which share the same G proteins (*R. Andrade et al., Science 234:1261, 1986*). PT abolished the inhibitory effect of the $GABA_B$ agonist baclofen on the firing activity of CA_3 neurons in control rats. In flesinoxan-treated rats, the reduction of the responsiveness to baclofen by PT was also attenuated. This suggests that the tonic activation of $5\text{-}HT_{1A}$ receptors prevents the PT-induced ADP ribosylation of the Gi/o α subunit and thereby protects an amount of G proteins sufficient to maintain the function of $GABA_B$ receptors.

46.4

EFFECT OF LOCAL INFUSIONS OF 8-OH-DPAT ON EXTRACELLULAR LEVELS OF 5-HT IN THE ANAESTHETISED GUINEA-PIG. M. Skingle*, A.J. Sleight and D.J. Rosser (SPON: Brain Research Association) Department of Neuropharmacology, Glaxo Group Research, Ware, Herts, SG12 0DP, UK

The effects of the $5\text{-}HT_{1A}$ receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) on extracellular levels of 5-HT in the CNS of the anaesthetised guinea-pig were studied using the technique of in vivo microdialysis with HPLC/ECD. Dialysis probes were inserted into frontal cortex or into the area adjacent to the median and dorsal raphe nuclei. 8-OH-DPAT was given systemically or infused directly via the probe. It is likely that the measured 5-HT in these experiments is from neurones since the sodium channel blocker tetrodotoxin (1 µM) markedly reduced basal levels of extracellular 5-HT when infused into frontal cortex.

Peripheral injections of 8-OH-DPAT, 0.01 and 0.5 mg/kg s.c., caused reductions in the levels of extracellular 5-HT in the frontal cortex (39% and 47% reduction respectively). When infused into the area adjacent to the median and dorsal raphe nuclei, which contain the somatodendritic $5\text{-}HT_{1A}$ autoreceptors, much lower doses of 8-OH-DPAT (1 and 10 nM) reduced cortical levels of 5-HT. By implanting microdialysis probes into the frontal cortex and raphe region of the same guinea-pig it was possible to demonstrate that intra-raphe injection of 8-OH-DPAT, 10 nM, caused a marked reduction in extracellular 5-HT in both frontal cortex (87 ± 13% inhibition) and in the raphe region (86 ± 14% inhibition).

These data suggest that $5\text{-}HT_{1A}$ receptors are involved in the control of extracellular levels of 5-HT in the guinea-pig and that, as in the rat, these receptors appear to be located in the cell body regions of the 5-HT neurones.

46.5

EFFECT OF ADRENALECTOMY ON BRAIN 5-HT1A RECEPTOR mRNA EXPRESSION. D.T. Chalmers*, S.P. Kwak, A. Mansour, H. Akil and S.J. Watson. Mental Health Research Inst., University of Michigan, Ann Arbor, MI 48109.

Corticosteroids have been shown to modulate hippocampal $5\text{-}HT_1$ receptor binding density. The present studies were designed to investigate the possibility of altered hippocampal $5\text{-}HT_{1A}$ receptor mRNA expression in response to acute or chronic adrenalectomy (ADX). After 24 hours ADX, $5\text{-}HT_{1A}$ receptor mRNA expression was significantly increased in all hippocampal subfields in ADX animals relative to SHAM. The magnitude of the increase was most pronounced within CA_2 (127%) and $CA_{3/4}$ (94%) subfields of dorsal hippocampus, intermediate in the dentate gyrus (73%) and least within CA_1 (60%). Administration of exogenous CORT at the time of ADX maintained the level of $5\text{-}HT_{1A}$ receptor mRNA expression within the range of SHAM animals. Autoradiographic analysis of $5\text{-}HT_{1A}$ receptors in adjacent sections indicated a simultaneous increase in $5\text{-}HT_{1A}$ binding throughout the hippocampus in response to ADX. $5\text{-}HT_{1A}$ binding increased uniformly (30%) in CA subfields and dentate gyrus but remained within SHAM levels in CORT replaced animals. Similar increases in both $5\text{-}HT_{1A}$ receptor mRNA expression and $5\text{-}HT_{1A}$ binding were evident after 1 week ADX treatment. Hippocampal $5\text{-}HT_{1C}$ receptor mRNA and dopamine D_1 receptor mRNA expression were not significantly altered by either acute or chronic ADX treatment. These data indicate that adrenal steroids may selectively regulate hippocampal $5\text{-}HT_{1A}$ receptors at the level of $5\text{-}HT_{1A}$ receptor mRNA expression. Supported by NIMH 422251.

46.6

THYROID HORMONE (TH) MODULATION OF SEROTONIN-1A (5-HT-1A) RECEPTORS IN RAT BRAIN. S.M Tejani-Butt,* J.Yang & A. Kaviani. Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Although a link between the hypothalamic-pituitary-thyroid (HPT) axis and affective disorder has been established, the mechanism underlying this relationship remains unclear. Since the 5-HT system appears to be involved in the pathophysiology of mood disorders, we examined the effects of thyroidectomy (Tx) with T4 replacement (euthyroid (EU), 15 µg/kg; hyperthyroid (HY), 200 µg/kg) and without T4 replacement (Tx) for 7, 14 or 35 days on the binding of 3H-DPAT to 5-HT-1A receptors in rat brain by quantitative autoradiography. Tx caused a significant decrease ($F=12.9$; $p<0.01$) in 3H-DPAT binding to 5-HT-1A receptors in the dorsal raphe nucleus (DRN) at 7 days, with a time-dependent recovery in 3H-DPAT binding 35 days post Tx. HY animals showed a significant increase ($F=6.8$; $p<0.05$) in 3H-DPAT binding to 5-HT-1A receptors in the DRN at 35 days. In contrast to the results seen in the DRN, Tx caused a significant increase ($F=11.7$; $p<0.01$) in 3H-DPAT binding to 5-HT-1A receptors in the molecular layer of the CA1 region of the hippocampus (HIP) at 7 days. At 14 days post Tx, the binding normalized to sham levels; however at 35 days post Tx, a significant increase ($F=5.5$; $p<0.05$) in 3H-DPAT binding to 5-HT-1A receptors was observed. No significant differences were seen in 3H-DPAT binding to 5-HT-1A receptors in the HIP in the HY or EU animals. These results suggest that a neuromodulatory link may exist between the HPT axis and 5-HT-1A receptor system in at least two distinct regions of the rat brain. (Research funds from USPHS grants MH 45472 and MH 44210).

46.7

DIFFERENTIAL EFFECTS OF NOVEL 8 OH-DPAT AND NAN-190 ANALOGUES ON CENTRAL $5\text{-}HT_{1A}$ RECEPTORS. H.Hecimovic*, J.P.Hodgkiss, I.M.Dawson, J.S.Kelly. Dept. of Pharmacology, Univ. of Edinburgh, 1 George Square, Edinburgh EH8 9JZ, Scotland.

We have examined the action of a variety of analogues of 8 OH-DPAT and NAN-190 on $5\text{-}HT_{1A}$ receptors in rat dorsal raphe (DR) and hippocampal CA1 pyramidal neurones usin intracellular recording in vitro.

The NAN-analogue compound, 5.20 [Iodo-NAN-190] (10-100µM, K_D=10.3nM) hyperpolarised and reduced input resistance (Rm) in DR and CA1 neurones. The hyperpolarisation of DR neurones elicited by 8 OH-DPAT (10µM) was reversed in a dose-dependent fashion by the same analogue.

We also tested the 8 OH-DPAT analogue 8.9 [Bis-C_6-8MeOPAT] (0.5-10µM, K_D=7nM). It caused a hyperpolarisation and fall in Rm in DR neurones and occluded the action of 5-HT (100µM). In CA1 neurones, in contrast, a small depolarisation was seen accompanied by either no change or a small fall in Rm; the hyperpolarisation elicited by 5-HT was unaffected by this compound.

The differences seen with these compounds support the idea that the populations of $5\text{-}HT_{1A}$ receptors on DR and CA1 pyramidal neurones possess different properties (Sprouse, J.S. & Aghajanian, G.K.: Neuropharmacol. 27:707-715, 1988.). (Supported by the Wellcome Trust.)

46.8

ACTION OF RU24969 ANALOGS ON THE INHIBITION OF FORSKOLIN-STIMULATED ADENYLATE CYCLASE IN RAT HIPPOCAMPAL MEMBRANES. H. B. Li, L. J. Cornfield, G. Lambert, A. A. Lettes*, J. W. Regan and A. R. Martin. Departments of Pharmacology & Toxicology and Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, AZ 85721

The serotonergic efficacy of several RU24969 analogs was evaluated in rat hippocampal membranes. Inhibition of forskolin-stimulated adenylate cyclase (FSC) was used to assay the functional activity of these putative $5\text{-}HT_{1A}$ agonists and antagonists. Compounds were chosen on the basis of having high binding affinity for the $5\text{-}HT_{1A}$ receptor using the displacement of [^{3}H]8-OH-DPAT. Most analogs produced complex inhibition curve consisting of a high potency component that could be shifted to the right by the antagonist pindolol. This sensitivity to pindolol is characteristic of agonist activity at the $5HT_{1A}$ receptor. For many of the RU24969 analogs, high concentrations of drug inhibited FSC greater than 10µM 5-HT itself. This low potency component could not be shifted by pindolol suggesting that it did not include the activation of $5HT_{1A}$ receptor. The compound, SN26 (5-carboxamido-(1-methyl-1,2,5,6-tetrahydro pyridin-4-yl)indole), had the highest binding affinity and cyclase potency. For most of the RU24969 analogs, the binding affinity and the cyclase activity had the same rank order. A few compounds including TD60 (5-carboxamido-(1-propyl-1,2,5,6-tetrahydro pyridin-4-yl)indole), however, displayed high binding affinities but low cyclase activities, and thus could be classified as weak agonists. Additional compounds related to these weak agonists are being examined to identify possible antagonists of the $5\text{-}HT_{1A}$ receptor.

46.9

EFFECT OF EEDQ TREATMENT ON THE RECOVERY OF 5-HT$_{1A}$ RECEPTORS AND THE LEVEL OF 5-HT$_{1A}$ RECEPTOR mRNA. D. Brousseau, R.A. Habboushe and P. McGonigle* Dept. of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

The density of post-synaptic 5-HT$_{1A}$ receptors in the CNS does not change significantly in response to standard pharmacological manipulations. Depletion of 5-HT does not increase the density of 5-HT$_{1A}$ receptors and chronic agonist treatment does not decrease receptor density. To further examine the plasticity of the 5-HT$_{1A}$ receptor system, male Sprague Dawley rats were treated with 10 mg/kg EEDQ to irreversibly inactivate a significant proportion of 5-HT$_{1A}$ receptors. The animals were sacrificed 1,2,4,7 and 14 days after treatment and coronal sections of brain at the level of the dorsal raphe and the hippocampus were labelled with [^{3}H]-8-OH-DPAT. Adjacent sections were labelled with a 183 base ^{35}S-labelled riboprobe complementary to rat 5-HT$_{1A}$ receptor mRNA. Quantitative autoradiographic analysis revealed that the percentage of receptors remaining compared to control ranged from 11% in the entorhinal cortex to 25% in the parietal cortex one day after the treatment. Seven days after the treatment, the density of receptors in the dorsal raphe had returned to control levels whereas the density in all other regions ranged between 55% and 75% of control levels. *In situ* hybridization histochemistry revealed that there were no significant changes in the level of mRNA for the 5-HT$_{1A}$ receptor at 1,2 or 4 days after treatment despite the significant increase in the density of receptors during this period. These results indicate that the mechanism by which neurons increase the expression of 5-HT$_{1A}$ receptors does not require increasing the level of receptor mRNA. These results also suggest that the mechanism regulating expression of autoreceptors in the dorsal raphe may be different from the mechanism in cells expressing post-synaptic receptors. (Supported by USPHS MH 43821)

46.10

CHARACTERIZATION OF THE 5HT-1C RECEPTOR BINDING USING A RAT RECOMBINANT EXPRESSED IN THE BACULOVIRUS SYSTEM. K. Barron, P. Payette', P. Dionne', J. Lancaster and P. Sweetnam'. 'Biosignal, Montreal, Quebec, Canada and NovaScreen®, Baltimore MD 21224.

When attempting to establish the 5HT-1C binding assay there are several technical issues which need to be addressed. First; the selectivity of drugs used to characterize the site demands a thorough examination of the numerous serotonin-like drugs and a comparison of their affinities at the 5HT-1C site. We have determined that eight drugs routinely used to pharmacologically define other serotonin receptor subtypes have K$_i$'s under 50 nM. Second; the use of either rat or pig choroid plexus will place a strain on animal and financial resources. We report here the use of a rat recombinant 5HT-1C receptor expressed in the baculovirus system as a means to reduce if not eliminate these technical difficulties. Binding characteristics for this clone using ^{3}H-mesulergine are similar to those reported for pig and rat choroid plexus (K_D = 2 nM; B_{max} = 75 pmol/mg prot). The rank order of potency established using select serotonin drugs was mesulergine > methysergide > mianserin > ketanserin > 5HT. For expression of the 5HT-1C receptor the baculovirus system provides a high yield of receptor protein with a binding profile similar to that reported for rat and pig choroid plexus.

46.11

EFFECTS OF THE 5-HT$_{1A}$ ANTAGONIST WAY-100135 ON MEDULLARY 5-HT CELL FIRING AND SYMPATHETIC NERVE DISCHARGE IN THE CAT N.A. Escandon, R.B. McCall* and D.C. Zimmermann. Cardiovascular Diseases Research, Upjohn Laboratories, Kalamazoo, MI 49001.

It has proven difficult to find a selective, pure 5-HT$_{1A}$ receptor antagonist. WAY-100135 (WAY), a novel phenylpiperazine derivative, has been reported to bind with high affinity and selectivity to the central 5-HT$_{1A}$ site and to display only 5-HT$_{1A}$ antagonist activity in both presynaptic and postsynaptic models (Neurosci. Abstr. 17:91,92). The present study utilized standard neurophysiological techniques to demonstrate that WAY acts as an agonist at the 5-HT$_{1A}$ autoreceptor site, but retains antagonist activity postsynaptically. We observed inhibitory effects of WAY on medullary raphe 5-HT cell firing in the cat with an ED_{50} of 105 μg/kg and with complete inhibition occurring at 300μg/kg. Neither pre nor posttreatment with WAY (100 μg/kg i.v.) had a significant effect on 8-OH DPAT-induced inhibition of unit firing. Inferior cardiac sympathetic nerve discharge was not significantly inhibited by WAY in doses up to 1 mg/kg i.v. WAY pretreatment (300 μg/kg) caused a fourfold rightward shift in the 8-OH DPAT dose response curve, while WAY posttreatment reversed the effects of 8-OH DPAT (100-300 μg/kg i.v.) up to 90% of baseline activity. These data indicate that although WAY is a 5-HT$_{1A}$ antagonist postsynaptically, it possesses agonist activity at the presynaptic autoreceptor site.

46.12

DIFFERENTIAL CONTRIBUTION OF PRE- AND POSTSYNAPTIC 5HT$_{1A}$ RECEPTORS TO THE ANXIOLYTIC, ANTIDEPRESSIVE AND DISCRIMINATIVE EFFECTS OF 8-OH-DPAT AND IPSAPIRONE IN THE RAT. T. Glaser, R. Schreiber, J.M. Greuel* and J.De Vry. Inst. for Neurobiology, Troponwerke GmbH & Co. KG, Berliner Str. 156,5000 Köln 80, Germany.

8-OH-DPAT (D) and ipsapirone (I) show high affinity for 5-HT$_{1A}$ receptors. These receptors are located presynaptically in the raphe nuclei and postsynaptically in the projection areas including lateral septum (LS) and hippocampus (HIP). In rats, predominantly presynaptic receptors appear to be involved in the anxiolytic (ANX) effects of D and I (shock-induced ultrasonic vocalization test and Geller-Seifter conflict test); whereas predominantly postsynaptic receptors appear to be involved in the antidepressive (AD) effects (forced swimming test) and, possible also, discriminative stimulus (DS) effects of rats trained on 8-OH-DPAT (0.1 mg/kg, i.p.). First, for D and I, the potency difference between IP and central application (dorsal raphe nucleus (DRN) is at least 10-times greater in case of the ANX effects, as compared with the AD and DS effects. In addition, the potency difference between administration in the DRN and in the HIP or LS is much greater in the case of the ANX effects, as compared with the AD and DS effects. Second, after application of I (0.3 - 10 mg/ kg, s.c.), the time-effect curves reflecting the ANX effects, those reflecting the 5-HT cell firing inhibitory effects (DRN) and those reflecting the release inhibitory effects (HIP), correlate well with each other. Third, depletion of brain 5-HT by means of 5,7-DHT (150 μg, i.c.v.) or pCPA (2 x 150 or 2 x 350 mg/kg, i.p.) induces ANX effects (50 %, 56 % and 97 %, respectively), but hardly AD effects (17 %, 25 % and 23 %, respectively). Fourth, pretreatment with the (postsynaptic) partial agonist I results in an attenuation of the AD effects of the full agonist D when both compounds are administered either i.p. or in the LS, but not after microinjection in the DRN. No attenuation of the ANX and DS effects were obtained after combined IP administration of both compounds.

46.13

REGULATION OF 5-HT$_{1A}$ RECEPTOR-MEDIATED INHIBITION OF ADENYLYL CYCLASE IN TRANSFECTED CELLS. J.G. Hensler* & P.B. Molinoff. Depts. of Psychiatry & Pharmacol., Univ. of Pennsylvania School of Medicine, DVA Med. Center, Phila., PA. 19104.

Changes in second messenger function linked to the 5-HT$_{1A}$ receptor, specifically the inhibition of forskolin-stimulated adenylyl cyclase activity, have not been consistently observed to follow antidepressant or agonist treatments in vivo. We have studied this phenomenon in vitro using human embryonic kidney (293) cells and Chinese Hamster Ovary (CHO) cells transfected with the human 5-HT$_{1A}$ receptor gene. Expression of the 5-HT$_{1A}$ receptor in either cell line was determined by measuring the specific binding of ^{3}H-DPAT (Kd = 1-2 nM). Bmax values were 100-800 fmol/mg protein for 293 cells, and 50-200 fmol/mg protein for CHO cells. 5-HT and DPAT inhibited forskolin-stimulated cyclic AMP formation in CHO cells with EC_{50} values of approximately 3 nM. Maximal inhibition by both agonists (1 μM) was approximately 90%. Incubation of CHO cells with 5-HT (100 nM) for 18 hrs resulted in an attenuation of the inhibition of forskolin-stimulated cyclic AMP formation by an EC_{50} concentration of DPAT (3 nM), with no change in the maximal inhibition produced by 1 μM DPAT. In 293 cells, 5-HT and DPAT also inhibited forskolin-stimulated cyclic AMP formation with EC_{50} values of approximately 1 nM. Maximal inhibition by both agonists was approximately 70%. Incubation of 293 cells with 5-HT (100 nM) for 18 hrs resulted in an attenuation of the maximal inhibition of forskolin-stimulated cyclic AMP formation by 5-HT. These data suggest that chronic exposure of the 5-HT$_{1A}$ receptor to 5-HT can result in desensitization of this second messenger response in CHO and 293 cells. (Supported by USPHS grant MH 48125).

46.14

DESENSITIZATION AND COUPLING OF CLONED HUMAN 5-HT1A AND 5-HT2 RECEPTORS. F. Van Huizen[1*], N.J. Stam[2], M. Bansse[1] and J.A.D.M. Tonnaer[1] Depts. of [1]Neuropharmacology and [2]Biotechnology & Biochemistry, Organon Intl. BV, PO Box 20, 5340 BH Oss, The Netherlands.

Swiss 3T3 cells were transfected with the human 5-HT$_{1A}$ or 5-HT$_2$ receptor genes. Sub-type specific pharmacological profiles were obtained for the competition of [^{3}H]8-OH-DPAT and [^{3}H]ketanserin, respectively. Buspirone and NAN-190 behaved as typical partial agonists in the cAMP assay: cAMP levels were inhibited with an intrinsic activity of about 70 % as compared to 5-HT, but the inhibition of cAMP levels by 5-HT itself was antagonized. The reducing effect of GTPγS on [^{3}H]8-OH-DPAT binding was directly related to the expression level of the 5-HT$_{1A}$ receptor, while EC_{50} values for the inhibition of forskolin-stimulated cAMP levels were inversely related. These data suggest that the coupling of the receptor to G-proteins is dependent on receptor number. Pre-incubation of the cells with 8-OH-DPAT generated a dose- and time-dependent decrease in [^{3}H]8-OH-DPAT binding, while the potency of 5-HT in the cAMP assay was diminished. Pilot experiments indicated that the 5-HT$_2$ receptor could also be desensitized by agonist pre-incubation. The present experiments indicate that desensitization and uncoupling of cloned human 5-HT$_{1A}$ and 5-HT$_2$ receptors occurs after agonist exposure.

46.15

AUTORADIOGRAPHIC LOCALIZATION OF 5-HT_{1A} RECEPTORS IN PIGEON BRAIN. David J. Bucci*[1], Cathy D. Mahle[1], Harvey J. Karten[2], and Richard B. Carter[1], [1]Department of Neuropharmacology, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492 and [2]Department of Neuroscience, University of California San Diego, La Jolla, CA 92093.

Behavioral paradigms used to predict the clinical anxiolytic efficacy of novel agents often employ pigeons because of the unique sensitivity of this species to compounds that act through 5-HT_{1A} mechanisms. Unlike rodents, however, little is known about the distribution and density of specific 5-HT receptors in pigeon brain. In the present study, pigeon brain slices (15 μM coronal sections) were incubated with 2 nM [^{3}H]-8-OH-DPAT in the presence or absence of 1 μM BMY 7378 to specifically label the 5-HT_{1A} receptor. Concentrations of 5-HT_{1A} receptors were highest in the medial caudal neostriatum (NC, excluding Field L); visual nuclei, including optic tectum (TeO), nucleus geniculatus lateralis - pars ventralis (GLv), and the nucleus pretectalis (PT); the dorsal raphe complex (R); and locus ceruleus (LoC). Densities of 5-HT_{1A} receptors in the NC, GLv, R, and LoC were 49.3, 24.3, 36.7, and 35.2 fmol/mg tissue, respectively. Other regions exhibiting 5-HT_{1A} binding include the rostral region of the nucleus et tractus descendens nervi trigemini (nTTD) and nucleus solitarius (S). These data will serve as the basis for further studies directed toward identifying the neuronal regions through which 5-HT_{1A} agents produce their anxiolytic-like actions in the pigeon, and thus may aid in elucidating the specific mechanisms by which this class of molecules produces their therapeutic effects.

46.16

^{3}H-8-OH-DPAT Labels a Heterogeneous Population of Serotonin$_{1A}$ Binding Sites in Rat Amygdala.

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A high density of 5-HT_{1A} sites are present in hippocampus, but a considerable number of ^{3}H-8-OH-DPAT sensitive sites are also present in other regions. Recent evidence suggests that biochemical (Yocca, *et al.*, 1992) and pharmacological (Radja *et al.*, 1992) properties of the 5-HT_{1A} receptor exhibit regional differences in the CNS, suggesting the possibility of multiple 5-HT_{1A} sites in the CNS . Autoradiographic examination of ^{3}H-8-OH-DPAT binding revealed a typical pattern of distribution, however while nearly all binding in the hippocampus was displaced by the 5-HT_{1A} partial agonist BMY 7378, a high percentage of the sites located in the amygdala were insensitive to BMY 7378 (1μM). We were therefore interested in examining the possibility of multiple 5-HT_{1A} sites in rat amygdala. Displacement studies in rat amygdala and hippocampal homognates, utilizing ^{3}H-8-OH-DPAT (1nM) and BMY 7378, resulted in a biphasic curve in amygdala with an almost even distribution of high and low affinity sites; while hippocampus yielded a near monophasic displacement. Earlier reports have shown ^{3}H-8-OH-DPAT to label the 5-HT_{Pre} site, concentrated in rat striatum (Gozlan, 1983). Pharmacologically, the 5-HT_{Pre} site has a reported lower affinity for the azapirone anxiolytics, is insensitive to guanine nucleotides, and its binding is inhibited in the presence of manganese (Mn^{2+}). GppNHp causes a concentration dependent inhibition of ^{3}H-8-OH-DPAT binding in both hippocampus and amygdala. No decrease in binding in either region was observed in the presence of 1 mM Mn^{2+}. Furthermore, this site displays no affinity for the selective 5-HT uptake inhibitor, fluoxetine. This raises the possibility that multiple subtypes of the 5-HT_{1A} receptor exist in CNS.

46.17

DORSAL RAPHE 5-HT_{1A} RECEPTORS REGULATE SOMATODENDRITIC AND HIPPOCAMPAL RELEASE OF SEROTONIN (5-HT). F.Fátima Matos*, Carolyn Brown and Frank Yocca, Department. of Neuropharmacology, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492.

It is well accepted that the effects of a 5-HT_{1A} agonist on 5-HT release in terminal areas are due to activation of 5-HT_{1A} receptors localized on 5-HT cell bodies and dendrites in the midbrain raphé nuclei. Previous microdialysis data have shown that 5-HT_{1A} agonists decrease extracellular 5-HT in the ventral hippocampus (Sharp et al. 1989). We tested whether buspirone (5-HT_{1A} agonist) effects upon hippocampal 5-HT release were mediated by somatodendritic 5-HT_{1A} receptors by directly perfusing (-) pindolol (ß, 5-HT_{1A} antagonist) into the dorsal raphé to block somatodendritic receptors. Two microdialysis probes were implanted in each rat, one in the dorsal raphé and one in the hippocampus, to allow the simultaneous measurement of 5-HT in both areas. Experiments were conducted the next day in awake rats. The dialysis probes were perfused at 1μL/min with Ringer (dorsal raphé) or Ringer containing 10 μM fluoxetine (hippocampus). Extracellular levels of 5-HT, 5-HIAA and HVA were simultaneously analyzed by HPLC-ECD. Buspirone (1.0-5.0 mg/kg, s.c.) induced a dose-related decrease in hippocampal 5-HT (maximal decrease 40% of control). Levels of 5-HIAA decreased by 20%, while HVA increased 2.5 fold. Extracellular 5-HT in the dorsal raphé changed in a similar fashion as in the hippocampus after 2.5 mg/kg buspirone; 5-HT decreased to 40%, 5-HIAA decreased by 30% and HVA increased 2.5 fold. Local perfusion of (-) pindolol (1 mM) into the dorsal raphé produced a sustained increase in 5-HT in the dorsal raphé and hippocampus (2.5 and 1.5 times basal levels, respectively). When buspirone (2.5 mg/kg) was injected during continuous perfusion with (-) pindolol into the raphé, the hippocampal and dorsal raphé decrease in 5-HT were completely antagonized; 5-HT levels remained elevated above controls. These data provide evidence that extracellular 5-HT in the dorsal raphé is under tonic control of the activity of 5-HT_{1A} receptors and that buspirone decreases hippocampal and dorsal raphé 5-HT release by direct activation of 5-HT_{1A} receptors in the dorsal raphé.

46.18

ONTOGENY OF 5-HT_{1A} RECEPTORS IN THE HUMAN BRAIN: AN AUTORADIOGRAPHIC STUDY. A. Pazos[1], E. del Olmo[1], A. Díaz[1], C. del Arco[1], M. Guirao-Piñeyro[3] and M. Lafarga[2]*. Depts of [1]Physiology and Pharmacol, and [2]Anatomy and Cell Biol., Univ. Cantabria, Santander, Spain; and [3]Dept. Morphology, Univ. of Granada, Spain.

The ontogenetic appearance of brain 5-HT_{1A} receptors was studied by autoradiography in 8 fetal brains (gestational ages between 25 and 40 weeks). ^{3}H-8-OH-DPAT was used as a ligand. At the gestational age 25, 5-HT_{1A} receptors were present at relevant densities in areas such as the neocortex, hippocampus and brainstem (raphé nuclei), among others, showing a good correlation with the distrition found in the adult brain. A progressive increase in the amount of receptors was observed along the prenatal period in these areas, up to reach adult levels. In contrast, high densities of these sites were found in the fetal cerebellar cortex, an area completely devoid of such receptors in the adult brain. These results illustrate on the developmental pattern of 5-HT_{1A} receptors in the human brain. In addition, the transient expression of these HT_{1A} receptors in the cerebellum support a role for 5-HT in central development, in line with previous physiological and biochemical findings.

Supported by DGICYT, Ministry of Education (PM88-0170).

46.19

UNCOUPLING OF THE RAT SEROTONIN-1A RECEPTOR BY A POINT MUTATION IN THE SECOND CYTOPLASMIC LOOP. P. Lembo, M. Quik*, P.R. Albert. Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada H3G-1Y6.

When expressed in fibroblasts, such as Ltk- cells, the serotonin-1A (5-HT1A) receptor couples negatively to adenylyl cyclase and positively to phospholipase C via pertussis toxin-sensitive G proteins. Activation of protein kinase C (PKC) by phorbol ester (TPA) phosphorylates the receptor, and selectively blocks receptor-mediated activation of phospholipase C and calcium mobilization, but not inhibition of forskolin-stimulated cAMP accumulation. To test whether putative PKC phosphorylation sites on the 5-HT1A receptor are involved in uncoupling the receptor, each of four sites (THR149 in the second cytoplasmic loop, and THR229, SER253, and THR343 in the third cytoplasmic loop) was mutated from SER—GLY or THR—ALA. Each mutant was transfected into Ltk- cells and stable transfectants expressing high levels of receptor RNA were isolated. In cells expressing the THR149 mutant, 5-HT induced calcium mobilization and inhibition of cAMP were abolished; on the other hand, specific [3H]-DPAT binding was present. However, for each of the other mutant receptors except THR149, 5-HT inhibited cAMP accumulation and enhanced calcium mobilization, as observed for the wild-type receptor. Acute (4-minute) pretreatment with TPA (10-100 nM) abolished the action of 5-HT to enhance calcium levels without altering cAMP accumulation, as for wild-type receptor. We conclude that the second cytoplasmic loop is crucial for coupling of the 5-HT1A receptor. Funded by the FCAR, FRSQ, and NCI, Canada.

47.1

CHEMICAL NEUROANATOMY OF THE CHOLINERGIC CONTROL OF RAT AND HUMAN STRIATAL NEURONS.

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Acetylcholine (ACh) plays a major role in the control of striatal neuronal activities. In order to bring informations about the cellular basis of such control, we have 1) characterized phenotypically the neurons expressing muscarinic receptor (mR) genes in rat and human, 2) identified in rats the neurons expressing c-fos protein after stimulation or inhibition of mR. By using several combinations of in situ hybridization (ISH) with immunohistochemistry, we demonstrated that in the striatum, several neuronal populations express one or several mR genes with specific patterns: most cholinergic neurons contain m1, m2 and m4 receptor (m1R, m2R and m4R) mRNA. All substance P (SP) neurons contain m1R and m4R mRNA. All enkephalinergic (Enk) neurons present a labelling with a m1R probe, but 39% of them only with m4R probe. The m1R mRNA is detected in most somatostatin (SRIF) and neurotensin (NT) neurons, but m4R mRNA is present in only a small part of them. These results show that ACh acts directly in a complex manner on the main cell populations of the striatum through mR. This suggests that ACh could mediate directly the release or synthesis of the neurotransmitters produced by these neurons. Use of several muscarinic agonists or antagonists demonstrated that neurons in the rat striatum react by c-fos expression to muscarinic receptor manipulation. After stimulation of the muscarinic receptors by oxotremorine, c-fos immunoreactivity (IR) was restricted to the striosomes. About 80% of the c-fos immunoreactive neurons was identified by ISH as Enk neurons and about 30% as SP neurons. This c-fos IR was inhibited by blockade of the muscarinic receptors by atropine. The c-fos induced seemed independent of the dopaminergic or opioïd influences. These results demonstrate that cellular and molecular basis exist for complex influences of ACh on the activities of the main neuronal populations of the striatum.

47.2

INTERACTIONS BETWEEN THE EFFECTS OF ENRIVONMENTAL MANIPULATIONS AND BENZODIAZEPINE RECEPTOR LIGANDS ON CORTICAL ACETYLCHOLINE RELEASE.

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In vivo microdialysis was used to measure the ability of benzodiazepine receptor (BZR) ligands to bidirectionally modulate acetylcholine (ACh) release in the frontoparietal cortex of freely moving rats. When animals were tested during the light phase, the BZR agonist chlordiazepoxide (CDP, 1-10 mg/kg, ip) did not inhibit ACh release, whereas the BZR selective inverse agonists ZK 93 426 (1 or 5 mg/kg, ip)and MDL 26,479 (1-10 mg/kg, ip) led to modest and insignificant increases in ACh release, respectively. It was found that a brief exposure to darkness during the light phase produced a 128% increase in cortical ACh efflux. Interestingly, CDP (10.0 mg/kg), which had no effect on ACh release during the light phase, inhibited the darkness-induced increase in ACh efflux. We are currently determining whether administration of BZR selective inverse agonists are able to potentiate the stimulating effects of exposure-to-darkness on cortical ACh release. Collectively, these experiments are designed to test the hypothesis that the ability of drugs presumably acting at the GABA-BZR complex to modulate cortical ACh release depends upon the state of activity in cortical cholinergic afferents.

47.3

CLASSICAL NONCHOLINERGIC NEUROTRANSMITTERS AND THE VESICULAR TRANSPORT SYSTEM FOR ACETYLCHOLINE

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Several classical noncholinergic neurotransmitters or their biosynthetic enzymes have been reported to be present in cholinergic nerve terminals. Potential interactions of classical noncholinergic neurotransmitters with cholinergic synaptic vesicles purified from electric organ were studied. No active transport of [^{3}H] serotonin, [^{3}H]noradrenaline or [^{3}H]glutamate occurred. Serotonin and noradrenaline inhibited active transport of [^{3}H]acetylcholine by the vesicles. Dopamine previously had been shown to inhibit transport. L-Glutamate and γ-aminobutyric acid did not. Noradrenaline was competitive with respect to active transport of [^{3}H]acetylcholine. The aromatic neurotransmitters also inhibited binding of [^{3}H]vesamicol to the vesicles, and dopamine was a competitive inhibitor. Vesamicol is an allosteric ligand of the acetylcholine transporter. The results indicate that dopamine, noradrenaline and serotonin bind to the acetylcholine site but are not transported.

47.4

ULTRASTRUCTURAL RELATIONS BETWEEN NIGROSTRIATAL DOPAMINERGIC NEURONS AND CHOLINERGIC NERVE TERMINALS IN THE HUMAN BRAIN.

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The connections between cholinergic nerve endings and nigrostriatal dopaminergic neurons were studied at ultrastructural level in the substantia nigra and the striatum in the human brain postmortem. Immunocytoperoxidasic reaction evidencing tyrosine hydroxylase (TH) was followed by ionic fixation of acetylcholine-like cation by silicotungstic acid in the form of punctiform precipitates in synaptic vesicles. Subsequent post-osmification with silicotungstic acid and embedding in araldite allowed TH immunoreactivity and punctiform precipitates of acetylcholine-like cation to be observed simultaneously on ultrathin sections. In substantia nigra, numerous synaptic contacts were observed between nerve endings with or without punctiform precipitates and TH-immunoreactive dendrites or neuronal cell bodies. In striatum, most of nerve endings displaying punctiform precipitates were not in contact with TH-immunoreactive nerve fibers. These results suggest that, in human, cholinergic nerve endings modulate the activity of nigrostriatal dopaminergic neurons essentially at the level of their dendrites and cell bodies in substantia nigra.

47.5

ENDOGENOUS DOPAMINE PRIMARILY CONTROLS D1 RECEPTOR MEDIATED FACILITATION OF IN VIVO ACh RELEASE FROM STRIATUM

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Striatal cholinergic activity is inhibited by stimulation of D2 receptors and is facilitated by stimulation of D1 receptors. In this study we show (1), that the D1 receptor regulation of striatal ACh release in vivo occurs locally in the striatum; (2), the D1 facilitatory control of DA on striatal ACh release is more sensitive to changes in DA transmission than is the inhibitory D2 mechanism. Thus,the D1 antagonist SCH 23390 (20 μM) infused or (0.45 nmol/side) locally injected, decreased striatal ACh release by about 35%, as it did after s.c. administration. Raising extracellular DA with d-amphetamine (2 mg/kg s.c.), or pargyline (75 mg/kg i.p.) resulted in a time-dependent increase in striatal ACh output. The effects were antagonized by SCH 23390 (10 μM) infused. Prolonged (16 h) depletion of DA by a-MpT treatment markedly decreased basal ACh release and prevented the effects of the D1 antagonist, SCH 23390 but not that of the D2 antagonist, REM, on ACh release.

47.6

MK-801 POTENTIATES THE d-AMPHETAMINE-INDUCED INCREASE IN STRIATAL EXTRACELLULAR DOPAMINE LEVELS.

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Numerous studies indicate an interaction, either direct or indirect, between striatal dopaminergic and glutamatergic systems. The present experiments analyzed this relationship pharmacologically by measuring, via *in vivo* microdialysis, the effects of the non-competitive NMDA receptor antagonist, MK-801, on basal and d-AMPH-induced efflux of dopamine (DA) and DA metabolites in striatum of freely-moving rats. MK-801 (0.5 mg/kg; i.p.) administration significantly increased extracellular DA and dihydroxyphenylacetic acid (DOPAC) levels by approximately 60% and 20%, respectively (n=7). Although not quantified, MK-801-induced increases in locomotor activity and rear limb ataxia were observed. Administration of d-AMPH (2.0 mg/kg; i.p.), produced a 20-fold increase in extracellular DA that was associated with a 70% decrease in DOPAC levels (n=7). When animals were pretreated with MK-801 30 minutes prior to d-AMPH, the magnitude of the subsequent d-AMPH-induced increase in extracellular DA levels was significantly potentiated to 35-fold (n=7). The d-AMPH-induced decline in extracellular DOPAC was not significantly affected by MK-801 pretreatment. Because d-AMPH acts primarily to increase release of newly synthesized DA from a cytoplasmic pool, these data suggest that MK-801 administration potently stimulates DA synthesis in striatum. In summary, the present data indicate that systemic MK-801 enhances both the spontaneous release and the synthesis of DA in nigrostriatal neurons. The synthesis-modulating effects of NMDA receptor blockade appear to be greater than its release-modulating effects under the present conditions. Finally, these results suggest that endogenous excitatory amino acids may exert a tonic inhibitory influence upon nigrostriatal DA synthesis and release. (Supported by USPHS Grant NS 19608)

47.7

STRESS INCREASES DOPAMINE SYNTHESIS IN STRIATUM AS MEASURED BY IN VIVO MICRODIALYSIS. S.L. Castro*, K.A. Keefe, A.F. Sved, E.D. Abercrombie[1], and M.J. Zigmond. Dept. of Cellular and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260 and [1]Center for Molecular and Behavioral Neuroscience, Rutgers Univ., Newark, NJ 07102.

Acute stressors increase dopamine (DA) release in striatum as evidenced by increases in extracellular DA and, in some cases, increases in the turnover of DA in striatal tissue. To delineate further the factors associated with the stress-induced release of DA in striatum, we examined the effects of 30 min of low intensity, intermittent tail shock on DA synthesis in striatum. A concentric microdialysis probe (4 mm) was implanted in rostromedial striatum and perfused with artificial cerebrospinal fluid containing NSD-1015 (100 μM), an inhibitor of aromatic amino acid decarboxylase. The concentration of DOPA in the striatum of freely moving rats after inhibition of the decarboxylase was used as an *in vivo* index of DA synthesis. In control rats, NSD-1015 increased extracellular DOPA from an undetectable level to 0.37 ± 0.04 ng/20 μl (mean ± SEM, n=10) 2 hours after the infusion of NSD was begun. Continued infusion of NSD did not significantly increase extracellular DOPA over the next 1.5 hours. Rats exposed to the stressor had a basal extracellular concentration of DOPA of 0.83 ± 0.37 ng/20 μl, which was not significantly different from controls. The application of the stressor 2 hours after the start of the NSD infusion significantly increased the amount of DOPA in striatal extracellular fluid by 64%. These data indicate that acute stress can increase not only DA release in striatum, but DA synthesis as well. Our recent studies suggest that stress-induced release of DA is mediated by activity in DA neurons rather than by excitatory amino acid input to the DA terminal (see Keefe et al., Soc. Neurosci. Abs., 1992). We currently are determining whether this also is the case for stress-induced changes in DA synthesis. (Supported in part by USPHS grants MH45156, MH43947, MH00058, and MH09972).

47.8

STRESS-INDUCED DOPAMINE RELEASE IN STRIATUM: EXCITATORY AMINO ACID STIMULATED OR ACTION POTENTIAL DEPENDENT? K.A. Keefe*, A.F. Sved, M.J.Zigmond, and E.D. Abercrombie[1]. Dept. of Cellular & Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260 & [1]CMBN, Rutgers Univ., Newark, NJ 07102.

Studies indicate that stress increases dopamine (DA) release in striatum, yet does not seem to alter the electrophysiological activity of nigrostriatal DA neurons. We find that stress also increases extracellular concentrations of glutamate and aspartate in striatum (+50% and +38%, respectively). Because excitatory amino acids (EAAs) can presynaptically stimulate DA release in striatum, we used *in vivo* microdialysis to examine whether DA release induced by 30 min of intermittent tail-shock stress is initiated locally by EAAs or is dependent on action potential propagation through nigrostriatal DA neurons. To this end, the EAA receptor antagonists APV or CNQX (0.1 mM) were administered via a microdialysis probe in striatum to block local effects of EAAs. In addition, tetrodotoxin (TTX; 10 μM) was infused via a microdialysis probe in the medial forebrain bundle (MFB) to block action potential propagation in nigrostriatal DA neurons. Stress alone increased the extracellular concentration of DA in striatum by 2.8 ± 0.7 pg/20 μl (+50%; mean ± SEM; n=6). During local infusion of APV or CNQX to striatum, stress increased extracellular DA by 2.9 ± 0.8 pg/20 μl (+80%; n=5) and 2.4 ± 0.6 pg/20 μl (+68%; n=6), respectively. The stress-induced increases in extracellular DA were not significantly different between the groups. In contrast, infusion of TTX into MFB reduced basal extracellular DA from 4.7 ± 0.7 pg/20 μl (n=6) to less than 0.5 pg (limit of detection) and eliminated the stress-induced increase in extracellular DA. The data therefore suggest that both the basal and stress-induced release of DA in striatum are largely, if not exclusively, determined by action potential propagation through the MFB.(Supported in part by USPHS grants MH45156, MH43947, MH00058, and MH09972.)

47.9

GABA RELEASE AND CORTICAL EXCITATION OF CELLS IN STRIATUM IS INFLUENCED BY NMDA AND AMPA RECEPTORS IN MULTIPLE WAYS. S. Bernath*, M.J. Zigmond, A.A. Grace, and T.W. Berger[1]. Univ. Pittsburgh, Pittsburgh, PA 15260 & [1]Univ. Southern California, Los Angeles, CA 90089

These experiments examined the contribution of the AMPA and NMDA subtypes of excitatory amino acid (EAA) receptors to excitation of putative GABAergic neurons and GABA efflux in striatum. Electrophysiological activity of single striatal cells evoked by direct electrical stimulation of corticostriatal afferents was recorded extracellularly in a corticostriatal slice. In parallel experiments [^{3}H]GABA efflux from striatal slices was measured. CNQX (1μM) abolished synaptically-evoked action potentials in striatal cells. APV (10 μM) had no inhibitory effects either in the presence or absence of external Mg^{2+} (1.5 mM), although NMDA (1 mM) elicited a rapid burst of spike firing. Quisqualic acid (QA, 10 μM) and NMDA (1 mM) promoted [^{3}H]GABA efflux in striatal slices that was not blocked by removal of external Ca^{2+} (2 mM). TTX (1 μM) increased the QA-evoked release of GABA 7-fold in the presence of Ca^{2+}, but not in its absence. Neither bicuculline nor phaclofen had any significant effects on QA-evoked release of GABA at 10 μM concentration. In contrast, sulpiride (0.5 μM) elevated QA-evoked GABA efflux 3-fold. Together, these results suggest that EAA receptors influence GABAergic cells in striatum in several ways: (a) corticostriatal excitation of striatal cells is mediated via AMPA receptors as previously described (Herrling, Neuroscience, 14, 417, 1985), although both AMPA and NMDA receptors are present on striatal neurons; (b) direct stimulation of AMPA or NMDA receptors on GABAergic neurons also elicits Ca^{2+}-independent GABA efflux; and (c) AMPA receptor stimulation triggers an inhibition of GABA release by dopaminergic afferents. (Supported by MH00343, MH00058, MH439547, and MH45156.)

47.10

K^+- AND METHAMPHETAMINE (METH)-INDUCED ENDOGENOUS DOPAMINE (DA) RELEASE FROM MOUSE NEOSTRIATAL SLICES: EFFECT OF ADENOSINE. K.T. DelleDonne* and P.K. Sonsalla. Dept. of Neurology, UMDNJ-RWJ Medical School, Piscataway, NJ 08854.

Proper regulation of DAergic activity in the neostriatum is critical for appropriate control of motor and mental functions. Adenosine is thought to act as a neuromodulator of this DAergic system. In order to evaluate the effect of adenosine on endogenous DA release, a method using a Brandel Superfusion 600 has been devised which permits the reliable discernment of even small drug effects on DA release. Inclusion of 10mM Mg^{2+} in the slice preparation buffer, increased flow rate, and the addition of a debubbler to the superfusion system were critical factors in maximizing reproducibility. Each chamber serves as its own control due to the application of three consecutive stimulations (S1,S2,S3) in which S2 is preceded by constant perfusion of the test drug; return of DA release in S3 to similar values elicited by S1 is assurance of tissue viability. The peak of K^+-induced DA release is found almost exclusively in a single 2.5 minute collection fraction whereas the peak of METH-induced DA release is found in two fractions. Amfonelic acid (10μM) blocked METH (35μM)-induced release; 35mM K^+-induced release was prevented by removal of Ca^{2+} and addition of 1mM EGTA and 10mM Mg^{2+}. Perfusion of slices with 0.5μM N^6-cyclopentyladenosine (CPA), a selective adenosine A_1 receptor agonist, for 30 minutes prior to S2 inhibited DA released by 35mM K^+ by about 20% but did not affect release by 35μM METH. In preliminary experiments, chronic treatment of mice with caffeine, an adenosine receptor antagonist, produced a slight increase in the inhibitory effect of CPA on 35mM K^+-induced DA release. These data are consistent with other findings using rat tissue and indicate a neuromodulatory role for adenosine on neostriatal DAergic activity.

47.11

IN VIVO DOPAMINE RELEASE IN THE ANTERIOR NUCLEUS ACCUMBENS OF THE RAT IS INCREASED BY STIMULATING CCK-B RECEPTORS IN THE VENTRAL TEGMENTAL AREA. R. Morgenstern, T. Reum, H. Fink and B. J. Cole*. Inst. of Pharmacology and Toxicology, Humboldt Univ. at Berlin and Dept. of Neuropsychopharmacology, Schering AG, Berlin, Germany.

The neuropeptide cholecystokinin-8s (CCK-8s) has been shown to modify the activity of mesolimbic dopamine neurones projecting from the ventral tegmental area (VTA) to the nucleus accumbens septi (NAS) of the rat. It is unclear whether its effects are mediated by CCK-A or CCK-B receptors.

Using differential pulse voltammetry with electrically pretreated carbon fiber electrodes placed in the anterior NAS of anesthetized rats (400 mg/kg chloralhydrate IP) we measured peak 2 after stereotaxic microinjection into the VTA of CCK-8s, CCK-4 and IP injection of selective CCK-A and CCK-B antagonists, with or without pretreatment with pargyline (75 mg/kg IP).

Whereas without pargyline CCK-8s did not produce any change of peak 2, following pargyline pretreatment CCK-8s increased peak 2 dose dependently (1, 10 and 100 ng) indicating no change in DOPAC but increase in dopamine release. The CCK-B agonist CCK-4 produced a smaller increase of peak 2. The CCK-8s induced increase of peak 2 could be blocked by the CCK-B antagonist L-365,260 (25 μg/kg IP) but not by the CCK-A antagonist L-364,718 (250 μg/kg IP).

Our results indicate that dopamine release from neurones projecting from VTA to the anterior NAS may be induced by activation of CCK-B receptors in the VTA.

47.12

SEROTONIN IN THE NUCLEUS ACCUMBENS SUPPRESSES ACETYLCHOLINE OUTPUT IN FREELY MOVING RATS. P.V. Rada, G.P. Mark and B.G. Hoebel*. Department of Psychology, Princeton University, Princeton, NJ 08544.

The purpose of this study was to characterize the interaction between 5-HT and ACh in the NAC of freely behaving animals. 5-HT and related agonists were either applied locally to the NAC by reverse microdialysis or injected systemically while extracellular ACh was simultaneously measured. Locally applied 5-HT or fluoxetine significantly decreased ACh in the NAC. This decrease was blocked by a β-adrenergic and 5-HT_1 antagonist, propranolol, but not by a 5-HT_2 antagonist, methysergide. The block by propranolol was probably due to its 5-HT_1 antagonistic properties because the β-adrenergic agonist isoproterenol did not change extracellular ACh. Systemic and local administration of 8-OH-DPAT dose-dependently decreased extracellular levels of ACh. These results suggest an inhibitory effect of 5-HT on ACh release in the NAC, and this effect is probably mediated, at least in part, through a 5-HT_1 receptor.

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47.13

SEROTONIN 5-HT_{1A} RECEPTORS MEDIATE INHIBITION OF TYROSINE HYDROXYLASE ACTIVITY IN RAT STRIATUM. E.A. Johnson*, C.E. Tsai, and A.J. Azzaro, Depts. of Behavioral Med./Psych., Neurology, and Pharmacology/Tox., West Virginia University School of Medicine, Morgantown, WV 26506

D_2 dopamine autoreceptor mediated control of dopamine synthesis, through modulation of tyrosine hydroxylase (TH) activity, has been well documented. However, recent data indicate that other presynaptic receptors also modulate dopamine synthesis. We have demonstrated 5-HT_{1A} receptor mediated inhibition of rat striatal TH in an in-vitro synaptosomal preparation and in in-vivo experiments measuring dihydroxyphenylalanine (DOPA) accumulation following inhibition of aromatic amino acid decarboxylase (NSD-1015, 100 mg/kg, i.p.). In synaptosomes, both 8-OHDPAT, the selective 5-HT_{1A} agonist and serotonin, exhibited dose-dependent inhibition, with EC_{50} values of 7.0 uM and 8.4 uM, respectively. The effects of 8-OHDPAT were antagonized with NAN-190, a 5-HT_{1A}/alpha$_1$ selective antagonist, propranolol, alprenolol, and pindolol ($p<0.01$) but not by (-)-sulpiride, a D_2 selective antagonist. The effects of serotonin were blocked by propranolol, but not by spiperone, a mixed 5-HT_2 > 5-HT_1 antagonist, or ketanserin, a 5-HT_2 antagonist. 8-OHDPAT was more potent as an inhibitor of rat striatal DOPA accumulation in-vivo (ED_{30} 0.3 mg/kg, s.c.). This effect was antagonized by pindolol (16 mg/kg, s.c.) and by NAN-190 (3 mg/kg, s.c.). These results support a role of 5-HT_{1A} receptors in modulation of dopamine synthesis in rat striatum.

47.14

CORTICAL REGULATION OF STRIATAL CATECHOLAMINE RELEASE IN THE RHESUS MONKEY Bhaskar S. Kolachana*, Richard C. Saunders and Daniel R. Weinberger, NIMH Neuroscience Center at St. Elizabeths, Washington, DC. 20032

Extracellular neurotransmitter levels in the **caudate nucleus (cd)** and the **dorsolateral prefrontal cortex (pfc)** of the Rhesus monkey were measured using intracerebral *in vivo* microdialysis coupled to microbore HPLC-EC. Adult Rhesus monkeys (N=3) were sedated with isofluorane gas anesthesia. Microdialysis probes, built in-house, were inserted into the **cd** and **pfc** through guide cannulae and were perfused continuously with artificial cerebrospinal fluid (csf). Perfusate was collected every 25-30 minutes at 1 ul/min flow rate and was assayed for catecholamines. After a 4-hr baseline d-amphetamine (amp) or cocaine hydrochloride (coc) dissolved in csf was infused into the **pfc** region for 25-30 min. Both amp and coc infusions into **pfc** resulted in elevation (150-400%) of cortical dopamine (DA) and reduction (20-40%) of metabolites Dopac, HVA and HIAA. In contrast, levels of DA, Dopac, HVA and HIAA in the **caudate nucleus** were markedly reduced (35-50%) following **pfc** infusion. Perfusate collected from the **pfc** was analyzed for aminoacids. Following coc infusion there were significant increases (250-400%) in cortical aspartate and glutamate levels as well as several other amino acids. These data demonstrate that in primates cortico-caudate projections regulate the release of striatal catecholamines and this cortico-striatal regulation may be mediated by excitatory amino acids.

47.15

STRIATAL DOPAMINE RECEPTORS INTERACTION IS LOST AFTER MPTP ADMINISTRATION. MR. Luquin*, J. Guillen, J. Laguna, J. Guridi, MT. Herrero, JA. Obeso. Movement Disorders Unit. Department of Neurology. Clinica Universitaria. Apdo. 192-31080 Pamplona-Spain.

We studied 3 previously normal monkeys and 1 parkinsonian monkeys. Hemiballism was induced by Kainic acid injection into the STN. Administration of a range of doses of SCH 23390 (0.8-3.2 mg/kg) abolished ballism in the MPTP-lesioned monkey while sulpiride (60 and 120 mg/kg) had no effect. In the 3 other animals neither SCH 23390 nor sulpiride given alone stopped ballism but co-administration of both drugs reduced it significatively. These results indicate that motor responses mediated D-1 and D-2 striatal receptors can only be dissociated after DA nigro-striatal lesion.

47.16

GABAergic INHIBITION OF DOPAMINE AND ACETYLCHOLINE OUTPUT IN THE NUCLEUS ACCUMBENS OF BEHAVING RATS. G.P. Mark*, P.V. Rada and B.G. Hoebel. Department of Psychology, Princeton University, Princeton, NJ 08544.

The influence of local application of GABA on dopamine (DA) and acetylcholine (ACh) output was studied by reverse dialysis in the nucleus accumbens (NAC). Addition of GABA (500 μM) to the perfusion medium caused an average 27% reduction in extracellular DA in the NAC. Perfusion with the $GABA_A$ receptor antagonist bicuculline (BIC; 50 μM) had the opposite effect, resulting in an average 89% increase in DA. Both the $GABA_B$ receptor agonist baclofen (1-50 μM) and the $GABA_A$ agonist muscimol (1-50 μM) dose-dependently decreased ACh output. The inhibitory effect of muscimol was more pronounced and more persistent than that of baclofen at all doses. Infusion of BIC increased ACh levels by 83% (10 μM) and 148% (50 μM). These results provide evidence for a local, inhibitory influence of GABA on the release of both ACh and DA within the nucleus accumbens.

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47.17

FUNCTIONAL INTERACTION OF DOPAMINE AND GLUTAMATE IN NUCLEUS ACCUMBENS IN THE REGULATION OF LOCOMOTION. M. Wu and S. M. Brudzynski*. Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

It has been shown that excitatory inputs from limbic structures to nucleus accumbens neurons, mediated by glutamate, implicate the initiation of locomotion (Yang & Mogenson, 1987; Yim & Mogenson, 1989). Earlier electrophysiological evidence suggests that these glutamatergic inputs were inhibited by dopamine (Yang & Mogenson, 1987; Yim & Mogenson, 1988). It was also shown that the locomotion induced by injecting glutamatergic receptor agonists, N-methyl-D-aspartic acid (NMDA) or α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) into accumbens may be dopamine-mediated (Boldry & Uretsky, 1988; Imperato et al., 1990). However, the site of this interaction between dopamine and glutamate in accumbens is still unclear. The objective of this study is to investigate the functional interaction of the dopamine and glutamate in accumbens in the regulation of locomotion.

The locomotion (measured in an Opto-Varimex-3 activity cage) induced by unilateral injections of NMDA or AMPA into accumbens was significantly reduced, in the dose-dependent manner, by the pretreatment of dopamine D_2 agonist quinpirole while the dopamine D_1 agonist SKF 38393 had little or no effect. When the axon terminals of mesolimbic DA neurons in accumbens were destroyed by injecting 6-OH-DA into the ventral tegmental area (VTA), NMDA- and AMPA- induced locomotion were reduced substantially. These results suggested that A_{10} DA terminals may be the sites for dopamine and glutamate interaction. By activating glutamate receptors located on DA terminals, NMDA and AMPA may influence DA terminals and subsequently increase locomotion.

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47.18

DOPAMINE GLUTAMATE INTERACTION IN THE ADAPTIVE RESPONSE TO REPEATED STRESSFUL EXPERIENCES. A. Mele*, S. Cabib, S. Puglisi-Allegra and A. Oliverio. Dip. Genetica e Biologia Molecolare, Dip. Psicologia, Universita' 'La Sapienza' Roma, I-00185 and Ist. Psicobiologia e Psicofarmacologia, C.N.R., I-00198 Roma, Italy.

Repeated exposure to a stressful event produces a modification in the response to DA agonists. Ten days of a two hours daily immobilization stress has been shown to increase the responsiveness of C57 mice to low, presynaptic, doses of Apomorphine in the climbing test.

The glutamatergic (Glu) system sends dense afferents to both the striatum and the n. accumbens, sites of afferents of the DA system. A close interaction between the two systems has been proposed in the regulation of the output of this two nuclei. Therefore the effects of systemic injections of the non-competitive NMDA antagonist, MK801, on the adapted response to low doses of Apomorphine in the C57 strain of mice were investigated. One daily injection of (+)MK 801 prior the exposure to the stressfull experience, is able to antagonize the effects of repeated stress on the sensitivity to Apomorphine. In a series of preliminary experiments we tested also the effects of chronic exposure to stress on the MK 801 induced locomotor activity. In this study no changes in the sensitivity of the Glu system by itself were found. Therefore if on one side the NMDA sensitivity does not change in response to repeated stressful events, on the other the blockage of the NMDA receptor with MK801 is able to exert a protective effect on the DA system.

48.1

LOCALIZATION OF ANDROGEN RECEPTORS IN THE BRAIN AND PITUITARY GLAND OF Xiphophorus maculatus. L. Magliulo Cepriano and M. P. Schreibman*. Graduate School-University Center and Brooklyn College Biology Department, C.U.N.Y., Brooklyn, New York 11210.

The distribution of androgen receptors in the brain and pituitary gland of X. maculatus, the platyfish, was studied, in animals of different ages and stages of development, by immunocytochemistry. Immunoreactivity to antisera generated against androgen receptors was observed in the nucleus lateralis tuberis (NLT) in pubertal and pre-pubertal animals but not in neonatal animals. Immunoreactivity in the NLT was restricted to nuclei. The NLT, an ir-GnRH containing nucleus, has a demonstrated role in the maturation and function of the reproductive system. In the pituitary gland, animals of all ages demonstrated an immune response in the pars intermedia (PI). Immunoreactivity was seen in both the cytoplasm and nuclei of reactive cells. In pre-pubertal and pubertal animals, immunoreactivity was also observed in cells in the ventral caudal pars distalis and at the caudal-rostral pars distalis boundary. [Supported by NASA (NAGW-1704), AID, and PSC-CUNY.]

48.2

NEURONAL TYPES AND SUBDIVISIONS OF THE HUMAN INTERMEDIATE RETICULAR ZONE. X-F. Huang, G. Paxinos and I. Törk* Schools of Anatomy and Psychology, University of New South Wales, P.O. Box 1, Kensington, N.S.W., 2033, Sydney, Australia.

The cytoarchitecture and chemoarchitecture of the intermediate reticular zone (IRt) of the medulla oblongata were examined in eight humans. Nissl stained preparations revealed that the majority of neurons in the IRt orient themselves in a dorsomedial to ventrolateral fashion, in line with the axis of the IRt. Four types of neurons were identified but there was no evidence that the specific types of neurons were differentially distributed within the IRt. Based on chemoarchitectonic examinations, the IRt was divided into two parts, external and internal. The external part is a narrow band lateral to the ambiguus nucleus while the internal part encompasses the ambiguus nucleus and extends dorsomedially to the dorsal motor nucleus of the vagus nerve. Both parts extend ventrolaterally to nearly the surface of the medulla. Both external and internal parts of the IRt displayed tyrosine hydroxylase immunoreactive fibers while only the external part displayed substance P-like immunoreactive fibers. Computer reconstructions of the cell populations enabled the determination of the relative positions of tyrosine hydroxylase- and substance P-like immunoreactive neurons within the subdivisions of the IRt. The substance P-like immunoreactive neurons were only found in the external part of the IRt rostral to the obex, while the tyrosine hydroxylase-like immunoreactive neurons were found throughout both segments of the IRt. The above findings indicate that the subdivisions of the IRt can best be revealed by chemoarchitectonic methods. The chemical differences between the subdivisions are likely to be important to the functional differences between the different regions of the IRt.

48.3

ENDOGENOUS COMPOUNDS FROM PORCINE BRAIN BIND TO THE CANNABINOID RECEPTOR AND BLOCK VAS DEFERENS CONTRACTION. W. A. Devane*, L. Hanuš, R. G. Pertwee^, L. A. Stevenson^, G. Griffin^, and R. Mechoulam. Dept. of Natural Products, Hebrew Univ., Jerusalem 91120, Israel and ^Dept. of Biomedical Sciences, Univ. of Aberdeen, Aberdeen AB9 1AS, Scotland.

To screen for endogenous cannabinoid compounds we employed a binding assay using tritiated 11-hydroxy-hexahydrocannabinol-dimethylheptyl ([3H]-HU-243). Beginning with an organic soluble extract from porcine brain, fractions from a silica gel column were tested for their ability to inhibit the binding of [3H]-HU-243 to rat synaptosomal membranes. Promising fractions were further purified using normal and reverse phase chromatography.

We have isolated a group of three compounds from porcine brain which were able to inhibit the specific binding of [3H]-HU-243 to synaptosomal membranes in a manner typical of competitive ligands. Two of the compounds have been tested for their ability to inhibit the electrically evoked twitch response of the mouse isolated vas deferens, a characteristic effect of psychotropic cannabinoids. Both compounds were active in the vas deferens, their IC-50's being similar to those observed in the binding assay. These findings strongly support the existence in the brain of pharmacologically active endogenous cannabinoids. Supported by NIDA.

48.4

PUTATIVE RECEPTOR FOR STEROID ANESTHETICS IDENTIFIED IN MOUSE BRAIN SYNAPTOSOMES. C.Bukusoglu and N.R.Krieger* Dept. of Anesthesia, Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115.

Binding of the anesthetic steroid 3α-hydroxy-5α-pregnan-20-one (3α) to synaptosomal membranes from mouse brain was specific, reversible, and saturable with respect to time and 3α concentration. The association of [^{3}H]3α (5 nM) was rapid and completed within 5 min. Incubations were carried out with 5-50 nM [^{3}H]3α (60 Ci/mmol) at 24°C in the absence and in the presence of unlabeled 3α. Binding was stopped by rapid centrifugation (12,000 g/3 min/4°C). Aliquots from the supernatant fraction were used to quantitate the amount of bound 3α.

The dissociation of [^{3}H]3α (5 nM) was rapid and completed within 5 min. [^{3}H]3α (10 nM) was displaced by unlabeled 3α (IC_{50} 60 nM) but not by 2 μM of progesterone, 3β-hydroxy-5α-pregnan-20-one, 5α-pregnan-3,20-dione or 5α-pregnan-3,20 diol. Receptor density estimated from displacement studies was twice as high in hindbrain as in forebrain. This work was funded by a grant (GM 42672) from the NIH.

48.5

DISTRIBUTION OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE AND ITS BINDING SITES IN THE SPINAL CORD AND SPINAL TRIGEMINAL NUCLEUS. G. Légrádi, T. J. Görcs and A. Arimura,* U.S.-Japan Biomedical Research Laboratories, Tulane Univ. Hebert Ctr. Belle Chasse, LA70037.

Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) is a new member of the secretin/glucagon/VIP family of peptides that exists in two forms with 38 (PACAP38) and 27 (PACAP27) residues, and it shows the greatest homology to VIP. PACAP is present in the brain and several peripheral tissues as demonstrated by radioimmunoassay and immunocytochemistry. There are two types of receptors for PACAP; Type I which is specific for PACAP, and Type II, which is shared with VIP. Anterior pituitary, brain, spinal cord, adrenal medulla and testis, contain Type I PACAP receptors, and lung, liver, gut and other peripheral tissues Type II receptors. In the present study, we investigated the distribution of PACAP and its binding sites in the rat spinal cord by means of immunocytochemistry, RIA and receptor autoradiography. Immunocytochemistry revealed a terminal-like arborization of nerve fibers in the spinal trigeminal nucleus, the laminae I-III and in lamina X of the spinal cord. RIA for PACAP38 showed extremely high concentrations of PACAP-like immunoreactivity in the spinal trigeminal nucleus (23 ng per mg protein) and in the dorsal horn (33 ng for cervical, 13 ng for thoracic, 19 ng for lumbar, and 17 ng for sacral levels per mg protein). The ventral horns contained much less PACAP (2 ng for cervical, 0.4 ng for thoracic, 10 ng for lumbar, and 1.6 ng for sacral levels). The trigeminal ganglion and the dorsal root ganglia contained lower concentrations of PACAP (2.6 ng for the trigeminal ganglion 0.8 ng for cervical, 2.8 ng for thoracic, 0.7 ng for lumbar, and 1 ng for sacral dorsal root ganglia per mg protein) Using [^{125}I]PACAP27 for autoradiography, the spinal trigeminal nucleus, laminae I-III and X throughout the spinal cord and the intermediolateral cell column of the thoracic part showed the highest density of binding. All the other areas of the spinal cord gray matter were moderately labeled, and the white matter was not labeled. One thousand-fold molar excess of unlabeled PACAP27, but not VIP, displaced the binding. These data suggest that PACAP is involved in the sensory information processing.

48.6

INOSITOL TRIPHOSPHATE (IP3) RECEPTOR AND PROTEIN KINASE C (PKC) LOCALIZATION IN THE HUMAN EYE

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Using *in vitro* autoradiography, Inositol 1,4,5 triphosphate, D-[inositol-1-3H(N)] ([3H]IP3) and Phorbol-12,13-dibutyrate, [20-3H(N)] ([3H]PDBu) were used to determine the distributions of IP3 receptors and PKC in post-mortem human eye sections. IP3 receptors were clearly localized in the trabecular meshwork and ciliary muscle, ciliary epithelium, iris, and corneal epithelium and endothelium, with the highest density in the ciliary muscle. In the posterior segment, the retinal pigment epithelium, the choroid and retina were each labelled. PKC was also present in each of these structures with highest concentrations in the ciliary muscle and retina. Both IP3 receptors and PKC were abundantly localized in the ciliary muscle. The presence of each of these molecules which identify a branch of the phospholipase C (PLC) second messenger pathway strongly suggests the involvement of PLC mediated signal transduction in human ciliary muscle. In our previous work, we localized the M3 muscarinic receptor subtype in ciliary muscle (21st Soc. Neurosci. Abst. 234.5, p.587, 1991). Co-localization of the IP3 receptor, PKC, and the M3 muscarinic receptor subtype in the ciliary muscle suggests that the M3 receptor subtype is involved in PLC pathway activation leading to ciliary muscle contraction.

48.7

NITRIC OXIDE (NO) IN THE THALAMUS AND UPPER BRAINSTEM: A HISTOCHEMICAL STUDY IN THE RAT AND THE CAT. G. Bertini, K.A. Koralek* and M. Bentivoglio, Institute of Anatomy, University of Verona, Italy. (Spon: EBBS)

The upper brainstem and thalamus have been investigated in the adult cat and rat with the histochemical staining for NO synthase (NOS). In the former region, a basic similarity was observed in the two species at the mesopontine junction, in which the cell bodies of both the laterodorsal tegmental and pedunculopontine nuclei displayed an intense NOS-staining. The distribution of fibers and terminals was also largely consistent in the thalamus of the rat and cat. Intensely positive fibers and terminals were observed in the anterior, midline and intralaminar nuclei, as well as in the reticular nucleus and in the cat perigeniculate nucleus. However, although the number of positive cell bodies was relatively limited, their distribution displayed species differences: stained neurons were present in the midline and intralaminar nuclei in the rat but not in the cat. In the latter species, however, small-sized positive cell bodies were seen in some domains, such as the pulvinar and lateroposterior nuclei, either scattered or grouped in clusters. By contrast, in both the rat and cat primary somatosensory and visual relay neurons do not appear to contain NO at thalamic level, whereas NO is expressed by prethalamic inputs, including the brainstem cholinergic one. Preliminary findings indicate a differential sequence of maturation of NOS expression in different systems during postnatal development of the rat thalamus.

48.8

Anatomical relations between NADPH-diaphorase (ND), tyrosine hydroxylase (TH) and serotonin (5-HT) containing neurons of the guinea pig mesopontine tegmentum: A quantitative light microscopic study. C.S. Leonard*, J. Kerman, G. Blaha and E. Taveras. Center for Neural Science, New York University. 6 Wash. Pl., New York, NY 10003.

Interactions between brainstem cholinergic and monoamine systems have long been hypothesized to control behavioral state yet little evidence regarding their interconnectivity has been available. We have begun to investigate this issue in guinea pig. Neurons containing ND (mesopontine cholinergic neurons), TH and 5-HT were labelled using diaphorase histochemistry in combination with immunochemical staining. Soma sizes, shapes and distributions as well as fiber patterns were measured and compared. ND positive cells in the laterodorsal tegmentum (LDT) had mean soma diameters of 23.9μ, mean areas of 459.8μ^2 and form factors of 0.644 which were similar to ND cells in the pedunculopontine tegmentum (PPT). The PPT also contained tightly packed clusters of ND neurons that were smaller (19.5μ diam.,303μ^2 area) than average. TH neurons in the locus coeruleus (LC) and rostral-LC were slightly smaller than ND cells (21.7μ diam., 395.1μ^2 area) and 5-HT cells within the dorsal raphe (DR) were smaller yet (16.8μ diam., 231.8μ^2 area). Cell locations were plotted in frontal sections to assess the degree of overlap in soma distributions. There was a striking degree of overlap in the distribution of TH cells and ND cells in medial PPT and LDT. As much as 78% of the area occupied by ND cells of LDT was contained within the area occupied by TH cells of rostral-LC. A large overlap between laterally displaced 5-HT cells and ND cells of LDT was also observed. TH and 5-HT immunoreactive processes were also observed in both LDT and PPT. Fine varicose fibers as well as thicker, possibly dendritic processes were often seen in close apposition to ND somata and proximal dendrites. These results suggest that ND cells of both the PPT and LDT receive input from catecholamine and 5-HT containing terminals. Moreover, given the restricted volume and high degree of overlap of these populations, a diffusion-bound messenger like nitric oxide, which is synthesized by NADPH-diaphorase could play a critical role modulating TH, 5-HT and ND containing neurons. Supported by NS27881.

48.9

GABAERGIC, CHOLINERGIC AND SEROTONINERGIC INPUTS TO THE ANTERIOR THALAMIC NUCLEI (ATN) OF THE ADULT RAT. A. Gonzalo-Ruiz*, M. Sanz and A.R. Lieberman. Dept. of Human Anatomy, School of Physiotherapy, Soria, Spain and Dept. of Anatomy, UCL, England.

We have examined the topographic organization and transmitter-related characteristics of projections to the ATN from the thalmic reticular nucleus (TRN), pedunculopontine nucleus (PPN), laterodorsal tegmental nucleus (LDTN) and dorsal and median raphe nuclei (DR, MR). Iontophoretic injections of HRP were placed in the anteroventral (AV) or anterodorsal (AD) subnuclei of the ATN in adult albino rats anaesthetized with Nembutal (45mg/kg). The animals were reanaesthetized 1-2d later, and perfused with fixative. Brains were sectioned coronally at 50μm using a Vibratome. The sections were reacted sequentially for HRP using DAB as the chromogen and with antibodies against GABA, ChAT, or serotonin, using the ABC method. (i) In every animal, all HRP-labelled cell bodies in ipsilateral TRN were also GABA+. (ii) After injections in AV, ChAT+ HRP-labelled neurons were present predominantly in the ipsilateral LDTN, with fewer in PPN. Between 60 and 70% of ChAT+ neurons in LDTN were HRP-labelled and 90-95% HRP-labelled cells in LDTN were ChAT+. In the contralateral LDTN, 30-40% of ChAT+ neurons were HRP-labelled. After injection confined to AD, no HRP-labelled cells were present in LDTN or PPN. (iii) Injections into AV and AD produced HRP-labelled 5HT+ cells throughout the rostrocaudal extent of DR and MR, predominantly in the ventromedial part of DR and also close to the midline in the MR. Thus we have shown that there are topographically precise (see Gonzalo-Ruiz et al., 1991) GABAergic inputs to ipsilateral AD and AV, that the brain stem cholinergic projection is predominantly from LDTN to ipsilateral AV, and that the serotonin projection is to the entire ATN and is derived predominantly from the ipsilateral DR.

48.10

SEROTONIN 1B RECEPTORS ON THALAMOCORTICAL AXONS FORM A VIBRISSAE-RELATED PATTERN IN THE SOMATOSENSORY CORTEX OF PERINATAL RATS. M.J. Leslie*, C.A. Bennett-Clarke, N.L. Chiaia and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Serotonin immunoreactive (5HT-IR) axons from nucleus raphe dorsalis (NRD) and perhaps the median raphe nucleus (MRN) transiently form a dense, patterned distribution in the primary visual (area 17) and somatosensory (SI) cortices of perinatal rats. The proposed effects that 5HT axons may have on the development of thalamocortical projections [Blue et al. (1991) Cerebral Cortex 1: 380-389] may be mediated by specific 5HT receptors. Receptor binding autoradiography using [^{125}I]-cyanopindolol (ICYP) in the presence of isoproterenol was used to determine the distribution of one 5HT receptor subtype, $5HT_{1B}$, in the cortices of perinatal and adult rats. In perinatal (P-8) rats, binding of ICYP is very dense in lamina IV of SI and area 17. It forms an ordered representation of the body surface which includes patches matching the distribution of the mystacial vibrissae in SI and a very dense patch which encompasses all of area 17. In adult rats ICYP binding was lower in SI and area 17 than in surrounding cortex even though patterned 5HT-IR is no longer present. Additional experiments were done to establish the location of these receptors. Subcutaneous injection of the neurotoxin 5,7-dihydroxytryptamine made on P-0 eliminated the dense and patterned 5HT-IR in area 17 and SI cortex of rats killed on P-8, but had no qualitative effect upon the distribution of $5HT_{1B}$ receptors. In contrast, lesion of the dorsal thalamus made on P-6 resulted in complete loss of the dense $5HT_{1B}$ receptor pattern on P-8. Thus, in perinatal rats, $5HT_{1B}$ receptors are present transiently on thalamocortical afferents in area 17 and SI and have a spatial distribution similar to that of serotonergic axons arising from NRD and MRN. EY08661

48.11

LOCALIZATION OF α_{2A}-ADRENERGIC RECEPTORS IN CULTURED CELLS AND RAT BRAIN USING A SUBTYPE-SPECIFIC POLYCLONAL ANTIBODY. D.L. Rosin*, D. Zeng, T. Riley, R. Stornetta, P.G. Guyenet, K.R. Lynch. Depts. of Pharmacology and Biochemistry, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

Three subtypes of the α_2-adrenergic receptor (α_2-AR) have been cloned to date. The amino acid sequence of these molecules is highly conserved with the exception of the third intracellular (i3) loop. Because of our interest in studying the function and distribution of α_2-AR in brain, we have developed a subtype-specific polyclonal antibody directed against the rat α_{2A}-AR. A nucleotide fragment encoding 50 amino acids of the α_{2A}-AR i3 loop was subcloned into the pGEX.KG vector downstream of the gene for a 26-kDa helmintic glutathione S-transferase (GST). The resulting GST/α_{2A}-AR i3 fusion protein was expressed in E. coli, purified, and used to immunize rabbits. The specificity of affinity-purified antibody was tested by western blot and immunocytochemical analysis of COS cells transfected with the gene encoding either the α_{2A}- or α_{2C}-AR subtype. Immunoreactivity was present only in α_{2A}-transfected COS cells and not in α_{2C}-transfected cells. The apparent molecular weight of the recombinant α_{2A}-AR decreased from 65 kDa to 45 kDa following treatment with N-Glycanase^R suggesting that the molecule is glycosylated in COS cells. Western blots of crude membrane fractions of rat brain cortex, medulla, and hypothalamus revealed a single immunoreactive band of 55 kDa which was still present following preabsorption of antibody with GST but not with fusion protein. We are currently investigating the utility of this antibody for immunocytochemical studies of the α_{2A}-AR in rat brain. (Supported by U.S. P.H.S. Grant DA 07216).

48.12

LOCALIZATION AND QUANTIFICATION OF THE DOPAMINE TRANSPORTER: COMPARISON OF [^{3}H]WIN 35,428 AND [^{125}I]RTI-55. C.L. Coulter, H.K. Happe, D.A. Bergman, L.C. Murrin*. Dept. of Neurology, Creighton Univ. Sch. Med., Omaha, NE 68131 and Dept. of Pharmacology, Univ. Nebraska Med. Sch., Omaha, NE 68198.

A number of radioligands have been identified that are useful for study of the dopamine transporter (cocaine-binding site). Data suggest that the ligands do not always label the same number of sites, leading to differences in the reported density of transporter. We here compare results using two ligand in use in our lab, [^{3}H]WIN 35,428 and [^{125}I]RTI-55, both with a high affinity for the transport site. Rat brain was sectioned at 16 ı and thaw-mounted onto subbed slides. Characterization of the binding of ligands to sections used the tissue-swipe method. Based on these studies, sections were incubated in 10 mM sodium phosphate buffer, 0.1 to 0.32 M sucrose, 120 mM NaCl, pH 7.4 at RT for 60-120 min. Tissue was rinsed 2 X 2 min in ice-cold buffer and sections apposed to Hyperfilm. Analysis of autoradiograms gave essentially the same localization for both ligands. Prefrontal cortex was somewhat higher in binding sites than frontal cortex, though both were relatively low. Highest binding was in the caudate-putamen, where there was a gradient decreasing from anterior to posterior and lateral to medial. The ventrolateral region of the posterior striatum was more heavily labelled than the dorsomedial region. The anterior to posterior gradient was less apparent with WIN. The nucleus accumbens had about twice as many sites as the n. accumbens shell. There was also an anterior to posterior gradient in the olfactory tubercles. Based on studies with one concentration of ligand, WIN gave approximately 5 times as many sites as RTI. A detailed analysis will be required to determine the reason for this discrepancy. Supported by NS23975.

48.13

MOSAIC DISTRIBUTION OF D_1 RECEPTOR mRNA IN PRIMATE STRIATUM. M.S. Rappaport*[1,3], J. H. Morrison[1], A. Prikhozhan[1], G.W. Huntley[1] and S.C. Sealfon[1,2]. [1]Fishberg Center for Research in Neurobiology and [2]Department of Neurology, The Mount Sinai School of Medicine, NY 10029; [3]NYS Psychiatric Institute, NY 10032

The distribution of dopamine D1 receptor (D1R) mRNA in monkey striatum (Macaca Fascicularis) was studied by *in situ* hybridization using a [^{35}S]-labeled monkey D1R cRNA probe. Film autoradiograms revealed a distinctly heterogeneous distribution of D1R mRNA in the caudate nucleus. Comparison with adjacent sections stained for the matrix-predominant 28 kd calcium-binding protein (calbinden), which was used to define striosomal and matrix compartments in striatum, showed that D1R mRNA was largely restricted to calbindin-poor striosomes. Moreover, emulsion autoradiography revealed that the D1R cRNA probe did not hybridize with all neurons in the caudate striosomes, possibly reflecting another level of compartmental organization. In the putamen, D1R mRNA-containing cells were not concentrated in calbindin-poor striosomes but were distributed more homogeneously. In the transition from putamen to ventral striatum the presence of D1R mRNA declined sharply. While generally sparse in the ventral striatum, D1R mRNA labeling usually appeared on film autoradiograms as dense, irregularly shaped islands (approx. 0.1-0.5 mm diameter) most prevalent in the nucleus accumbens and adjacent basal forebrain, particularly in the olfactory tubercule. Nissl counter-stained sections revealed these to be clusters of closely packed D1R mRNA-containing cells. Given the known differences in chemoarchitecture and connectivity of the dorsal and ventral striatum as well as of the striosomal and matrix compartments, the distribution of D1R mRNA suggests that this receptor subtype subserves a unique function in intrastriatal neuronal processing in the nonhuman primate.

(Supported by the Aaron Diamond Foundation and NIH Schizophrenia Fellowship).

48.14

MULTIPLE DOPAMINE RECEPTOR SUBTYPE mRNAs IN HUMAN AND MONKEY MOTOR CORTEX AND STRIATUM. G.W. Huntley*, S.C.Sealfon, A.Prikhozhan, & J.H.Morrison Fishberg Res. Ctr. for Neurobiology, The Mount Sinai School of Medicine, New York, NY 10029.

Dopamine is thought to play a critical role in motor and cognitive function through actions mediated by specific receptors, multiple subtypes of which have recently been identified. *In situ* hybridization was used to examine the expression and distribution of mRNAs encoding D_1, D_2 and D_5 receptors in the motor cortex of humans and in the motor cortex and striatum of macaque monkeys. Hybridization to each receptor probe yielded labeled cells throughout layers II-VI in the motor cortices from both primate species, with virtually all of the large pyramidal (Betz) cells showing significant hybridization to each of the three receptor probes. Thus, many different functional classes of motor cortex output neuron may be directly involved in dopamine circuits. In monkey striatum, in contrast to motor cortex, only the D_1 and D_2 receptor probes showed significant hybridization. Both probes gave a distinct mosaic pattern of hybridization. Thus, primate neocortex possesses a broader representation of the dopamine receptor subtype mRNAs examined in comparison with striatum. Moreover, the unexpected presence and widespread distribution of D_2 and D_5 receptor mRNAs in cortex suggests that all three receptors play a crucial role in the dopaminergic modulation of cognition and motor behavior and in dopamine dysfunction associated with neuropsychiatric disorders.

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48.15

DISTRIBUTION AND REGULATION OF DOPAMINE RECEPTORS IN THE NUCLEUS ACCUMBENS OF THE RAT. **A.L. Jongen-Rêlo, H.J. Groenewegen* and P. Voorn. Dept. of Anatomy and Embryology, Vrije Universiteit, Amsterdam, The Netherlands.**

The distribution of dopamine D-1 and D-2 receptors in the nucleus accumbens (Acc) was investigated by means of in vitro receptor autoradiography using [3H]-SCH-23390 or [3H]-raclopride, respectively. Both D-1 and D-2 receptors were heterogeneously distributed in the Acc. D-1 binding in rostral areas was higher in the shell than in the core, whereas caudally in the Acc binding was higher in the core than in the shell. D-2 binding was lower in the shell than in de core throughout the rostrocaudal extent of the nucleus. A rostrocaudal high-to-low gradient for both D-1 and D-2 binding was found over the Acc as a whole. The effects of unilateral 6-OHDA lesions of the ascending dopaminergic system on D-1 and D-2 mRNA levels and the ligand binding to the dopamine receptors were studied by means of quantitative in situ hybridization and receptor autoradiography, respectively. Two weeks after the lesion D-1 mRNA levels were 15% lower in both the Acc and the caudate-putamen (CP) in the lesioned side compared to the control side. The levels of D-2 mRNA had increased 13% in the Acc and 36% the CP. Preliminary results show similar changes in the D-1 and D-2 binding, viz. a decrease in D-1 and an increase in D-2 binding in the lesioned side, both in the Acc and the CP. These findings indicate that the D-1 and D-2 receptors are differentially distributed in the subregions of the Acc and that dopaminergic inputs from the midbrain may exert opposite regulatory effects on the tow receptor subtypes.

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48.16

DIFFERENTIAL ALTERATIONS OF D1, D2 DOPAMINE AND A2 ADENOSINE RECEPTOR mRNA IN RAT STRIATUM FOLLOWING A VOLKENSIN LESION OF STRIATONIGRAL NEURONS.

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and Univ. of Virginia Health Sci. Center, Charlottesville, VA 22908.

A2 adenosine receptor (A2R) mRNA is abundantly co-expressed in the same striatal neurons as D2 receptor (D2R) mRNA; A2R mRNA is never expressed in the same cells as D1 receptor (D1R) mRNA (Mol. Brain Res., in press). Using the retrogradely transported toxic lectin volkensin injected unilaterally in the substantia nigra (SN), Harrison et al. (Brain Res. 1990, 528: 317) showed that striatal D1Rs are predominantly localized to striatonigral neurons. We used the same technique to test the hypothesis that A2R-expressing striatal neurons were not part of the striatonigral pathway. Volkensin (2.5ng) was injected unilaterally into the SN of six rats. At 10 days post lesion, 10-12u sections were processed for in situ hybridization using ^{35}S-labeled riboprobes for D1R, D2R, and A2R mRNAs. Striatal D1R mRNA ipsilateral to the volkensin injection was reduced 24% while D2R and A2R mRNA were reduced 2 and 3%, respectively. D1R mRNA was reduced 43% in the "maximally affected area" while D2R and A2R mRNA were reduced 14 and 12%, respectively. The parallel changes in D2R and A2R mRNA after SN volkensin lesion support the co-localization of A2 and D2 receptors in the striatum. The differential changes in D1R, D2R and A2R mRNAs confirm D1R localization on striatonigral neurons, and suggest that a small population of striatonigral neurons express D2R and A2R mRNAs.

48.17

DISTRIBUTION OF [125I]NCQ 298 BINDING TO DOPAMINE D2 RECEPTORS IN HUMAN BRAIN. A M Murray*[1], K A Neve[2] and JN Joyce[1]. Departments of Psychiatry and Pharmacology, Univ Penn Sch Med, Philadelphia, PA[1]; Research Service, VA Medical Center, Portland, OR[2].

The binding of D2 receptors in human brain was measured using quantitative receptor autoradiography with the substituted salicylamide [^{125}I]NCQ 298 and was compared to the distribution observed with [^{125}I]epidepride as previously described (Joyce et al., *JPET*, **235**;1253,1991). Tissue sections were incubated with 250 pM [^{125}I]NCQ 298 at RT for 60 min. Non-specific binding was similar with butaclamol or raclopride. The pattern of [^{125}I]NCQ 298 distribution was very similar to that seen with [^{125}I]epidepride and is expressed as fm/mg protein. The highest binding was seen in the striatum (77 caudate, 85.5 putamen) and in the pallidal complex, with binding in the external segment (33.2) higher than the internal segment (12), and is equivalent to the binding of 80 pM [^{125}I]epidepride. Within the midbrain the binding distribution of [^{125}I]NCQ 298 correlated well with the distribution of DA containing cell bodies and was approximately 50% of epidepride binding. The SNpc (21), SNpl (13) and A10 (6.3) exhibited higher binding than the A8 (2.3) or central grey (4.8). Binding of [^{125}I]NCQ 298 in the superior colliculus (10.6) equaled 30% of epidepride binding in this region. Binding in the frontal cortex was highest in the internal layers(I-II 1, III-IV 1.29, V-VI 2.4). Binding in the motor cortex was highest in lamina IIIb (I-II 0.9, IIIb 1.8 , V-VI 1.2) and low throughout somatosensory cortex (I-II 0.6, III-IV 1.2, V-VI 0.7). Highest levels of cortical binding were seen in the temporal cortex (I-II 3.9, III-IV 3, V-VI 3.6). Binding was higher in external layers of perirhinal cortex (I-II 8.1, III-IV 4.1, V-VI 4.5). Within the hippocampal complex, binding was present in subiculum (3.2), CA3 (3.5) and dentate gyrus (3.3). Binding in cortical regions was 2-3 fold lower than that observed with 80 pM [^{125}I]epidepride. Further characterization experiments of NCQ 298 are in progress. **This work was supported by the NPF, MH43880 and AG09215.**

48.18

THE PATTERN OF DOPAMINE D2 RECEPTORS IN MEDIAL TEMPORAL LOBE OF HUMANS AS COMPARED TO RAT AND CAT. S.K. Goldsmith* and J.N. Joyce, Dept. Psychiatry, Lab Chemical Neuroanatomy, Univ. Pennsylvania School of Medicine, Philadelphia, PA, U.S.A.

In our intial reports of the distribution of D2 receptors in human temporal lobe we described a complex pattern with unique features to humans (Goldsmith et al, 1991; Joyce et al, 1991). Further study of the D2 family of receptors using ^{125}I epidepride autoradiography, immunohistochemical (IHC) localization of dopaminergic innervation (comparative anti-TH & anti DBH), and nissl and Timms histochemistry revealed new observations. Timms stain for zinc and heavy meatals revealed a comlementary pattern to that of D2 receptors in cat and human, but not rat. D2 receptors are visible in the facia dentata, region of the dendrites of the granule cells, capping the dense Timm's stain of the DG granule cells. Timms staining is intense throughout hilus, whereas D2 binding is low in external hilus and higher where mossy fibers exit the hilus. D2 receptor numbers are greater in CA regions more distal to the DG, whereas Timms stain ends obliquely at the CA3-CA2 border. In human tissue only, dense bands of D2 receptors are observed in temporal association cortex. The bands either cross all cortical layers, or show a paucity of binding in the mid-layers. They have a mean width of 2.75mm (+/-0.62), and are continuous for an A-P distance of at least 1500 μm. The location of the bands are in good concordance with the observations of Penfiled and Perot (1963) on regions giving auditory experiential responses upon stimulation. In the 8 cases studied to date, the majority of bands were observed in the lateral and inferior aspects of the superior temporal gyrus, with occassional bands observed on the lateral surface of the middle temporal gyrus and on the parahippocampal cortices (Brodmann's 22,41,42 and 20,21,37). No bands of D2 binding were observed in any cases in anterior Heschl's gyrus, with bands found in one case in the posterior primary auditory cortex. IHC of adjacent slabs of temporal cortex, reveal minimal levels of dopmanergic innervation, with no increase in the regions of the bands. This suggests a possible site of antispychotic action of D2 antagonists Further studies will compare these bands in controls and dementing neuropsychiatric diseases (Joyce et al, this meeting). **Funded by MH43852, MH 43880, AG 09215.**

48.19

D3 DOPAMINE RECEPTOR GENE EXPRESSION IN RAT AND HUMAN STRIATUM. C. Le Moine*; I. Aubert; M.P. Martres+, P. Sokoloff+; J.C. Schwartz+ and B. Bloch. URA CNRS 1200- Laboratoire d'Histologie-Embryologie, Université de Bordeaux II, 146 rue Léo Saignat, 33076 Bordeaux, France; + Unité de Neurobiologie et Pharmacologie, INSERM U 109, Centre Paul Broca, 2 rue d'Alesia, 75014 Paris, France.

The D3 dopamine receptor genes from rat and human have been recently cloned and it has been demonstrated that the D3 receptor displays both specific pharmacology and pattern of expression, as compared to others identified dopamine receptor genes, particularly the D1 and D2 receptors (Sokoloff et al.,1990; Bouthenet et al., 1991). We used rat and human D3 RNA probes labeled with S^{35}-UTP to study D3 receptor gene expression in rat and human striatum. We developed various strategies of double in situ hybridization on same sections and/or 3µm-thick adjacent sections to identify the phenotype of neurons expressing the D3 mRNA in the rat and human striatum, especially with respect to the main striatal populations, enkephalin/GABA and substance P/GABA neurons. In addition, comparison with D1 and D2 receptor gene expression were performed on adjacent sections in order to study potential co-localization of several receptor mRNAs within same neurons. In the rat striatum, D3 mRNA were detected in the accumbens nucleus, the islands of Calleja and the olfactory tubercle. Double in situ hybridization experiments show the presence of D3 mRNA in part of the enkephalin neurons of the olfactory tubercle.

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS: PEPTIDES AND GABA

49.1

SUBSTANCE P BINDING IN THE GUT: DIFFERENCES BETWEEN CAT AND BB RAT. Q. Yu* and A. Ouyang. GI Division, Department. of Medicine., University of Pennsylvania, Philadelphia, PA 19104.

Substance P (SP) plays an important role in the control of gut motility in health and disease. With the aid of emulsion and film autoradiography, we investigated the distribution of SP binding in the gut of cat and BB rat. The binding of 100 pM ^{125}I-SP to 10 µm sections was performed at 20 °C for 2 hr.

In the cat gut, SP binding was dense in the ileum, ileocecal sphincter and colon. Lower level of binding was observed in the pylorus and duodenum. A negligible amount of binding was observed in the esophagus, lower esophageal sphincter, cardiac and antral areas of the stomach, jejunum and rectum. Emulsion autoradiography demonstrated dense patches of binding in the myenteric plexus of several gut regions (antrum, pylorus, duodenum, ileum, and colon). In the muscle layers, SP binding was confined to the circular muscle and to a much lesser extent the longitudinal muscle. Some binding was observed in the deep muscular plexus of the ileum and colon. A negligible amount of binding was observed in the muscularis mucosa, submucus plexus, and mucosa. These results are in contrast to those found in the rat gut, where dense binding of SP was found from the stomach to the colon. The binding was negligible in the esophagus. In the rat antral pyloric area, SP binding was found mainly in the circular muscle, with a lesser amount in the myenteric plexus and muscularis mucosa, and even less in the longitudinal muscle. SP binding to small intestine was primarily in the deep muscular plexus, and to a lesser extent in the circular muscle and myenteric plexus. SP binding in the colon was confined to the circular muscle, the myenteric plexus, and deep muscular plexus. Therefore, in different gut regions of these two animal species, the innervation of SP neurones may be different and SP may contribute differently to the control of gut motility.

49.2

PATTERNS OF ENKEPHALIN EXPRESSION IN THE AVIAN BRAIN. M. Molnar, G. Fontanesi, G. Casini, B.M. Davis, P. Bagnoli, N. Brecha*, Depts. Anat. & Cell Biol. and Med., BRI, CURE, UCLA and VAMC-West Los Angeles, Dept. Physiol. & Biochem., Univ. Pisa, Univ. Kentucky, & Dept. Environ. Sci., Univ. Tuscia.

Enkephalin (ENK) peptides are widely distributed in the vertebrate central nervous system. Using *in situ* hybridization histochemistry with a chick prepro-ENK RNA probe, we studied the distribution of ENK peptides in the visual system, limbic system and basal ganglia of chick and pigeon brains. We also mapped ENK immunoreactivity in the pigeon brain using a polyclonal antibody directed to met-ENK. In the pigeon visual system, ENK mRNA was present in a few scattered cells in the hyperstriatum accessorium of the Wulst, in the n. lateralis anterior thalami and in the optic tectum (TeO). In the limbic system, cells expressing ENK mRNA were found in the hippocampal complex, septum and n. of the diagonal band. Finally, hybridization signal was very high in the basal ganglia, in the n. tegmenti peduncolopontinus pars compacta, and in the n. spiriformis lateralis, which is a relay nucleus for the basal ganglia efferents to the TeO. Immunohistochemical studies confirmed the presence of ENK containing cell bodies in these areas. In addition, immunolabeled fibers were observed in the hyperstriatum dorsale (HD) of the Wulst and in the superficial layers of the TeO. In the chick brain, the distribution of the ENK mRNA showed the same general pattern observed in the pigeon, with the addition of strong hybridization signal in the HD. The pattern of ENK expression in avian brains appears remarkably similar to that of mammals.
Supported by NEI grant EY04067 and VA Medical Research Funds.

49.3

LOCALIZATION OF ENKEPHALINASE (EC 3.4.24.11) mRNA IN RAT BRAIN BY *IN SITU* HYBRIDIZATION. F. Gaudoux, G. Boileau, G. Lavigne* and P. Crine. Département de biochimie, Faculté de médecine, Université de Montréal, Montréal, Québec, Canada. H3C 3J7.

The enkephalinase (EC 3.4.24.11) messenger RNA (mRNA) in adult rat brain presented a markedly heterogeneous localization among various brain regions. A strong signal was observed in the glomerular layer of the olfactory bulb, the olfactory tubercle, the caudate putamen, the habenular, anterior pretectal, interpeduncular, red, dorso tegmental, pontine and vestibular nuclei, the mammillary bodies, the Purkinje cells and the choroid plexus of the fourth ventricle. A large number of areas such as the cortex, the Dentate gyrus, the accumbens and the arcuate nuclei, the superior and inferior colliculi and a few regions in the thalamus at the mesencephalic level provided a moderate or low signal of hybridization. The majority of these regions are also known to contain the enkephalinase protein. On the other hand, the globus pallidus, the substantia nigra and the central gray matter which show a high or a moderate amount of enkephalinase respectively did not contain any enkephalinase mRNA. Comparison of the regional distribution of enkephalinase mRNA with that of its translation product provides insight into enkephalinase neuronal pathways in the central nervous system.

49.4

DIFFERENTIATION OF BOMBESIN RECEPTOR SUBTYPES IN THE RAT GASTROINTESTINAL TRACT. E.E. Ladenheim*, R.T. Jensen, S.A. Mantey, J.E. Taylor and T.H. Moran. Dept. Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205, NIDDKD, NIH, Bethesda, MD 20892 & Biomeasure, Inc., Hopkinton, MA 01748.

Two distinct bombesin (BN) receptor subtypes have recently been identified. One subtype, characterized in pancreatic tissue, has a high affinity for gastrin-releasing peptide (GRP) and a low affinity for neuromedin B (NMB) (GRP-preferring). The other, characterized in esophagus, has a higher affinity for NMB than for GRP (NMB-preferring). We have previously demonstrated the presence of both receptor subtypes in rat brain. To determine whether BN receptor heterogeneity is also present in the rat gastrointestinal (GI) tract we examined the competitive inhibition of ^{125}I-(Tyr^4)BN binding by NMB and [D-F$_5$-Phe^6,D-Ala^{11}]BN(6-13)O-methyl ester (ME) using receptor autoradiography. Although ^{125}I-(Tyr^4)BN binds equally to both receptor subtypes, NMB inhibits binding to NMB-preferring receptors while ME inhibits binding to GRP-preferring receptors. In the stomach, high densities of BN binding sites were observed in the circular muscle of the fundus and corpus. This binding was completely inhibited by ME but only partially inhibited by NMB. A similar pattern of binding was observed in the descending colon. In contrast, binding in the duodenum, ileum and jejunum was localized to the submucosal layer and this binding was completely inhibited by NMB. Therefore, BN receptors in the circular muscle layer were predominantly GRP-preferring, while those in the submucosal layer were NMB-preferring. Our results show that, as in rat brain, BN receptor heterogeneity is present in the rat GI tract.

49.5

PROLACTIN-RECEPTOR LOCALIZATION IN THE BRAIN BY *IN SITU* HYBRIDIZATION. S. Chiu, R.F. Bulleit* and P.M. Wise. Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201.

Recent evidence shows that prolactin may mediate parental behavior or influence pituitary hormone secretion by direct actions on the brain. We demonstrated previously using PCR that hypothalamic and pituitary tissues express prolactin-receptor mRNA (Endocrinology 130:1747, 1992). To localize the expression of prolactin-receptor mRNA at the individual cell level in the brain, we performed *in situ* hybridization. Two separate regions (180 and 247 bp long) of the prolactin-receptor cDNA (gift of PA Kelly), both of which are common to the long and short forms of the receptor, were subcloned and used to transcribe ^{35}S-labeled cRNA probes. We maximized the sensitivity and specificity of the *in situ* hybridization by altering several steps including increasing (a) specific activity of the probe, (b) hybridization stringency, (c) wash stringency and (d) wash time. Our preliminary results reveal that the choroid plexus contains the highest level of prolactin-receptor mRNA expression. Scattered cells in the suprachiasmatic nucleus, dorsomedial hypothalamic nucleus, ventromedial hypothalamic nucleus and subfornical organ also contain prolactin-receptor mRNA. Therefore, prolactin may mediate its actions on the brain by a known prolactin-receptor. Supported by NIH HD 15955 and AG02224.

49.6

COMPARISON OF THE LOCATION OF VITAMIN D RECEPTOR SITES AND CALBINDIN 28kD-IMMUNOREACTIVITY IN BASAL FOREBRAIN REGIONS OF THE SIBERIAN HAMSTER. I.M. Musiol*, M. M. Perez-Delgado, H.-J. Bidmon, A. Bartke and W.E. Stumpf. Dept. Cell Biology & Anatomy, Univ. of North Carolina, Chapel Hill,NC 27599-7090 and Dept. Physiology, Southern Illinois University, Carbondale, IL.

The present study aims at investigating wether or not vitamin D receptor sites correlate with the topography of neurons producing calbindin 28 kD [28kD CaBP] in the brain of the Siberian hamster (*Phodopus sungorus*), a species strongly adapted to the annual cycle. For the autoradiographic experiment adult animals were injected sc with tritiated 1,25-dihydroxyvitamin D_3 (Musiol et al., Neuroscience,1992). To locate 28kD CaBP-immunoreactive cells, a monoclonal mouse antibody (Sigma) was used on 50 μm vibratome sections. Regions in which both vitamin D receptor sites and calbindin 28kD-like cells are present include the diagonal band of Broca and the bed nucleus of the stria terminalis in the anterior basal forebrain and the intralaminar and midline nuclei in the thalamus. There are several regions containing calbindin 28kD-positive neurons which apparently do not contain vitamin D target neurons, e.g. the reticular thalamic nucleus (although a target in rat and mouse) and the mediodorsal thalamic nucleus. In general there is only limited regional correspondence between vitamin D target neurons and calbindin 28kD-immunoreactive neurons, which are more widely distributed.

49.7

FIRST EXTENSIVE VISUALIZATION OF GLYCINE IMMUNOREACTIVE FIBERS AND PERIKARYA IN THE RAT BRAIN. P-H. Luppi*, P. Fort and M. Jouvet, CNRS UA 1195, INSERM U52, Dept. Med. Exptl., Fac. Med., Univ. C. Bernard, 8 Av. Rockefeller, 69373, LYON, CEDEX 08, France

There is strong evidence that glycine (GLY) is a major inhibitory neurotransmitter in the mammalian central nervous system (CNS). However, extensive description regarding the distribution of GLY-immunoreactive (IR) elements within the CNS is surprisingly lacking. By combinational means of a highly specific antiserum to GLY (a gift from Dr WENTHOLD) and a very sensitive immunohistochemical method with streptavidin-HRP, we attempted to fill this gap in the rat brain. Animals under deep nembutal anesthesia were perfused with a fixative containing 4% paraformaldehyde, 0.25% glutaraldehyde and 0.2% picric acid. Cryostat (20µm) free floating sections were incubated 3 days in rabbit GLY antiserum (1/2000) followed by a biotinylated donkey-anti-rabbit IgG (1/2000) and streptavidin-HRP (1/40 000) overnight at 4°C. The sections were then immersed in 0.02% DAB and 0.6% nickel ammonium sulfate in Tris-HCl buffer (0.05M, pH 7.6) with 0.003% H_2O_2. The antiserum has been characterized elsewhere (*Neuroscience, 1987, 22:897-912*) and by us with immunoblot analysis. We visualized a considerable number of GLY-IR fibers at all medullary levels. The highest density was observed in the cranial motor nuclei, followed by the bulbar reticular formation, the nucleus of the solitary tract and the spinal trigeminal nucleus. More rostrally, dense plexus of GLY-IR fibers were seen in the ponto-mesencephalic reticular formation, the periaqueductal gray, the parabrachial area and the locus coeruleus. Finally, a number of GLY-IR fibers appeared in the raphe nuclei, the lateral preoptic and hypothalamic areas and the basal forebrain. With regard to the perikarya, in addition to the cellular groups already described, GLY-IR neurons were observed in a large number of structures such as gracilis and cuneatus nuclei, the area postrema, the nucleus of the solitary tract, the trigeminal sensory complex and the ventral nucleus of the lateral lemniscus. We observed for the first time a large number of positive cells in the magno- and parvicellular bulbar reticular nuclei, the nucleus raphe magnus, the ventral part of the pontine and mesencephalic reticular formation, the ventral part of the periaqueductal gray matter and reticular areas lateral to it. These results suggest that, GLY might be a basic inhibitory neurotransmitter not only in the spinal cord and the medulla but also in the mesencephalon, the hypothalamus and basal forebrain.

49.8

Differential expression of Glutamate decarboxylase and GABA Transporter messenger RNAs in rat brain.
Catherine Jomary*, Dawn Savery, Pei-Ying Wu, Glen Wotherspoon, Sharon Averill, John V. Priestley and Marcus Rattray. Divisions of Biochemistry and Physiology, United Medical and Dental Schools, University of London, St Thomas's Hospital, London SE1 7EH, U.K.

Molecular cloning has produced several markers which can be used to elucidate the distribution and regulation of presumptive GABA neurones such as the rate-limiting enzyme of GABA synthesis, glutamate decarboxylase (GAD65 and GAD67) and a GABA transporter (GAT1). The relative distributions of expression of these genes can be used to reveal phenotypic differences which may underlie differences in physiological function and regulation of GABA neurones.

Using 35-S labelled oligonucleotide probes directed against rat GAD67 and GAT1 messenger RNAs, we have carried out in situ hybridization analyses using liquid emulsion techniques to detect mRNA in presumptive GABA neurones. GAT1 mRNA was found many neurones. In some brain regions, including hippocampus, and cerebral cortex, the distribution of expression of both genes was similar, as expected. However, in other regions gross differences in gene expression were observed. For example, in the thalamic reticular nucleus, striatum, and cerebellar purkinje cells high levels of GAD67 mRNA were expressed in the absence of significant GAT1 expression. Conversely, high levels of GAT1 mRNA with low levels of GAD67 mRNA were found in other regions, including the molecular layer of the cerebellum.

The study shows that neurones differ in their expression of mRNAs which produce proteins involved in presynaptic aspects of GABA neurotransmission. The consequences of this phenotypic subdivision of GABA neurones is not clear, but may offer insight into the selective regulation of GABA neurones by drugs such as benzodiazepines.

49.9

EARLY CO-EXPRESSION OF $GABA_A$ RECEPTOR SUBUNIT mRNAs IN THE DEVELOPING RAT SPINAL CORD AND DORSAL ROOT GANGLIA. W. Ma, M. Poulter, P. Saunders and J.L. Barker, Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

The anatomical localization and relative abundance of diverse $GABA_A$ receptor subunit (α1, ,α2, α5, β2, β3 and γ2) mRNAs were examined in neurons of the spinal cord and dorsal root ganglia (DRG) of embryonic and postnatal rats by *in situ* hybridization histochemistry with oligonucleotide probes. During embryogenesis, the mRNAs encoding α2, β3 and γ2 subunits were the most abundantly expressed of those examined, β2 subunit mRNA was moderate, while α1 and α5 subunit mRNAs were barely detected in both the spinal cord and DRG. The α2, β3 and γ2 subunit mRNAs were clearly expressed at E13 in motoneurons of the cervical cord, but were not detectable in the germinal zone. The mRNAs then extended from the ventral to dorsal portions of the mantle zone and became abundant in all types of spinal neurons between E17-20. After birth, all mRNAs declined in abundance. A marked reduction in α2 and γ2 subunit mRNAs was observed in the dorsal horn, whereas β2 and β3 subunit mRNAs were eliminated in the ventral horn. Four weeks after birth, α2 and γ2 subunit mRNAs were highly expressed in motoneurons, whereas β2 and β3 subunit mRNAs were more concentrated in the superficial dorsal horn. The expressions of most mRNAs in DRG roughly paralleled those in the spinal cord, but appeared 1-2 days latter. β3 subunit mRNA expression, however, was detected in the cervical DRG at E13. These results reveal a heterogeneous and transient co-expression of specific $GABA_A$ subunit mRNAs during the development of the spinal cord and DRG.

49.10

NEURONS WITH GLUTAMIC ACID DECARBOXYLASE mRNA IN THE HUMAN HIPPOCAMPUS. G. Zhang*, W.S. Young, III and R.E. Powers. UAB Brain Resource Program, University of Alabama at Birmingham, Birmingham, Alabama 35294 and NIMH, Bethesda, MD 20892.

The distributions of neurons that contained mRNA encoding gamma amino decarboxylase (GAD) were mapped in hippocampus from five normal human subjects using hybridization histochemistry performed with a ^{35}S-oligodeoxynucleotide probe. Neurons with GAD transcripts were identified in multiple rostrocaudal levels of human hippocampus and entorhinal cortex. Many neurons in the granule cell layer and the polymorphic layer of the dentate were labeled. Approximately 20% of granule cells were labeled. A modest number of medium sized and small multipolar and bipolar neurons was present in the CA_4 region. Few pyramidal neurons were labeled in the CA_3 through CA_1, but a modest number of small fusiform and large basket shaped neurons were present in the stratum oriens. A modest population of labeled neurons was present in the subiculum and entorhinal cortex.

The types and distribution of these neurons are similar to those stained with antisera to GAD or GABA in monkey and rodent hippocampus in previous studies. These data support GABA as a hippocampal neurotransmitter in the human hippocampus.

49.11

AN ELECTRON MICROSCOPIC QUANTITATIVE ANALYSIS OF GABA AXON TERMINALS ON CELL BODIES, PROXIMAL DENDRITES AND INITIAL SEGMENTS OF ABDUCENS MOTONEURONS IN THE RAT. G.Chazal*, H. Bras, F. Lahjouji and A. Barbe, CNRS UPR 418, 280 Bd Ste Marguerite, 13009 Marseille (France).

Electrophysiological investigations suggested that ipsilateral abducens motoneurons receive an inhibitory GABAergic input from the vestibular formation. The aim of this study was to describe the location, frequency and synaptic covering of GABAergic and unlabeled axon terminals (ATs) on identified abducens motoneurons. We developed a double labeling technique: the motoneurons were stained retrogradely or intracellularly with HRP, GABA ATs were visualized with a post-embedding procedure. The somata were serially cut and we analyzed 7 to 12 levels per soma.

We identified 1560 ATs on 76 levels of 9 somata and 1176 ATs on 63 proximal dendritic profiles. At each level, all the ATs were randomly distributed. The relative frequency of GABA ATs greatly varied between levels (ranging between 0 to 47%) but the mean average for each somata varied between 5 to 25%. This frequency was slightly lower on the proximal dendrites. The synaptic covering for the labeled ATs was in the range of 2.5 to 14% for the somata while it varied from 4.2 to 8.9% for the dendrites. The lowest values were always found on the somata and dendrites located in the rostral part of the nucleus. The postsynaptic membrane length devoided of apposition was about 60% for both somata and dendrites. GABAergic ATs filled with pleomorphic vesicles were observed making symetrical synaptic contacts on axons. They were more numerous on the axon hillock than on the initial segment.

This study demonstrated that the distribution of GABAergic ATs is higher on the somata than on the proximal dendrites. The presence of such ATs on the initial segment raises the question of inhibitory input on the modulation of efferent messages.

49.12

DISPARITY BETWEEN DIAZEPAM-INSENSITIVE $GABA_A$ RECEPTOR BINDING AND mRNA DISTRIBUTIONS

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Molecular biological experiments have previously shown that non-neuronal cells transfected with cloned $GABA_A$ receptor α4 or α6 subunits express $GABA_A$ receptors which bind [^{3}H]RO 15-4513 with high affinity but not classical benzodiazepines (Wisden et al. FEBS 289:227, 1991; Luddens et al. Nature 346:648, 1990). The reported distribution of [^{3}H]RO 15-4513 binding to benzodiazepine-insensitive sites in the rat brain (Sieghart et al. J. Neruochem. 48:46 1987), however, was consistent with the localization of α6 subunit in the cerebellum but not the broader distribution of the α4 subunit. In order to clarify the disparity, *in situ* hybridization signals of DNA probes against the α4 and α6 subunit mRNAs were compared with [^{3}H]RO15-4513 binding on sections from the same adult male rat. The α4 mRNA was highly expressed in thalamus, cerebellum, hippocampus, and striatum. The α6 subunit mRNA was found exclusively in the cerebellum. Autoradiograms of [^{3}H]RO15-4513 binding showed strong labelling of many structures in the rat brain. Diazepam-insensitive binding of [^{3}H]RO15-4513 was measured by the addition of 10 uM diazepam to the incubation solution. Autoradiograms of diazepam-insensitive binding produced intense graining over the cerebellar granule cell layer, but structures which were positive for the α4 but not the α6 subunit mRNA showed very little if any graining. These data suggest that the α4 subunit is found in combination with other subunits which mask its diazepam-insensitive characteristics.

49.13

EXPRESSION OF GAD ISOFORMS IN THE ENDOCRINE PANCREAS. C. Sternini*, R. De Giorgio, M. Lai and K. Anderson. CURE, Dept. of Medicine, Brain Research Institute, UCLA, and VAMC at Wadsworth, Los Angeles, CA 90073.

GAD, the GABA biosynthetic enzyme, consists of two major forms, GAD_{65} and GAD_{67} that are derived from separate genes. In addition to neurons, GABA is found in endocrine structures, including the pancreas. We examined the expression of GAD_{65} and GAD_{67} isoforms in the rat endocrine pancreas using specific RNA probes and antibodies. *In situ* hybridization histochemistry with ^{35}S-labeled rat antisense GAD_{65} and GAD_{67} RNA probes showed that both isoforms are expressed in the core of the islets, where B-cells are located. In general, the GAD_{65} probe gave a stronger signal than the GAD_{67} probe. Hybridization signal above background levels was not observed with sense RNA probes. When using an RNA probe complementary to somatostatin (a marker for D-cells), labeling was observed on cells at the peripheral margin of the islets, where D-cells are located, but not over the core of the islets. This further supports the specificity of the GAD labeling. Antibodies specific for each GAD isoform labeled B cells; staining was stronger with the GAD_{65} than with the GAD_{67} antibody. Double-labeling confirmed the colocalization of GAD and insulin. The present results, together with the identification of GAD_{65} as a key autoantigen in type I diabetes, suggest a role for the GABA system in islet cell function and dysfunction.

Supported by NIH grant DK38752. We thank Drs. A.J. Tobin & D. L. Kaufman for the GAD cDNAs and GAD_{67} antibody.

49.14

MEASUREMENT OF REGIONAL KINETICS OF LIGAND BINDING TO THE $GABA_A$ RECEPTOR BY QUANTITATIVE AUTORADIOGRAPHY. A. M. Mans*and K. M. Kukulka. Dept. Physiology and Biophysics, The Chicago Medical School, North Chicago, IL 60064.

The $GABA_A$/benzodiazepine receptor mediates inhibitory GABA neurotransmission throughout the brain. Earlier studies led to the conclusion that the affinity of binding to the GABA and benzodiazepine sites is regionally homogeneous; however in these studies only a few regions were analyzed. We used quantitative autoradiography to measure ligand binding density (B_{max}) and affinity (K_d) in more detail. Consecutively cut brain sections containing common regions were incubated in a range of different concentrations of each ligand to enable kinetic parameters to be calculated. The ligands used were a GABA site agonist (^{3}H-muscimol) and two benzodiazepines (^{3}H-flunitrazepam and ^{3}H-Ro15-1788). The affinity of binding was clearly regionally heterogeneous with all three ligands measured. The range of values among areas was three- to eight-fold and appeared to be continuous. The densities, as expected, also showed differences among brain regions. The results are consistent with the presence of multiple subtypes of the $GABA_A$/benzodiazepine receptor distributed heterogeneously throughout the brain.

Supported by NIH grant NS 16389.

SECOND MESSENGERS I

50.1

HUMAN MELANOMA (M-6) CELLS EXPRESS HIGH-AFFINITY RECEPTORS FOR MELATONIN. L.P. Niles*, S.-W. Ying and C. Tenn. Department of Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

A high-affinity receptor for melatonin has been localized and partially characterized in vertebrate brain and other tissues. This receptor exhibits both high- and low-affinity states, and it is sensitive to guanine nucleotides and monovalent cations. Moreover, melatonin signalling via this receptor is transduced by a pertussis toxin-sensitive G protein, resulting in inhibition of forskolin-stimulated adenylate cyclase activity in brain membranes. In order to further study this receptor, we have examined various cell lines for its presence. We now report that human malignant melanoma (M-6) cell membranes bind [^{125}I]melatonin with a pharmacological profile (IMEL>6-Cl-MEL≥MEL>>NAS>5-HT) which is similar to that of the above-mentioned receptor. Saturation binding experiments revealed the presence of two affinity states (K_{d1}=290 ± 85 pM, B_{max1}=2.5 ± 0.4 fmol/mg protein; K_{d2}= 6.5 ± 1.1 nM, B_{max2}=24 ± 5 fmol/mg protein), with abolishment of the high-affinity state in the presence of GTP. Similarly, GTP caused a rightward shift in agonist competition curves, with conversion of all high-affinity sites to a low-affinity state. More importantly, in intact M-6 cells, melatonin caused a significant suppression of forskolin-stimulated cAMP accumulation with an EC_{50} value of ~5 nM.

These findings indicate that the M-6 melatonin receptor is pharmacologically and functionally similar to that in the brain. The M-6 cell line should be useful for further characterization of this G protein-coupled melatonin receptor.

Supported by the OMHF and NSERC Canada.

50.2

METABOTROPIC GLUTAMATE RECEPTORS COUPLED TO ADENYLYL CYCLASE DECREASE FORSKOLIN-STIMULATED cAMP FORMATION IN CEREBELLAR GRANULE CELLS. J. T. Wroblewski*[1], M. Majewska[1] and B. Wroblewska[2]. [1]Fidia-Georgetown Institute for the Neurosciences and [2]Department of Biology, Georgetown University, Washington D.C. 20007.

In primary cultures of cerebellar granule cells, glutamate was shown to enhance the accumulation of cGMP mediated through the formation of nitric oxide. Now we report that these neurons express glutamate receptors which contribute to the regulation of cAMP concentrations. Incubation of granule cells with forskolin caused a concentration-dependent increase in cAMP accumulation which was decreased in a dose-dependent manner by the simultaneous addition of either glutamate, ibotenate, *trans*-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD) or quisqualate. The inhibition reached about 50% of the forskolin-stimulated cAMP accumulation and was not prevented by antagonists of ionotropic glutamate receptors. The nonselective phosphodiesterase inhibitor 3-isobutyl-1-methylxantine increased the forskolin-stimulated cAMP production but failed to reduce the inhibition elicited by glutamate receptor agonists. This indicates that glutamate decreases cAMP levels by inhibiting its production rather than enhancing its degradation and suggests an inhibitory coupling of glutamate receptors with adenylyl cyclase. This possibility was further investigated in a preparation of washed membranes isolated from granule cells where the activity of adenylyl cyclase was stimulated by GTP and measured by cAMP accumulation in presence of phosphodiesterase inhibitors. Under these conditions glutamate, quisqualate and ACPD decreased the GTP-stimulated adenylyl cyclase activity. These results suggest that cerebellar granule cells in primary culture express metabotropic glutamate receptors which are negatively coupled through G proteins to adenylyl cyclase and show an agonist pharmacology similar to that reported for the cloned mGluR2 receptor (Tanabe et al., Neuron 8:169, 1992).

50.3

CYCLIC ADENOSINE 3'5'-MONOPHOSPHATE POTENTIATES EXCITATORY AMINO ACID AND SYNAPTIC RESPONSES OF RAT SPINAL DORSAL HORN NEURONS. M. Randic*, R. Cerne, G. Gerber and M.C. Jiang. Dept. of Vet. Physiology and Pharmacology, Iowa State University, Ames, IA 50011.

Intracellular recordings were made from rat dorsal horn (DH) neurons in the in vitro slice preparation to study the actions of cyclic adenosine 3',5'-monophosphate (cyclic AMP). In the presence of TTX, bath application of the membrane permeable analogue of cyclic AMP, 8-Br cyclic AMP (25-100μM) caused a small depolarization of the resting membrane potential, a long-lasting increase in the spontaneous synaptic activity and the amplitude of presumed monosynaptic excitatory postsynaptic potentials (EPSPs) evoked in the substantia gelatinosa neurons by orthodromic stimulation of a dorsal root. In the presence of TTX, 8-Br cyclic AMP enhanced in a reversible manner, the depolarizing responses of a proportion of DH neurons to N-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, quisqualic acid and kainic acid. The effects of 8-Br cyclic AMP on the resting membrane potential and the NMDA responses of DH neurons were mimicked by reducing phosphodiesterase activity with 3-isobutyl-1-methylxanthine, but not by cyclic AMP applied extracellularly. Intracellular application of a protein inhibitor of cyclic AMP-dependent protein kinase (PKI) into DH neurons prevented the 8-Br cyclic AMP-induced potentiation of the NMDA response of these cells. Moreover, we have found the enhancement of the NMDA-induced current responses upon i.c. application of the catalytic subunit of protein kinase A in the whole-cell voltage-clamped isolated rat DH neurons. These results suggest that in the rat spinal dorsal horn the activation of the adenylate cyclase-cyclic AMP-dependent protein kinase system may be involved in the regulation of the sensitivity of postsynaptic excitatory amino acid (NMDA, AMPA, KA) receptors and primary afferent neurotransmission, including nociception. (Supported by BNS and USDA).

50.4

NAAG DECREASES FORSKOLIN-STIMULATED cAMP FORMATION IN CEREBELLAR GRANULE CELL. B. Wroblewska* and J.H. Neale, Department of Biology, Georgetown University, Washington, DC 20057.

N-Acetylaspartylglutamate (NAAG) fulfills several criteria for action as a peptide neurotransmitter. We tested the ability of NAAG to activate metabotropic "glutamate" receptors in cultured cerebellar granule cells under conditions of negligible extracellular conversion of NAAG to glutamate and NAA. In contrast to glutamate, NAAG failed to stimulate PI turnover in these cells. However, NAAG produced a dose-dependent decrease in forskolin-stimulated cAMP, while having no effect on basal levels of this cyclic nucleotide. Similar inhibition of cAMP levels was obtained with L-glutamate, ibotenate, and trans-ACPD, consistent with activation of mGluR2 or a related receptor. The potencies of glutamate and NAAG were similar. These effects were obtained in the presence of NMDA receptor blockade and phosphodiesterase inhibition. The NAAG-induced reduction in forskolin-stimulated cAMP formation was restricted to the alpha-linked peptide, beta-NAAG did not affect cAMP levels. These data support the hypothesis that NAAG is an agonist for the subclass of metabotropic "glutamate" receptor(s), acting via G-protein to inhibit adenylate cyclase, while not activating directly those related glutamate receptors which are coupled to PI turnover (supported by NS 28130).

50.5

MOLECULAR CHARACTERIZATION OF A 63 kDa CALMODULIN-DEPENDENT PHOSPHODIESTERASE THAT IS HIGHLY EXPRESSED IN STRIATUM. Joseph W. Polli and Randall L. Kincaid*. Section on Immunology, NIAAA, Rockville, MD 20852.

Two classes of calmodulin-dependent phosphodiesterase (CaM-PDE) have been identified biochemically in neuronal tissue (i.e. 61 and 63 kDa isoforms). We have cloned a cDNA for the 63 kDa isoform from mouse brain by using PCR of mRNA isolated from micropunched Purkinje and granule cell layers. Deduced protein sequence showed ≈60% homology with that of the 61 kDa isoform, indicating that these proteins are products of different genes that diverged long ago. Southern blot analysis suggests high nucleotide sequence conservation of the 63 kDa CaM-PDE among mammalian and avian species. Northern blots showed a single 3.6 kb transcript in all brain regions; weak hybridization also was seen in lung, spleen, thymus and testes. Remarkably, striatum contained an 8-fold higher CaM-PDE mRNA concentration than cortex, hippocampus, olfactory bulb and midbrain, and 40-fold more than cerebellum. This finding, in conjunction with electron microscopy studies demonstrating a postsynaptic localization of CaM-PDE, would suggest that the 63 kDa isoform has an important role in Ca^{2+}-modulated regulation of cyclic nucleotides in striatal neurons. Studies to determine which subpopulation of striatal neurons express the 63 kDa isoform and how this isoform may be regulated by neuronal input are in progress.

50.6

CHARACTERIZATION OF cAMP EFFLUX FROM RAT STRIATUM AS MEASURED BY IN VIVO MICRODIALYSIS K.Suyama, M.I.Masana, I.N.Mefford*, H.Manji and W.Z.Potter. Section on Clinical Pharmacology, ETB, NIMH, Bethesda, MD, 20892

It has been demonstrated that activation of postsynaptic receptors can be monitored by in vivo microdialysis of adenosine 3'5'monophosphate (cAMP). We report the application of this technique to examine the effect of dopaminergic and adrenergic agents on the efflux of cAMP from rat striatum. Microdialysis probes with a 3 mm membrane tip were stereotaxically inserted into the anterior striatum (AP +0.7 mm, L +2.7 mm, V -7.0 mm) of chloral hydrate-anesthetized rats. Probes were perfused with artificial cerebrospinal fluid at a flow rate of 2 µl/min. Two hours after insertion of the probe, samples were collected every 20 min and analyzed for cAMP by RIA. Basal cAMP was taken as the average cAMP concentration for the 1 h prior to drug administration (1.00 ± 0.09 fmol/min, n=29). As reported by others, infusion of dopamine (up to 100 µM) did not significantly alter cAMP efflux, whereas a D1 receptor agonist (+) SKF 38393 (100 µM) increased. We also observed that a D2 receptor antagonist (-) sulpiride (100 µM) modestly increased cAMP. Interestingly, the beta-adrenergic receptor agonist (-) isoproterenol produced a concentration-dependent increase in cAMP efflux. These data raise the possibility that non-neuronal elements and/or stimulation of extrasynaptic receptors contribute to the cAMP response observed by in vivo microdialysis in the striatum.

50.7

REMOXIPRIDE ACTION ON DOPAMINE D2 RECEPTOR MEDIATED INHIBITION OF CYCLIC AMP IN RAT STRIATUM. A.I. Westlind-Danielsson*, K. Gustafsson and I. Andersson. Department of Neuropharmacology, CNS R&D, ASTRA ARCUS AB, S-151 85 Södertälje, Sweden.

The ability of the novel neuroleptic compound remoxipride to block dopamine (DA) D2 receptor mediated inhibition of cyclic AMP (cAMP) formation was studied in rat striatal tissue pieces. The action of remoxipride was compared to the effect of the high affinity D2 antagonists, haloperidol, (-)sulpiride, raclopride and NCQ 298. In addition, four of the main metabolites of remoxipride, identified in rat but not man, were also examined. The compounds were assessed either in the presence of (1) DA (100 µM) (2) SKF 38393 (100 µM) + pergolide (1 µM) or (3) DA (100 µM) + forskolin (1 µM). Remoxipride along with one of the metabolites (up to 100 µM) did not prove as potent in blocking the D2 mediated inhibition of cAMP accumulation as the other compounds tested. Furthermore, neither remoxipride or any of the metabolites had any significant action (100 µM) on either basal or SKF 38393 stimulated cAMP levels, making antagonistic or agonistic properties of these compounds at e.g. the DA D1 receptor unlikely.

The inclusion of a D2 antagonist in (3) was found to be expressed as a large (>2-fold) augmentation of cAMP formation. Block of the D2 receptor could be thought to liberate a growing D2 mediated inhibitory constraint following increased DA receptor-effector coupling provided by (3). Thus, the span available for carrying out pharmacological manipulation of D2 receptor mediated inhibition of cAMP provided by this system greatly facilitates the study of D2 antagonistic properties of different compounds.

50.8

ATTENUATION OF AFTERHYPERPOLARIZATION IN LOCUS COERULEUS NEURONS BY cAMP IS INDEPENDENT OF PROTEIN KINASE ACTIVATION. G. Aston-Jones* and R. Shiekhattar, Department of Mental Health Sciences, Division of Behavioral Neurobiology, Hahnemann University, Broad and Vine, Philadelphia, PA 19102, U.S.A.

Afterhyperpolarizations (AHPs) that follow action potentials are a prominent mechanism for the control of neuronal excitability. The AHP amplitude of locus coeruleus (LC) neurons recorded in brain slices was reduced by superfusion of 20 µM forskolin (FSK; AHP amplitude=17.0±0.7 mV before vs 11.5±0.6 mV following 10-20 min FSK, $p<0.001$, n=11), an activator of adenylate cyclase, or by membrane permeable analogs of adenosine 3',5'-cyclic monophosphate (cAMP, $p<0.01$; n=7). Furthermore, superfusion of the phosphodiestrase inhibitor, Ro20-1724, also attenuated the AHP ($p<0.05$, n=4). In contrast, superfusion of 1,9-dideoxyforskolin (DFSK), the FSK analog that does not activate adenylate cyclase, had no effect on these AHPs (16.7±1.5 mV before vs 16.7±2.3 mV following DFSK, n=4). Co-application of a protein kinase inhibitor (H-8, KT 5720, or RpcAMPS) with either FSK or 8-Br-cAMP failed to block the reduction of AHP amplitude, but blocked the cAMP-dependent protein kinase (PKA)-mediated enhancement of opiate responses in the same LC neurons (reported in Shiekhattar and Aston-Jones, this volume). Furthermore, the AHP amplitude was significantly reduced (to 88% of control, n=11 for each treatment) in LC neurons taken from chronically morphine-treated rats, a treatment known to increase adenylate cyclase activity. These results indicate that elevation of intracellular cAMP reduces the AHP amplitude in LC neurons through a mechanism independent of PKA. Such a mechanism may underlie the activation of LC neurons during local opiate withdrawal (Kogan et al., Eur. J. Pharmacol. 211 ;47 (1992) and Hirata et al., this volume). Supported by PHS grants NS 24698 and DA 06214.

50.9

CYTOSKELETAL REGULATION OF NEURONAL SIGNAL TRANSDUCTION: TRANSFER OF GTP FROM TUBULIN TO Gα IN A RECONSTITUTED SYSTEM M.M. Rasenick* and S. Roychowdhury, Dept. of Physiology and Biophysics, U. Illinois College of Medicine, Chicago, IL 60680

This laboratory has suggested that synaptic membrane tubulin elicits a receptor-independent activation or inhibition of adenylyl cyclase. Previous studies have shown that tubulin binds specifically to the signal transducing proteins, Gsα and Giα1, and a direct nucleotide transfer from tubulin to G-protein is the mechanism for adenylyl cyclase regulation (*Biochemistry 30*: 110957-10965, 1991). In order to identify the participants in the nucleotide transfer process in detail, the tubulin-AAGTP was incubated with purified Gi/o or recombinant αi1 in solution. Transfer of the hydrolysis-resistant photoaffinity GTP analog, AAGTP, from tubulin to αi was observed, and AAGTP was bound to tubulin or Gα (no AAGTP was released into the medium). Free Gpp(NH)p in 10-100 fold excess of tubulin-AAGTP did not affect the transfer significantly. The addition of βγ subunits to α, did not influence the transfer. Two well defined monoclonal antibodies against tubulin, whose epitopes are located near the C-terminal of α and β subunits respectively, partially blocked the transfer of nucleotide to the G protein, but did not disrupt the physical complex formed between these proteins. This suggests multiple sites of interaction between tubulin and G protein may be required for the nucleotide transfer process. The results also suggest that the complex formation between tubulin and G protein α subunits allows for the direct transfer of GTP from tubulin to Gα. It is suggested that synaptic membrane tubulin, with GTP bound, is capable of bypassing the receptor to activate G protein mediated cellular processes.

50.10

ISOFORMS OF NEURONAL PROTEIN PHOSPHATASE 1 E.F. da Cruz e Silva and P. Greengard*. Laboratory of Molec. & Cellular Neuroscience, The Rockefeller University, New York, 10021

DARPP-32 (dopamine- and cAMP-regulated phosphoprotein, M, = 32,000) is a member of a family of related proteins which includes inhibitor-1 (I1) and G-substrate. Whereas I1 is found throughout most brain regions and peripheral tissues, DARPP-32 is particularly enriched in the medium spiny neurons of the striatum and G-substrate is most abundant in the cerebellum. Both DARPP-32 and I1 can be phosphorylated in response to increased intracellular cAMP, and thus become potent inhibitors of protein phosphatase 1 (PP1). Given the central role of reversible protein phosphorylation in the control of neuronal function, it is important to determine which phosphatases are expressed in brain, and in particular, which isoforms of PP1 are co-expressed with DARPP-32 in the striatum. To this end, a rat striatum cDNA library was screened at low stringency using a rabbit cDNA encoding the alpha isoform of protein phosphatase 1 (PP1α). Clones encoding two different isoforms of PP1 were isolated and characterized. One was identical to PP1α, whereas the other was identical to PP1γ. Isoform-specific peptides were designed based on the deduced amino acid sequences which were used to raise antibodies. The availability of isoform-specific antibodies allowed us to investigate the distribution of the two PP1 isoforms by Western blotting. Although detected in all brain regions and peripheral tissues analyzed, both were found to be particularly abundant in the striatum. Their distribution was similar in various brain regions with only three notable exceptions. That is, PP1γ was relatively more abundant than PP1α in the olfactory bulb, olfactory tubercule and pineal gland. The PP1γ-specific antibody also identified an alternatively spliced isoform in the testis which could not be detected in any of the other tissues or brain regions tested. We have also recently isolated a PP1β cDNA from a rat cortex library.

50.11

PHOSPHORYLATION OF RAT DARPP-32 BY CASEIN KINASE I. F. Desdouits, D. Cohen, P. Greengard*, and J.-A. Girault*
INSERM U114, Chaire de Neuropharmacologie, Collège de France, Paris, and *The Rockefeller University, New York, 10021 NY.

DARPP-32 (dopamine- and cAMP-regulated phosphoprotein, apparent Mr~32 000) is an inhibitor of protein phosphatase 1, enriched in striatonigral neurons, and activated by cAMP-dependent phosphorylation on Thr_{34}. On immunoblots prepared with SDS extracts of rat striatum, DARPP-32 migrates as a doublet. The lower band of the doublet disappears when the tissue is incubated at 30°C before homogenization. When DARPP-32 is immunoprecipitated from ^{32}Pi-labeled striatal slices, the lower band of the doublet contains an acidic phosphopeptide, phosphorylated on serine, and not found in the upper band. Recombinant rat DARPP-32 has been subjected to site-directed mutagenesis, expressed in *Escherichia coli*, and purified to homogeneity. When it is phosphorylated *in vitro* by casein kinase I, wild type DARPP-32 migrates as a doublet on SDS-PAGE. The lower band of this doublet contains an acidic phosphopeptide, which has the same migration on 2-dimensional peptide maps as the phosphopeptide derived from the lower band of DARPP-32 labeled in striatal slices. When mutated DARPP-32, in which Ser_{137} has been replaced by an alanine, is phosphorylated by casein kinase I, the appearance of a doublet and the acidic phosphopeptide are not observed. These results demonstrate that rat DARPP-32 is phosphorylated on Ser_{137} by casein kinase I, and that this phosphorylation is responsible for a slightly faster migration of the protein on SDS-PAGE. Partial phosphorylation by casein kinase I in intact neurons is likely to account for the migration of striatal DARPP-32 as a doublet.

50.12

PHOSPHORYLATION OF DARPP-32 BY FORSKOLIN IS POTENTIATED BY GABA IN STRIATAL SLICES. G.L.Snyder*, G. Fisone, and P. Greengard.The Rockefeller Univ., NY, NY 10021

DARPP-32, a dopamine and cAMP-regulated phosphoprotein, Mr= 32,000) is highly enriched in the medium spiny-type striatal neurons that comprise the striato-nigral projection. Phosphorylation of DARPP-32 at a single threonine residue is cAMP-dependent and is increased in striatal slices by forskolin, an activator of adenylyl cyclase. Recently, we reported that GABA (100 μM) increased the threonine phosphorylation of DARPP-32 either alone, or in combination with l-DOPA (100 μM), precursor of dopamine which in turn activates dopamine D-1 receptors that are linked to adenylyl cyclase (Snyder et al.,Soc. Neurosci. Abst.21:988). Using an antibody able to selectively detect the phosphorylated form of DARPP-32, we are examining the mechanism by which GABA increases phosphorylation of DARPP-32. We have found that GABA (100 μM) also potentiates DARPP-32 phosphorylation in striatal slices in which cAMP-dependent phosphorylation is increased by forskolin (10 μM). Since GABA is known to hyperpolarize neurons, thereby decreasing calcium influx, and since DARPP-32 is dephosphorylated by a calcium-dependent phosphatase (i.e.,calcineurin), these data are consistent with the hypothesis that GABA may increase DARPP-32 phosphorylation by inhibiting calcium-dependent dephosphorylation.

50.13

REGULATION OF CYCLIC AMP-DEPENDENT PHOSPHORYLATION IN RAT FRONTAL CORTEX BY CHRONIC LITHIUM: IDENTIFICATION OF DARPP-32 AND OTHER LITHIUM REGULATED PHOSPHOPROTEINS. X. Guitart* and E.J.Nestler. Lab. of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06508.

In contrast to other psychotherapeutic drugs, lithium acts on postreceptor components of signal-transduction pathways. Most studies of lithium concern its acute actions, with relatively little information available on its chronic effects on the nervous system. As chronic lithium treatment has been shown to alter adenylate cyclase and G-protein expression in brain (Colin et al., *PNAS* 88, 10634, 1991), we studied the next step in the cyclic AMP pathway: cyclic AMP-dependent protein phosphorylation.

By use of back phosphorylation and immunoblotting techniques, we found that among a number of lithium-regulated phosphoproteins, chronic lithium increases by 25% the levels of DARPP-32 in rat frontal cortex. This effect was also seen using other chronic antidepressant treatments (imipramine, tranylcypromine) and was specific to the frontal cortex. In contrast, chronic treatment with haloperidol, morphine, or cocaine did not alter DARPP-32 levels. Chronic lithium was also shown to alter the subcellular distribution of cyclic AMP-dependent protein kinase activity in frontal cortex, as observed previously for other antidepressants (Nestler et al., *J. Neurochem.* 53, 1644, 1989). These findings suggest that regulation of the cyclic AMP pathway, including increased levels of DARPP-32, could reflect a common functional effect of long-term exposure to lithium or other antidepressant treatments.

50.14

EFFECT OF ORAL-ADMINISTRATION OF UCB 29427, A NEW POTENTIAL ANTIDEMENTIA DRUG, ON THE CYCLIC AMP (cAMP) GENERATING SYSTEM IN RAT BRAIN. A. El Tamer*, K. Raikoff, E. Wulfert* and I. Hanin. Loyola Univ. Chicago Stritch Sch. Med., Dept. Pharmacology, Maywood, Il. 60153 and *UCB s.a., Pharmaceutical R&D, Brussels, Belgium.

Ucb 29427 (2-cyclopropyl-4-cyclopropyl amino-6-morpholino-1,3,5-triazine), is a close structural analog of ucb 11056. Since ucb 11056 has been shown to have a strong potentiating effect on the cAMP generating system in rat brain (El Tamer et al. Soc. Neurosci. Abstr. 17:605, 1991), we decided to investigate whether ucb 29427 has similar activity. Fifteen min. post-oral administration of ucb 29427 (3 mg/Kg) we observed a significant increase in vivo, in cAMP levels in the hippocampus (26%, $P<0.05$), septum (9.2%, N.S), hypothalamus (19%, $P<0.05$) and cortex (10%, N.S). In-vitro studies showed that ucb 29427 (0.1 and 1 μM) induced 50% and 120% of an additional increase, respectively, in norepinephrine (NE)-stimulated response of cAMP in hippocampal slices. Acute, oral treatment of rats with ucb 29427 (3 mg/Kg) or longer periods of treatment for 4, 7, 14 and 28 days, twice a day did not alter or modify NE-stimulated cAMP formation in hippocampal slices. These combined data demonstrate that: 1) ucb 29427 has a strong potentiating effect on the cAMP generating system in the rat brain; 2) this effect is measured at doses 3 times (in-vivo) and 100 times (in-vitro) lower than ucb 11056; and 3) long-term oral treatment with ucb 29427 does not alter the cAMP generating system in the rat brain.

50.15

ROLE OF THE NEUROSPECIFIC CALMODULIN SENSITIVE ADENYLYL CYCLASE IN NEUROPLASTICITY. D. R. Storm*, Z. Xia, and E. J. Choi. Department of Pharmacology, University of Washington, Seattle, WA 98195.

Mammalian tissues express at least eight distinct adenylyl cyclases including two calmodulin sensitive enzymes, type I and III. One of these enzymes, the type I adenylyl cyclase is thought to be important for learning and memory. The present study demonstrates that expression of mRNA for this enzyme is limited to neural tissues, including brain, retina, and adrenal medulla. *In situ* hybridization indicates that mRNA for this enzyme is expressed in specific areas of brain that have been implicated in learning and memory, including the neocortex, the hippocampus, and the olfactory system. Although it has been generally assumed that calmodulin sensitive adenylyl cyclases can function to couple increases in cellular free calcium to elevation in cAMP *in vivo*, this had not been experimentally demonstrated. This question was examined by expressing the type I adenylyl cyclase in human 293 cells. Cellular cAMP in 293 cells expressing type I adenylyl cyclase was markedly elevated by a calcium ionophore, A23187. Furthermore, the muscarinic agonist carbachol also increased cAMP in 293 cells expressing the type I enzyme, due to increases in cellular free calcium. These data indicate that the type I adenylyl cyclase can be stimulated by calcium *in vivo*. We propose that the calcium stimulation of type I adenylyl cyclase may provide synergistic regulation by calcium and cAMP of a variety of neuronal functions, either because of convergence of cAMP and calcium stimulated phosphorylations, or because of stronger or more persistent cAMP signals caused by increased calcium level.

50.16

HUMAN FETAL BRAIN ADENYLYL CYCLASE: CLONING, CHROMOSOMAL MAPPING, AND EXPRESSION. E.C. Villacres,[1,2] L. H. Bookbinder,[1] Z. Xia,[1*] S. Edelhoff,[3] D.A. Adler,[3] C.M. Disteche,[3] and D.R. Storm[1]. Departments of Pharmacology[1], Psychiatry & Behavioral Sciences[2], and Pathology[3], Univ. of Washington School of Medicine, Seattle, Washington 98195.

The neural specific calmodulin-sensitive adenylyl cyclase (Type I), which was first cloned from bovine brain, has been implicated in memory and learning. A 2.2 kb fragment from the bovine cDNA was used to screen a human fetal brain cDNA library. A resulting clone with a 3.8 kb insert, contains sequence which coincides with the 3' end 2522 basepairs of the bovine open reading frame. This clone has been fully sequenced and shows 87% nucleic acid and 92% translated amino acid sequence identity to the bovine clone. Comparison of the amino acid sequence from our human clone to the bovine clone shows that most differences are in the carboxy-terminal 100 amino acid residues. This region contains the only putative cyclic AMP-dependent protein kinase phosphorylation site in the molecule. To determine if these sequence differences reflect differences in the regulation of the human cyclase activity, a chimera was constructed which contains the 5' half of the bovine type I cyclase and the 3' half corresponding to the human type I cyclase. The activity of this chimera has yielded no significant difference in calmodulin and calcium sensitivity when compared to the wild type bovine adenylyl cyclase. *In situ* hybridization was used to localize the human type I adenylyl cyclase to the proximal portion of the short arm of chromosome 7, using both the human 3.8 kb insert, and a bovine probe which is specific for the brain type I calmodulin-sensitive adenylyl cyclase.

50.17

DISTRIBUTION OF ADENYLATE CYCLASE mRNA SPECIES IN RAT BRAIN. M.N. Gannon* and B.S. McEwen. Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

We have previously shown that manipulation of the hypothalamic-pituitary-adrenal axis modulates the activity of calmodulin (CaM) -dependent adenylate cyclase(s) (AC), in a regionally selective manner (e.g. J.Neurochem. 55:276-284,1990). We are interested in determining whether parallel changes occur in AC mRNA species, since many effects of glucocorticoids are ascribed to a genomic site of action. Towards this end, we are developing selective oligonucleotide probes to identify the distribution of CaM-dependent (AC1), CaM-independent (AC2) and olfactory-selective (AC3) mRNA species for different isoforms of AC. Preliminary northern analysis suggests that high levels of AC1 mRNA are present in the cortex, hippocampus, cerebellum and olfactory bulb: In contrast, high levels of AC2 mRNA are only observed in the olfactory bulb, with moderate levels in the hippocampus. Lower levels of both AC1 and AC2 mRNA were observed in other brain regions. By northern analysis, AC3 mRNA was only present in olfactory bulb. Preliminary *in situ* hybridization analysis confirms the heterogeneous distribution of different AC mRNA species, and suggests that AC3 mRNA might be also be present in a discrete population of hippocampal neurons.

Supported by a NARSAD Junior Investigator Award (MG) and MH41256 (BM).

50.18

PROTEIN KINASE A AND PROTEIN KINASE C REGULATE THE TYROSINE HYDROXYLASE mRNA LEVEL IN HYPOTHALAMIC NEURONS. W. Kedzierski, N. Aguila-Mansilla, G.P. Kozlowski*, and J.C. Porter. Depts. of Obstetric and Gynecology and Physiology, University of Texas Southwestern Medical Center, Dallas TX.

Long-term regulation of catecholamine synthesis is based on changes in the synthesis of tyrosine hydroxylase (TH). TH synthesis is, in turn, determined by the level of TH mRNA. The TH mRNA level may be influenced by extracellular factors, which mediate their actions through second messenger pathways. The purpose of these studies was to investigate the roles of protein kinase A and protein kinase C in regulating TH mRNA level in hypothalamic neurons cultured in serum-free medium. TH mRNA was quantified by an S1 nuclease protection assay. Activation of the protein kinase A pathway by treatment of cultured neurons with 8-bromo cyclic AMP, forskolin or 3-isobutyl-1-methyl-xanthine caused a 1.5-2 fold increase in TH mRNA level. TH mRNA was also increased by 12-0-tetradecanoylphorbol 13-acetate or diacylglycerols, synthetic and natural activators of protein kinase C, respectively. Inhibition of protein kinase C for 16h with 0.05 μM or 0.5 μM calphostin C decreased the amount of TH mRNA to 67% or 56%, respectively, of that in unstimulated cells. These data suggest that both protein kinase A and protein kinase C pathways are involved in the regulation of the TH mRNA level in hypothalamic neurons. Supported by DK-01237, AG-08173, AG-04344, and DK-01237.

50.19

LOCALIZATION OF cGMP-STIMULATED PHOSPHODIESTERASE mRNA IN RAT BRAIN. J.G Corbin, D.R Repaske, M. Conti, and M.F. Goy*. Departments of Physiology and Pediatrics, and Neuriobiology Curriculum, University of North Carolina, Chapel Hill, NC 27599.

The cyclic GMP stimulated phosphodiesterase (cG-PDE) is a member of a larger family of enzymes that break down cAMP and cGMP to their respective 5'-nucleotide monophosphates. The cG-PDE preferentially hydrolizes cAMP, and is powerfully stimulated by cGMP (thereby producing cAMP decreases in response to cGMP increases).

Using PCR amplification, with PDE-specific primers, a 350bp cDNA clone of part of the cG-PDE gene was obtained from rat liver mRNA. The sequence of this clone is 93% homologous to the corresponding region of a previously cloned bovine brain and cardiac cG-PDE (Sonnenburg et al., J. Biol. Chem. **266**: 17655-17661 (1991)). A labelled probe derived from the clone reveals a 4.4 kb mRNA transcript in Northern blots. This transcript is highly enriched in rat brain. In agreement with this result, in situ hybridization analysis demonstrates the presence of cG-PDE mRNA in the hippocampal formation, basal ganglia, habenular nucleus, and pyriform, entorhinal and cerebral cortices. This suggests that within these specific populations of neurons, the cG-PDE is important in the regulation of intracellular levels of cAMP, and that this enzyme (perhaps via the nitric oxide/cGMP pathway) may play a role in the synaptic plasticity that is a prominent feature of several of these regions.

50.20

EFFECTS ON cAMP REGULATED GENE EXPRESSION BY TRANSFECTED G-PROTEIN SUBUNITS AND MUSCARINIC ACETYLCHOLINE RECEPTORS. J.C. Migeon, K.A. Hegewald, and N.M. Nathanson*. Department of Pharmacology, University of Washington School of Medicine, Seattle, WA 98195.

We have used a luciferase reporter gene under the transcriptional control of a cAMP response element (CRE) as a monitor of intracellular cAMP levels and cAMP regulated gene expression. Treatment of transfected JEG cells with forskolin to activate adenylate cyclase (AC)resulted in a greater than 10 fold increase in luciferase activity. Transfection with the oncogenic $G_i\alpha 2$ mutation in which glutamine 205 is replaced with leucine decreases forskolin-stimulated expression from the CRE- luciferase gene by up to 75%. This is consistent with previous experiments demonstrating constituitive activation of $G_i\alpha 2$ by this mutation. In addition, mutation of glycine 43 (corresponding to glycine 12 in p21 ras (G43V) to valine also decreases forskolin stimulated expression from the CRE luciferase gene by a maximum of 50%.

Treatment with the muscarinic agonist carbachol resulted in an increase in luciferase activity in JEG 3 cells transiently transfected with mouse m1 (8-10 fold) and chick m4 (3-5 fold) mAChRs. Treatment with the calcium ionophore A23187, the phorbol ester PMA, or both together increases luciferase activity by only 1.7, 1.5, and 2.4-fold suggesting that calcium-regulated pathways are not responsible for the observed increase in reporter gene activity. These data demonstrate that the $G_i\alpha 2$ G43V mutation can constituitively inhibit expression at CRE regulated genes while various transfected mAChR subtypes can increase expression of these genes in a carbachol dependant manner.

50.21

LEUCINE ZIPPER MUTATIONS IN CREB CAUSE SELECTIVE PATTERNS OF HETERODIMERIZATION. M.M. Loriaux, R.P. Rehfuss, R.G. Brennan, R.H. Goodman*. Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201.

Transcription factors in the CREB gene family have been implicated in virtually all aspects of second messenger mediated gene expression. These factors have three functional domains, an activator region, a basic DNA-binding domain, and a leucine zipper region which functions as a dimerization interface. CREB can form homodimers as well as heterodimers with related factors, and, in some instances, the process of heterodimerization can dramatically alter the biological properties of the transcription factor complex. Because of the ability of these transcription factors to combine in various patterns it is difficult to ascertain the functional capabilities of selected heterodimer combinations. Our studies are directed toward engineering factors that will pair in predictable patterns so that their functional contribution to the regulation of neuroendocrine gene expression can be determined.

The recently solved crystal structure of the yeast transcriptional activator GCN4 suggests that the Asn residue in the a3 position of the leucine zipper, which is conserved in all CREB related factors, functions by destabilizing the hydrophobic dimer interface. Presumably this destabilization is important in allowing CREB-like factors to exchange partners under physiological conditions. By using molecular modeling, we predicted that substitution of this Asn in the CREB leucine zipper with His would further destabilize homodimer formation while enhancing heterodimer formation. Gel shift assays in which full length mutant CREB was mixed with a truncated wild-type protein showed an elimination of mutant His homodimers and an enhancement of His:Asn heterodimer formation. This strategy of directing selective transcription factor pairing should be generally applicable to all CREB-related factors and should enable us to ascribe specific transcriptional properties to specific heterodimer combinations.

50.22

A FUNCTIONAL EXPRESSION CLONING STRATEGY FOR G-PROTEIN COUPLED, ADENYLYL CYCLASE ACTIVATING RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES -- USING THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR) GENE AS A SENSOR. Y. Uezono*, J. Bradley, J.R. Riordan#, N. Davidson, H.A. Lester and K. Zinn Division of Biology, Caltech, Pasadena, CA 91125; # Hospital for Sick Children, Tronto, Ontario, Canada

Functional expression cloning is widely used in isolating 7-helix G protein-activating receptor cDNAs with the *Xenopus* oocyte expression system. These receptors are coupled to 2nd messenger pathway such as the phospholipase C/IP_3/DAG or the adenylyl cyclase/cAMP pathway. The *Xenopus* oocyte system is remarkably sensitive for electrophysiological assays of receptors that couple to the phospholipase C pathway, because of the robust Ca^{2+}-activated Cl^- current. However there has been no comparably sensitive assay for changes in cAMP concentration. Here we show a fast and sensitive electrophysiological assay for changes in cAMP concentration by expressing the human cystic fibrosis transmembrane conductance regulator (CFTR) gene in oocytes. The CFTR acts as a Cl^- channel which is opened by phosphorylation via cAMP-dependent protein kinase. Several days after a CFTR transcript is injected into oocytes, Cl^- currents can be detected after activation of adenylyl cyclase by forskolin. Furthermore, after coinjection of CFTR and human β_2 adrenergic receptor transcripts, stimulation with the β_2 agonist isoproterenol elicits a robust Cl^- current.

This assay for activation of the cAMP pathway is currently being used in studies of the cloning of functional olfactory receptors (see abstract J. Bradley et al.). In addition, this method may be useful for cloning other cAMP-G protein-coupled receptors. (Support: GM-29836, NF-28182, CFF, Drawn Foundation)

50.23

Immunohistochemical Localization of Calcium/Calmodulin Dependent Protein Kinase-Gr in Mouse Brain. K. F. Jensen* and M. M. Bland, Neurotoxicology Division, HERL, US EPA, Research Triangle Park, NC 27711 and Cell Biology Division, Wellcome Research Laboratory, Research Triangle Park, NC 27709

Calcium/calmodulin dependent protein kinases are involved in the regulation of a variety of neuronal processes including neurotransmitter release, cytoskeletal alterations and gene expression. One such kinase, CaM kinase-Gr, is particularly abundant in the rat cerebellum and is present in both the nuclei and axons of granule cells. To determine if a similar pattern of distribution occurs in other species we extended our investigation to the mouse brain. We have used a rabbit polyclonal antibody made against a peptide of a highly conserved sequence at the carboxyl terminus of human CaM kinase-Gr. We observed a pattern of staining in the mouse brain similar to what we have previously reported in the rat using an polyclonal antibody against rat CaM kinase-Gr. Immunoreactivity was apparent in all major brain regions and was most prominent in the perikaryon of small neurons. As in the rat, the immunoreactivity was most dramatic in the granule cells of the cerebellum and was most prominent in the nucleus of these neurons. Immunoreactivity was also apparent in the molecular layer, presumably due to staining of the parallel fibers of granule cells. No nuclear staining was visible in Purkinje cells. The similarity in the pattern of distribution in mouse and rat brain suggests that CaM kinase-Gr may mediate calcium signaling in similar neuronal circuits in the two rodent species.

50.24

DIFFERENT DISTRIBUTION OF CA2+/CALMODULIN-SENSITIVE AND INSENSITIVE ADENYLATE CYCLASE mRNAs IN RAT HIPPOCAMPUS. N. MONS[1,2], M. YOSHIMURA[2], P. MOLLARD*[,3] and D.M.F. COOPER[2].
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2. UCHSC, Dept of Pharmacology, 4200 East Ninth Avenue. Denver (CO) 80262, USA. 3. URA-CNRS 1200, 146 rue Leo Saignat, 33076 Bordeaux cedex, France.

The pattern of Ca2+/calmodulin (CaM)-sensitive (Type 1) and Ca^{2+}/CaM-insensitive (Type 2) adenylate cyclase (AC) mRNAs expression has been examined in rat hippocampal formation using in situ hybridization. Cryostat sections were hybridized with ^{35}S-labeled synthetic oligonucleotide rat probes. A quantitative comparison among the signals obtained with the two probes was done after using the same concentration of probes and the same exposure protocol. Both Type 1 and Type 2 AC mRNAs were seen to be predominantly expressed in the granular cell layer of the dentate gyrus., but Type 1 AC mRNAs were much more expressed than type 2 AC mRNAs. The most notable difference between the signals obtained with the two probes occurs specifically in the pyramidal cell layers of CA1-CA4 fields. Examination of Type 1 AC mRNAs expression showed a moderate labeling that was restricted to the pyramidal cells of CA1-CA2 layers, as neither CA3 nor CA4 displayed appreciable specific labeling. By contrast, Type 2 AC mRNAs are expressed along CA1-CA4 fields, with no significant variation of hybridization density observed across the different CA fields and the dentate gyrus. These results provide an anatomical basis for studying factors that regulate expression of these isoforms, such as hormonal status, or in association with learning.

50.25

EXTRACELLULAR CYCLIC AMP, SECRETED BY ASTROCYTES IN RESPONSE TO BETA-ADRENERGIC STIMULATION, IS A SOURCE OF EXTRACELLULAR ADENOSINE IN CORTICAL CULTURES. P.A. Rosenberg* and Ya Li, Children's Hosp & Harvard Med Sch, Boston MA 02115.

We have previously shown that stimulation of cortical cultures with the beta-adrenergic agonist isoproterenol results in the efflux of significant quantities of cAMP into the extracellular medium, and an increase in extracellular adenosine concentrations. In the current studies, we address the question of whether the adenosine that accumulates in response to beta-adrenergic stimulation of cortical cultures actually derives from extracellular cAMP.

We found in a total of thirty-four experiments in which astrocyte-rich cultures were stimulated with 1 μM isoproterenol, and medium was assayed at thirty minutes, a significant increase ($p < 0.05$) of 65 $\pm$ 53% in the extracellular adenosine concentration in addition to the appearance of extracellular cAMP. If the adenosine that accumulates in response to beta-adrenergic stimulation was actually derived from extracellular cAMP, then one would expect that agents which block cAMP secretion, such as probenecid, should block this accumulation of adenosine. We found that 1 mM probenecid blocked both the extracellular accumulation of cAMP as well as the accumulation of adenosine in cultures stimulated with isoproterenol. This suggests that the extracellular adenosine which accumulates in response to beta-adrenergic stimulation is derived from extracellular cAMP.

Supported by PHS grant NS26830.

50.26

STIMULATION OF THE cAMP SYSTEM POTENTIATES THE EXCITATORY EFFECTS OF NMDA IN THE RAT HIPPOCAMPUS, *IN VIVO*. N. Ludvig*, S.M. Lasley and P.C. Jobe, Dept. of Basic Sciences, University of Illinois College of Medicine at Peoria, Peoria IL 61656.

The combined EEG - intracerebral microdialysis technique (Ludvig et al., *Synapse*, in press) was used to determine whether the predominantly postsynaptically localized cAMP system (Ludvig et al., *Neuroscience*, 44 [1991] 491-500) modifies the NMDA receptor-mediated excitation in the hippocampus of freely behaving rats. The microdialysis parameters were: probe tip: 1 mm; flow rate: 10 µl/min; drug efflux rate: 15%; and dialysis area: 1 mm^3, as determined with (^{3}H)cAMP autoradiography.

Intrahippocampal microdialysis with 0.1 mM NMDA was performed for 5 min, followed by a 30 min washout period, and a subsequent 30 min microdialysis with 1.0 mM dibutyryl cAMP (dcAMP). These pharmacological manipulations caused no EEG changes. However, a subsequent 5 min microdialysis with the combination of 0.1 mM NMDA and 1.0 mM dcAMP resulted in repetitive EEG spike-waves, which were associated with limbic type behavioral seizures. Additional experiments showed that 0.1 mM NMDA alone was not able to produce such events, even if it was microdialysed repeatedly. Also, 1.0 mM dcAMP alone was unable to induce seizures, even if it was microdialysed for 60 min.

This study supports the hypothesis that in the hippocampus, *in vivo*, the cAMP system may amplify NMDA receptor actions.

51.1

STATE-DEPENDENT OPERANT STIMULUS CONTROL: CUEING PROPERTIES OF ETHANOL "HANGOVER" IN RATS. D.V. Gauvin*, K.L. Goulden & F.A. Holloway. Univ. of Okla. Hlth. Sci. Cntr., Dept. Psychiat. & Beh. Sci., Oklahoma City, OK 73190.

Twelve Sprague-Dawley rats were trained in a two-choice food-reinforced drug discrimination task (FR-10, 10 min sessions) using the state-dependent interoceptive stimulus properties of *experimentally-induced ethanol (ETOH) hangover vs "normal" basal homeostasis*. Rats were injected with either: a) 4 g/kg ETOH, or b) equiv. vol. of saline (SAL) 18 hrs prior to training sessions. Each rat was injected with an additional 1 ml/kg injection of SAL 15 min prior to the sessions. "Hangover" training sessions were always followed by a "day-off". SAL sessions were conducted from between 36 to 90 hrs after a "hangover" training session.

Rats demonstrated >90% discriminative performance accuracy. Test sessions showed a time-dependent, cyclic, return from the "hangover" to the "normal" state, by 48 hrs. The acute (immediate) effects of ETOH (15 min pretreatments) did not cross-generalize with the "hangover" state. Both low dose ETOH and chlordiaze-poxide pretreatments blocked the stimulus properties of "hangover". All subjects responded exclusively on the "hangover"-appropriate lever at one of a selected range of pentylenetetrazole doses. The data demonstrated the "anxiogenic" dimensionality of experimentally-induced "hangover" in rats.

51.2

DIFFERENTIAL TOLERANCE TO THE ANTICONVULSANT, HYPOTHERMIC, AND ATAXIC EFFECTS OF ETHANOL. C.K. Kim, S. Dalal, J.P.J. Pinel*, G.J. Payne and L.E. Kalynchuk. Departments of Anatomy and Psychology, University of British Colombia, Vancouver, B.C., Canada.

The amygdala-kindling model of epilepsy was used to examine contingent tolerance to the anticonvulsant, hypothermic, and ataxic effects of ethanol (1.5 g/kg, IP) in adult male Long-Evans rats. Rats were assigned to one of three experimental conditions: ethanol 1 hr before a convulsive stimulation and saline 1 hr after the stimulation (Before-group), saline 1 hr before a stimulation and ethanol 1 hr later (After-group), or saline 1 hr before and after a stimulation (Saline-group). Following 10 such bidaily tolerance-development trials, all rats received a tolerance test in which ethanol was administered 1 hr before a convulsive stimulation. Tolerance to the anticonvulsant effect of ethanol was observed only in the Before-group. In contrast, tolerance to the hypothermic and ataxic effects of ethanol was observed in both the Before- and After-groups. These results support the drug-effect theory of drug tolerance: Tolerance does not develop to drug exposure per se, but rather to the effects of the drug; and the development of tolerance to a particular drug effect is facilitated by the expression of that drug effect. In this experiment, tolerance developed to the anticonvulsant effect of ethanol in the Before-group only: These rats had convulsive stimulations delivered in the drugged state on each tolerance-development trial, whereas those in the After-group did not. Tolerance to the hypothermic and ataxic effects of ethanol developed in both the Before- and After-groups: These rats experienced these drug effects on each tolerance-development trial.

51.3

ANIMAL-TO-HUMAN EXTRAPOLATION: I. ETHANOL EFFECTS ON COMPENSATORY TRACKING PERFORMANCE IN RHESUS MONKEYS. D. W. Blick*[1], S. A. Miller[2], G. C. Brown[1], and M. R. Murphy[2]. Systems Research Laboratories, Inc.[1] & U.S. Air Force Armstrong Laboratory[2], Brooks AFB, TX 78235

This is the first in a series of experiments in which animals and humans doing essentially identical tasks will be tested under the influence of the same pharmacological agents. We hope to develop and validate a set of models for extrapolating from animal performance data to changes in human performance induced by agents and other stressors that cannot be tested directly in human subjects.

Twenty adult male (4.8-7.5 kg) rhesus monkeys (*M. mulatta*) ingested various doses of ethanol mixed in orange-flavored drink (10% by volume). After each dose, 4-5 venous blood samples were analyzed to determine peak blood alcohol level (BAL), so as to estimate a dose-response function for each animal. Then, in different (but balanced) random orders, each animal received ethanol doses estimated to produce BALs of 0.00, 0.08, 0.12, 0.16, and 0.20%. Performance of a well-trained compensatory tracking task, the Primate Equilibrium Platform (PEP) task, was tested for 2 hr, commencing 30 min after the beginning of ethanol ingestion (which was completed in <15 min). BAL was determined at 30 min intervals before, during, and after PEP testing. The significant and dose-related performance decrements induced by alcohol varied in severity from nearly undetectable at the lowest dose to periods of incapacitation in many subjects at the highest.

51.4

SENSITIZATION TO ETHANOL DEMONSTRATED IN PLACE-PREFERENCE AND LOCOMOTOR ACTIVATION. K.R. Goldstein, D.J. Knapp, E.I. Saiff*, L.A. Pohorecky, and D. Benjamin. Center of Alcohol Studies, Rutgers University, Piscataway, NJ 08855.

As opposed to the place preference (PP) for cocaine, PP for ethanol (ET) does not occur after a single exposure, but must be acquired. To characterize this, 4 groups of male Long-Evans rats were tested for conditioned PP to ET using a new one-trial conditioning procedure. The groups were: naive, 3 days forced drinking, 6 days forced drinking and 1 month forced drinking followed by 1 month 6% ET choice drinking. 24h after removing ET, rats were given tap-water (3ml) by gavage and placed in one side of a 3 chambered PP apparatus for 30 min. Rats were then given ET by gavage (1g/kg 20% v/v) and placed in the opposite chamber for 30 min. 24 hrs later the subject was placed in the center chamber and allowed free access to all chambers for 30 min, the time spent in each side was recorded. To aid discrimination, the opposite chambers differed in visual cues To control for side bias, the side paired with ET was switched for each rat. ET-naive rats showed no PP while rats given a 4% ET solution as their drinking water for 3 days showed a nonsignifican t PP to the ET side, while rats given 4% ET for 6 days showed a significant PP for the ET side ($p<0.05$). Long term drinking rats also showed a significant PP ($p<0.05$). Locomotor activity, increased over time of ET pre-exposure during ET conditioning sessions (controls vs. long term drinkers $p<0.05$). These results do not suggest tolerance to the aversive effects of ET, as rats with 6 days pre-exposure to 4% ET show no change in performance on a roto-rod test 10, 30, 60 and 120 min after administration of 2g/kg (20% v/v) ET. These results demonstrate sensitization to ET-induced PP and suggest that the development of PP (as opposed to aversion) is not the result of conditioning *per se*, but reflect underlying neurochemical changes (see Benjamin *et al.*, this volume). (Supported by Smithers Prevention and NIAAA grants AA05306 and AA08499)

51.5

EFFECT OF ETHANOL ON LOCOMOTOR ACTIVITY INTIATED BY MESOLIMBIC ACTIVATION. G.V. MILTON, C.K. ERICKSON* AND P.K. RANDALL. Division of Pharmacology and Toxicology, College of Pharmacy, University of Texas, Austin, TX 78712.

Our objective was to study the interaction of a low dose of ethanol (0.5 gm/kg, i.p.) with drugs that increase locomotor activity by acting through DA, GABA and opiopeptidergic neurotransmitter systems in the mesolimbic system (MLS). After drug treatments the locomotor activity was measured in a doughnut shaped circular arena for 1 hr in male Sprague-Dawley rats. Amphetamine (0.25-1.0 g/kg, i.p.) increased locomotor activity in a dose-related manner and ethanol attenuated this amphetamine-induced locomotor activity. In bilaterally cannulated rats, the $GABA_A$ antagonist picrotoxin (0.025-0.1 µg/side) and mu receptor agonist DAGO (0.025-0.1 µg/side) given into the ventral tegmental area (VTA) increased locomotor activity in a dose-dependent manner. Ethanol attenuated picrotoxin-induced locomotor activity, but did not modify DAGO-induced locomotor activity. Thus, ethanol modified DA agonist-and $GABA_A$ antagonist-induced locomotor activity, but did not modify opiopeptide-induced locomotor activity. These results suggest that ethanol may act directly to decrease DA levels in the MLS or indirectly by increasing GABA levels in the VTA. (Funded by NIDA grant 7355.)

51.6

GABAERGIC REGULATION OF MALE SEXUAL BEHAVIOR IN RATS. R. C. Eaton*, L. A. Lumley, L. Matuszewich, D. S. Lorrain, and E. M. Hull. Department of Psychology, Program of Behavioral Neuroscience, State University of New York, Amherst, NY. 14260 USA.

The amino acid gamma-aminobutyric acid (GABA) is typically an inhibitory neurotransmitter of the mammalian brain and has been implicated in the regulation of masculine sexual behavior (Fernandez-Guasti et al., 1986). We studied the effects of the $GABA_A$ antagonist (-)-bicucculline methiodide (BIC), microinjected into the MPOA, on male rat sexual behavior.

BIC (0, 30, 60 ng / 1 µl) produced a biphasic effect on ejeculation latency. BIC (30 ng) significantly reduced the ejaculation latency ($p<.05$), while BIC (60 ng) was similar to the control value. Several animals exhibited stereotyped behavior after injections of the higher dose. Both doses of BIC decreased the number of intromissions required to achieve ejaculation ($p<.05$). In addition, BIC reduced the post ejaculatory interval ($p<.05$).

These experiments confirm and expand the role of GABA in regulating sexual behavior in the male rat. Further experiments are presently underway to determine the effects of the GABA agonist THIP, and the possible interaction of GABA with the neurotransmitter dopamine (DA) in the regulation of male sexual behavior.

Supported by NIMH grant 40826 to EMH.

51.7

DISCRIMINATIVE STIMULUS EFFECTS OF THE DIRECT $GABA_a$ AGONIST MUSCIMOL. Doreen M. Grech* and Robert L. Balster, Dept. of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

The discriminative stimulus effects of a direct $GABA_a$ agonist, muscimol, were compared to those of other GABAergic drugs. Ten male Sprague-Dawley rats were trained in 2-lever operant chambers to discriminate 1.0 mg/kg muscimol from saline. Responding was maintained under an FR-20 food reinforcement schedule. Subjects required a mean of 73 training sessions before achieving criterion. Substitution testing was completed with doses of muscimol (0.03-3.0 mg/kg), the direct $GABA_a$ agonist THIP (0.30-10 mg/kg), pentobarbital (0.03-17.3 mg/kg) and the $GABA_b$ agonist baclofen (1-10 mg/kg). Doses of muscimol produced dose-dependent substitution for the training dose. THIP substituted for muscimol and decreased response rates at doses greater than 5.6 mg/kg. Baclofen failed to substitute for muscimol, producing no greater than 24% muscimol-lever responding. Pentobarbital produced, at best, partial substitution for muscimol with a maximum of 45% muscimol-lever responding. These data suggest that the discriminative stimulus properties of muscimol are mediated by selective $GABA_a$ receptor activation. The lack of complete substitution with pentobarbital is consistent with the results of other studies showing a lack of complete substitution by direct $GABA_a$ agonists in rats trained with indirect agonists, showing that these classes of GABAergics can be distinguished in drug discrimination models.(Research supported by NIDA grants DA 01442 and DA 07027).

51.8

INVOLVEMENT OF GABAERGIC MECHANISMS IN ENHANCED SENSITIVITY TO NALTREXONE IN RATS? M. Gewiss, R. J. Marley, S. R. Goldberg and C. W. Schindler*. Beh. Pharmacol. and Genetics Lab., NIDA Addiction Research Center, Baltimore, MD 21224.

Rats treated weekly with cumulative doses of naltrexone develop enhanced sensitivity to the operant response rate decreasing effects of naltrexone (JPET **252**: 8-14). As naltrexone at high doses can function as a GABA antagonist and the development of enhanced sensitivity is associated with an up-regulation in GABA receptor function (Life Sci. **50**: PL1-PL6), we investigated whether the enhanced sensitivity could be altered by specific GABA agonists or antagonists, or whether the effects of those GABA agents would be altered by the development of enhanced sensitivity to naltrexone. Rats were trained to respond on a fixed-ratio schedule of food reinforcement and then were made sensitive to the response rate decreasing effect of naltrexone by treatment once per week with cumulative doses (1-100 mg/kg) of naltrexone for a period of 8 weeks. Initially, only the 100 mg/kg dose decreased operant responding, however, by the end of 8 weeks a dose as low as 10 mg/kg produced clear decrements in response rate. Pretreatment prior to the determination of the naltrexone dose-effect function with either the $GABA_A$ agonist muscimol (1 mg/kg) or the $GABA_A$ antagonist bicuculline (1 mg/kg) failed to alter the enhanced sensitivity. Further, the dose-effect functions for neither bicuculline (0.03-2 mg/kg) nor the $GABA_B$ agonist baclofen (0.05-5 mg/kg) were altered by the development of enhanced sensitivity to naltrexone. However, the dose-effect function for muscimol (0.03-2 mg/kg) was shifted to the left by the development of enhanced sensitivity. This result suggests that the function of $GABA_A$ receptors is altered by the development of enhanced sensitivity to naltrexone. However, the potential role for GABA in the development of enhanced sensitivity to naltrexone remains unclear.

51.9

THE β-CARBOLINE, DMCM, INHIBITS SHOCK-INDUCED AGGRESSION AND POSITIVELY MODULATES A POPULATION OF GABA-A RECEPTORS. A. H. Vaidya, R. P. Shank, and B. Dubinsky*. CNS Research, The R. W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477-0776.

DMCM (methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate) traditionally has been regarded as having inverse agonist (negative modulatory) properties at the GABA-A receptor complex. Recent evidence, based on recombinant receptor expression (Puia et al., Mol. Pharmacol. 39: 691, 1991) indicates that DMCM may negatively or positively modulate GABA-A receptor function depending on the subunit composition. Results of the present study suggest that DMCM may exert a positive modulatory effect at some GABA-A receptors, in vitro, which may be expressed, in vivo, as an inhibition of aggressive behavior. Using a shock-induced aggression paradigm, pairs of rats received DMCM (0.63 to 10 mg/kg, s.c.) 20 min prior to testing and were placed on a metal grid floor through which a mild footshock (0.7 mA) was delivered for a one minute test duration. The number of pairs of animals displaying aggressive behavior (converging abruptly, and standing face-to-face on their hind limbs, in an upright boxing posture) was recorded. DMCM blocked shock-induced aggression in rats (ED_{50} = 2.5 mg/kg, s.c.), an effect consistent with positive modulation of a population of GABA-A receptors. Unlike DMCM, abecarnil, an anxiolytic β-carboline, did not block shock-induced aggression when administered alone (1-30 mg/kg, p.o.). However, abecarnil pretreatment reversed (ED_{50}=0.3 mg/kg p.o.) DMCM (2.5 mg/kg s.c.) blockade of aggression suggesting a competitive interaction. Potent (ED_{50}=0.1 mg/kg i.p.) reversal of DMCM (2.5 mg/kg s.c.) blockade of aggression by RO-15-1788, a benzodiazepine antagonist, suggests the involvement of benzodiazepine receptors. Furthermore, DMCM concentration-inhibition curves (complex 20 point curves) in flunitrazepam binding studies revealed that DMCM produced a biphasic GABA shift indicative of mixed agonist/inverse agonist activity.

51.10

SEX DIFFERENCES IN THE DELAYED ANTI-CONFLICT EFFECT OF MK-801 IN RATS. Z.C. Xie* and R.L. Commissaris. Dept. Pharmaceut. Sci., Wayne State Univ., Detroit, MI 48202.

In female rats, MK-801 exerts anti-conflict effects when tested 24 hours after administration, but not when tested at pretreatment (preTx) intervals of 10 min - 12 hr (Neurosci. Abstr. 17:1493, 1991). In the present study, a thorough time course (10 min - 48 hr in 2-hr intervals) for the effects of MK-801 on conflict behavior was determined in both male and female rats. In daily 10-min sessions, thirsty rats drank from a tube which was occasionally electrified (0.5 mA shocks signaled by a tone). Acute challenges with MK-801 were conducted at weekly intervals. Overall, MK-801 was found to be 3 - 4 times more potent in females when compared to males. In females, 0.1 mg/kg MK-801 failed to exert anti-conflict effects when tested after preTx intervals of 10-min - 12 hr; this dose did increase punished responding at preTx intervals of 14 - 32 hr, but not at 34 - 48 hr. A qualitatively similar, but much more abbreviated, time course was produced by 0.4 mg/kg MK-801 in males: i.e., no anti-conflict effect at preTx intervals of 10 min - 2 hr, significant anti-conflict effects for preTx intervals of 4 - 14 hr, followed by no effect for preTx intervals of 16 - 48 hr. In both sexes, the peak and duration of the anti-conflict effect increased with increasing dose of MK-801. The mechanism for the delayed anti-conflict effect of MK-801 remains undetermined. (USPHS MH 47181).

51.11

TOLERANCE AND CROSS-TOLERANCE STUDIES TO μ OPIOID STIMULUS EFFECTS IN PIGEONS. S.A. Vanecek* and A.M. Young, Department of Psychology, Wayne State University, Detroit, MI 48202.

Experiments were conducted to assess the ability of repeated treatment with morphine to produce tolerance and cross-tolerance to the stimulus effects of morphine (MS) and the MS-like effects of fentanyl (FE). Pigeons (N=13) were trained to discriminate among i.m. injections of saline (SAL), 1.8 mg/kg MS, and 10 mg/kg MS under FR 30 schedules of food reinforcement. Prior to repeated treatment, MS (.18 to 56 mg/kg) and FE (.001 to .56 mg/kg) evoked dose-dependent generalization with both the low and the high MS training doses during tests of cumulative doses. Following two weeks of repeated treatment with 56 mg/kg MS, b.i.d., the doses of MS and FE required to generalize with both the low and the high training doses were increased approximately 10-fold. After this treatment, the dose of MS required to suppress rates increased 3- to 5-fold, whereas the dose of FE required to suppress rates increased only 2-fold. Repeated treatment with 10 mg/kg MS, b.i.d., for two weeks produced no increase in the doses of MS or FE required to evoke generalization with either training dose, however, changes in the sensitivity to rate-altering effects were comparable to those obtained following repeated treatment with the higher MS dose. These results demonstrate that repeated treatment with a large dose of MS induces tolerance and cross-tolerance to the stimulus effects of MS and MS-like effects of FE. (Supported by DA03796 and K02 DA00132)

51.12

BEHAVIORAL AND PHARMACOLOGICAL EFFECTS OF PHENCYCLIDINE (PCP) METABOLITES ON RAT. A. Baba[1], T. Yamamoto[1], H. Yamamoto[1], H. Nagase[1], T. Hori[1], T.Moroji[1] and H.Nakata*[2]. [1]Dept of Psychopharmacology, Tokyo inst. of Psychiatry, Tokyo 156, [2]Dept. of Mol. Biol., Tokyo metropolitan Inst. for Neurosci., Tokyo 183, Japan.

In order to better understand the prolonged delirious state beyond the drug half-life in human, which caused by phencyclidine (PCP), we evaluated behavioral and pharmacological activities of PCP metabolites, (trans)-4OH-cyclohexyl PCP, (cis)-4OH-cyclohexyl PCP, (trans)-3OH-cyclohexyl PCP and (cis)-3OH-cyclohexyl PCP. Systemic injection of (trans)-4OH-cyclohexyl PCP (10-50mg/kg i.p.), major metabolite of PCP, induced an increased locomotor activity and stereotyped behaviors on rats. Additionally, *in vitro* binding experiments to *sigma* and NMDA/PCP-receptor were performed using [^{3}H]DTG, [^{3}H]SKF10047, [^{3}H]TCP and [^{3}H]3OH phrnyl PCP as their ligands to examine the exact site of action. However, we failed to demonstrate that these metabolites have the affinities to *sigma* and NMDA/PCP site. On the other hand, (trans)-4OH-cyclohexyl PCP have an activity of the dopamine uptake inhibition similar to PCP. These observation indicate that the major metabolite of PCP, (trans)-4OH-cyclohexyl PCP, may produce some of the psychotomimetic effects of PCP not through the binding site of PCP/NMDA receptor and *sigma* site but through the dopamine uptake inhibition.

51.13

EFFECT OF INTRACAROTID INJECTIONS OF LIDOCAINE ON THE RAT VISUAL SYSTEM AS COMPARED WITH AMOBARBITAL. K.Nakai*, T.Terada, M.Nakai, T.Itakura, K.Komai, Dept. Neurol. Surg. Wakayama Med. Col., Wakayama, Japan 640

Effects of two kinds of anesthetic agents, lidocaine and amobarbital a non-specific blocking agent of nerve conduction and depressant of nervous activity in the central nervous system, respectively, were examined electrophysiologically using the rat visual system.

Under urethane anesthesia, male Sprague-Dawley rats weighing 500-600g were fixed to the stereotactic frame. Various doses (lidocaine: 0.5, 1.0, 2.0, 4.0 mg/kg, amobarbital: 1.0, 2.0, 4.0, 10.0 mg/kg) of either chemicals were injected through the cannula placed in the left carotid artery. Before and after the injection, single unit recordings were done from the left lateral geniculate body (LGN) for recording antidromic activity from the visual cortex.

Latencies of antidromic spikes in the LGN from the visual cortex were prolonged significantly (-20 %) for 10 minutes by the injection of lidocaine (1.0 - 4.0 mg/kg). Whereas those of animals injected with amobarbital (1.0 - 4.0 mg/kg) were unchanged. Spontaneous firing rate of LGN neurons were suppressed significantly by the injectoin of amobarbital (2.0, 4.0, 10 mg/kg) for 10 minutes, whereas the lidocaine injection showed valid reaction from excitation to inhibition even under high doses.

The present study not only indicates that intravascularly applied lidocaine can prevent conduction as was demonstrated by the perineural application in the peripheral system, but also that the technic is a useful method to examine the effect of intravascularly applied chemicals in the central nervous system.

51.14

AGONISTIC ENCOUNTERS BETWEEN MALE RATS SUBSTITUTE FOR THE PENTYLENETETRAZOL CUE. J.A. Vivian* and K.A. Miczek. Department of Psychology, Tufts University, Medford, MA 02155.

The drug discrimination paradigm involving pentylenetetrazol (PTZ) has been proposed as a preclinical model of anxiety. However, questions of validity have arisen as primarily compounds which are proconvulsant generalize to the PTZ "cue" and the nature of the PTZ cue remains unclear. Attack and threat of attack encounters between conspecifics may produce an anxiety-like state which engenders PTZ responding and would provide further support for the PTZ cue as an anxiety cue, as well as provide evidence that anxiety is a component of agonistic interactions. Male Long-Evans rats (*Rattus norvegicus*) were trained to discriminate 20 mg/kg PTZ from saline in a two-choice drug-discrimination task. After two brief defeats consisting of defensive and submissive postures as well as audible and ultrasonic vocalizations (Ss serving as intruders), administration of saline engendered approximately 80 and 56% PTZ-appropriate responding. Subsequently, during four exposures to the threat of attack (Ss protected from physical contact with a wire mesh cage), saline generated 54, 47, 59 and 34% PTZ-appropriate responding. These results suggest that an anxiety-like state: (1) is induced during attack and threat of attack encounters and (2) appears to be the primary component of the PTZ cue.

51.15

EVIDENCE THAT CHOICE BEHAVIOR IN DRUG-DISCRIMINATION STUDIES REFLECTS A CONTINUOUS RATHER THAN A QUANTAL PROCESS. W. F. Caul*, R. J. Barrett, R. L. Smith, and E. M. Huffman, Dept. Psych. and Pharmacol., Vanderbilt Univ., and V. A. Medical Center, Nashville, TN 37240.

Debate continues as to whether stimulus generalization reflects underlying stimulus-response relationships that are quantal or continuous. This issue is especially important in determining the appropriate interpretation of the results of drug-discrimination studies that rely on choice behavior to assess the nature of drug-induced interoceptive stimuli.

The data consistent with the quantal view were generated by animals trained to respond using FR schedules of reinforcement. This outcome may have been produced by constraints on choice behavior imposed by such schedules. The use of Variable schedules of reinforcement may be more appropriate because the pattern of responding does not preclude results consistent with either of the competing interpretations.

Data from studies that used VI schedules were analyzed: Steranka & Barrett, 1983, Amphetamine vs. Saline; Friedman, Barrett, & Sanders-Bush, 1981, 5-HTP vs. Saline; Barrett & Smith, 1988, Diazepam vs. Pentylenetetrazol; Barrett, Caul, & White, 1992, Amphetamine vs. Haloperidol. In every case, when experimental conditions produced a group mean intermediate to that for the training drugs, the distribution of scores for individual animals was normally distributed. This result is in contrast to the bimodal distribution that would be observed if the underlying stimulus-response process was quantal.

51.16

BEHAVIORAL EFFECTS OF NITROUS OXIDE (N_2O) IN THE CONDITIONED DEFENSIVE BURYING (CDB) TEST. D.A. Czech,*[1] R.M. Quock,[2] and J.S. Sundstrom.[1] [1]Dept. of Psychology, Marquette Univ., Milwaukee, WI 53233 and [2]Dept. of Biomedical Sciences, Univ. of Illinois College of Medicine, Rockford, IL 61107.

The present study was conducted to determine whether an anxiolytic effect of N_2O was demonstrable in the CDB test, a paradigm that exploits a species-typical propensity of rats to bury objects associated with aversive stimulation (Treit et al., Pharmac Biochem Behav 15:619, 1981). A single, brief electric "shock" was delivered to male hooded rats on contact with a wire-wrapped prod in a conditioning chamber. Rats were subsequently tested (15 min) under several concentrations of a N_2O and O_2 mixture (0-40% N_2O), or room air, in a polycarbonate test unit with an inactive prod. Measures included height and duration of prod-directed burying with floor bedding material. Both behaviors were attenuated in a dose-related manner. Pretreatment with 20 mg/kg of the benzodiazepine (BZP) receptor blocker, flumazenil, which alone had no effect, did effectively antagonize 30% N_2O-induced burying. Locomotion and rearing in an open field were not affected at concentrations of N_2O that attenuated burying. Tests with chlordiazepoxide (CDP) (0-10 mg/kg) also resulted in dose-related attenuation of burying. All behaviors were videotaped for "blind" review. These findings suggest that N_2O can induce an anxiolytic effect and implicate a BZP mechanism.

(Supported by NIH Grant DE-09378)

51.17

BUSPIRONE - EVIDENCE OF AN ANTIPSYCHOTIC-LIKE PROFILE IN THE RAT C Ryan, J Evenden, M. Nielsen* and K. Ensler
Behavioural Pharmacology, Astra Arcus, S- 15 185 Södertälje, Sweden

Buspirone , which is both an agonist at 5-HT_{1A} receptors and an antagonist at dopamine D_2 receptors, has been successfully used in the clinic as an anxiolytic (see Taylor et al, 1985). Although buspirone blocks dopamine receptors, it is not a clinically effective antipsychotic (Cimino et al 1983). To examine the behavioural profile of the drug after repeated administration, it was compared to a series of more selective 5-HT_{1A} agonists or a D_2 antagonist in active avoidance and schedule induced polydipsia (SIP). Rats were first trained to stable performance and then treated daily with a range of doses of each drug just before testing for 16-18 days. In the above tests, 5-HT_{1A} receptor agonists had an activating effect after repeated administration. Thus, flesinoxan, isapirone and 8-OH-DPAT, increased avoidance of shocks in the active avoidance test, where rats are trained to avoid an electric shock (0.2 mA) by shuttling from one side of a chamber to another in response to the conditioned stimulus (CS, a tone). In direct contrast, buspirone reduced the number of avoidances, revealing a profile similar to that induced by antipsychotics. When buspirone was compared to raclopride or 8-OH-DPAT in SIP, a similar picture emerged. All three drugs reduced excessive drinking induced by a FT-60 secs schedule of food delivery, at high doses, but 8-OH-DPAT increased investigatory entries into the food tray after repeated treatment. In contrast, both buspirone and raclopride reduced entries into the food tray weakly following acute treatment but to a greater degree following repeated treatment. In conclusion buspirone acts like an antipsychotic in both active avoidance and schedule induced polydipsia and thus, although the drug may have 5HT_{1A} agonist activity in selective procedures, its D_2 antagonist properties appear to predominate in the rat.

Cimino et al (1983) Biochem Pharmacol, 32 , 1069-1074
Taylor et al (1985) Pharmacol Biochem Behav, 23, 687-694

52.1

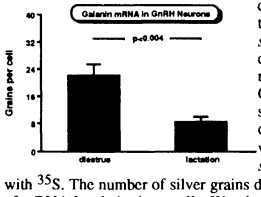
STIMULATION OF LH SECRETION BY CCK: POSSIBLE RELATION TO VISCERAL MALAISE. D. A. Schreihofer*, G. A. Golden, J. G. Verbalis and J. L. Cameron. Departments of Behavioral Neuroscience, Medicine, and Psychiatry. University of Pittsburgh, Pittsburgh, PA 15260.

We have recently shown that systemic administration of cholecystokinin (CCK; a peptide released from the gut and the hypothalamus in response to a meal) causes a rapid large magnitude release of luteinizing hormone (LH) in adult male rhesus monkeys (Schreihofer, et. al., Endo. Soc. Abst. 191, 1992). CCK also causes visceral malaise and emesis in humans and monkeys, and it has been proposed that these chemotoxic-like effects of CCK are responsible for causing the CCK-induced secretion of other peptide hormones, such as AVP and ACTH. We therefore sought to determine whether the chemotoxic-like effects of CCK are important for the CCK-induced stimulation of LH secretion by comparing the LH response to CCK and two other chemical agents which also can cause visceral malaise and emesis. CCK, LiCl, and cupric acetate (CuAc) were administered to monkeys via indwelling venous catheters. Three baseline blood samples were taken at -30, -15, and -1 min prior to i.v. drug administration. Samples were then taken at frequent intervals for 165 min following drug administration to characterize LH secretion. CCK (15μg/kg) caused emesis in 5 of 7 monkeys and produced a significant elevation in LH secretion (from 11.55±0.54 to 30.47±6.42 ng/ml) with a peak response 15 min post-CCK. LiCl (3 mEq/kg) caused emesis in 4 of 4 monkeys and produced a smaller, but significant, rise in LH secretion (from 14.38±0.57 to 19.2±1.92 ng/ml) with a peak response 15 min post-LiCl. CuAc (2.5 mg/kg) caused emesis in 3 of 5 monkeys and produced a large magnitude LH release (from 12.74±0.47 to 45.29±7.68 ng/ml) with a peak response 105 min post-CuAc. In comparison, 6 animals receiving saline vehicle showed no increase in LH secretion after vehicle administration (from 14.71±1.18 to 13.14±1.18 ng/ml). These results suggest that the CCK-stimulated release of LH may be related to activation of neural centers involved in the sensation of visceral malaise.

52.2

SUPPRESSION OF GALANIN GENE EXPRESSION IN GNRH NEURONS DURING LACTATION. D.L. Marks, M.S. Smith, D.K. Clifton,& R. Steiner*. OB/GYN & PBio, U. WA, Seattle WA 98195 & Phys, U. Pitt., Pittsburgh PA 15261.

Lactation in rats is associated with the relative absence of pulsatile GnRH activity, and GnRH neurons are refractory to exogenous excitation. The decrease in GnRH neuronal activity may be associated with either an inhibition of GnRH synthesis or the loss of other colocalized cellular products that enhance GnRH release or efficacy. Galanin is colocalized with GnRH and its expression in these neurons is enhanced at proestrus, a time of activation of GnRH neurons. We tested the hypothesis that the expression of both the GnRH and galanin genes in GnRH neurons decrease during lactation. We sacrificed groups of adult female rats at 1000 h on diestrus-1 (n=5) and on the tenth day of lactation (n=4) and utilized double and single *in situ* hybridization and image analysis to compare the level of galanin and GnRH mRNA in GnRH neurons. For double *in situ*, GnRH mRNA was detected with an antisense cRNA probe labeled with the hapten digoxigenin, while the galanin cRNA probe was labeled with ^{35}S. For single-label *in situ*, the GnRH cRNA probe was labeled with ^{35}S. The number of silver grains deposited over a cell body provided an index of mRNA levels in these cells. We observed a 60% reduction in signal for galanin mRNA in the GnRH neurons of lactating animals compared with those of diestrus animals, although levels for GnRH mRNA were not different between these two groups (133.1±14.6 diestrus vs.127.7±8.32 lactation; p>0.7). Conclusion: Galanin gene expression in GnRH neurons of the rat is lowered during lactation, but GnRH expression is not. We infer that the extremely low plasma estrogen levels during lactation cannot support galanin gene expression. This decrease in galanin gene expression may lead to the decreased synthesis and secretion of galanin, which, in turn, could diminish the secretion of GnRH or its activity at the pituitary.



52.3

EFFECTS OF STEROID REPLACEMENT ON NEUROPEPTIDE Y (NPY) GENE EXPRESSION IN THE ARCUATE NUCLEUS (ARC) OF OVARIECTOMIZED (OVX) RATS. J.H. Urban*, A.C. Bauer-Dantoin, J.E. Levine. Dept. Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208.

We have recently demonstrated that NPY mRNA levels in the ARC are decreased by castration and restored by steroid treatment in male rats. The purpose of the present study was to determine whether estrogen (E) and progesterone (P) similarly modulate NPY gene expression in the ARC of female rats. OVX rats (8d) received sham capsules or doses of E (1x14mm capsule containing 50μg estradiol/ml oil) or E+P (3x20mm caspules; crystalline P) that were comparable to steroid levels observed on the day of diestrous. NPY mRNA levels were assessed in the ARC using in situ hybridization and quantitative autoradiography. Plasma LH levels were significantly decreased in the OVX+E ($p<0.05$) and OVX+EP ($p<0.01$) treated groups when compared with the OVX group. NPY mRNA levels in the ARC, were significantly increased in the OVX+EP group (70.0± 3.0; $p<0.01$) but only slightly elevated in OVX+E treated animals when compared with values from OVX animals (OVX:47.9±3.0; OVX+E:59.5±5.8). These results suggest that E and P provide a facilitatory stimulus for NPY gene expression in the ARC and that, as in male rats, steroid effects on NPY gene expression are not directly related to negative feedback regulation of LH secretion. Studies are underway to determine if the effects of E and P on NPY gene expression may instead comprise a component of positive feedback regulation of the LH surge. (NIH HD20677, HD00879 & HD21921)

52.4

ESTROGEN-DEPENDENT NEUROTENSIN IMMUNOREACTIVITY IN THE PREOPTIC AREA OF THE FEMALE RAT. M.J. Alexander* and S.E. Leeman, Department of Pharmacology & Experimental Therapeutics, Boston University School of Medicine, Boston, MA 02118.

Neurotensin (NT) neurons in the rostral preoptic area may mediate stimulatory effects of estrogen on preovulatory secretion of gonadotropin-releasing hormone in the female rat. Estrogen markedly increases the abundance of mRNA encoding NT and neuromedin N in the anteroventral periventricular nucleus (AVPv), periventricular preoptic nucleus (PvPO), and medial preoptic nucleus (MPN). To determine if estrogen-dependent expression of NT/N mRNA is indicative of increased NT biosynthesis, we investigated whether estrogen can induce NT-immunoreactivity (NT-ir) in these same cell groups. Ovariectomized (OVX) adult rats were left untreated or were treated with supraphysiological levels of 17ß-estradiol (E2) for 2 wks, and a subset of each group received an intracerebroventricular injection of colchicine (100 μg) 48 hr before transcardiac perfusion. NT-ir was detected in brain sections with a polyclonal antiserum exhibiting negligible cross-reactivity with neuromedin N. E2 caused a dramatic increase in the number of NT-ir cell bodies specifically in the AVPv, rostral PvPO, and extreme medial part of the rostral MPN. There were more than eight times as many NT-ir cell bodies (101 ± 5 vs. 12 ± 1 in a 40-μm bilateral section) in the caudal AVPv of E2-treated OVX rats (n=7) as in the same region of OVX rats (n=7), and a difference of similar magnitude was observed in the adjacent PvPO. Although NT-ir cell bodies were undetectable when colchicine treatment was omitted (n=5 per group), E2 nevertheless caused a pronounced increase in the number of NT-ir fibers in these same regions. The concordant effects of estrogen on steady-state levels of NT/N mRNA and NT-ir in specific neuronal groups of the female preoptic area indicate that estrogen increases NT biosynthesis in these neurons and suggest that estrogen-associated change in NT/N mRNA abundance is a reliable sign of altered NT biosynthesis in this brain area. (Supported by DK-29876)

52.5

LACTATION BUT NOT PROLACTIN INCREASES THE LEVELS OF PRE-PRONPY MRNA IN THE RAT ARCUATE NUCLEUS. G. Pelletier, Y. Tong and A. Dupont*. MRC Group in Molecular Endocrinology, CHUL Research Center and Laval University, Québec, G1V 4G2, Canada.

Evidence mostly obtained from pharmacological studies shows that neuropeptide Y (NPY) is involved in neuroendocrine regulation of reproduction functions. In the present report, we have studied the influence of lactation, a physiological condition associated with high plasma levels of prolactin (PRL), as well as the effects of hyperprolactinemia on pre-proNPY gene expression in the rat arcuate nucleus. The amounts of pre-proNPY mRNA were measured by *in situ* hybridization using a [^{35}S]-labeled cRNA probe encoding for pre-proNPY. In lactating animals killed 4 days after parturition, pre-proNPY mRNA levels were 50% higher than those detected in virgin adult females. Twenty-four hours after pup-removal the mRNA levels returned to those observed in virgin animals. Hypophysectomy performed 2 weeks previously decreased by 50% pre-proNPY mRNA levels. Chronic haloperidol treatment which induced high levels of circulating prolactin increased the amounts of pre-proNPY mRNA by 70% and 66% in intact and hypophysectomized animals, respectively. Intracerebroventricular injections of PRL produced no significant changes in the hybridization signal. Chronic hyperprolactinemia obtained by pituitary implants under the kidney capsule did not induce any modifications in the pre-proNPY mRNA levels. These data demonstrate that pre-proNPY gene expression in the rat arcuate nucleus is increased during lactation and that this effect is probably not a consequence of hyperprolactinemia. Moreover, the stimulatory effect of haloperidol treatment on pre-proNPY mRNA levels does not appear to be mediated by the pituitary gland.

52.6

NMDA STIMULATES QUIESCENT MU-OPIOID AFFERENTS WHICH MODULATE GNRH RELEASE IN THE INTACT MALE MOUSE. G.M. Miller* and M.J.Gibson, Division of Endocrinology, Mount Sinai School of Medicine, N.Y., N.Y., 10029.

We have previously shown that NMDA stimulates LH release in male and female mice (Endocr.128: 2432,'91) and our recent work suggests that NMDA's effect on GnRH secretion is modulated by other afferents. The present study examines mu-opioid interaction with NMDA on LH release. We now report that naloxone (3mg/kg) failed to stimulate LH release in male mice (n=23), yet significantly potentiated NMDA (20mg/kg,iv)-stimulated LH release ($p<0.01$;n=8). Naloxone methiodide, which does not cross the blood-brain barrier, had a similar effect when administered at the same dose (n=8). Neither opioid antagonist affected GnRH (50ng)-stimulated LH release, suggesting that mu-opioid modulation is central.

These data suggest that NMDA stimulates otherwise quiescent mu-opioid afferents to GnRH neurons, thereby attenuating LH release by decreasing the amount of GnRH released at the median eminence.

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52.7

GLUCOCORTICOIDS STABILIZE CALMODULIN ASSOCIATION WITH SYNAPTOSOMAL PLASMA MEMBRANE. Zafar Iqbal and Paul Y. Sze.* Dept. of Pharmacol. & Mol. Biol., The Chicago Medical School, North Chicago, IL 60064, and Dept. of Neurology, Northwestern University Medical School, Chicago, IL 60611.

The synaptic plasma membrane (SPM) from brain is known to have specific binding sites for glucocorticoids. Knowledge about biochemical consequences of the steroid-membrane interaction has only begun to emerge. In the present study, we determined the effects of glucocorticoids on the stability of membrane association of calmodulin (CaM) *in vitro* in purified SPM from rat cerebral cortex. SPM was first washed with EGTA to deplete endogenously bound CaM, and then reloaded with [^{125}I] CaM. At 37°C, [^{125}I] CaM was found to dissociate rapidly from the membrane, even in the presence of 10 uM Ca^{2+}. A fast and a slow component were identified in the first-order dissociation kinetics. In SPM preincubated with 1 uM corticosterone, the fast dissociation of [^{125}I] CaM was markedly decreased, whereas the slow dissociation was not affected. The effect of the steroid was concentration-dependent. In another experiment, RIA was used to determine the dissociation of endogenously bound CaM. Preincubation of SPM with corticosterone showed a similar stabilizing effect against the rapid dissociation of endogenous CaM from the membrane. Our results support the notion that one of the glucocorticoid actions on neuronal plasma membrane is to stabilize the association of CaM with the membrane. Since CaM is involved in a variety of biochemical activities in the plasma membrane, such an action could lead to the regulation of membrane functions.

52.8

NEUROTRANSMITTER REGULATION OF GNRH GENE EXPRESSION: ROLES OF NMA AND OPIATES. Andrea C. Gore* & James L. Roberts. Fishberg Ctr. for Neurobiology, Mt. Sinai Med. Ctr., NY, NY, 10029.

Numerous neurotransmitters such as N-methyl-D,L-aspartate (NMA) and opiates regulate GnRH release. We were interested in examining whether these neurotransmitters exert their effects by altering GnRH gene expression, particularly transcription, by measuring changes in GnRH primary transcript (PT) and processing intermediate (INT) levels, as well as mRNA levels. NMA (14 mg/kg, i.p.), naloxone (NX, 2 mg/kg i.p.) or saline were injected in intact male or ovariectomized female rats. Rats were decapitated 15 or 60 min later, the brains removed on ice and the preoptic area dissected and frozen. Cytoplasmic and nuclear RNA were extracted and assayed for GnRH mRNA, INT and PT by RNase protection assay. For NMA, cytoplasmic mRNA levels increased significantly 60 min, but not 15 min, after NMA injection compared to control (no treatment or saline) levels. In the nucleus, no significant changes in PT or INT were observed. For NX, a small increase (non-significant) in mRNA was observed in the cytoplasm 60 min after injection. Again, no changes in INT or PT were observed in the nucleus. These studies indicate that GnRH mRNA levels in the cytoplasm can be rapidly increased by NMA and NX, while PT and INT in the nucleus do not change. The results suggest that mRNA levels can increase without concomitant changes in gene transcription, possibly as a result of alterations in mRNA turnover.

52.9

PULSATILE SECRETION OF LHRH AND β-ENDORPHIN INTO PITUITARY PORTAL BLOOD DURING DIFFERENT DAYS OF THE ESTROUS CYCLE IN RATS. D.K. Sarkar, S. Minami, Q-W Xie, J.P. Advis* and B.M. Prasad. Dept of VCAPP, Washington State University, Pullman, WA 99164, Dept of Animal Sciences, Rutgers University, New Brunswick, NJ 08903.

Hypothalamic β-endorphin (β-EP) has been implicated in the negative control of LHRH secretion by acting both at the preoptic-anterior hypothalamus and the median eminence during the rat estrous cycle. We have previously shown that a large amount of β-EP from the median eminence is secreted into the portal blood of female rats. Using Saffan anesthetic (which does not inhibit preovulatory LH and PRL release) and long surgical recovery (60-90 min), we have previously noted that the portal levels of β-EP were negatively associated with the levels of LHRH immediately prior to and during the preovulatory LH surge in female rats. The inverse relationship in the pattern of median eminence β-EP and LHRH secretion prior to the preovulatory LH release has recently been confirmed in conscious female ewes and has provided a physiological evidence for the inhibitory role of β-EP on LHRH secretion. In order to further characterize the dynamic interaction between LHRH and β-EP at the median eminence, we determined the levels of LHRH and β-EP in portal plasma samples collected at 10 min intervals between 1000-1800 h on the day of diestrus and proestrus in cyclic female rats. Both LHRH and β-EP are secreted in portal blood in a pulsatile manner on the day of diestrus and proestrus. The pulsatile secretion of β-EP were highest between 1000-1500 h and lowest between 1600-1800 h of proestrus. The pulsatile secretion of LHRH was low during the elevated β-EP secretion, but increased remarkably on the proestrous afternoon. The secretory profiles of these two peptides also showed a diurnal rhythmicity and an inverse relationship on the day of diestrus, although the levels of β-EP and LHRH were in general lower on the this day than on the day of proestrus. These data suggest for the first time that there is a diurnal rhythm in LHRH and β-EP secretion in the cyclic female and that the interaction between these two peptides may be important in governing the timing of this rhythmic β-EP and LHRH release.

52.10

STEREOLOGICAL AND MORPHOMETRIC EVIDENCE FOR SELECTIVE BETA-ENDORPHIN NEURON LOSS FOLLOWING LONG-TERM ESTRADIOL EXPOSURE. G. Clarissa Desjardins* James R. Brawer and A. Beaudet, Departments of Anatomy and Neurology & Neurosurgery, McGill U., Mtl., Quebec H3A 2B4.

We have previously shown that the long-term exposure to physiological concentrations of estradiol that follows a single 2 mg IM injection of estradiol valerate (EV) results in the loss of more than 60% of all hypothalamic beta-endorphin immunoreactive (ir) neurons while sparing, somatostatin (SRIF), neurotensin (NT) and tyrosine hydroxylase-(TH) immunoreactive neuronal populations. In order to determine whether this decrement in beta-endorphin-ir neuron number was due to actual cell loss or to a decrease in ir-peptide content, unbiased stereological methods (Gundersen et al., 1988) were used to estimate the total number of neurons in the arcuate nucleus of control and EV treated (n=10) animals. A systematic sampling scheme resulting in a coefficient of error of less than 7% was used. In addition, the morphometry (form factor, area, diameter) of immunolabeled beta-endorphin neurons was analyzed. Mean number of neurons (coefficient of variation) in controls was 23 820 (0.07) consistent with what was previously reported using Golgi staining or classical stereology. In estradiol-treated animals neuron numbers were reduced to 20 249 (0.07), indicating that significant neuronal cell loss had occurred. Furthermore, this neuronal loss (3500 cells) corresponded precisely with the total estimated loss of beta-endorphin neurons (3600), thus confirming the idea that chronic exposure to estradiol results in selective beta-endorphin neuron destruction. Morphometric analysis of beta-endorphin neuron surface area revealed a relatively greater loss of small (25-30μm^2) versus large (45-50μm^2) neurons in EV-treated animals and a significant decrease in form factor with respect to controls (0.45±.02 vs. 0.52±.005; $p<0.05$). Such alterations could reflect early changes associated with neuronal degeneration.

52.11

OPIOID AND DOPAMINERGIC REGULATION OF GNRH GENE EXPRESSION IN THE MALE RAT HYPOTHALAMUS. S. Li, G. Pelletier* MRC Group in Molecular Endocrinology, CHUL Research Center and Laval University, Québec, G1V 4G2, Canada.

Compelling evidence indicates that opioid peptides have an inhibitory effect of GnRH secretion while the role of dopamine in the regulation of GnRH secretion is still controversial since both stimulatory and inhibitory effects have been reported. The role of these transmitters on GnRH biosynthesis was investigated by quantitative *in situ* hybridization involving use of a [^{35}S]-labeled 48 base oligonucleotide complementary to the GnRH coding region of the GnRH DNA. The corresponding mRNA levels were assessed by counting the number of silver grains per labeled neuron. A 14-day treatment with the dopaminergic agonist bromocriptine increased by 67% the number of silver grains overlying GnRH-producing neurons while the dopamine antagonist haloperidol decrased by 31% the value of this parameter. A 24-hour treatment with the opioid drug morphine produced a 33% decrease in the hybridization signal. Conversely naloxone, an opiate receptor antagonist, also administered during a 24-hour period, induced a 22% increase in the number of grains/GnRH neuron. The present data clearly indicate that GnRH mRNA levels are positively and negatively regulated by dopamine and opiates, respectively. They suggest that these transmitters can not only modified the release of GnRH by also the biosynthesis of this peptide.

52.12

FEED RESTRICTION DECREASES ARCUATE mRNA AND IN VIVO MEDIAN EMINENCE RELEASE OF ß-ENDORPHINE IN EWE LAMBS. B.M. Prasad, A. C. Gore, J.L. Roberts, D.K. Sarkar, J. Rabii* and J.P. Advis. Department of Animal Sciences, Rutgers University, New Brunswick, NJ 08903, VCAPP, Washington State University, Pullman, WA 99164, Department of Neurobiology, Mount Sinai Sch Med, NY City, NY 10029, and Dept of Biological Sci, Rutgers University, Piscataway, NJ 08855.

Feed restricted (FR) lambs fail to reach puberty and show lower plasma LH values than full fed (FF) age-paired controls. We analyzed: **a)** steady state levels of ßEND mRNA in arcuate nuclei using a solution hybridization - RNase protection assay; and **b)** ME in vivo release of LHRH, ßEND, and NPY by push-pull cannula (PPC). FF and FR lambs were sampled at the time each FF age-paired control was in the follicular phase of its 2nd and 4th estrous cycle. FR lambs had decreased in vivo ME-LHRH release (0.87±0.04 vs 0.26± 0.04 pg/100 µl PPC perfusate, sampled at 10-min intervals for 4h, FF vs FR, mean ±sem, n=12), lower plasma LH (6.0±1.6 vs 1.3±0.3 ng/ml), and failed to reach puberty when compared to age-matched FF controls. The low LHRH and LH release was caused by a decrease in pulse amplitude (LHRH=1.91± 0.19 vs 0.62±0.14; LH= 7.60±2.42 vs 0.54± 0.22) rather than alterations in pulse frequency. These changes occurred in the presence of decreased ß-END (246±44 vs 46± 13) and unchanged NPY release. Exogenous infusion of ß-END in ME decreased in vivo LHRH (in FF but not in FR) and NPY (in FF and in FR) release (LHRH= 2.15±0.67 vs 0.67±0.1; NPY= 331±50 vs 172±42 before vs during infusion) and plasma LH (3.8±0.3 vs 2.09±0.3) in FF lambs. Steady state levels of ßEND message were lower in FR than in their FF paired controls (0.83±0.18 vs 0.48±0.10 pg POMC/µg RNA). Thus, FR lowers LH release and prevents pubertal onset by decreasing the amplitude of pulsatile LHRH release. The decreased ßEND synthesis and release might represent unsuccessful compensatory mechanisms. The lack of effect of FR on ME-NPY release, supports the specificity of changes induced on ME-LHRH and ß-END release (NJAES-Hatch 06108 & USDA 89-37240-4587 to JP Advis).

52.13

MEDIAN EMINENCE IN VIVO RELEASE, INFUNDIBULAR/ARCUATE PEPTIDE CONTENT, AND ARCUATE STEADY-STATE mRNA OF ß-ENDORPHINE, BEFORE, DURING AND AFTER A PREOVULATORY LH SURGE IN EWES. C.D. Conover*, A.C. Gore, J.L. Roberts, D.K. Sarkar, J. Rabii and J.P. Advis. Dept of Animal Sciences, Rutgers Univ, New Brunswick, NJ 08903, VCAPP, Washington State Univ, Pullman, WA 99164, Dept of Neurobiology, Mount Sinai Sch Med, NY City, NY 10029, & Dept Biological Sciences, Rutgers Univ, Piscataway, NJ 08855.

An involvement of ß-endorphine (ßEND) at the median eminence (ME) has been suggested to play a role in the neuroendocrine control of preovulatory release of luteinizing hormone (LH) - releasing hormone (LHRH) and LH. Infusion of ßEND into the posterior-lateral ME of follicular ewes decreases in vivo release of ME-LHRH and plasma LH. We analyzed: **a)** ME in vivo release of ßEND and LHRH by push-pull cannula (PPC) sampling; **b)** steady state levels of ßEND mRNA in arcuate nuclei by a solution hybridization-RNase protection assay; and **c)** content of ßEND in the infundibular/arcuate area by RIA, before, during, and after an induced follicular phase in adult cycling ewes. Follicular content of tissue ßEND was twice ($P<0.01$) that observed in early luteal ewes (25±3 vs 52± 5 pg/µg protein, mean±sem, n=26). The low ME-in vivo pulsatile ßEND release (27±6 pg/100 µl PPC perfusate, n=6, sampled at 10-min intervals for 4h) increased a thousand fold to peak levels immediately before the LH surge (2713±2047, n=4), and was lower in ewes undergoing an LH surge (92±46, n=4). In vivo pulsatile ME-LHRH release increased during the follicular phase (2.8±0.3 vs 24.5±22.2). Increases of in vivo peptide release were associated predominantly with changes in amplitude (ßEND= 26.9 ±13.9 vs 81.3 ± 23.7; LHRH= 1.92 ± 0.29 vs 4.29 ± 0.89). Steady state ßEND mRNA levels increased gradually during the follicular phase (0.55± 0.06 vs 0.86 ± 0.08 pg/µg RNA, n=25, start vs end, $P<0.05$). In conclusion, ßEND secretion might exert an increasing tonic inhibition on LHRH release until the onset of the preovulatory surge of ME-LHRH and plasma LH (supported by NJAES - Hatch 06108 & USDA 89-37240-4587 to JP Advis).

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION: STEROIDS

53.1

WITHDRAWN

53.2

MELATONIN SUPPRESSION OF ESTROGEN RECEPTOR PROTEIN AND MESSENGER RNA IN OVARIECTOMIZED OUTBRED LVG SYRIAN HAMSTERS. N.O. Lawson[1]*, B.E.F Wee[2], J.G. Drane[1] and S.M. Hill[1]. [1]Depts. of Anatomy & [2]Psychology, Tulane University, New Orleans, LA 70112.

Melatonin, the major pineal hormone, is a key regulator of the reproductive system of photoperiodic animals such as the hamster. We have previously reported that melatonin suppresses estrogen receptor (ER) expression in the medial preoptic area (MPOA) of inbred LSH/SsLak hamsters. The current study looks at whether this effect on ER is strain specific and the biological level at which this is mediated. Ovariectomized outbred LVG Syrian hamsters held under long photoperiod (14L:10D), were treated with either melatonin or vehicle for 12 weeks. Immunocytochemistry was performed on coronal brain sections of the MPOA, using the H222 monoclonal antibody to the human ER. To examine ER mRNA, total RNA was isolated from blocked hypothalami and RNase protection analysis performed using a cRNA probe generated from a 300 nucleotide cDNA fragment encoding the ligand binding domain of the human ER. The results show that melatonin induced a statistically significant 39% decrease in the number of cells/mm² showing ER-immunoreactivity in the MPOA of these outbred hamsters. This decrease is less than what was observed in the inbred animals (50-70%). Melatonin treatment also induced a significant 31% decrease in steady state ER mRNA levels, suggesting that the effects of melatonin on ER expression may be mediated at the transcriptional level. Thus, it appears that the effects of melatonin are not strain specific, in that both inbred and outbred hamsters responded to melatonin with decreased ER expression and that this effect is at least partially mediated at the level of gene transcription.

53.3

PLASTICITY IN THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS OF THE CYCLING AND OVARIECTOMIZED FEMALE RAT. M.C. Langub Jr.* and R.E. Watson Jr. Department of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, Kentucky 40536.

Estrous cycle-related plasticity has been documented in the preoptic-hypothalamic region (Naftolin, et al., 1990). For example, arcuate nucleus neurons are contacted by 31% fewer axosomatic synapses during estrus than during proestrus 24 h earlier (Olmos, et al., 1989). The present study was conducted to determine whether another sexually dimorphic area, the anteroventral periventricular nucleus (AVPv), also exhibits estrous cycle-related changes in morphology and synaptic input. The AVPv is a key site for control of steroid-mediated gonadotropin secretion (Wiegand and Terasawa, 1982) and contains abundant estrogen receptor immunoreactive (ER-ir) cells. Tissue sections through the AVPv from cycling and ovariectomized Sprague-Dawley rats were processed for the immunolocalization of ER-ir using monoclonal antibody H222 (Abbott Laboratories) and tetramethylbenzidine (TMB). ER-ir and nonER-ir neurons were photographed on a Hitachi H7000 transmission electron microscope. No differences in perikaryal area of ER- and nonER-ir cells were present across the estrous cycle or following ovariectomy. However, significant decreases in nuclear circumference were present in ER-ir cells at proestrus relative to other cycle stages and to ovariectomized females. Nuclear area was unaffected by cycle stage or ovariectomy in ER- and nonER-ir cells. Significant differences were also present in the number of axosomatic synapses upon AVPv neurons. More synapses were present on nonER-ir cells than ER-ir cells, particularly at proestrus. When data from ER- and nonER-ir neurons was pooled, there was no significant difference in the number of axosomatic contacts across the estrous cycle. However, significantly more synapses were present on ER-ir cells following ovariectomy than during proestrus. This effect was not seen in nonER-ir cells. Thus, collectively, these data support the hypothesis that the degree of axosomatic afferent input to ER- and nonER-ir cells is different and that the steroidal environment alters synaptic input to ER-ir neurons in the AVPv. [Supported by NIH HD 29050]

53.4

DISTRIBUTION OF ESTROGEN RECEPTOR-IMMUNOREACTIVE CELLS IN THE FOREBRAIN OF THE FEMALE SIBERIAN HAMSTER C.M. Meyers, M.J. Duncan, M.B. Nikitovitch-Winer*, and R.E. Watson, Jr. Dept. of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, KY 40536.

Gonadal steroids have profound effects on reproduction, thermoregulation, and body weight in Siberian hamsters. To identify potential sites of action of estrogen in this species, we investigated the distribution of estrogen receptor - immunoreactive (ER-ir) cells in the forebrain. Ovariectomized hamsters (2 weeks) were perfused with Zamboni's fixative and sections through the forebrain were incubated in monoclonal antibody H222 (Abbott Laboratories) and reacted using nickel-intensified diaminobenzidine. ER-ir cells were identified by the presence of dense reaction product that was localized predominantly in cell nuclei. Many heavily labeled cells were present in the medial preoptic region at its anterior pole immediately adjacent to the third ventricle and at more posterior and lateral levels. ER-ir cells were also present in the ventral part of the lateral septal nucleus and in the adjacent bed nucleus of the stria terminalis (BNST). Further caudally, abundant labeled cells were present in the encapsulated part of the BNST. Fewer ER-ir cells were present in the anterior hypothalamus and the periventricular nucleus at this level, and none were present in the suprachiasmatic, supraoptic, or paraventricular nuclei. Many ER-ir cells were present in the retrochiasmatic area, arcuate nucleus and in the ventrolateral pole of the ventromedial nucleus and immediately adjacent to it. Only sparse ER-ir cells were present in the dorsomedial nucleus. Abundant ER-ir cells were present in the premammillary nuclei. In the amygdala, many heavily labeled cells were present in the medial and cortical nuclei, and posteriorly, the amygdalohippocampal area contained abundant ER-ir cells. Finally, labeled cells were also present in the midbrain central gray. These results demonstrate that the overall pattern of ER-ir in this species is very similar to that reported previously for a number of other species, including the rat, guinea pig, and opossum. [Supported by NIH DK 42056 (MJD) and HD 29050 (REW)]

53.5

VASOPRESSINERGIC SYNAPTIC INPUT UPON ESTROGEN RECEPTIVE NEURONS IN THE ANTERIOR PREOPTIC REGION OF THE RAT: SUPRACHIASMATIC NUCLEUS ORIGIN?

R.E. Watson, Jr.*, and M.C. Langub, Jr. Dept. of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, KY 40536.

Feedback of increasing titers of estrogen and progesterone on the brain during the follicular phase of the estrous cycle stimulates the preovulatory surge of pituitary luteinizing hormone (LH) secretion. Steroid receptive neurons located at the anterior pole of the preoptic area in the anteroventral periventricular nucleus (AVPv) are believed to play a critical role in transduction of the steroid feedback (Wiegand and Terasawa, 1982). The timing of the preovulatory LH surge is evidently governed also by a circadian signal since it occurs at a characteristic time of day relative to the light-dark cycle to which the rat is entrained. Thus, the possibility exists that estrogen receptive neurons in the AVPv receive synaptic input from the suprachiamatic nucleus (SCN). To test this possibility we have employed ultrastructural double label immunocytochemistry to determine whether vasopressin (VP) - immunoreactive (ir) fibers in the AVPv, which arise predominantly in the SCN, provide synaptic input to estrogen receptor-ir (ER-ir) neurons. Sections through the AVPv from cycling female rats were stained sequentially for ER-ir using monoclonal antibody H222 (Abbott Laboratories) using tetramethylbenzidine (TMB), while VP-ir was demonstrated with rabbit polyclonal antisera (ICN) and diaminobenzidine (DAB). Ultrastructurally, TMB and DAB reaction products can be readily distinguished from one another. Abundant examples of synaptic interactions between VP-ir terminals and ER-ir neurons throughout the AVPv were present. Thus, these results strongly support the hypothesis that VPergic neurons in the SCN are capable of regulating the activity of ER-ir AVPv neurons, thereby potentially providing a means by which circadian information from the SCN could be integrated with estrogen feedback signals. To further explore synaptic relationships between the SCN and AVPv, PHA-L injections have been made into the SCN and PHA-L-ir synapses documented ultrastructurally in the AVPv. [Supported by NIH HD 29050]

53.6

RESTRICTED COLOCALIZATION OF PROGESTIN RECEPTORS (PR) AND OXYTOCIN (OT) IN HYPOTHALAMIC NEURONS OF STEROID-TREATED MONKEYS

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Progesterone (P4) increases PRL secretion in estrogen (E)-primed monkeys, but lactotropes do not have PR. OT directly stimulates PRL secretion. We questioned whether OT neurons are progestin target cells which could mediate the effect of P4 on PRL secretion. Female rhesus monkeys were spayed and implanted with either empty silastic capsules [**SPAY**], E-filled capsules for 28 days [**E**], or E-capsules supplemented with P4 capsules for the last 14 of 28 days [**E+P**]. Hypothalamic sections (10μ) were double-labeled for PR and OT using B39 (2μg/ml) and nickel intensified DAB to visualize PR in the nucleus followed by anti-OT (W.K. Samson) plus DAB to visualize OT in the cytoplasm. Nuclear staining for PR in the magnocellular SON is slightly more diffuse than in other PR+ neurons. Distributions of PR+ cells and OT+ cells overlapped in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON). However, colocalization of PR and OT in the same cells was less than 5% in the PVN and the medial SON regardless of steroid treatment. E-treatment did not signficantly alter PR in the SON. Rather, a signficant population of lightly staining OT cells with PR was observed in the lateral SON due to an apparent induction of OT immunoreactivity with E. Because PR was evident in other magnocellular neurons, the colocalization of PR and arginine vasopressin (AVP) was explored. Preliminary evidence suggests that the majority of PR-positive magnocellular neurons contain AVP. Hypothalamic OT content by RIA was higher in rostral, medial basal, dorsomedial and mamillary areas of adult male and female monkeys than in juveniles of both sexes. Supported by HD17269, HD18185.

53.7

PROGESTERONE RECEPTOR mRNA EXPRESSION IN THE FEMALE RAT HYPOTHALAMUS.

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We have previously reported that progesterone receptor (PR) mRNA is transiently expressed in granulosa cells of the rat ovary during the estrous cycle. Unlike PR mRNA in other target tissues for progesterone action (e.g. uterus and mammary gland), ovarian PR mRNA is not induced by estrogen but rather is increased by the pituitary hormone LH. The brain is known to be a target for progesterone action. In the present study, we addressed the question of whether PR mRNA is regulated in the female rat brain during the estrous cycle. We localized PR mRNA using *in situ* hybridization with ^{35}S-labeled antisense RNA to the rat PR. Many areas throughout the brain express PR mRNA but they appeared to be categorized into two groups; Levels of PR mRNA in areas including the cortex and hippocampus appeared not to change throughout the estrous cycle; However, levels of PR mRNA in the medial preoptic nucleus (MPN), arcuate nucleus (Arc), and ventromedial hypothalamus (VMH) were regulated during the estrous cycle. PR mRNA levels in these areas were low on estrus and metestrus, high on diestrus and proestrus, and down regulated during the transition from proestrus to estrus, suggesting that PR mRNA expression in these brain areas may be regulated by circulating estrogen. We studied effects of estrogen on PR mRNA expression in these brain areas using ovariectomized (OVX) and ovariectomized, estrogen-primed (OVX+E) rats. Ovariectomy was for 7 days and estrogen treatment was for 2 days. *In situ* hybridization showed that PR mRNA levels in the MPN, Arc, and VMH of OVX+E rats were much higher than those of OVX rats. We also utilized a reverse transcription-polymerase chain reaction (RT-PCR) assay to compare PR mRNA levels in dissected hypothalamic fragments of OVX and OVX+E rats. PR mRNA levels in OVX+E rats were about 2.5-fold higher than those in OVX rats. Taken together, our data strongly suggest that PR mRNA expression in the MPN, Arc, and VMH regions of the brain is induced by estrogen.

53.8

IMMUNOCYTOCHEMICAL COLOCALIZATION OF PROGESTIN RECEPTORS (PR) AND SUBSTANCE P (SP) IN HYPOTHALAMIC NEURONS OF STEROID-TREATED MONKEYS

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Progesterone (P4) increases PRL secretion in estrogen (E)-primed monkeys, but lactotropes do not have PR. Substance P is found in high concentrations in the external zone of the median eminence in primates and it stimulates PRL secretion. We questioned whether SP neurons are progestin target cells which could mediate the effect of P4 on prolactin secretion. Female rhesus monkeys were spayed and implanted with either empty silastic capsules [**SPAY**], E-filled capsules for 28 days [**E**], or E-capsules supplemented with P4 capsules for the last 14 of 28 days [**E+P**]. Hypothalamic sections (10μ) were double-labeled for PR and SP using B39 (2μg/ml) and nickel intensified DAB to visualize PR in the nucleus followed by anti-SP (R.L.Eskay) plus DAB to visualize SP in the cytoplasm. SP+ neurons were located in a small caudal periventricular group, in the arcuate/ median eminence and underneath the capsules of the mamillary nuclei in spayed monkeys. In addition, there was a significant induction of SP+ neurons in the VMN and ARC of the E-treated monkey which diminished in the E+P monkey. In the external zone of the median eminence, IR-SP decreased with E and then increased with E+P.

%SP+PR	Perivent	VMN	ARC/ME	SUBMAM
Spay	110/265 (41%)	19/40(47%)	7/29(3%)	0/22(0%)
E	31/57 (62%)	1495/1495(100%)	224/230(97%)	1/60(0%)
E+P	3/218 (1%)	16/21 (76%)	35/36 (97%)	0/93(0%)

Hypothalamic SP content by RIA was higher in rostral, medial basal, dorsomedial and mamillary areas of 6 intact female monkeys than in 3 spays, 3 juveniles or 1 pregnant female. Supported by HD17269, HD18185.

53.9

CYTOPLASMIC ESTROGEN RECEPTORS IN RAT BRAIN: IMMUNOCYTOCHEMICAL EVIDENCE USING THREE DIFFERENT ANTIBODIES AND EFFECTS OF ESTRADIOL INJECTION.

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The existence of cytoplasmic estrogen receptors has been reported in the brains of a variety of species using immunocytochemical techniques. Because all previous studies used the same antibody (H 222; Abbott Laboratories), it is possible that this finding is idiosyncratic to one antibody. Therefore, three antibodies directed against diverse epitopes on the estrogen receptor protein were used to immunostain estrogen receptors in rat brain. With each antibody, estrogen receptor-immunoreactivity (ER-IR) was observed in the hypothalamus, preoptic area, amygdala and midbrain central gray. While the darkest reaction product was seen within cell nuclei, in most areas extensive ER-IR was also observed in perikaryal cytoplasm and cytoplasmic processes. Estradiol injection caused a rapid, large decrease in ER-IR when the H 222 antibody was used. This decrease was not due to movement of the receptors to the cell nucleus, as cell nuclear ER-IR decreased too. Furthermore, the extent of loss of immunostaining was greatly influenced by the particular antibody used, suggesting that the decrease in ER-IR is due to a conformational change in the receptor. This study demonstrates the existence of neural ER-IR in perikaryal cytoplasm and cytoplasmic processes in rat brain, it suggests that the cytoplasmic ER-IR is not just a small fragment of the receptor protein, and it suggests that the decrease in ER-IR seen immediately after estradiol injection with some antibodies is due to a conformational change in the receptor.
(Supported by NIH NS 19327, RSDA MH 00885, and NSF BNS 11464)

53.10

IN VITRO **EXCHANGE AUTORADIOGRAPHY OF ESTROGEN RECEPTORS: REGIONAL DISTRIBUTION OF ESTROGEN RECEPTOR OCCUPATION IN THE BRAIN OF THE ADULT MALE RAT.**

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Conversion of testosterone to estradiol in the diencephalon and limbic system plays an important role in neurochemical and behavioral responses to circulating androgen. The extent to which locally-synthesized estrogen impacts on the estrogen receptor (ER) systems in different regions of the brain has, however, been difficult to determine because of methodological problems. Using a recently-developed *in vitro* autoradiographic method (Walters *et al.*, Soc. for Neurosci.,17:1410, 1991), we re-examined the distribution of occupied ER in the brain of the male rat. Cryostat sections (20 μm) through the brain were incubated with 11β-methoxy-16α-[^{125}I]iodoestradiol, then washed and exposed for 16-24h against Amersham Hyperfilm. The regional distribution of estrogen binding was assessed from the autoradiograms by computerized densitometry. In orchidectomized rats, very little labelling was observed. In intact males, very high levels of ER occupation - 50% or more of the available binding capacity - were observed in the periventricular preoptic area, ventromedial nucleus, amygdala and bed nucleus of the stria terminalis. Much lower levels of occupation were observed in the medial preoptic, arcuate and supraoptic nuclei, all of which have previously been reported to contain aromatase based on biochemical and/or immunocytochemical studies. These studies demonstrate a remarkable specificity in the extent of ER occupation in the brain of the intact male rat. In some regions of the brain, ER occupation in the male is comparable to that observed in cycling females at proestrus. The regional pattern of ER occupation in the male differs in important respects from the predictions of previous studies based on measurement of aromatase activity. [Supported by grants MT-11235 (to TJB) and PG 11115 (to NJM) from MRC Canada, and CA 37799 (to RBH)].

53.11

LOCALIZATION AND MEASUREMENT OF OCCUPIED ANDROGEN RECEPTORS IN THAW-MOUNTED TISSUE SECTIONS. T.J. Brown*, M. Sharma, and N.J. MacLusky. Dept. of OB/GYN, Univ. of Toronto, Toronto, ON M5G 1L7.

The study of androgen action in the brain has been severely hampered by the lack of a suitable method to quantify occupied androgen receptors with the necessary degree of anatomical resolution. We now report the development of a quantitative autoradiographic method that uses a binding exchange reaction in thaw-mounted tissue sections (10-20 μm). This method enables the selective measurement of occupied androgen receptors with anatomical resolution approaching the cellular level. The appropriate conditions for the method were established in frozen ventral prostate sections from intact, castrate, or testosterone-treated (500 μg) rats. Sections were incubated with 2.5 nM [^{3}H]R1881 for 0.5-120 hr at either 4, 14, 22, or 37C. All incubates contained triamcinolone acetonide to suppress binding to the progestin receptor and parallel incubations were conducted in the presence of unlabeled R1881 to assess nonspecific binding. After incubation, sections were briefly rinsed before fixation in 4% paraformaldehyde and washed to remove unbound [^{3}H]R1881. After drying, the sections were scraped from the slides and radioactivity and protein content were determined. Optimum binding was achieved with a 72 hr incubation at 4C. Essentially no androgen receptor binding was detected in castrate animals whereas levels in intact and testosterone-treated rats were 79.3 and 143.6 fmol/mg protein respectively, indicating that the method is selective for the occupied receptor. Saturation binding analysis revealed binding to a single class site with a Kdiss = 0.546 nM. Autoradiographic images of androgen binding in the brain were obtained by treating brain sections as described and placing them against emulsion-coated film for a period of 2.5 mo. Essentially no binding was observed in the brain of castrate animals while specific binding was observed in the preoptic and hypothalamic areas of the intact and testosterone-treated animal. This method will enable, with a high degree of anatomical resolution, the quantification of active androgen receptor in specific regions of the brain (or any other tissue) under various physiological conditions. Supported by Canadian MRC grants MT-11235 (to TJB) and PG 11115 (to NJM).

53.12

COLOCALIZATION OF ESTROGEN-CONCENTRATING CELLS AND CRF-LIKE IMMUNOREACTIVE NEURONS IN THE MOUSE PVN. K.D.R. Morton*, M.S. Brownfield and N.J. MacLusky. Dept. of OB/GYN, Univ. of Toronto, Toronto, Ontario M5G 1L7 and Dept. Comp. Biosciences, Univ. of Wisconsin, Madison,WI.

Stimulation of hypothalamic corticotropin-releasing factor (CRF), by stress or other factors, suppresses gonadotropin release. This is mediated, in part, via opioid inhibition of GnRH from the hypothalamus. CRF mRNA in the parvocellular paraventricular nucleus (PVN) varies during the estrous cycle of the female rat, with a significant increase in CRF mRNA levels during the afternoon of proestrus, corresponding with the estrogen-induced ovulatory surge of LH (Bohler et al., 1990). Our studies (Walters et al., 1990) have shown populations of estrogen-concentrating cells in the PVN in mouse brain. These results suggest a role for estrogen as a neuromodulator for CRF. Female CD-1 mice were OVX and given i.c.v. injections of colchicine (4 μg/μl). After 48 hrs, mice were injected with 11β-methoxy-16-[^{125}I]-estradiol (MIE_2; 2 μg/kg) via the external jugular vein 1 hr before intracardial perfusion of saline followed by 4% paraformaldehyde in 0.1 M PB. The control received an injection of DES (1 μg/μl) prior to MIE_2. Coronal sections (10 μm) were thaw-mounted onto slides precoated with Kodak NTB-3 photographic emulsion. The slides were exposed for 7 days, developed with Kodak D-170 (neutral pH), then immediately immunostained using a primary antibody for CRF (gift of Dr. Mark Brownfield, Madison, WI) and a Vectastain Elite kit. Our findings do not show an extensive colocalization of estrogen-concentrating cells and CRF-like immunoreactive neurons throughout the PVN. However, there are scattered cells within the medial and lateral parvocellular subdivisions of the PVN which show colocalization, and a substantial number in the ventral, periventricular aspects of the PVN. These findings are in agreement with Bohler et al., where the descibed changes in CRF mRNA message were restricted to the ventral regions of the PVN. Supported by MRC Canada PG11115.

53.13

DIFFERENTIAL EFFECTS OF CASTRATION ON ANDROGEN RECEPTOR DYNAMICS IN GUINEA PIG BRAIN. J.V.A. Choate*, P.B. Connolly and J.A. Resko. Dept. of Physiology, Oregon Health Sciences Univ., Portland, OR 97201, Oregon Reg. Primate Res. Ctr., Beaverton, OR 97006.

We studied the effects of androgen removal on cytosolic (ARc) and nuclear (ARn) androgen receptor dynamics by measuring ARc and ARn levels in preoptic area, septum, medial basal hypothalamus (MBH), amygdala and cortex at different times after castration (Cx, 12 hrs, 1, 2, 3, 5, 7, 10 and 14 days). We compared these results with those from other androgen sensitive tissues, prostate and seminal vesicle. Receptor content was measured by a binding assay using ^{3}H-dihydrotestosterone as a ligand and radioinert methyltrienolone to determine nonspecific binding. In accessory organs of reproduction, ARc was elevated over intact levels at all time periods studied ($p<0.05$). In brain, only MBH showed elevated ARc content at one time period (3 days post castration). ARn levels, however, declined significantly ($p<0.05$) in peripheral tissues and all brain areas, except cortex, at all times after castration. These data provide evidence for a differential effect of androgen withdrawal on AR in target tissues. Differences in the response of brain and peripheral tissues to Cx show up primarily in ARc dynamics. Supported by NIH grants: T32 HD07133 and HD18196

53.14

ETHANOL EFFECTS ON NUCLEAR ANDROGEN RECEPTORS IN THE HYPOTHALAMUS AND PITUITARY GLAND IN MALE RATS. K.W. Chung and D. J. Gower.* Dept. Anat. & Surgery, Univ. Okla., Okla. City, OK 73190

The present study was undertaken to investigate ethanol-induced and castration-induced changes in the concentration of nuclear androgen receptors in the hypothalamic(H)-pituitary(P) unit. Young adult male rats were pair fed for 3 months a nutritionally complete liquid diet containing ethyl alcohol (36% of total calories) or an identical liquid diet with the isocaloric substitution of sucrose for ethanol. Rats were castrated and implanted subcutaneously with a 50 mg testosterone pellet 2 weeks prior to being sacrificed. The nuclear androgen receptor contents of the P and H were measured by cell nuclear exchange assay. Scatchard analysis revealed that mean values of androgen receptors of the P and H were significantly altered in ethanol-fed rats (93.4 ± 8.7 and 72.3 ± 6.3 fmol/mg DNA, respectively), when compared to values from the isocaloric controls (182.4 ± 16.7 and 116.8 ± 10.3 fmol/mg DNA). Also, castration decreased the nuclear receptor binding of the P (3.4 ± 0.3 fmol/mg DNA) and the H (6.8 ± 0.5 fmol/mg DNA) in controls. Treatment of castrated control animals with testosterone increased binding sites to 167.6 ± 15.4 fmol/mg DNA for the P and 104.7 ± 9.8 fmol/mg DNA for the H. However, testosterone given to castrated ethanol-fed rats did not significantly alter ethanol-induced reduction of the nuclear receptor sites. Dissociation constants of the ^{3H}-R1881-receptor complex are similar in all groups. These findings indicate that ethanol not only exerts a suppressive effect on the nuclear receptor contents of the H and P but also inhibits the castration-induced change in the receptor binding sites.

NEUROENDOCRINE REGULATION: PROLACTIN

54.1

REGULATION OF PROLACTIN-RECEPTOR AND PROLACTIN-RECEPTOR mRNA BY HUMAN GROWTH HORMONE AND GLUCOCORTICOID IN MOUSE LIVER. J.R. Dave, T. Dang, P. Bednarek, E.W. Mougey* and E.W. Bernton. Departments of Medical Neurosciences and Bacterial Disease, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Earlier studies have demonstrated an up-regulation of hepatic prolactin binding sites with ovine prolactin (oPRL) or human growth hormone (hGH) treatment and down-regulation with glucocorticoid (GC) treatment *in vivo*. We have demonstrated that the up-regulation of hepatic prolactin binding following lactogenic hormone treatment may be due to an increase in membrane fluidity. The objective of this study was to determine if hGH and GC treatment had any effect on prolactin receptor mRNA in mouse liver. Adult female C3H mice were treated with vehicle, hGH (24 μg/day) or dexamethasone (50 μg/day) for 72 hr. Using the reverse transcriptase/polymerase chain reaction (RT/PCR) technique and specific forward and reverse oligonucleotide PCR primers (representing a total of 390 nucleotides including the entire transmembrane region of the prolactin receptor gene), hepatic RNA samples were amplified, and the Southern blots hybridized with a ^{32}P-labeled oligonucleotide probe. Glucocorticoid treatment decreased both prolactin binding activity and prolactin receptor mRNA levels in liver. However, hGH treatment increased prolactin binding activity without affecting prolactin receptor mRNA levels. These findings suggest that the effect of GC on down-regulation of prolactin binding activity may be at the transcription level, however, hGH mediated up-regulation of prolactin binding activity may be a membrane-associated phenomenon.

54.2

EFFECTS OF FEED RESTRICTION ON MAMMARY TUMOR GROWTH AND HYPOTHALAMIC NEUROTRANSMITTERS IN RATS. S. Thyagarajan* and S.K. Quadri. Kansas State University, Manhattan, KS 66506.

Restriction of feed intake inhibits the growth of dimethylbenzanthracene (DMBA)-induced mammary tumor in rats and this effect can be blocked by treatment with estrogen (E) and haloperidol (HAL). Although feed restriction is known to affect the neurotransmitter (NT) metabolism in the brain, the exact mechanism of this effect on mammary tumor growth and its blockade by E and HAL are not known. The purpose of this study was to investigate the long-term effects of feed restriction with or without simultaneous treatment with E and HAL on hypothalamic NT and mammary tumor growth. Sprague-Dawley female rats with DMBA-induced mammary tumor were full fed (FF), half fed (HF), or HF and treated with estradiol benzoate (EB) and/or HAL for a duration of 15 weeks. Mammary tumor diameter was determined every week. The concentrations of NT were determined in the medial basal hypothalamus at the end of the treatment period by HPLC-EC. They are expressed in terms of per μg protein. The average tumor diameter increased by 213±88% (Mean±S.E) in the FF rats during 15 weeks period whereas, it decreased by 81±13% in the HF rats ($p<0.001$). In contrast to HF rats, the tumor diameter increased by 83±36% in HF+EB, 42±23% in HF+HAL, and 43±11% in HF+EB+HAL rats ($p<0.05$). The hypothalamic dopamine (DA) concentration increased ($p<0.01$) in the HF (21.5±0.5 pg) compared to that in the FF (19.1±0.4 pg) rats. In contrast to HF rats, DA concentration decreased to 18.5±0.5 pg in the HF+HAL and to 15.7±0.5 pg in the HF+EB+HAL ($p<0.01$) rats. The concentration of serotonin was 133.4±17.6 pg in the FF and 78.2±10.7 pg in the HF ($p<0.05$) rats. Compared to the HF rats, serotonin concentration increased to 120.2±12.2 pg in the HF+HAL rats ($p<0.05$). These results suggest that long-term treatment with E and HAL increases the growth of mammary tumors in dietary restricted rats through alterations in the concentration of hypothalamic NT. (Supported by NIH grant AG 05980)

54.3

STIMULATION OF A PROTEIN TYROSINE PHOSPHATASE (PTP) AS A POSSIBLE INTRACELLULAR MECHANISM FOR DOPAMINE ANTIPROLIFERATIVE EFFECTS. T. Florio, M.-G. Pan, O. Civelli* and P.J.S. Stork. Vollum Institute for Advanced Biomedical Research, OHSU, Portland, OR 97201, USA.

Dopaminergic agonists are largely used in the pharmacological treatment of GH or PRL secreting pituitary adenomas. In this report we studied the possible mechanisms of action of DA in the control of cell proliferation in a transfected tumor pituitary cell line, GH_4ZR7, expressing high levels of the short form of dopamine D_2 receptor. Dopamine (DA) (100 nM-10 μM) inhibited DNA synthesis, measured as [^{3}H]-thymidine uptake, in a dose-dependent manner. This effect was completely abolished by pretreating the cells with the D_2 receptor antagonist haloperidol, with pertussis toxin or with vanadate, a selective PTP inhibitor. These results suggest that the stimulation of a PTP activity could be responsible for the DA effects on DNA synthesis. In fact, in GH_4ZR7 membranes, DA (50 nM-10 μM) stimulated a PTP activity, as measured by using the synthetic substrate para-nitrophenol phosphate. This effect was also reverted by haloperidol, pertussis toxin and vanadate.

These results show that DA is able to decrease cell proliferation in GH_4ZR7 cells and suggest that DA activation of PTP activity could represent one of the intracellular mechanisms mediating this effect.

54.4

PROLACTIN TREATMENT DURING DEVELOPMENT IN AMES DWARF MICE INCREASES DOPAMINE HISTOFLUORESCENCE AND TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN VENTRAL HYPOTHALAMUS. M.I. Romero, C.J. Phelps.* Neurosciences Training Program and Department Anatomy, Tulane University School of Medicine. New Orleans, LA 70112.

Under physiological conditions, pituitary prolactin (PRL) secretion is tonically inhibited by hypothalamic dopamine (DA). Dwarf mice (Snell and Ames) do not produce PRL, are nearly totally DA deficient in the median eminence (ME), and arcuate nucleus (ARC) tyrosine hydroxylase (TH) immunoreactivity is reduced compared with normal phenotypic littermates. PRL treatment in adult dwarfs has failed to increase DA levels in the ME (Morgan et al., Endocrinology,1981) or significantly increase the number of TH neurons in ARC (Morgan and Besch, Neuroendocrinology, 1990). In the present study we tested the possibility that PRL feedforward effect on the DA neurons in the tuberoinfundibular region is essential during development in order for the neurons to synthesize DA. Ames dwarf and normal sibling mice were treated daily with 50 μg of PRL i.p. for 30 days starting at 12 days old; at 42 d anesthetized animals were perfused transcardially with paraformaldehyde (4%)-glutaraldehyde (0.5%) to induce histofluorescence; brains were sectioned coronally at 30 μm using a vibratome. TH immunocytochemistry was performed on floating sections and cell numbers in ARC were recorded. In agreement with previous studies, untreated dwarf mice showed almost no DA fluorescence in ME external zone and TH cells in ARC were about 40% of normal siblings, accompanied by a decrease in TH staining intensity in ME. PRL treatment increased the intensity of DA fluorescence in ARC and ME to a level comparable to normal mice, and TH-positive cell numbers in ARC were similar to normal values. Although TH ARC cell numbers in PRL-treated normal mice did not show a significant change compared with untreated mice, the staining intensity of the TH fibers in ME was clearly increased. No differences according to sex were found. The data suggest that target hormone secretion is necessary for full development of CNS pituitary regulatory neurons. Supported by PHS grant NS25987 (CJP).

54.5

QUANTIFICATION OF TYROSINE HYDROXYLASE-IMMUNOREACTIVE TUBEROINFUNDIBULAR NEURONS IN THE HYPOTHALAMUS OF ADULT AND DEVELOPING AMES DWARF MICE. C.J. Phelps, M.Y. Vaccarella, D.L. Hurley*. Dept. Anatomy, Tulane Univ. Sch. Med., New Orleans, LA 70112.

Dopaminergic (DA) neurons in the hypothalamic arcuate nucleus (ARC) tonically inhibit pituitary prolactin (PRL) secretion. Ames dwarf (df/df) mice, which produce no PRL, have a reduced number of ARC tyrosine hydroxylase (TH)-immunoreactive neurons (Morgan and Besch, Neuroendocrinology, 1990). In order to quantify whether this deficit in adult dwarfs is present at birth or regresses during development, immunocytochemically stained DA/TH cells were counted in zona incerta (ZI, A13), periventricular region (A14), and ARC (A12) at 180 μm intervals in coronal sections through the hypothalamus of dwarf and normal sibling (DF/?) mice 7, 14, and 21 days, and 2-16 months, of age. At 7, 14, and 21d, TH+ cell numbers were comparable for DF/? and df/df and increased dramatically (to 2-3X at 21d compared with counts at 7d) in A13, A14, and A12; the developmental increase was smallest in ZI. Between 21d and 2 mos, both DF/? and df/df showed a decrease in A12 TH+ neurons, which was significant in dwarfs ($p<0.02$), and at 2 mos, ARC TH+ cells in df/df were significantly less ($p<0.005$) in number than in DF/?. Total ARC TH+ cell numbers are shown below.

mouse type / age:	7d	14d	21 d	2-4 mo.
DF/? (n=6-10)	918±137	2107±311	3306±572	2387±136
df/df (n=6-10)	1095±200	1425±251	2707±437	1176±277

In adults (4 -16 m.o.), both A14 ($p<0.05$) and A12 ($p<0.001$) groups showed fewer TH+ cells in dwarfs; TH cells in ZI numbers were not significantly reduced. Gender differences were not significant. Adult total TH+ neurons are shown below.

mouse type / neuron group:	A13	A14	A12
DF/? (n=17)	1818±186	4245±485	2710±292
df/df (n=13)	1443±134	2580±434	1315±147

The results imply that absence of PRL is related to decreased TH expression in A12; A14 may also be involved in this developmental regression in DA PRL-regulating neurons. Supported by PHS grant NS25987 (CJP).

54.6

USE OF RECEPTOR ANTIBODIES TO CHARACTERIZE PROLACTIN RECEPTORS IN THE RING DOVE BRAIN. C. Li[1], P. A. Kelly[2], and J. D. Buntin.[1]* Dept. of Biological Sciences, UW-Milwaukee, Milwaukee, WI 53201[1], and INSERM Unite 344 (Endocrinologie Moleculaire), 75730 Paris Cédex 15, France[2].

Central administration of prolactin(PRL) has been shown to inhibit gonadotropin secretion and to facilitate food intake and parental behavior in ring doves. In addition, PRL receptors have been mapped in ring dove brain by in vitro autoradiography. In the present study, the characteristics of PRL receptors were examined in binding studies using a polyclonal antiserum generated in rabbits against rat liver prolactin receptors. A purified gamma globulin (IgG) fraction of the antiserum was first obtained using protein A affinity chromatography. Frozen dove brain sections were then slide mounted and incubated with ^{125}I-ovine PRL plus receptor antibody, ^{125}I-ovine PRL plus an IgG fraction obtained from normal rabbit serum, or ^{125}I-ovine PRL plus excess unlabelled PRL and normal rabbit serum IgG. After washing, sections were dried and apposed to Kodak SB-5 film. Densitometric analysis of autoradiographs revealed that receptor antibody concentrations of $1.2x10^{-6}$ M to $5.76x10^{-7}$ M inhibited the specific binding of labelled PRL by 40% to 50% in the choroid plexus, lateral septal area, the ventromedial, suprachiasmatic, and paraventricular nuclei of the hypothalamus, and the area surrounding the paraventricular organ. The tuberal region of the hypothalamus showed approximately 20% inhibition of specific binding with the same antibody concentration. No significant inhibition was observed with $1.2x10^{-7}$ M or $5.76x10^{-8}$ M concentrations of antibody. This study suggests structural similarities between the dove brain and rat liver PRL receptors and offers a methodological tool for examining the action of centrally administered PRL in vivo (supported by NIMH grant MH41447).

54.7

SEROTONIN-INDUCED INHIBITON OF TYROSINE HYDROXYLASE (TH) ACTIVITY AND STIMULATION OF PROLACTIN (PRL) RELEASE ARE ABOLISHED AT MIDPREGNANCY. Joanne R. Mathiasen* and James L. Voogt Department of Physiology, University of Kansas Medical Center Kansas City, KS 66103

We investigated the effect of central serotonin (5-HT) administration on hypothalamic tuberoinfundibular dopamine (TIDA) neurons and related the changes to plasma PRL levels in two physiological models: 1) pregnant rats on days 8, 11 and 16; and 2) ovariectomized (OVX) rats injected under the kidney capsule with rat choriocarcinoma (Rcho) cells, which secrete placental lactogen-I. Rats were given 5-HT (20 μg/6 μl) through a lateral ventricular cannula and blood samples were taken at various times for PRL RIA. NSD 1015 (25 mg/kg i.a.), a dihydroxyphenylalanine (DOPA) decarboxylase inhibitor, was injected at 20 min. Ten min later, rats were killed and the stalk-median eminence (SME) was dissected. The rate of DOPA accumulation as a measure of TH catalytic activity was determined by measuring DOPA levels in the SME by HPLC. DOPA accumulation in 5-HT-treated rats on day 8 of pregnancy was significantly ($p<0.05$) reduced to 57% of vehicle-treated controls. This decrease in DOPA accumulation correlated with a 13-fold increase in PRL levels. In contrast, on days 11 and 16 of pregnancy, SME DOPA accumulation and PRL levels were unchanged following 5-HT treatment. In vehicle-treated OVX rats, DOPA accumulation in the SME was 41.9±8.5 ng/mg protein. 5-HT treatment significantly ($p<0.05$) decreased DOPA accumulation in the SME to 18.2±2.0 ng/mg protein, and increased PRL levels 26-fold. In OVX rats injected with Rcho cells, 5-HT treatment produced no changes in either SME DOPA accumulation or circulating PRL levels compared to OVX controls. These data suggest that the presence of placental lactogens beginning at mid-pregnancy, may terminate the 5-HT-induced decrease in TIDA neuronal activity and corresponding increase in plasma PRL levels present in early pregnancy.

54.8

EFFECTS OF DOPAMINE (DA) ON PROLACTIN (PRL) SECRETION AND CYTOSOLIC CALCIUM CONCENTRATIONS IN A CLONAL PITUITARY CELL LINE EXPRESSING THE SHORT FORM OF THE D2 RECEPTOR. T.P.Burris* and M.E.Freeman. Dept of Biol Sci, Fl St Univ, Tallahassee, 32306.

GH_4C_1 cells are rat somatomammotrophs which lack DA receptors. We have examined the ability of DA to modulate both basal and TRH stimulated PRL secretion and cytosolic calcium concentrations in GH_4C_1 cells stably transfected with the cDNA for the short form of the rat DA D2 receptor (GH_4ZR_7 cells). Cells were plated on 96-well microtiter plate and challenged with various concentrations of DA and/or TRH for 6 hrs. Fura-2 was used to estimate changes in cytosolic calcium concentrations by single cell microfluorimetry. Twenty to 30 cells were recorded simultaneously and challenged with various concentrations of DA and/or TRH. DA (0.1 pM - 10 μM) had no effect on PRL secretion or cytosolic calcium in GH_4C_1 cells. However, DA at concentrations of 1 and 10 μM significantly inhibited basal PRL secretion 25% and 36% respectively in GH_4ZR_7 cells. Ten μM DA completely reversed the stimulatory effects of TRH (10 nM - 1 μM) on PRL secretion and 1 μM DA significantly attenuated the effects of TRH in these cells. Concentrations of DA lower than 1 μM had no effect on basal or stimulated PRL secretion. Basal cytosolic calcium concentrations were undisturbed by any concentration of DA. However, DA at concentrations of 100 nM and greater completely inhibited TRH induced increases in cytosolic calcium. A D2 receptor antagonist, Eticlopride (1 μM), completely blocked the ability of DA to inhibit the TRH-induced increase in cytosolic calcium. These data indicate that the short form of the D2 receptor when expressed in GH_4C_1 cells is able to mediate DAergic inhibition of basal PRL secretion by a mechanism which does not acutely alter basal cytosolic calcium concentrations. This receptor also mediates DAergic inhibition of TRH-stimulated PRL secretion by a mechanism which inhibits TRH-stimulated increases in cytosolic calcium. Supported by NIH, DK-43200.

54.9

THE 5-HT_3 ANTAGONIST ONDANSETRON ATTENUATES P-CHLOROAMPHETAMINE-INDUCED ELEVATION OF PROLACTIN, BUT NOT CORTICOSTERONE, RENIN, OR OXYTOCIN SECRETION. A.D. Levy*, P.A. Rittenhouse, J.E. Kerr, M.S. Brownfield & L.D. Van de Kar. Dept. Pharmacology, Loyola Univ. Chicago, Maywood, IL 60153 and Dept. Comparative Biosciences, Univ. Wisconsin, Madison, WI 53706.

Serotonin (5-HT) containing neurons in brain mediate the secretion of many hormones. The roles of the 5-HT receptor subtypes 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_2 have been previously studied, and have differential influence on the secretion of ACTH/corticosterone, prolactin, renin and oxytocin (see Van de Kar Ann. Rev. Pharmacol. Toxicol. 31:289, 1991 for review). This study examined whether pretreatment with the 5-HT_3 antagonist ondansetron (GR 38032F) would modify the stimulation of corticosterone, prolactin, renin, or oxytocin secretion, induced by the 5-HT releaser p-chloroamphetamine (PCA). Adult, male Sprague-Dawley rats (N=8/group) were administered ondansetron (0, 0.1, or 1 mg/kg i.p.) 30 minutes prior to PCA (0, 3, or 8 mg/kg i.p.) injection. Rats were sacrificed 1 hr after PCA for blood collection. Plasma concentrations of corticosterone, prolactin, renin, and oxytocin were determined by radioimmunoassay. The 5-HT releaser PCA increased secretion of all of these hormones ($p<.001$). The 5-HT_3 antagonist ondansetron (1 mg/kg) reduced the PCA (3 mg/kg)-induced stimulation of plasma prolactin concentration by 51% ($p<.05$, Duncan's post-hoc test). In contrast, the PCA-induced stimulation of corticosterone, renin and oxytocin secretions were not altered by ondansetron pretreatment. Furthermore, ondansetron administration alone (i.e. in rats receiving the 0 dose of PCA) did not alter plasma concentrations of any of the hormones examined. The data suggest that the serotonergic stimulation of prolactin secretion is partially mediated by 5-HT_3 receptors. The data also suggest that 5-HT_3 receptors are not likely to be involved in the serotonergic stimulation of corticosterone, renin or oxytocin secretions. (Supported by DA04865 and MH45812).

54.10

NEURONS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) MEDIATE THE SEROTONERGIC STIMULATION OF PROLACTIN (PRL) SECRETION. P.A. Rittenhouse*, A.D. Levy, Q. Li, & L.D. Van de Kar. Dept. Pharmacology, Loyola University Chicago, Maywood, IL 60153.

These studies examined the hypothalamic site regulating serotonergic (5-HT) control of PRL secretion in male rats. Initially, we characterized the pharmacology of the drugs utilized. The 5-HT uptake inhibitor fluoxetine significantly impaired the ability of the 5-HT releaser *p*-chloroamphetamine (PCA, 8 mg/kg, ip) to stimulate PRL secretion, demonstrating that PCA increases PRL *via* a 5-HT mechanism. Next, the ability of the 5-HT agonist, RU 24969, (0.5, 1, 5, 10 mg/kg, ip) to elevate PRL secretion was potentiated by pretreatment with the 5-HT synthesis inhibitor, *p*-chlorophenylalanine, suggesting that RU 24969 stimulates PRL secretion through post-synaptic action. RU 24969 was then injected either centrally through chronic intracerebroventricular (icv) cannulae or peripherally (ip). Rats were pretreated with the 5-$HT_{1C/2}$ antagonist LY53857 (icv). The PRL response was significantly inhibited by LY53857 if RU 24969 was injected ip, but not icv. However, RU 24969 injected icv significantly stimulated PRL secretion at doses 500 fold lower than the peripherally effective doses (10 μg/kg *vs* 5 mg/kg), suggesting a role for central 5-HT receptors in the regulation of PRL regulation. Lastly, a series of lesion experiments were performed using the cell-selective neurotoxin ibotenic acid. Ibotenic acid was injected (3 μg/0.3 μl) bilaterally into the hypothalamic PVN, dorsomedial, ventromedial, or supraoptic nuclei. The PRL response to PCA (8 mg/kg, ip) was unaltered in rats with dorsomedial, ventromedial or supraoptic nuclei lesions. In contrast, rats with histologically confirmed lesions in the PVN had a significantly lower PRL response to both RU 24969 (5 mg/kg, ip) and PCA (8 mg/kg, ip). These data suggest that cell bodies in the PVN are necessary to mediate the 5-HT regulation of PRL. Supported by AHA Chicago, MH45812 and DA04865.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION: LHRH AND LH I

55.1

GnRH-GnRH Neuronal Associations in Primate and Rodent Brain. J.W. Witkin*, M. Ferin and A.-J. Silverman. Dept. Anatomy and Cell Biology, Columbia Univ. Coll.. P&S., New York, NY 10032.

Gonadotropin-releasing hormone (GnRH) is secreted in rhythmic bursts, a mode which is essential to drive the pulsatile secretion of gonadotropins. There is increasing evidence that GnRH neurons themselves may be intrinsically pulsatile, however the synchronization of their release is not understood. We hypothesize an anatomical substrate. In a light microscopic study the brains of cycling rats (n=17) and ovariectomized rhesus monkeys with gonadal steroid replacement (n=3) were examined for the occurrence of GnRH neurons which were in apposition. In the rats the frequency was between 1.7% and 6.8% and in monkeys, between 3% and 9.3%, with examples throughout the anatomical distribution of GnRH neurons in both species. The frequency was independent of the phase of the estrous cycle. To examine the nature of these appositions, two GnRH neurons from the MBH of one monkey were serially sectioned and examined ultrastructurally. A computerized reconstruction revealed that each cell received a modest synaptic input (5 and 9 terminals,respectively), none of which was GnRH+. There were several regions where other neuronal or glial elements were not interposed between the processes of the GnRH cells. In some of these areas it appeared that cell membranes no longer intervened between the processes, suggesting that the two cells formed a syncytium. A neural network formed by anastomosing processes, highly novel within the CNS (and requiring confirmation), could provide the basis for synchronization of neurohormonal release. NIH DK42323

55.2

THE NUMBER OF LUTEINING HORMONE RELEASING HORMONE (LHRH)- CONTAINING NEURONS INCREASES IN RESPONSE TO GLUCOCORTICOID ADMINISTRATION. X. Huang and R. E. Harlan*, Dept. of Anatomy, Tulane Med. School, New Orleans, LA 70112.

Endogenous or exogenous glucocoticoid excess can lead to the development of hypogonadism. The present study investigated the effects of glucocoticoid on the number of LHRH-containing neurons in the central nervous system of 10 adult intact male rats. Glucocorticoid capsules (200 mg releasing 10 mg/day) were implanted subcutaneously in 5 rats and placebo capsules in another 5 rats for 1 week. Immunocytochemistry with the LR1 antibody (gift of R. Benoit) was used to determine the number of LHRH-containing cells in the brain. Sections which contained LHRH neurons were examined and the number of immunopositive neurons per section was determined. It was found that the total number of LHRH-immunoreactive neurons in glucocorticoid-treated rats increased by 20% when compared to immunoreactive neurons in placebo-treated rats. The increase in the number of immunoreactive neurons was predominantly localized in the region surrounding the organum vasculasum lamina terminalis (OVLT), where the cell number doubled. These results suggest that glucocorticoid regulates LHRH content. However, whether the production and/or inhibition of release of LHRH is modified by glucocorticoid has yet to be established. In order to characterize the effects of glucocorticoid on LHRH immunoreactivity in females, 5 pairs of adult female rats were similarly implanted with glucocorticoid or placebo capsules one week after ovariectomy. No differences were found either in the total number of LHRH-containing neurons or the number of neurons in the OVLT regions of females that received glucocorticoid or placebo treatment. While this study is limited to examing these effects in ovariectomized rats, further investigation of this phenomenon in intact female rats may support the differential regulation of LHRH in males and females by glucocorticoids. Supported by Grant NS-24148.

55.3

ROSTRAL MEDIAN EMINENCE IS THE SITE OF DYNAMIC CHANGES IN THE ULTRASTRUCTURE OF GONADOTROPIN-RELEASING HORMONE (GNRH) TERMINALS DURING THE GNRH SURGE IN THE EWE. J.-J. Xiong*, S.M. Moenter, F.J. Karsch, and M.N. Lehman. Univ. Cincinnati, Dept. Anat. & Cell Biol., Cinti, OH 45267; Univ. Mich., Reprod. Sci. Prog., Ann Arbor, MI 48109.

Direct measurement of GnRH in portal blood in sheep has shown that the preovulatory gonadotropin surge is due to a simultaneous explosive release of GnRH by axon terminals in the median eminence (ME). We previously found that following the GnRH surge there is a striking decrease in the number and mean diameter of immunoreactive GnRH terminals in the median eminence (Xiong et. al., Biol. Reprod., 42, suppl.1:102). Since earlier studies implicated GnRH terminals in the rostral-mid ME as being specifically required for the surge (Polkowska et al., Cell. Tiss. Res. 208:327), we compared the ultrastructure of GnRH terminals in the rostral, mid, or caudal ME between luteal phase and post-surge ewes. Intact cycling Suffolk ewes were cranially perfused during either days 5-13 of the luteal phase (n=4) or 11-32 hours after the onset of the preovulatory LH surge (n=4). Hypothalami with pituitary stalk attached were embedded in gelatin, an' vibratome sections were processed for electron microscopic immunodetection of GnRH. Region-specific ultrastructural differences were observed between groups. In the rostral ME there was a reduction in the mean area of GnRH terminals, the number of GnRH secretory granules per terminal, and the percentage of GnRH terminals containing secretory granules in post-surge compared to luteal phase ewes. In the mid ME the difference was confined to a reduction in the percentage of GnRH terminals containing secretory granules in the post-surge group. In the caudal ME no group differences were observed. These results suggest that dynamic changes in GnRH terminal ultrastructure accompany the GnRH surge, changes that are largely confined to terminals in the rostral ME. [Supported by NIH R01 HD18337 to FJ.K, HD21968 to M.N.L. and HD18258]

55.4

EFFECT OF CASTRATION ON PUBERTAL DEVELOPMENT OF GnRH PROJECTIONS TO THE MEDIAL BASAL HYPOTHALAMUS OF THE MALE DJUNGARIAN HAMSTER. K.L. Buchanan* and S.M. Yellon. Div. Perinatal Biol, Depts. Physiol, Peds, Anat, Loma Linda Univ Sch of Med, Loma Linda, CA 92350.

In the male Djungarian hamster, evidence suggests a decrease with sexual maturation in the number of GnRH neurons that directly project to the medial basal hypothalamus *(Biol Reprod 46 Suppl: #499, 1992; Soc Neurosci Abstr 17: #387.15, 1991)*. To test the hypothesis that the decline in GnRH neuronal input to the medial basal hypothalamus at the onset of puberty does not depend upon the testes, prepubertal males were castrated (n=6) or sham-operated (n=6) at 12d of age. At 25d, hamsters were perfused intracardially with 4% paraformaldehyde. Brains were removed and crystals of DiI, a fluorescent tract tracer, implanted directly into the median eminence. After 8 weeks, brains were sectioned (60 μm) and GnRH cells labelled by immunofluorescence. Similar to earlier reports, GnRH-immunofluorescent cells were morphologically bipolar or unipolar and present in a 2:1 ratio; both subtypes were labelled with DiI, i.e., projected to the medial basal hypothalamus. GnRH soma that contained DiI were located primarily in the medial preoptic area (28% of 150), septum (50% of 44), and diagonal band of Broca (51% of 40). Fewer DiI-labelled GnRH cells were found in lateral preoptic and hypothalamic areas. Irrespective of treatment, the number, subtype ratio, and neuroanatomical distribution of GnRH cells and those labelled with DiI were similar in castrated and sham-operated males. The data suggests that both bipolar and unipolar GnRH neurons in the rostral medial forebrain constitute the major GnRH input to directly control pituitary gonadotropin secretion in the male Djungarian hamster. Furthermore, removal of the testes, i.e., absence of gonadal steroid feedback, does not influence the development of GnRH innervation of the medial basal hypothalamus during sexual maturation. (Supported by NIH HD22479)

55.5

SOCIAL/BEHAVIORAL REGULATION OF GNRH-IR. E.F. Rissman* and T.L. Dellovade. Departments of Biology and Psychology, NSF Center for Biological Timing, University of VA, Charlottesville, VA 22901.

Gonadotropin releasing hormone (GnRH) is produced by a small and discreet population of neurons in the brain. Its production and pulsatile secretion from terminals located in the median eminence are regulated by the brain-pituitary-gonad feedback loop. In addition, synthesis and release of GnRH are influenced by environmental variables (eg photoperiod, nutrition, pheromones). We examined the role of the social/behavioral environment on GnRH immunoreactivity (GnRH-ir) in the female musk shrew, *Suncus murinus*. Virgin females exposed to a male across a screen barrier for 48 hours, had 40% more positive GnRH-ir neurons in the forebrain as compared to control females housed alone. If females mate once and are sacrificed 48 hours later, counts of GnRH-ir neurons differ depending upon whether or not ovulation occurred. Mated females that did not ovulate had 40-50% more GnRH-ir neurons in the preoptic area of the forebrain as compared to mated, ovulated females, and virgins. These data suggest that exposure to a male primes the GnRH producing neurons for subsequent synthesis and release of GnRH before ovulation. This degree of environmentally induced plasticity in the production of GnRH has not been observed before in a mammal. Supported by NSF grants BNS 9021226 and DIR 8920162.

55.6

IMMUNOHISTOCHEMICAL LOCALIZATION OF cGnRH I AND cGnRH II IN THE BRAINS OF PHOTOSENSITIVE AND PHOTOREFRACTORY EUROPEAN STARLINGS AND JAPANESE QUAIL G.F. Ball*, T.S. Juss & D. M. Parry Dept of Psychology, Johns Hopkins Univ. Baltimore, MD 21218 USA; AFRC Research Group, Dept. of Zoology, Univ. of Bristol, Bristol, U.K.

The avian brain is known to contain two forms of gonadotropin-releasing hormone (chicken GnRH I and chicken GnRH II). With the use of three antisera, (two that recognize both cGnRH I and II, and one that only recognizes cGnRH II), we examined the distribution of these two peptides in the brains of photosensitive and photorefractory European starlings and Japanese quail. In both species, immunoreactive perikarya for cGnRH II were limited to a region in the mesencephalon just dorsal to N III. In European starlings, in some cases, these cells were present in the ventral part of the midbrain central gray. Immunoreactive fibers for cGnRH II were observed in a variety of areas including the neostriatum, the dorso-medial forebrain, the lateral hypothalamus and diffuse fibers were observed in the lateral and medial parts of the intercollicular nucleus. In no case were fibers for cGnRH II observed in the median eminence, nor were cells bodies observed in regions known to project to the median eminence. cGnRH I positive cell bodies were observed in the preoptic area, the lateral septum and the rostral-dorsal part of the paraventricular nucleus. A fiber plexus was apparent in the median eminence . In starlings, as has been previoulsy reported, there was a striking difference in the number immunoreactive cell bodies and in the density of the immunoreactive fiber plexus in the median eminence, between the photosensitive and the photorefractory birds. There was no evidence for such a difference for either fiber tract density or number of immunoreactive cells for cGnRH II. Thus, cGnRH II, as has been reported for other avian species, is not regulated seasonally and does not appear to play a role in the control of the pituitary.

55.7

ELECTRON MICROSCOPIC STUDIES ON CO-CULTURES OF IMMORTALIZED HYPOTHALAMIC LHRH NEURONS AND PRIMARY OR IMMORTALIZED ANTERIOR PITUITARY CELLS. Zs. Liposits[1,2]*, W. Wetsel[2], J.J. Reid[2], I. Merchenthaler[2], P.L. Mellon[3], A. Negro-Vilar[2]. [1]Dept. of Anat., Univ. Med. Sch., Pécs, Hungary, H-7643; [2]Lab. of Molec. & Integrative Neurosci., NIEHS, Research Triangle Park, NC, 27709; [3]Dept. of Reprod. Med., UCSD, La Jolla, CA 92092.

Recently, genetic targeting of tumorigenesis has been used to immortalize hypothalamic LHRH neurons (GT1-7 cells; Neuron 5:1, 1991) and pituitary cells (αT3-1 cells) which express the α subunit of the glycoprotein hormones (Mol. Endocrinol. 4:597 1990). GT1-7 cells were co-cultured with either primary pituitary or αT3-1 cells to study the architecture, organization and interaction among these cells. All cells contained well-developed, active protein synthesizing apparatus and displayed mature secretory granules. GT1-7 neurons established direct contacts (primarily tight junctions) with gonadotrophs and αT3-1 cells. Co-cultures with GT1-7 cells stimulated hypertrophy of rough endoplasmic reticulum in both gonadotrophs and αT3-1 cells, presumably due to a stimulatory effect of LHRH. These data indicate that (1) immortalized LHRH cells are compatible with both primary and immortalized pituitary cells, (2) gonadotroph and LHRH cells attract each other and make contacts, and (3) the immortalized LHRH cells stimulate cellular responses within gonadotrophs which are compatible with their underlying physiological role in regulating mammalian reproduction. Co-culture of immortalized LHRH and pituitary cells may provide a useful model to study the morphology, physiology and electrophysiology of cell-to-cell communication between hypophysiotropic neurons and pituitary cells. (Supported by grants from the NIH Intramural Program, and DK44838 & HD20377.)

55.8

REGULATION OF LHRH GENE EXPRESSION, PRO-LHRH PROCESSING AND SECRETION FROM IMMORTALIZED LHRH NEURONS. W.C. Wetsel*[1], S. Eraly[2], D.B. Whyte[2], A. Negro-Vilar[1], and P.L. Mellon[2]. [1]LMIN., NIEHS, Res. Tri. Park, NC 27709 and [2]Dept. of Reprod. Med., UCSD, La Jolla, CA 92093.

Activation of protein kinase A and C stimulates LHRH secreton in vitro. Since this hypothalamic preparation is heterogeneous, other neurons could mediate these effects. Our study sought to determine whether these agents can influence directly LHRH mRNA levels, pro-LHRH processing and secretion from an immortalized hypothalamic LHRH neuronal cell line (GT1-7 cells). Thirty min of 12-0-tetradecanoylphorbol 13-acetate (TPA) stimulated LHRH secretion in a dose-dependent manner; dibutyryl cAMP (1-500,000 nM) or forskolin (0.5-100 μM) produced only a slight enhancement. By contrast, 24 hrs of 100 nM TPA or 10 μM forskolin stimulated secretion of both LHRH and gonadotropin-releasing hormone-associated peptide (GAP). Compared to unstimulated controls, the TPA and forskolin responses were evident within the first 30 and 120 min, respectively. The molar ratio of GAP/LHRH was ~15 with TPA or forskolin; while that from controls reached 60 over 24 hrs. A 4-fold suppression of LHRH mRNA levels occurred at 16 hrs with TPA while forskolin had no effect. These data indicate that relationships among LHRH gene expression, pro-LHRH processing and secretion are complex and that regulation may occur at multiple levels. (Supported by NIH grant DK44838 and the NIEHS Intramural Program.)

55.9

CHARACTERIZATION OF CYTOSOLIC Ca^{2+} DYNAMICS IN CULTURED GnRH SECRETING GT1-7 NEURONS. **Martin A. Javors,* Thomas S. King, Stephen D. Samuelson, Xiaoying Chang and Robert S. Schenken, Departments of Psychiatry, Pharmacology, CSB and OB-GYN, The University of Texas Health Science Center, San Antonio, TX 78284.**

Gonadotropin releasing hormone is a decapeptide that is synthesized and released from neurons in the hypothalamus. By regulating the secretion of pituitary gonadotropins, GnRH plays a central role in the regulation of reproductive function. Recently, immortalized GT1-7 neurons containing the promotor of the GnRH gene coupled with the coding region for the large T-antigen of the SV-40 virus have been shown to synthesize and secrete GnRH in response to depolarization (*Neuron* 5: 1 - 10, 1990). In view of the importance of Ca^{2+} in secretion, we sought to characterize cytosolic Ca^{2+} dynamics in response to K^+-induced depolarization and norepinephrine (NE). GT1-7 neurons were grown and maintained in modified DMEM (Gibco, Grand Island, NY). Twenty four hours prior to the experiments, the neurons were plated on poly-lysine coated glass cover slips. The cells were then loaded with the Ca^{2+}-chelating dye fura-2 for measurement of changes in cytosolic Ca^{2+} ($[Ca^{2+}]_c$). Our results showed that K^+ ranging from 15 to 60 mM increased $[Ca^{2+}]_c$ in a concentration-dependent manner. Resting $[Ca^{2+}]_c$ was 69.7 ± 4 nM (n = 69). Fifteen mM K^+ increased $[Ca^{2+}]_c$ by 102 ± 16 nM (n = 10); 60 mM K^+ increased $[Ca^{2+}]_c$ by 367 ± 59 nM (n = 7). Increased $[Ca^{2+}]_c$ in response to 22.5 mM K^+ together with 100 μM NE (206 ± 13; n = 13) was greater than 22.5 mM K^+ alone (160 ± 18; n = 18). These results suggest that K^+-induced depolarization, which stimulates GnRH release from these neurons, increases cytosolic Ca^{2+}. The effect of NE and K^+ on $[Ca^{2+}]_c$ appears to be additive. The relationship between increases cytosolic Ca^{2+} and GnRH release in these cells is currently being studied. (Supported by NIDA grant DA 06039.)

55.10

PROGESTERONE BLOCKS FOS EXPRESSION IN GONADOTROPIN-RELEASING HORMONE (GnRH) NEURONS DURING THE ESTRADIOL-INDUCED GnRH SURGE IN THE EWE. M.N. Lehman , S.J. Berriman, X. H. Gu, A.S.W. Shih, S.M. Moenter†*, N. P. Evanst, G.E. Dahlt, and F.J. Karscht. Univ. Cincinnati Coll. Med., Dept. Anat. & Cell Biol., Cincinnati, OH 45267; †Univ. Mich., Reprod. Sci. Prog., Ann Arbor, MI 48109.

GnRH cells in the sheep express Fos during the estradiol-induced GnRH surge (Moenter et al., Soc. Neurosci. Abstr. 361.6, 1991). Elevated progesterone blocks the GnRH surge in the ewe. We tested the hypothesis that blockade of the GnRH surge by progesterone is associated with an alteration of Fos expression in GnRH cells. To produce a GnRH surge a physiological model for the follicular phase of the estrous cycle was used; an estradiol rise to a late follicular phase level was given after progesterone removal. Animals were cranially perfused with 2% paraformaldehyde either 1) 9 hrs. following estradiol rise (pre-surge, n = 3), 2) 30 hrs. after estradiol rise (presumed surge, n = 3) or 3) 30 hrs. after estradiol rise in ewes in which an elevation in circulating progesterone was maintained, as this treatment is known to block the GnRH surge (n = 3). Nuclear Fos was immunostained using nickel-enhanced diaminobenzidine (DAB) (black) and GnRH with unenhanced DAB (brown). Fos expression in GnRH cells was seen in 2 of 3 animals of the surge-induction group. Fos expression in GnRH cells was not seen 9 hrs. following the estradiol rise or in animals in which elevated progesterone levels were maintained. These results support the conclusion that a progesterone treatment known to block the GnRH surge alters expression of Fos in GnRH neurons and that the expression of Fos in GnRH neurons is correlated to their increased neurosecretory activity during the surge. [Supported by NIH R01 HD21968 and USDA9102515 to M.N.L., HD18337 to F.J.K., and HD 18258].

55.11

ENHANCED AFTERNOON SECRETION OF LH IN MALE SYRIAN HAMSTERS IS NOT ASSOCIATED WITH THE INDUCTION OF FOS-RELATED PROTEIN IN LHRH NEURONS. A. Doan* and H.F. Urbanski. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Although proestrous female hamsters show a pronounced afternoon increase in plasma LH levels, it is unclear whether the pattern of LH secretion in the male also has a diurnal component. In the present study, male Syrian hamsters were housed under long days (lights on 5:00 - 19:00 h) and blood samples were collected at 3:00, 9:00, 15:00, and 21:00 h. RIA of the plasma revealed a significant peak of LH secretion at 15:00 h. Interestingly, this diurnal pattern persisted after the animals were transferred to short days (lights on 9:00 - 15:00 h), even though the mean daily plasma LH levels had markedly decreased. To investigate whether the afternoon LH peaks reflected activation of LHRH neurons, paraformaldehyde-fixed brains were obtained from male hamsters at 3:00, 9:00, 15:00, 21:00, and 24:00 h. They were sectioned (50 μm) using a vibratome and then immunocytochemically-stained, first using a rabbit antibody generated against amino acids 127-152 of the Fos protein (Tom Curran, Roche), and secondly, using a mouse monoclonal LHRH antibody (HU4H); diaminobenzidine and benzidine dihydrochloride were used as the two chromogens. Although intense Fos staining was observed in brain areas such as the cerebral cortex, at no time was staining detected within LHRH neurons. Thus, males appear to differ radically from proestrous females which show an afternoon neuronal coexpression of Fos and LHRH. *(Supported by NIH Grants HD-24312 and RR-00163)*

55.12

LUTEINIZING HORMONE RELEASING HORMONE (LHRH) ALTERS THE ACTIVITY OF NEURONS IN THE HYPOTHALAMIC ARCUATE NUCLEUS. M.D. Loose* and D.A. Jacobs, Neuroscience Program, Oberlin College, Oberlin, OH 44074

The neuronal circuit that generates the episodic pattern of release of LHRH is not understood. Neurons of the arcuate nucleus (ARC) are known to affect episodic LHRH output. To explore the hypothesis that LHRH may alter its own output by acting as a neuromodulator in the ARC, we examined the effects of LHRH on spontaneous electrical activity of ARC neurons in vitro.

Single unit activity was recorded from ARC neurons in coronal brain slices prepared from female guinea pigs in their luteal phase. Stable cells firing at >0.5 Hz were perfused for 5 - 20 min with ACSF containing LHRH. Most ARC neurons (>70%) had a significant change (>30% from baseline) in firing rate during LHRH perfusion. Responses were often complex with periods of excitation and inhibition that outlasted the period of application. The majority of responders had an overall increase in firing rate. Approximately half of the cells exposed to 100 pM LHRH responded within 4 min of perfusion onset. Other cells responded only after 8-14 min had elapsed. Ten pM LHRH induced only excitatory responses during the first 14 min of perfusion. Subsequently a majority of cells stopped firing. On the basis of these findings, we propose that physiological concentrations of LHRH modulate the activity patterns of ARC neurons.

55.13

DISRUPTION OF ESTROUS CYCLICITY FOLLOWING ADMINISTRATION OF AN LHRH ANTAGONIST TO THE PREOPTIC AREA OF THE RAT. G.D. Weesner and D.W. Pfaff*, Laboratory of Neurobiology and Behavior, The Rockefeller University, New York, NY 10021.

We sought to determine if anatomical connections observed between LHRH neurons (Leranth et al., Brain Res 345:332; Witkin et al., Peptides 6:263) in the preoptic area (POA) are of physiological importance for estrous cyclicity. Bilateral cannulae were implanted just dorsal to the POA. Estrous cycles were monitored daily by vaginal smears. Antide, a long-acting LHRH antagonist, was infused bilaterally (2.5 µg/side) in the POA or the hypothalamus on the mornings of diestrus I and II. As controls, at separate times, rats also received similar infusions of either vehicle or a bombesin antagonist (Sigma #B0650). Collection of daily vaginal smears continued, and the number of days from the first infusion to the next day of Estrus which preceded a normal cycle was recorded. Following infusion of Antide into the POA (n=13), rats demonstrated varying durations of interrupted cycles ranging from 11 days to more than 100 days. These periods of disruption were characterized by either long periods of diestrus, long periods of estrus, or an extended period of diestrus followed by an extended period of estrus. Following infusion of Antide into the dorsomedial (n=4), ventromedial (n=2), or anterior (n=2) hypothalamic areas, rats had either a 4 or 5 day estrous cycle and continued to cycle normally. Likewise, infusions into the septum (n=5) had no effect. Infusion of vehicle (n=24) or bombesin antagonist (n=10) into any of the hypothalamic or preoptic area sites tested also resulted in no interruptions in the cyclic activity of the rats. Therefore, it appears that functional LHRH receptors on POA neurons are important for mediating the LHRH release required to drive the estrous cycle.

55.14

EFFECTS OF AN ACUTE STRESSOR ON LH AND CORTISOL SECRETION IN RHESUS MONKEYS: COMPARISON TO HORMONE SECRETORY RESPONSES INDUCED BY FASTING. D.L. Helmreich* and J. L. Cameron, Depts. of Behavioral Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We have previously demonstrated that one day of fasting can cause a suppression of pulsatile LH secretion in adult male rhesus monkeys (*Macaca mulatta*), and that the slowing of pulsatile LH secretion is apparent within the first 6 h after the missed meal (1100-1700 h). Accompanying this rapid slowing of LH secretion is a moderate increase in adrenal axis activity, indicated by increases in plasma ACTH and cortisol (rising from 17.86 ± 2.3 to 25.14 ± 2.7 µg/dl), and an increase in agitated behavior by the animals. To begin to determine if the rise in adrenal axis activity occurring at the time of the missed meal plays a causal role in the suppression of LH secretion, we used another stimulus to cause a similar rise in adrenal axis activity and determined its effects on LH secretion. The stimulus we chose was presentation of a leather catch glove that is generally used to catch unanesthetized monkeys and causes most animals in our colony to become quite agitated. Blood samples were collected at 15 min intervals from 8 adult male rhesus monkeys with chronic indwelling venous catheters from 1100-2400 h on a control day, and from 1100-2400 h on a day in which the glove was randomly presented 12 times between 1100 h and 1300 h. On the experimental day, the presentation of the glove caused mean cortisol levels to be significantly elevated from 1200-1600 h, with a rise that was similar in magnitude to that observed on a day of fasting (24.22 ± 2.4 µg/dl). However, in contrast to fasting, there was no suppression of LH secretion from 1100-1700 h on the day of glove presentation (LH pulse frequency: 1.5 ± 0.38 pulses/6 h on the control day, 1.6 ± 0.53 pulses/6 h with glove presentation). We conclude that the degree of activation of the adrenal axis observed during short-term fasting is not always associated with a slowing of pulsatile LH secretion. It therefore seems unlikely that the activation of the adrenal axis plays a causal role in fasting-induced suppression of LH secretion.

55.15

STUDIES ON THE ROLE OF ADRENOCEPTOR SUBTYPES IN THE SUPPRESSION OF PULSATILE LH RELEASE. F. Tacconelli & C.W. Coen*. Division of Biomedical Sciences, King's College, London, UK.

The effect on pulsatile LH release of agonists or antagonists for adrenoceptor subtypes has been studied in bilaterally ovariectomised rats with chronically implanted cannulae; these permitted the administration of drugs into the third cerebral ventricle (ICV) or intraperitoneal cavity (IP) and the removal of blood samples at frequent intervals from the right cardiac atrium without disturbing the animal during the course of the experiment. The pulsatile release of LH was suppressed by treatment with α_1 antagonists (alfuzosine, 0.03μmoles, ICV; prazosin, 0.25, 0.5, 1.0mg/kg, IP) or α_2 antagonists (SKF 86466-A, 0.03 or 0.3μmoles, ICV; idazoxane, 2mg/kg, IP; piperoxane, 50mg/kg, IP); no effect was observed following treatment with the β_1 antagonist atenolol (0.06 or 0.12μmoles, ICV) or the β_2 antagonist ICI 118551 (0.06μmoles, ICV). Treatment with α_1 agonists (methoxamine, 0.03μmoles; phenylephrine, 0.03 or 0.3μmoles), α_2 agonists (clonidine, 0.3μmoles; guanabenz 0.03 or 0.06μmoles) or the β_2 agonist clenbuterol (0.15μmoles) suppressed the pulses when administered ICV; no effect was found following treatment with prenalterol (0.3μmoles, ICV), a β_1 agonist. However, the suppressive effects of an α_2 or a β_2 agonist (clonidine, 0.3μmoles, ICV or clenbuterol, 0.15μmoles, ICV, respectively) were enhanced if the animals were concomitantly treated with a β_1 agonist (prenalterol, 0.3μmoles, ICV). All appropriate vehicle treatments failed to affect the LH pulses. These results suggest that the ability of ICV adrenaline or noradrenaline (both non-selective α and β agonists) to suppress LH release in ovariectomised rats may involve the concomitant activation of α_1, α_2, β_1 and β_2 adrenoceptors. The study also demonstrates that LH pulses can be disrupted by treatment with selective agonists or antagonists of α_1 or α_2 adrenoceptors; the apparent paradox concerning these two receptor subtypes remains to be resolved.

55.16

EFFECT OF PHARMACOLOGICAL DOSES OF NICOTINE ON ANTERIOR PITUITARY HORMONES C.A. Hodson, W.K. Bowman, A.T. Davenport and H.W. Burden*. Departments of Obstetrics and Gynecology and Anatomy, East Carolina University, School of Medicine, Greenville, NC 27858

Previous studies have shown that acute administration of pharmacological doses of nicotine inhibits luteinizing hormone secretion and stimulates prolactin secretion. The present study investigated the effect of nicotine treatment for 7 days on serum and pituitary gland hormone content. Adult male Sprague-Dawley rats were given 1.25 or .312 mg of nicotine per kg bw or the saline injection vehicle for 7 days. On day 7, one hour after the final injection of nicotine, blood samples and anterior pituitary glands were collected for hormone assay. LH, TSH and prolactin concentrations were determined by radioimmunoassay. Data were statistically analyzed using random design analysis of variance and means were compared at the α=.05 level using the Student-Newman-Keul's test.

Daily nicotine treatment for seven days at .312 but not at 1.25 mg/kg body weight resulted in decreased anterior pituitary gland prolactin TSH and LH content. Serum TSH and LH concentrations were decreased in response to daily administration of 1.25 mg nicotine/kg bw. These results suggest that nicotine has pronounced effects on anterior pituitary gland function. Studies are currently in progress to determine the effect of nicotine on endogenous opioids in the anterior pituitary gland and hypothalamus. We hope to determine whether endogenous opioids mediate the effects of nicotine on anterior pituitary gland hormones.

55.17

MONAMINE OXIDASE INHIBITION MODIFIES LUTEINIZING HORMONE BUT NOT PROLACTIN RELEASE. KM Ogilvie* and MH Stetson. Sch. of Life and Hlth. Sci., Univ. of Del., Newark, DE 19716.

The female golden hamster is a seasonally reproducing rodent in which anestrus is evoked by exposure to short days (photoperiod-induced anestrus; PIA). PIA hamsters are characterized by low levels of prolactin (PRL) and a daily surge of gonadotropins. We injected animals with saline or pargyline (a mononamine oxidase inhibitor) on diestrus day 1 (DIE), proestrus (PRO) or to PIA animals at 2 hour (h) intervals during the day. Animals were killed 10 minutes after injection and blood collected. We found pargyline affected LH levels in PRO and PIA, but not DIE hamsters. In PRO hamsters (14L:10D), pargyline decreased LH levels at 1800h; in PIA hamsters (12L:12D), LH was decreased at both 1600h and 1800h (lights off at 2000h in both). Conversely, pargyline given to PIA hamsters at 2000h increased LH. PRL levels were not changed, indicating brain dopamine levels were probably unaffected by this treatment. These data support the idea that serotonin, which is dramatically increased by pargyline, plays a role in the control of gonadotropin surges.
Supported by NSF research grant DCB87-14638.

55.18

LACK OF EFFECT OF ADRENALECTOMY ON THE PROLACTIN-INDUCED SUPPRESSION OF POSTCASTRATION LH SECRETION. S.-K. Park, D.R. Grattan, L.A. Martin and M. Selmanoff*, Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201-1559.

We contrasted the inhibitory effects of acute hyperprolactinemia on postcastration LH and FSH secretion in adrenal intact and adrenalectomized (ADX) rats with or without physiological corticosterone (B) replacement. Adult male rats were administered purified ovine prolactin (oPRL) every 12h (2,400µg/sc injection) for 10 days, beginning at the time of castration in three groups of rats: orchidectomized (ORCH) rats, ORCH+ADX rats, and ORCH+ADX+B rats. In ORCH rats, oPRL suppressed mean plasma LH and FSH levels from about 18 to 72h postcastration when the effect spontaneously reversed in the face of elevated oPRL levels. In ADX rats, the LH and FSH postcastration increases were delayed as previously reported by Ringstrom and Schwartz (Endo 114:880, 1984), and this delay was not prevented by B replacement. In the case of FSH, the delay of postcastration secretion due to ADX was very striking, and appeared to override any oPRL-induced suppression. Due to this delaying effect, the inhibition of LH by oPRL in ORCH+ADX and ORCH+ADX+B rats was less pronounced than in ORCH rats. We conclude that the oPRL-induced suppression of postcastration LH secretion occurs with or without the adrenal glands. The suppression is most prominent in adrenal intact animals, apparently because the postcastration LH rise is most rapid in these rats and not because of an adrenal-mediated effect of oPRL on the hypothalamic-pituitary axis. (Supported by NIH grant HD21351).

55.19

EFFECT OF COCAINE ON GnRH-STIMULATED PITUITARY GONADOTROPIN SECRETION *IN VIVO*. Thomas S. King,* Ting-Ting Gu, Melin S. Canez, Martin A. Javors and Robert S. Schenken, Depts. of CSB, OB-GYN & Psychiatry, The University of Texas Health Science Center, San Antonio, TX 78284.

We have shown previously that cocaine administration disrupts estrous cyclicity and normal rates of ovulation in rats (*Neuroendocrinology 52*: 15 - 22, 1990). While the primary effects of cocaine appear to involve the regulation of hypothalamic GnRH release, a direct effect of this drug on pituitary gonadotropin secretion has not been ruled out. Thus, we sought to characterize the effects of cocaine on GnRH-stimulated pituitary LH and FSH secretion. Ovariectomized (OVX), 17β-estradiol benzoate (E_2B)-primed rats were injected s.c. every day for one week with saline or 10 mg/kg of cocaine HCl (*i.e.*, "chronic" exposure). On the last day of these injections, a single blood sample was collected from each rat via an indwelling jugular vein cannula. This was followed by i.v. infusion of 100 ng/kg of synthetic GnRH in saline vehicle. Twenty min later a second blood sample was collected. Chronic cocaine had no effect on either basal or GnRH-stimulated LH or on FSH levels. To examine the effects of "acute" cocaine exposure on GnRH-stimulated gonadotropin secretion, a single blood sample was collected from OVX-E_2B, cocaine-naïve rats followed by i.v. injection of (1) 100 ng/kg of synthetic GnRH in saline vehicle, (2) GnRH + 4 mg/kg of cocaine HCl in saline vehicle or (3) GnRH + 1 mg/kg of lidocaine HCl in saline vehicle. Twenty min later a second blood sample was collected. Acute exposure either to cocaine or lidocaine significantly blunted GnRH-stimulated increases in LH, but not FSH, levels. These results suggest that acute, but not chronic, exposure to cocaine attenuates GnRH-stimulated LH secretion. The former effect may reflect cocaine's activity as a local anesthetic, acting on Na^+ influx required for GnRH-stimulated pituitary gonadotropin release. (Supported by NIDA grant DA 06039 and NIH grant HD 02012.)

NEUROENDOCRINE REGULATION: HYPOTHALAMUS/PITUITARY

56.1

THE ENKEPHALIN ANALOG [D-MET^2,PRO^5]-ENKEPHALINAMIDE STIMULATES MELANOCYTE-STIMULATING HORMONE SECRETION. J.A. Carr*, L.C. Saland, A. Samora, D. Tejeda. Dept. of Biol. Sci., Texas Tech Univ., Lubbock, TX 79409-3131 and Dept. of Anatomy, Univ. of New Mexico Sch. Med., Albuquerque, NM 87131 (USA)

We used a combination of *in-vivo* and *in-vitro* techniques to investigate enkephalinergic modulation of pituitary intermediate lobe (IL) secretory activity in the rat using the super-active met-enkephalin analog [D-Met^2, Pro^5]- enkephalinamide (EA). Plasma titers of alpha-melanocyte-stimulating hormone (α-MSH) were measured by radioimmunoassay to gauge IL secretory activity. Systemic administration of EA resulted in dose- and time-dependent elevations in plasma α-MSH levels. The stimulatory effects of EA were blocked by pretreatment with the μ-selective opiate antagonist naltrexone. *In-vitro* treatment of neurointermediate lobes (NILS) with 10 nM EA caused a rapid 3.7-fold increase in α-MSH release. *In-vitro* α-MSH release in response to EA was maximal 15 min after administration but had returned to baseline levels 90 min after administration, suggesting desensitization of the response. Treatment with a dose of naltrexone sufficient to block EA-induced secretion of α-MSH had no effect on stress-induced secretion of α-MSH but completely blocked the rise in plasma prolactin levels caused by stress. Our results indicate that systemic administration of EA stimulates α-MSH secretion, possibly by acting through μ-selective opiate receptors at the level of the NIL. The inability of naltrexone to reduce stress-induced secretion of α-MSH suggests that endogenous met-enkephalin does not play a role in secretion of α-MSH during stress. Supported by NIH NS08447 (JAC) and NS21256, GM08139 (LCS).

56.2

COLOCALIZATION OF ß-ENDORPHIN AND FLUOROGOLD IN THE ARCUATE NUCLEUS OF THE FEMALE GUINEA PIG. J.E. Thornton*, N. Khaleeli and M.D. Loose, Neuroscience Program and Biology Dept, Oberlin College, Oberlin OH 44074.

Although ß-endorphin (BE) is found in the cells of the arcuate nucleus (ARC) and in the portal blood, it is unclear which subpopulation of ARC BE cells project to the median eminence (ME). The present study used fluorogold (FG) to determine which BE cells project outside the blood brain barrier, presumably to the ME. Adult ovx guinea pigs were injected ip with 20mg/kg FG. Five days later females were injected with 25ug EB or oil. Females were sacrificed 24h later and brains were postfixed in 4% paraformaldehyde. Cryostat sections (20um thick) were stained with ß-endorphin antibody (R13, E. Weber, 1:1000) and with donkey anti-rabbit IgG Texas Red (Jackson, 1:50). Only a small proportion (less than 10%) of BE cells contained FG. However, in parts of the anterior and medial arcuate, approximately 40% of the FG cells also contained BE. ß-endorphin released at the median eminence by arcuate cells may have a neuroendocrine role and/or may modify neurosecretion at the median eminence.

56.3

MELANIN CONCENTRATING HORMONE (MCH) AND NEUROPEPTIDE-E-I (NEI) ACTIVATE HYPOTHALAMIC CRF NEURONS IN CONSCIOUS RATS. D.G.Parkes, S.Rivest, C.Rivier, P.Sawchenko+ & W.Vale*. The Clayton Foundation Laboratories for Peptide Biology and +Laboratory for Neuronal Structure and Function, The Salk Institute, La Jolla CA 92037.

MCH and NEI are neuropeptides both processed from the MCH precursor hormone and are localized within the dorsolateral region of the rat and human hypothalamus. In teleost fish, MCH acts directly on the pituitary to suppress ACTH release. The physiological relevance of MCH within the mammalian CNS is at present unclear. However, recent studies in rats have suggested contrasting neuroendocrine actions compared to those in fish, as icv injection of MCH into rats produces a CRF-dependent increase in pituitary ACTH release. The present study investigated the effect of acute icv infusion of MCH, NEI and CRF on the neurophysiological expression of the proto-oncogene *c-Fos* in the rat CNS, as a marker of neuronal activation. Guide cannulae were implanted in the lateral ventricle of adult male rats one week prior to experimentation. On the day of experimentation, 1µg of MCH, NEI or CRF was infused over 2 min. Sixty min following infusion, rats were anaesthetized and perfused with 4% paraformaldehyde. Frozen brains were cut into 40µm sections and tissue slices were stained for *c-Fos* protein, and either CRF or LHRH by means of a double immunocytochemistry method. ICV artificial CSF did not measurably alter *c-Fos* expression in either the PVN or the MPoA. ICV MCH and NEI significantly increased the expression of *c-Fos* protein in the parvocellular subdivision of the PVN. In this hypothalamic nucleus *c-Fos* was mainly located within the nuclei of CRF-like immunoreactive perikarya. In addition, both NEI and CRF markedly increased *c-Fos* expression within the medial preoptic area (MPoA), although the activated cells were shown not to be LHRH-containing perikarya. This study provides the first evidence of direct activation of hypothalamic CRF perikarya by both MCH and NEI, and together with *in vivo* findings, suggest that these neuropeptides may be involved in regulating the HPA in mammals. Furthermore, icv NEI and CRF appear to influence activity of neurons located within preoptic regions of the hypothalamus.

56.4

PRETREATMENT WITH SOMATOSTATIN ANALOG SMS 201-995 POTENTIATES GH RESPONSIVENESS TO GRF. J. Turner, A. Beaudet and G.S. Tannenbaum*. Departments of Neurology & Neurosurgery and Pediatrics, McGill University, Montreal, Quebec, H3H 1P3.

Our recent studies suggest that the temporal pattern of hypothalamic somatostatin (SRIF)/GRF signaling to pituitary somatotropes is an important determinant for pulsatile GH secretion, however the mechanism remains unclear. In the present investigation, we used the long-acting SRIF analog, SMS 201-995, to further elucidate the nature of the SRIF/GRF interaction in GH regulation. Administration of SMS 201-995 (in doses ranging from 25-100 µg iv) to free-moving adult male rats resulted in obliteration of the spontaneous GH pulses for 3-6 h. Rats (n=6) pretreated with SMS 201-995 (25 µg iv) and subsequently challenged with GRF (1 µg iv) exhibited reduced GRF-induced GH release 1 h post treatment; in contrast, preexposure to SMS 201-995 for 3 h markedly enhanced GH responsiveness to GRF (354.7 ± 71.0 vs. 166.0 ± 43.8 ng/ml; P<0.05), compared to controls (n=10) pretreated with normal saline. Animals iv injected with 50 µg SMS 201-995 (n=6) or saline (n=5) 3 h prior to the concomitant administration of native SRIF-14 (50 µg iv) and GRF (1 µg iv) exhibited similar SRIF-14-mediated inhibition of GRF-induced GH release; however, the post-inhibitory rebound release of GH 15 min after SRIF-14 administration was 12-fold higher in SMS 201-995-treated rats than in saline controls (347.8 ± 53.9 vs. 27.6 ± 9.0 ng/ml; P<0.001). These results demonstrate that a 3-h period of exposure to SMS 201-995 is sufficient to enhance pituitary sensitivity to GRF. The findings suggest that this effect is not due to down regulation of SRIF receptors but rather to a SRIF-mediated build-up of pituitary GH stores in a readily releasable pool. Such a synergistic interaction between SRIF and GRF may be necessary to optimize pulsatile GH release.

56.5

INVOLVEMENT OF CALCIUM IN SOMATOSTATIN RELEASE FROM THE BOVINE INFUNDIBULUM. P.G. Harms*, P.L. Chen, J.P. Walter and N.H. McArthur, Departments of Animal Science and Veterinary Anatomy & Public Health, Texas A&M University, College Station, TX 77843.

Bovine infundibular (INF) explants were cultured to study calcium (Ca) involvement in somatostatin (SS) release. INF were excised, mid-sagitally sectioned, and each half randomly assigned to either control or treated (EGTA or Verapamil) groups. Individual halves were incubated in 600 µl of Krebs-Ringer bicarbonate medium (KRB) (4.7 mM K^+, 10mM glucose, 0.1% bovine serum albumin, pH 7.3 at 37° C and saturated with 95% O_2, 5% CO_2) for 180 min. At 30 min intervals, 500 µl aliquots were removed from each incubate and replaced with fresh KRB. Basal (BAS) and depolarization induced (DEP) (substitution of 60 mM K^+ for sodium in KRB) SS release was evaluated by RIA of SS content in the media at 90-120 and 120-150 min of culture, respectively. An analysis of variance was used to determine differences in BAS and DEP SS release between control and treated halves. Differences between BAS and DEP contents were used to evaluate response to depolarization. INF halves from steers treated with 1.25 mM EGTA (Ca binding agent) in the KRB released less (P<.02) SS during DEP than control halves. Similarly, INF halves from steers and heifers treated with 50 or 100 µM Verapamil (Ca L-channel blocker) in the KRB released less (P<.02) SS in response to DEP than control halves. BAS release was not affected (P>.10) by EGTA or Verapamil. These results demonstrate that extracellular Ca is involved in DEP induced SS release and suggest that Ca is involved in the release (exocytosis) of SS from the bovine INF.

56.6

GALANIN SECRETION FROM PITUITARY CELLS IS REGULATED BY ESTRADIOL AND GHRH. J.F. Hyde* and A. Hemmer, Dept. Anatomy & Neurobiology, Univ. of Kentucky, Lexington, KY 40536.

The peptide galanin is localized in lactotrophs, somatotrophs and thyrotrophs of the rat anterior pituitary gland (AP). *In vivo* treatment with estradiol (E2) increases galanin mRNA and peptide levels in the AP, particularly within lactotrophs. Studies using E2-treated AP cells showed that galanin secretion is regulated by dopamine, somatostatin and TRH. Our objectives were to 1) determine if E2 increases galanin release from AP cells *in vitro*, and 2) examine if growth hormone-releasing hormone (GHRH) alters galanin secretion from cells not exposed to E2. AP cells from male and ovariectomized F344 rats were cultured under low-E2 conditions. The cells were challenged with varying concentrations of 17β-estradiol (.01-10 nM) or GHRH (1-10 nM). Hormone levels were measured by RIA. E2 increased galanin release in a dose-dependent manner. Cells from ovariectomized rats released more galanin than cells from males. Immunocytochemistry was used to quantify the number of galanin-positive AP cells *in vitro*. E2 (10 nM) increased the number of galanin cells 2-fold. GHRH caused a dose-dependent increase of galanin release, which was greater in cells from ovariectomized rats. Conclusions: 1) E2 increases galanin release from AP cells *in vitro* in a sex-dependent manner. 2) E2 increases the number of AP cells synthesizing galanin *in vitro*. 3) GHRH stimulates galanin release from AP cells when E2 is absent. These data indicate that the effects of E2 on galanin synthesis and release are mediated, in part, by a direct action at the level of the AP. Moreover, the regulation of galanin release is dependent on the steroid environment.
(Supported by ACS grant #IN-163 and UKMC Research Fund.)

56.7

INDUCTION OF FOS AND FOS RELATED PROTEINS IN THE RAT PITUITARY FOLLOWING CORRESPONDING RELEASING-FACTOR TREATMENT J. KONONEN1, J. HONKANIEMI1, L. KIVIPELTO2*, H. ALHO1, M. PELTO-HUIKKO1 1.) Dept. of Biomed. Sci., Univ. of Tampere, 33101 Tampere 2.) Dept. of Anatomy, Univ. of Helsinki, FINLAND

Transcription factors Fos and Jun might act as intranuclear messengers in the cascade of events concluding in increased pituitary hormone synthesis following releasing factor treatment. In order to test this hypothesis we treated adult male rats with i.p. injections of either CRF, TRH, LHRH, GRF or physiologic saline. Pituitaries were prepared for immunocytochemistry and incubated with antibodies directed against either the N-terminal or M-peptide sequence of Fos.

Injection of CRF resulted in outstanding induction of Fos-immunoreactive (Fos-IR) cells in both anterior and intermediate lobes (AL and IL) of pituitary when compared to animals injected with physiologic saline. Double-labelling verified that majority of cells expressing Fos-IR after CRF treatment contained ACTH-IR. Injections of TRH, LHRH and GRF resulted in induction of Fos-IR in TSH-, LH-, FSH-, and GH- cells of the AL. Injection of physiologic saline induced Fos-IR in AL and IL when compared to untreated animals. Consecutive double staining revealed that these cells also contained ACTH-IR. Similar inductions of Fos-IR were obtained in all experiments with both Fos-antibodies used, although more stained cells were always observed with the Fos antibody directed against the M-peptide. Our results demonstrate the induction of Fos and Fos-related proteins in the rat pituitary after treatment of the corresponding releasing factor, suggesting a role for AP-1 binding proteins in the control of pituitary hormone synthesis.

56.8

CORTICOTROPIN RELEASING FACTOR GENE EXPRESSION IN JEG-3 CELLS. J.W. Kasckow*, M.R. Montminy and W.W. Vale, The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92131

Corticotropin releasing factor (CRF) production in the hypothalamus is known to be regulated by activators of the protein kinase A (PKA) and C (PKC) pathway. We have studied CRF gene expression induced by these pathways in transient transfection assays of JEG-3 cells. The rat CRF promoter (base pairs -337 to +25 bp) linked to a luciferase reporter plasmid was transfected into placental JEG-3 cells using standard $CaPO_4$ precipitation methods. Following a 5-6 hour incubation, cells were glycerol shocked and the CRF gene was expressed for 16 hours in the presence or absence of various agents. Following this, cells were harvested, tested for luciferase activity and normalized to β-galactosidase activity. Forskolin enhanced basal CRF gene expression approximately 20 fold. The phorbol ester 12-o-tetradecanoyl phorbol 13-acetate (TPA) was also able to enhance CRF transcription approximately 5 fold. Addition of both TPA and forskolin lead to a synergistic activation. The presence of a palindromic cAMP responsive element (CRE) in the CRF promoter and the lack of a AP1 consensus sequence suggests that both PKA and PKC pathways may act through the CRE to activate transcription. Supported by NIMH 5T22, MH 17140 and DK 26741.

56.9

SPONTANEOUS AND AGONIST-INDUCED CYTOSOLIC CALCIUM TRANSIENTS IN FURA-2-LOADED CULTURES OF ANTERIOR PITUITARY CORTICOTROPE CELLS (AtT-20/D16v).
J. F. Fiekers*, L. M. Konopka[1] and V. May. Dept. Anatomy & Neurobiology., Univ. Vermont Coll. Med. Burl., VT 05405, [1]Hines Veterans Administration Hosp., Hines, IL 60141.

Spontaneous, corticotropin releasing factor (CRF)- and galanin-induced changes in $\{Ca^{2+}\}_i$ were examined in cultures of anterior pituitary (AtT-20/D16v) cells. $\{Ca^{2+}\}_i$ was monitored in individual corticotropes by dual excitation microspectrofluorometry using fura-2. Spontaneous Ca^{2+} oscillations were observed in >80% of single cells. The frequency, but not the duration or amplitude of Ca^{2+} oscillations, was significantly increased with elevated $\{Ca^{2+}\}_o$. The spontaneous transients were reversibly abolished by Co^{2+} (2mM) or nifedipine (100uM), but were unaffected by tetrodotoxin (1μM), suggesting that the Ca^{2+} transients are caused by Ca^{2+} entry through L-type voltage-gated Ca^{2+} channels. CRF and galanin caused transient increases in $\{Ca^{2+}\}_i$ and increased the frequency of spontaneous Ca^{2+} transients. Galanin responses were abolished by Cd^{2+} (150μM) or Ca^{2+}-free solutions. CRF produced a rapid transient increase $\{Ca^{2+}\}_i$ in Ca^{2+}-free solutions. These results suggest that Ca^{2+} entry through voltage-gated Ca^{2+} channels is an important pathway for regulating $\{Ca^{2+}\}_i$ and that hormonal activation may involve calcium entry and/or calcium mobilization from intracellular store(s). (Supported by NS27319).

56.10

CHARACTERIZATION OF ENDOTHELIN RECEPTORS ON THE PITUITARY BY A NOVEL ENDOTHELIN ANTAGONIST.
B. Kanyicska* and M.E. Freeman. Dept. of Biol. Sci., Florida State University, Tallahassee, FL 32306.

Members of the endothelin-like peptide family are capable of modifying hormone secretion of different pituitary cells *in vitro*: they attenuate prolactin (PRL) and enhance luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH) release in a dose-dependent manner. Since a remarkable difference has been found in terms of relative potencies of different ET-like peptides on PRL and TSH vs. LH and FSH, we proposed that two different ET receptors are present on lactotrophs and thyrotrophs compared to that of gonadotrophs. In this study, a new competitive ET_A receptor antagonist, BQ123 (M. Ihara et al., Life Sci. 50:247, 1992) was used for further characterization of ET receptors in the pituitary. In order to establish the inhibitory potency of BQ123, primary monolayer cultures of anterior pituitary cells originated from female rats were preincubated for 30 min without or with 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M antagonist followed by challenge with ET-1 or ET-3, respectively. After either four or 24 hours of incubation, the media were collected and the amounts of secreted PRL, TSH, LH and FSH were determined by radioimmunoassays. Pretreatment with BQ123 markedly antagonized the effects of ET-1 and ET-3 on PRL and TSH secretion without effecting the ET-induced changes on LH and FSH. The concentrations of ETs necessary for the half maximal responses (EC_{50}) with or without the antagonist were calculated using non-linear regression analysis of dose-response curves. In the case of PRL and TSH, the pA_2 values for BQ123 were calculated using Schild's method (Tallarida et al., Life Sci. 25:637, 1979). The pA_2 and K_i (inhibitory constant) values on PRL and on TSH either with ET-1 or ET-3 were similar (pA_2 ~9 and K_i ~1 nM). These data strongly suggest that the lactotroph and thyrotroph cells possess the same type of ET receptors (ET_A) which mediate the effects of both ET-3 and ET-1. In contrast, the ET's effects on gonadotroph cells are likely involve ET_B (or ET_C?) receptors. Supported by NIH, HD-11669.

56.11

THE EFFECT OF GROWTH HORMONE-RELEASING FACTOR (GHRF) ON CYCLIC AMP (cAMP) PRODUCTION AND GROWTH HORMONE (GH) RELEASE FROM PITUITARY CELL CULTURES OF DEVELOPING AND MATURE RATS.
L. Cuttler* and B.J. Collins. Depts. Pediatrics/Pharmacology, Case Western Reserve Univ., Cleveland, OH 44106; Dept. Pediatrics, Univ. of Chicago, Chicago, IL 60637.

Circulating GH levels and the GH response to GHRF are heightened in perinatal mammals, compared with adults. In order to assess the mechanisms underlying this characteristic ontogenic pattern, we tested the effect of GHRF (0.01-10 nM GHRF, for 5-180 min) on cAMP production and GH release by pituitary cell cultures of newborn (2-day-old), juvenile (12-day-old), and adult male (3-4 months) rats (n= 3 experiments/age group). GHRF stimulated intracellular cAMP accumulation in a dose and time- dependent manner in all age groups, with peak cAMP production occurring at 30 min after GHRF application. Although GHRF-induced fractional GH release was greatest from pituitary cell cultures of newborn rats and least from those of adults (ANOVA for age effect: $P < 0.001$; similar to our earlier observations, Endocrinology 118:69), GHRF-induced cAMP production was similar in all age groups (ANOVA, NS). These findings suggest that GHRF stimulates cAMP production in pituitaries of immature as well as mature rats, and support the concept that cAMP mediates the effect of GHRF throughout development. The dissociation between the ontogenic effects of GHRF on cAMP production from those on GH release suggest that the heightened GH secretory response to GHRF in perinatal animals reflects, at least in part, immaturity of cellular processes distal to the GHRF receptor and cAMP generation.

56.12

IMMUNOCYTOCHEMICAL LOCALIZATION OF GAP-43 IN NERVE FIBERS OF THE ADULT RAT NEUROINTERMEDIATE PITUITARY.
L.C. Saland*, N. Perrone-Bizzozero, A. Samora and P. Sanchez. Depts. of Anatomy and Biochemistry, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131.

GAP-43 is a neuronal phosphoprotein associated with developing and regenerating nerve fibers. Here, we demonstrate that nerve fibers of rat pituitary neurointermediate lobes (NILS) contain immunoreactive (IR) GAP-43. Male rats were injected I.V. with 150 mg/kg 6-hydroxydopamine (6-OHDA), then halothane anesthetized one week later and perfused intracardially with 4% paraformaldehyde in 0.1M phosphate buffer. Alternatively, NILS were incubated in vitro for 90 minutes in control media or dopamine (DA). Paraffin sections were immunostained for GAP-43 with a polyclonal antiserum (1:1500, Benowitz et al, '88, J. Neurosci. 8: 339). Small numbers of GAP-43-positive fibers were observed in NILS of perfused rats. More intense staining was observed after in vitro DA treatment. GAP-43 is associated with release of DA in PC-12 cells (Ivins et al, '91, Soc. Neurosci. Abst. 17: 576). Here, DA may interact with nerve fibers, perhaps by uptake, to increase GAP-43 IR in pituitary. Support by NIH NS21256, GM08139(LS) and NS30255(NPB).

56.13

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND BASIC FIBROBLAST GROWTH FACTOR (bFGF) IN HUMAN PITUITARY ADENOMAS.
D.F. Brown, E.G. Stopa, A. Baird, J. Daucher, A. Tatum, D.I.Friedman* and G.S. Rodziewicz. Depts. of Pathology, Neurology and Neurosurgery, SUNY Health Science Center, Syracuse, NY 13210 and The Whittier Institute for Diabetes and Endocrinology, La Jolla, CA 92037

In addition to the classical pituitary hormones, other proteins have been found to be involved in the regulation and maintenance of pituitary function. Included among these are the heparin-binding proteins Basic Fibroblast Growth Factor (bFGF) and Vascular Endothelial Growth Factor (VEGF). In this study we examined human pituitary adenomas to determine if VEGF and bFGF may be elaborated by these neoplasms. Adenoma specimens and normal gland were fixed in Zamboni's fixative and embedded in paraffin.The tumors varied in their hormonal secretion and included ACTH secreting (n=2), prolactin secreting (n=1), GH secreting (n=1), prolactin and GH secreting (n=2), and non-secreting (n=1) tumors. The immunocytochemical procedures were performed on 5μm sections using the ABC method and specific polyclonal antibodies directed against the amino terminus of VEGF (#61,67) and bFGF (#773). VEGF-like immunoreactivity showed no apparent specificity for adenoma type, and could be found in all specimens examined (n=7). bFGF-like immunoreactivity was observed only within the single non-secreting adenoma . Our data suggest that VEGF is increased and bFGF reduced as a function of pituitary neoplastic transformation . The possibility that bFGF content is reduced due to rapid secretion,however,cannot be excluded. AG10682, DK18811.

57.1

EFFECT OF ESTROGEN ON IMMEDIATE EARLY GENE EXPRESSION IN THE RAT BRAIN. R.Miyoshi*, E.Fujii, T. Muraki and S. Kito, Department of Pharmacology, Tokyo Women's Medical College, Tokyo 162, *Division of Health Sciences, University of the Air, Chiba 261, Japan.

It has been demonstrated that female steroid hormones can act in the mammalian central nervous system to regulate endocrine functions and sexual and affective behaviors. In this study, we examined the effect of estrogen on c-fos and zif/268 mRNA expression in the rat brain using an in situ hybridization technique. Female Fischer 344 strain rats were ovariectomized and allowed to recover for 2 weeks. β-estradiol was subcutaneously administered. The basal level of c-fos and zif/268 expression was very low throughout the brain. From 30 to 60 min after β-estradiol injection, c-fos and zif/268 mRNAs were increased in the hippocampus and cerebral cortex. In addition, at 60 or 90 min following the injection, it was detected in the midbrain such as the superior colliculus, central gray matter and interpeduncular nucleus. It has been reported that estrogen receptors richly exist in the hippocampus of ovariectomized rats. It is considered that the induction of c-fos and zif/268 mRNA plays some role in the action of estrogen in the central nervous system.

57.2

AGE- AND STRAIN-RELATED LOSS OF mRNA FOR MINERALOCORTICOID RECEPTORS IN THE BRAIN. S. Boutelle, A.K. Johnson, S.J. Lewis, W.W. Kaelber* and B. Kasson. Depts. Pharmacol., Psychol., Cardiovasc. Ctr., Univ. of Iowa, Iowa City, IA 52242.

It has been established that mineralocorticoid receptors (MR) that mediate the actions of steroids by functioning as ligand-dependent nuclear transcription factors exist within the brain. We have previously reported that MR mRNA in the hippocampi of aged 24 - 30 month) old spontaneously hypertensive (SHR) and stroke-prone hypertensive (SHRsp) rats is markedly less than in young (5 or 10 week old) rats. This study examined whether the age-related reduction in MR mRNA is due to a loss of MR mRNA production per cell or due to a net loss of neurons within the hippocampi of these rats. Within the hippocampus of young SHR and SHRsp, heavy MR mRNA labelling was found in the pyramidal layer throughout Ammon's horn and the intensity of labelling was higher in field CA3 than in CA1. The aged rats had markedly less MR mRNA throughout the hippocampus. This was evident in areas which were as neuronally rich as the young rats. However, there was a marked loss of cells in some areas of the hippocampi of the aged rats. These results suggest that the loss of MR mRNA in the hippocampi of aged rats is due to both a net loss of MR mRNA per cell and a net loss of neurons per se.

57.3

EFFECT OF MINERAL CORTICOID ON IN VIVO RELEASE OF NEUROTRANSMITTERS FROM THE RAT HIPPOCAMPUS. M.Shimoyama*, R.Miyoshi, S.Kito, A.Kimura, M.Hayashi, R.Shimada and Y.Maruyama, Division of Health Sciences, University of the Air, Chiba 261, *Department of Pharmacology, Tokyo Women's Medical College, Tokyo 162, Japan.

The presence and biosynthesis of steroids within the mammalian central nervous system is now accepted. Steroids exert both genomic and non-genomic effects on neurons and the hippocampus contains high densities of mineral corticoid receptors of the brain. In order to directly assess changes of release of endogenous acetylcholine (ACh), GABA and aspartate from the rat hippocampus induced by systemic administration of deoxycorticosterone acetate (DOCA), a mineral corticoid, we measured release of these substances using an intracerebral dialysis technique. A dialysis probe of Carnegie Medicine was inserted into the hippocampus of a Wistar strain rat. ACh was assayed by HPLC with an electrochemical detector and amino acids with a fluorescence detector. Subcutaneous injection of DOCA caused short-latency decreases of both glutamate and GABA, and increase of ACh. Aspartate was also decreased. These effects of mineral corticoid are considered to be direct actions on neuronal receptors. It is presumed that mineral corticoid is playing roles in regulating hippocampal functions.

57.4

EFFECT OF GLUCOCORTICOID ON IN VIVO RELEASE OF NEUROTRANSMITTERS FROM THE RAT HIPPOCAMPUS. S.Kito,* R.Miyoshi, M.Shimoyama, Y.Kimura, M.Hayashi, R.Shimada, and Y.Maruyama, Division of Health Sciences, University of the Air, Chiba 261, *Department of Pharmacology, Tokyo Women's Medical College, Tokyo 162, Japan.

It has been demonstrated that glucocorticoids specifically bind to neuronal receptors in the hippocampus and the hippocampus contains the highest densities of glucocorticoid receptors of the brain. In order to directly assess changes of release of endogenous acetylcholine (ACh), GABA, glutamate and glycine from the rat hippocampus induced by systemic administration of dexamethasone, a glucocorticoid, we measured release of these substances using an intracerebral dialysis technique. A dialysis probe of Carnegie Medicine was inserted into the hippocampus of a Wistar strain rat. ACh was assayed by HPLC with an electrochemical detector and amino acids with a fluorescence detector. Subcutaneous injection of dexamethasone caused short-latency decreases of both ACh and GABA and increases of glutamate and glycine. These effects of dexamethasone are considered to be direct actions on neuronal receptors. It has been reported that glucocorticoid has a cytotoxic effect in the hippocampus. The increase of glutamate release and decrease of GABA release induced by dexamethasone may have some relation to neuronal damage by the steroid.

57.5

DECREASED CORTICOSTEROID RECEPTOR NUMBERS IN THE HIPPOCAMPUS ASSOCIATED WITH DEXAMETHASONE RESISTANCE IN THE PRIMATE. S. Brooke[1], A. de Haas-Johnson [1], J. Kaplan [2], S. Manuck [3] J. Miller[1], R. Sapolsky[1]. [1] Dept. of Biol Sci, Stanford University, Stanford, CA 94305 and [2] Bowman Gray Sch Med, Winston Salem, NC. 27103 [3] Univ. Pittsburgh, Pittsburgh, PA

Neural corticosteroid receptors and their relationship to glucocorticoid negative feedback regulation have been studied in rats; we present data from the primate brain which support those findings. Subjects were 51 male *Macaca fasicularis* monkeys with known behavioral histories and who had been given dexamethasone (DEX) suppresion tests a few weeks prior to killing. Receptor analysis of tissue from hippocampi of frozen brains consisted of incubating cytosol (stripped of unbound cortisol) at 4 °C with ^{3}H cortisol (20nM) ± 80nM RU28362 or 250 nM dexamethasone; pilot studies with this assay showed Type I and II receptors in the monkey brain to have Kd's similar to that seen in the rat. We compared the subset of monkeys who were most DEX responsive (post-DEX cortisol values of 3 ± .2 ug/dl) versus a DEX resistant subset (cortisol values of 9 ± .6ug/dl). DEX resistant monkeys had significantly fewer Type II receptors in the hippocampus; they did not differ in numbers of Type I receptors. Animals had resided for a number of years in social groups that were either stable or were repeatedly destabilized by changing of group membership; the latter has been shown to constitute a sustained stressor. DEX resistant animals disproportionately came from such unstable social groupings. Rodent studies have shown that a depletion of hippocampal corticosteroid receptors is associated with glucocorticoid hypersecretion and feedback resistance, and that sustained stress can bring about such a down-regulatory depletion. The present data demonstrate similar associations in the primate.

57.6

CORTICOSTERONE ALTERS MEMBRANE TIME CONSTANT THROUGH TYPE I RECEPTOR ACTIVATION S.G. Beck*, T. List and K. Choi, Department of Pharmacology, Loyola University Chicago Stritch School of Medicine, Maywood, Il 60153.

The hippocampus is integral in the feedback inhibitory control of the hypothalamic-pituitary-adrenal axis control of serum corticosterone (CT) levels. The hippocampus contains the highest density of both Type I and Type II CT receptors and mRNA. Standard intracellular recording techniques were used to measure the effects of Type I and Type II receptor activation on CA1 hippocampal pyramidal cell active and passive properties. Rats were adrenalectomized (ADX) or ADX and treated with a 12.5 mg CT pellet for two weeks. During the experiment either 1 nM CT and 1 nM RU486 for Type I receptor activation or 10 nM RU 28362 for Type II receptor activation were included in the perfusion buffer. Type I receptor activation significantly increased the time constant of cells recorded from either ADX or ADX+CT treated animals as compared to cells from ADX or ADX+CT without Type I receptor activation. Action potential height and duration were significantly increased, fAHP amplitude reduced and the decay time of the sAHP reduced by Type I receptor activation. Type II receptor activation did not significantly alter membrane properties. These effects of Type I receptor activation result in an increase in pyramidal cell excitability. We conclude that CT has a significant effect on active and passive hippocampal pyramidal cell properties at concentrations normally present during basal conditions. Supported by KO2 MH00880 and NS28512.

57.7

CELLS IN THE MEDIAL AMYGDALA ARE ESSENTIAL FOR ADRENAL-STEROID-INDUCED SODIUM INGESTION.

D.M. Zhang, A.N. Epstein and J. Schulkin.* Departments of Biology and Anatomy. University of Pennsylvania., Philadelphia PA 19104-6048

Mineralocorticoid hormones play an important role in the regulation of body sodium both in terms of physiology and behavior. Adrenal-steroid hormones are known to bind to forebrain sites; including TYPE I receptors of the medial amygdala. In previous work with electrolytic lesions we have found that damage to the medial nucleus of the amygdala abolished mineralocorticoid-induced sodium intake. In the present study, cell body damage in the medial amygdala was induced by ibotenic acid in mature Sprague-Dawley rats. Following recovery, the rats were given 3%NaCl in addition to their food and water. They were then treated with either aldosterone alone, corticosterone alone, or both. We found that the lesion abolished both aldosterone-induced sodium ingestion and the potentiation of aldosterone appetite by corticosterone. By contrast,we also later found that sodium depletion-induced and captopril-induced sodium intake were left intact. These results provide further evidence that the medial region of the amygdala is importantly involved in both mineralocorticoid-induced and corticosterone potentiation of mineralocoticoid-induced sodium appetite. Supported by NIMH 43787 and NSO 3469.

RESPIRATORY REGULATION: CENTRAL NETWORKS/PATTERNS

58.1

CARDIORESPIRATORY EFFECTS OF TRIGEMINAL (V) AND SUPERIOR LARYNGEAL NERVE (SLN) STIMULATION IN THE PIGLET.

R.A. Darnall* and D.Berard, Dept. of Physiology, Dartmouth Medical School, Lebanon, NH 03756.

To explore whether sensory inputs from the face and nose can augment the apnea and bradycardia produced by laryngeal stimulation (stim), we evaluated the effects of electrical stim of the maxillary branches of V and the SLNs. Piglets were anesthetized, paralyzed, and respirated either at a constant rate (CV) or were allowed to be ventilated in proportion to their intrinsic phrenic nerve activity (SV). Nerves were stimulated (500 ms train) using constant current during early to mid phrenic inspiration. In the CV animals stim of V increased phrenic amplitude (Pmax) (122.3 ± 3.9 and 150.0 ± 14.4) (intact vs vagotomy, % control ± SEM) whereas SLN stim decreased Pmax (51.3 ± 3.1 and 61.3 ± 4.4). Ttot was decreased by V stim (82.5 ± 3.3 and 98.6 ± 1.9) and increased by SLN stim (134.1 ± 5.3 and 108.9 ± 2.2). There were similar decreases in Ti after both V and SLN stim. When both nerves were stimulated together there was little net change in Pmax, TTot, or Ti. In the SV animals the changes in Pmax and TTot after V and SLN stim were similar to those in the CV animals. Ti, however, increased after V stim and decreased after SLN stim. BP changed little after either V or SLN stim in both CV and SV animals. HR fell after both V and SLN stimulation in the SV animals but not when CV continued despite a decrease in phrenic nerve output. There was no further decrease in HR when V and SLN were stimulated together. We conclude that stimulation of the maxillary branch of V, augments phrenic activity and decreases the inhibitory effects of SLN stimulation. The HR effects of both SLN and V stim appear to be decreased by chest movement.

58.2

SOME SIGNS OF SHORT TERM MEMORY AT THE CENTRAL GENERATOR OF BREATHING.

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In pentobarbital anesthetized vagotomized cats it wes observed that: a) Repeated stimulation of medium threshold vagal afferents (.2 msec 4-10v) applied at the end of inspiration, which have an off-switch effect therefore increasing slightly the breathing rate. This controlled faster rate persisted for several cycles after stopping the stimulation. The effects were not accompanied by correlated changes in end-tidal CO_2. b) The bradypnea induced by laryngeal distension overlasted also the period of stimulation. c) Brief trains of stimuli (4-6 Hz) applied at the middle of inspiration reduced transiently the diaphragmatic tension and lengthened the inspiratory movement. By repeating the trains for ten or more successive cycles, a similar pattern of the response appeared at the following inspirations after ceasing the stimuli, extinguishing gradually. Shortly after extinction, one single similar train could induce identical behavior. The results are interpreted as short-term memory mechanisms present at the central generator of breathing. The phenomena were not affected by strychnine, diazepam or amino-phosphono-valeric acid (APV) administration.

58.3

PULMONARY C-FIBRE ACTIVATION OF VAGAL MOTONEURONES AND RESPIRATORY CELLS IN THE BRAINSTEM OF ANAESTHETIZED RATS AND CATS.

James F.X. Jones and David Jordan* Department of Physiology, Royal Free Hospital Medical School, Rowland Hill Street, London NW3 2PF, UK.

The pulmonary chemoreflex is a triad comprising apnoea, bradycardia and systemic hypotension (Coleridge & Coleridge Rev.Physiol.Biochem.Pharmacol. 99: 1-110, 1984). We have recently become interested in the central mechanisms underlying this reflex. The responses of vagal motoneurones and respiratory neurones evoked by right atrial injection of Phenylbiguanide (PBG, 5-50μg/kg) were studied in chloralose/barbiturate anaesthetized rats and barbiturate anaesthetized cats. In the two species PBG evoked a short latency inhibition of both inspiratory (n=5) and expiratory neurones (n=6) located in both dorsal and ventral respiratory groups. In contrast, antidromically activated post-inspiratory laryngeal motoneurones (n=4) were strongly excited by PBG. However, the responses of different vagal motoneurones in the dorsal vagal motor nucleus (n=9) and nucleus ambiguus(n=8) to administration of PBG are less consistent than the responses of the different classes of respiratory neurone. The elicitation of this simple, primitive pulmonary chemoreflex is proving to be useful in dissecting central respiratory and cardiovascular mechanisms.

This work was supported by M.R.C.

58.4

NEURONS OF THE PARAPYRAMIDAL REGION MODULATE RESPIRATORY ACTIVITY AND REFLEX RESPONSES TO STIMULATION OF PULMONARY C-FIBER RECEPTORS.

B. Erokwu, K.P. Strohl , N.S. Cherniack* and M.A. Haxhiu. Case Western Reserve University, Cleveland, OH 44106.

It has been shown that ventral medullary neurons close to the surface and just lateral to pyramidal tract (parapyramidal region-pp) project to spinal cord respiratory and cardiovascular related neurons. However, their role in regulation of cardiopulmonary responses is not well established. In the present experiments we assessed the effects of a) direct activation of pp on breathing activity and b) cardiorespiratory changes induced by stimulation of pulmonary c-fiber receptors, produced by phenylbiguanide (PBG) given into right atrium (5-30 μg/kg). Experiments were performed in α-chloralose-anesthetized and artificially ventilated cats with O_2 at end tidal CO_2 above apneic threshold. Direct activation of pp neurons by topical application or microinjection of N-methyl-D-aspartate (NMDA; 10-100 nmol) increased diaphragm electrical activity from 16±2 to 28±3 units (p< 0.05; n=12), and caused an elevation of arterial blood pressure (BP) by 16±4% (mean±SEM; p<0.05); but had no effect on heart rate. Activation of pp neurons significantly decreased duration of apnea resulting from stimulation of pulmonary c-fiber receptors (n=8). In a control period administration of PBG caused cessation of inspiratory activity, on average lasting 14.6±2.6s (p<0.05), decreased systolic blood pressure by 27±5% (p<0.05) and caused slowing of the heart rate, on an average by 33±9% (p<0.05). Activation of pp neurons significantly reduced inhibitory effects on respiratory activity reducing duration of apnea to 4.1±1.7s (control vs NMDA: p< 0.005), but had no significant effects on cardiovascular responses. These data indicate an involvement of pp neurons in control of breathing activity, blood pressure, and respiratory reflex responses. Supp: HL-35830.

58.5

RESPIRATORY PHASE-DEPENDENT VARIATIONS IN CORRELATED SPIKE TRAINS OF BRAIN STEM CARDIORESPIRATORY NEURONS. B. G. Lindsey*, Y. M. Hernandez, A. Arata, K. F. Morris, and R. Shannon. Dept. Physiol. & Biophysics, Univ. South Florida Med. Ctr., Tampa, FL 33612.

The regulation of gas exchange requires coordination of the respiratory and cardiovascular systems. One component of this control mechanism may be a central "irradiation" of inspiratory drive from respiratory neurons of the ventrolateral medulla to cardiorespiratory neurons of the brain stem midline (*Soc. Neurosci. Abst.* 17:339, 1991; Morris et al., these proceedings). We sought evidence for the respiratory phase-dependent modulation of effective connectivity among cardiorespiratory neurons that could reflect one consequence of the irradiation and act cooperatively with baroreceptor feedback. Spike trains were recorded simultaneously in the rostral ventrolateral medulla (RVL) and regions of the medullary raphe nuclei of 10 anesthetized, vagotomized, paralyzed, artificially ventilated cats. Respiratory and cardiac related neurons were identified by cycle triggered histograms. Baroreceptors were stimulated by local pressure changes in the carotid sinus or increased blood pressure caused by occlusion of the descending aorta or i.v. phenylephrine hydrochloride. Correlated spike trains of 52 pairs of neurons were analyzed with the normalized Joint Peristimulus Time Histogram (*J. Neurophysiol.* 61:900, 1989) triggered by the onset of a respiratory phase. Diagonal "bands" of significant bins indicative of impulse synchrony (*S*) or asynchrony (*A*) throughout the respiratory cycle were most common. Phase-dependent variations were found in 12 pairs: 4 of 5 *S* bands were greater during the inspiratory phase; 5 of 7 *A* bands were more pronounced during expiration. The results provide evidence for respiratory phase-dependent modulation of functional links between baroresponsive and respiratory related midline and RVL neurons. Supported by NS19814 & BRSG S07 RR05749.

58.6

MEDULLARY VENTRAL RESPIRATORY GROUP (VRG) NEURON RESPONSES DURING FICTIVE COUGH R. Shannon*, K.F. Morris and B.G. Lindsey. Physiol. & Biophy., Col. Med., Univ. So. FL., Tampa, FL 33612

The central neural network mechanisms that produce the cough motor pattern are poorly understood. Experiments were conducted to determine if activities of bulbospinal inspiratory and expiratory VRG neurons change appropriately during cough for roles in transmission of cough patterns to spinal motoneurons. Anesthetized cats (n=4) were decerebrated (mid-collicular), thoracotomized, paralyzed and ventilated (phrenic driven). Extracellular single neuron activity and phrenic and lumbar nerve efferent neurograms were monitored during fictive cough, produced by mechanical stimulation of the intra-tracheobronchial tree. Bulbospinal neurons were identified by antidromic stimulation at T1 (collision test). The firing rates of inspiratory (n=10) and expiratory (n=10) bulbospinal neurons increased during the respective phases of the cough cycle. Putative propriobulbar neurons (n=32), active in different phases of the respiratory cycle and located in the caudal and rostral VRG, also responded in ways consistent with roles in cough generation. Altered discharge patterns were similar to those observed during simulated fictive cough generated by enhanced versions of a previously described respiratory network model (Soc. Neurosci. Abst.17:620, 1991). The results suggest 1) that premotor I and E VRG neurons provide descending drive during both eupnea and cough, and 2) that the respiratory network is dynamically reconfigured to generate the cough motor pattern. (BRSG RR05749)

58.7

RESPIRATORY NEURONS IN THE VENTROLATERAL RETICULAR FORMATION OF OSCILLATING MEDULLARY SLICES FROM NEONATAL RAT. G.D. Funk*, J.C. Smith, S.M. Johnson, & J.L. Feldman. Systems Neurobiology Lab., Dept. Physiological Science, UCLA, Los Angeles, CA, 90024-1527.

Medullary slices (300-600 µm thick) from neonatal rat containing the pre-Bötzinger Complex (preBötC), generate respiratory oscillations in cranial nerves IX and XII (Smith *et al., Science*, 254,'91), allowing analysis of mechanisms of rhythm generation in an isolated, active respiratory circuit. We have constructed maps of the spatiotemporal patterns of neuronal activity in the region of preBötC. Extracellular and whole-cell patch-clamp recordings identified 5 major cell types with distinct temporal discharge patterns. Inspiratory neurons (*I*) discharge coincident with nerve XII output, and receive large amplitude, rhythmic synaptic drive potentials (10-20 mV). Late *I* neurons discharge starting at the peak of XII output and continuing through *I*. Tonic expiratory neurons (*E*) discharge tonically during expiration and receive rhythmic, inhibitory synaptic potentials (2-10 mV) during *I*. Beating neurons discharge tonically at 3-10 Hz. Oscillatory neurons, proposed to be the kernel of the respiratory rhythm generator, discharge throughout *I*, and exhibit voltage-dependent pacemaker-like properties, post-inhibitory rebound, and receive small, rhythmic synaptic drive potentials (5-8 mV) during *I*. Lucifer yellow fills of *I* and *E* neurons revealed complex cellular morphologies. Dendrites of both cell types extend ventrally 200-300 µm to within 50 µm of the ventral medullary surface. These studies indicate that there are complex, respiratory-related, synaptic interactions in medullary slices *in vitro*, and provide information essential for understanding the cellular and synaptic processes underlying respiratory rhythm and pattern generation. Supported by NIH Grants HL40959 & HL02204 (JCS) and NSERC of Canada (GDF).

58.8

SPATIAL ORGANIZATION OF PREMOTONEURONS IN A LOCAL RESPIRATORY CIRCUIT STUDIED BY TRANSSYNAPTIC NEURONAL LABELING WITH PSEUDO-RABIES VIRUS (PRV). R. Van Bibber, C.L. Barry, E.G. Dobbins, J.C. Smith, & J.L. Feldman*. Systems Neurobiology Lab, Dept. of Physiological Science, UCLA, Los Angeles, CA 90024-1527.

We have proposed that respiratory rhythm is generated in the pre-Bötzinger Complex (pre-BötC) of the ventrolateral medulla, and that rhythmic respiratory drive is transmitted through local interneuronal circuits to cranial respiratory motoneurons including hypoglossal (XII) motoneurons (Smith et al., Science 254: 726, '91). To establish the organization of premotor and interneurons in this local circuit, we used the transsynaptic tracer PRV. Injections of PRV were made into respiratory-modulated musculature of the tongue of neonatal rats, and the neuronal labeling pattern analyzed at different survival times. XII motoneurons were the main population labeled after the shortest survival times (30-35 hrs). With survival times of 48-52 hrs, discrete populations of hypoglossal premotoneurons were labeled predominantly in the tegmental field (TF), just lateral and ventral to XII. With 70 hr survival times, dense neuronal labeling occurred in pre-BötC and more rostral Bötzinger Complex. Less dense neuronal labeling occurred in more caudal ventrolateral reticular formation (rostral ventral respiratory group). These results suggest a spatial segregation of interneurons in the transmission pathway from pre-BötC neurons through more dorsal TF premotoneurons to XII motoneurons. The results are consistent with our previous studies (*ibid*) which indicate a dense concentration of propriobulbar interneurons within the pre-BötC that are the substrate for transmission of the rhythm (generated locally) to cranial (pre)motoneurons. We are grateful to J.P. Card, Du Pont Merck Pharmaceutical Co. for his generous gift of PRV and antibody. Supported by NIH Grants NS24742, HL40959 & HL02204.

58.9

ORGANIZATION OF THE VENTROLATERAL MEDULLA: DISCRETE PROJECTIONS TO TWO RESPIRATORY NUCLEI IN NEONATAL AND ADULT RATS. E.G. Dobbins* & J.L. Feldman. Systems Neurobiology Lab., Dept. of Physiological Science, UCLA, Los Angeles, CA 90024-1527.

The medullary lateral tegmental field (LTF) has long been considered critical for the control of respiration. Recent developments suggest a high degree of regionalization among respiratory related LTF neurons (Ellenberger & Feldman, JCN 294 ('90): 202). To establish whether premotoneurons, or afferents to premotoneurons, projecting to cranial and spinal cord respiratory nuclei were located in overlapping populations in LTF, we used the transynaptic tracer pseudorabies virus (PRV). Injections into the phrenic nerve of adult, or the right hemidiaphragm of neonatal, rats labeled premotoneurons in a discrete region of LTF, just rostral to obex. Injections into respiratory modulated musculature at the base of the tongue labeled neurons premotor to the hypoglossal nucleus (XII) predominantly in the medial reticular formation, just lateral to XII. Neurons afferent to either premotor population are contained within rostral regions of LTF, known as the Bötzinger (BötC) and the preBötzinger Complex (preBötC). The populations do not appear to overlap. The density of neuronal label in BötC and preBötC is greater following tongue injections, compared to a more caudal concentration of neuronal label following diaphragm injection. These results indicate that there is a separation of control elements, at least two synapses prior to motoneurons of the tongue and diaphragm, within a highly circumscribed region of the brainstem. We are grateful to J.P. Card, Ph.D., Du Pont Merck Pharmaceutical Co., for his generous gift of PRV and antibody. Supported by NIH Grant NS24742.

58.10

FLUORESCENT DYE TRACING REVEALS RECIPROCAL CONNECTIONS BETWEEN RESPIRATORY NUCLEI AND THE MEDIAL PONTINE RETICULAR FORMATION (mPRF). L.H. Lee, J.G. Veino, and R.Lydic * Department of Anesthesia, Penn. State University, College of Medicine, Hershey Pa 17033.

Microinjection of cholinergic agonists into the mPRF of intact, unanesthetized cats causes a REM sleep-like state and state-dependent respiratory depression. Since there are no major clusters of respiratory neurons in the mPRF, these potent respiratory effects suggest connectivity between the mPRF and brain stem respiratory nuclei. The present study is characterizing these connections by injecting the mPRF and the pontine respiratory group (PRG) with 50 or 100 nl of the retrograde tracers True Blue (5%), Fluro-Gold (2%), and Diamidino Yellow (3%). To date, we have studied 8 cats following 6 bilateral injections into the mPRF and 2 bilateral injections into the PRG. After mPRF injections fluorescent labeling was observed in nuclei comprising the dorsal, ventral, and pontine respiratory groups. Bilateral mPRF injections produced double labeled cells in the PRG and bilateral PRG injections double labeled mPRF neurons. The mPRF injections consistently labeled cells in the retrofacial, retrotrapezoid and pedunculopontine tegmental nuclei. These data demonstrate connections between cholinoceptive regions of the mPRF and brain stem respiratory groups. Such connections could mediate state-dependent respiratory depression evoked by cholinoceptive mechanisms within the mPRF. *Support: Department of Anesthesia, HL47749, HL40881(RL).*

58.11

SUB-POPULATIONS OF NEURONS IN THE ROSTRAL PONS MEDIATE DISTINCT FUNCTIONS IN THE CONTROL OF RESPIRATION IN CATS. M.-L. Fung, R. A. Maue* and W. M. St. John. Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756.

The rostral pons has been shown to be involved in difference functional roles in the control of respiration in mammals. Here we examined the hypothesis that sub-populations of neurons in the rostral pons mediate distinct functions in the control of respiration. Experiments were performed on decerebrate, vagatomized and paralyzed animals. Kainic acid (KA) (4.69 mM) was microinjected (10-60 nl) into loci in the rostral pons in which electrical stimulation had caused an on- or off-switch of phrenic activity. Most of the effective microinjections of KA caused changes in the length of different phases of the respiratory cycle. In particularly, microinjections of KA in a medial region (2-3 mm from midline) increased the length of expiratory phase and delayed the onset of expiratory activity of spinal nerves. In a lateral region (4-5 mm from midline), prolongations of the inspiratory phase followed microinjections of KA into the rostral portion (0-1 mm rostral to inferior colliculus). In contrast, KA injections into the caudal portion (1-3 mm caudal to inferior colliculus) increased the length of expiratory phase and changed the onset time of spinal neural expiratory activity. In addition, the frequency response to hypercapnia was diminished significantly after KA was applied in the caudal portion of the lateral region. Results confirmed that the rostral pons plays an important role in the control of the respiration. We conclude that groups of neurons with differential functional roles in the control of respiration are located in the rostral pons. (Supported by HL 26091)

58.12

DYNAMIC RESPIRATORY RESPONSES TO ANTERIOR HYPOTHALAMIC WARMING IN THE SLEEPING CAT. H. Ni*, J. Zhang, and R.M. Harper. Brain Research Institute and Department of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024.

Local warming of the hypothalamus modifies respiratory rate and elicits panting. This effect is state-dependent, with rapid eye movement sleep greatly diminishing the respiratory response. We examined the respiratory responses following anterior hypothalamic warming during different sleep states; the objective was to determine the mechanisms by which respiratory patterns change to thermal challenge in each state. The anterior hypothalamus was warmed (0.5MHz, 80-150mW) bilaterally by two electrode pairs (2mm inter-tip distance, 2 mm bared tip, and 0.3 mm in diameter). Anterior hypothalamic temperatures were recorded by a thermocouple placed 1 mm deeper than the tip of one heating electrode. Electrodes were also placed for recording EEG, ECG, diaphragmatic and neck EMG, and core body temperature. Warming of the anterior hypothalamus by 1-2°C elicited polypnea during awake and quiet sleep states, but not in rapid eye movement sleep. As total respiratory cycle time decreased, the ratio of inspiratory time to expiratory time (Ti/Te), and inspiratory time to total time (Ti/Ttot) remained constant, although increased variation in these ratios appeared during the warming period, particularly during REM sleep. The amplitude of diaphragmatic EMG activity decreased with extreme tachypnea. These results suggest that descending thermal influences selectively modify inspiratory/expiratory switching thresholds rather than enhancing inspiratory drive systems. *Supported by HD22506.*

58.13

DEVELOPMENTAL CHANGES IN RESPIRATORY NEURAL OUTPUT IN LARVAL FORM OF *RANA CATESBEIANA*. R. Galante, E. Smith, L. Kubin and A.I. Pack*. Center for Sleep and Respiratory Neurobiology, Univ. of Pennsylvania, Philadelphia, PA.

Studies have been conducted to examine the changes in respiratory neural output at different stages of development in the larval form of the amphibian *Rana catesbeiana*. We used an isolated *in vitro* brainstem preparation superfused with an oxygenated physiological solution. Extracellular multiunit activity was recorded from the 7th motor nucleus at various stages of development, both in normal solution and when chloride ion was replaced in the superfusate by gluconate. When lung ventilation first appeared (stages VI-VII), the neural activity consisted of brief, fast-rising bursts superimposed on the ongoing rhythmic activity reflecting the gill ventilation. During developmental stages XX-XXI, the lung burst rise time lengthened and a distinct oscillation appeared on the burst rising slope; following the lung burst, there appeared a brief period without gill activity. Still later (stages XXIV-XXV), the gill rhythm disappeared and there was a further lengthening of the bursts for lung breathing. At all stages of development, removal of chloride abolished the bursts corresponding to gill ventilation, while the bursts for lung ventilation persisted, albeit with an increased duration and amplitude. There were no obvious developmental changes in the pattern of lung ventilation bursts in the absence of chloride. These data suggest that chloride ion-mediated inhibition is necessary for generation of the gill but not the lung breathing rhythms. The generator for the latter appears early and shows alteration with development that, at least in part, depend on chloride channels. (Supported in part by HL-39775)

58.14

RESPIRATORY MODULATION OF LOCUS COERULEUS (A6) AND A5 NORADRENERGIC (NE) CELLS. D. Huangfu*, N. Koshiya, A.J.M. Verberne and P.G. Guyenet. Dept. Pharmacology, Univ. of Virginia, Charlottesville, Va 22908.

The present experiments were designed to test the hypothesis that A5 and A6 NE cells receive central respiratory information from the rostral ventrolateral medulla (RVL), a region of the brain primarily involved in autonomic and respiratory control. A5 and A6 cells were recorded in urethan-anesthetized vagotomized rats along with the discharge of the phrenic (PND) and splanchnic nerves (SND). Carotid chemoreceptor stimulation (CCSt) was performed with 10 s N_2 inhalation or 3 min hypoxia (12% O_2). Both A5 and A6 neurons were activated by CCSt and displayed respiratory patterns of discharge at rest which was enhanced by CCSt. A6 cells displayed one of the two central respiratory patterns found in RVL neurons previously: i) an inspiratory (I) pattern characterized by peak firing coinciding with the PND peak (202±39 ms after PND onset) and nadir during early expiration, or ii) a postinspiratory (post-I) pattern characterized by increased firing probability at the end of the phrenic burst (590±44 ms after PND onset) and a nadir coincident with the peak of PND. Most A5 cells displayed a post-I pattern which was enhanced by CCSt. RVL neurons with direct projections to A6 were identified with antidromic mapping techniques (depth threshold curves). The vast majority of active RVL cells with projections to LC (19/27) also had central respiratory patterns reminiscent of that of A5 and A6 cells. The patterns were enhanced by CCSt. These RVL cells were frequently inhibited by raising arterial pressure but were distinct from RVL sympathetic premotor neurons since they did not exhibit detectable pulse synchrony and could not be backfired from the thoracic spinal cord. Support: HL 28785 and HL 39841 from NIH.

58.15

RESPIRATORY RELATED NEURONS SUPPLYING BILATERAL INNERVATION IN THE RAT. R. Pásaro* and P.A. Núñez-Abades. Dept. of Animal Physiology and Biology, University of Sevilla, 41012-Spain.

The respiratory motoneurons of the nucleus ambiguus and the cervical spinal cord are organized as a bilateral column of cells. These motoneurons project exclusively ipsilateral with no evidence of axonal branching or axons crossing the midline, in the rat. Which cells are implicated in the synchronization of the motoneuronal activity in both pools? To address this issue, we have looked for the neurons supplying bilateral innervation by means of the multiple fluorescent retrograde neuronal labeling technique. Our results indicated that a discrete population of neurons within the rostral Ventral Respiratory Group (rVRG) is the main source (90%) of neurons that sent axonal collaterals to both phrenic nuclei. Furthermore, the rVRG and the Bötzinger Complex concentrate the highest number of neurons (50%) that sent axonal projections by collaterals to both VRG. These results suggested that multiple pathways (instead of only one) could contribute to the synchronization of the bilateral respiratory motoneuronal networks and, that the interconnections of the VRG could satisfy similar purposes.

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59.1

CENTRAL NERVOUS SYSTEM NEURONS LABELLED FOLLOWING THE INJECTION OF PSEUDORABIES VIRUS INTO THE RAT URINARY BLADDER

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Pseudorabies virus (PRV) was injected into the wall of the urinary bladder and, following incubation times of 2, 3, 4 and 4 1/2 days, central nervous tissue was processed immunohistochemically for the presence of virus. Infected neurons were initially visualized in the sacral parasympathetic nucleus and other parts of the spinal cord (mainly lumbosacral). Following longer survival times, infected neurons were found in the raphe nuclei, reticular area, pontine micturition center (PMC), locus coeruleus (LC), parabrachial nucleus, red nucleus, hypothalamus, preoptic area, amygdala and cortex. Sections from the medullary dorsal tegmental region (DLT) of a three-day animal which included the PMC and the LC were immunohistochemically processed for PRV and acetyl cholinesterase (AChE). Neurons in the locus coeruleus stained for AChE whereas neurons in the PMC stained only for PRV. There were also some neurons medial to the PMC that stained lightly for AChE. There were no doubly labelled neurons. Therefore, since we found no AChE in the PRV-labelled PMC neurons, it is unlikely that PMC neurons are cholinergic. In conclusion, these data define a multisynaptic circuit of neurons whose ultimate output influences urinary bladder function.

59.2

ANALYSIS OF THE MICTURITION REFLEX PATHWAY IN THE IN VITRO BRAIN STEM – SPINAL CORD – BLADDER PREPARATION OF THE NEONATAL RAT. K. Sugaya*, M.N. Kruse and W.C. de Groat. Dept. Pharmacology, Univ. Pittsburgh, Pittsburgh, PA 15261.

Micturition in the neonatal rat is induced by a spinal reflex pathway activated when the mother licks the perineum of the pup. Experiments were undertaken to determine whether this reflex could be elicited in isolated brain stem-spinal cord-urinary bladder (B-SC-B) preparation of the neonatal rat (1-7 days of age). B-SC-B were removed from hypothermic anesthetized rats and perfused with Kreb's Ringers solution at 26-30°C. Bladder activity was monitored by measuring intravesical pressure with a needle inserted into the bladder lumen. Following distension of the bladder with 0.1-0.2 ml of saline tactile stimulation of the perineal region (PS) induced reflex bladder contractions (mean 9 cmH_2O) which were almost the same amplitude as the contractions induced by electrical stimulation (ES) of the bladder surface. The response to ES or PS were blocked by TTX (1μM), however, rhythmic intrinsic contractions of the bladder were unaffected by TTX. Removal of the spinal cord blocked PS-contractions, but enhanced intrinsic contractions. CNQX (5μM) but not MK-801 (50μM) decreased the amplitudes of PS-contractions but not intrinsic contractions. In some preparations, bicuculline methiodide (BCMI; 10μM) increased the amplitude of PS-contractions. NMDA (10μM) transiently increased the frequency of rhythmic bladder contractions.

It is concluded that glutamic acid acting via AMPA receptors may be involved as a transmitter in the perineal-bladder reflex in neonatal rats. The enhancement of the reflex by BCMI indicates that the reflex pathway is tonically inhibited by a GABAergic mechanism.

59.3

Identification of the Central Nervous System Pathways Controlling Bladder Function in the Neonatal Rat Using Transneuronal Tracing with Pseudorabies Virus (PRV). J.R. Roppolo*, P. Card, K. Sugaya, N. Yoshimura and W.C. de Groat. Dept. Pharm., Univ. Pittsburgh and DuPont Inc., Wilmington, DE.

During the early postnatal (PN) period (day 0-6) in the rat, micturition (MT) is mediated by a spinal reflex pathway (SRP), whereas in older animals 12-15 days of age MT occurs via a supraspinal reflex pathway. Axonal tracing studies using PSV were conducted to examine the changes in the MT reflex pathway during the PN-period. PSV (1-2 μl, 10^6p/μl) was injected into one side of the urinary bladder of anesthetized rat pups ranging from 2 to 14 days in age. After transport time of 1-4 days, animals were perfused with fixative and tissues were sectioned and processed using immunocytochemical techniques to identify PSV. In young rats (3-6 days) versus older rats (12-17 days) virus labeled cells in the L_6-S_1 spinal cord were present in greater numbers and occurred in a wider distribution. In rats of both ages, labelled cells were present in the area of the sacral parasympathetic nucleus (SPN) and in the dorsal commissure (DCM). In young rats, labelled neurons were also present in lamina I of the dorsal horn and in large numbers in medial laminae (V-VII). In older rats, labelled cells were located predominately ipsilateral to the injection site whereas in young rats the cells were distributed bilaterally. Brain stem labelling however was more prominent and had a wider distribution in the older animal. Labelled neurons were seen in the area of the locus ceruleus, periaqueductal grey (PAG), and pontine reticular formation. In young animals (5 days), few or no labelled neurons were seen in these areas. In conclusion, these experiments revealed a more extensive spinal interneuronal system associated with the bladder innervation in day 3 to day 6 rats. The apparent elimination of this interneuronal system in older rat pups is consistent with the reorganization of the micturition reflex and emergence with supraspinal mechanisms during postnatal development.

59.4

THE EFFECTS OF LY-274614, A COMPETITIVE NMDA RECEPTOR ANTAGONIST, ON THE MICTURITION REFLEX IN THE RAT. M. Yoshiyama*, J.R. Roppolo, K.B. Thor and W.C. de Groat. Dept. of Pharmacol., Univ. of Pittsburgh, Sch. of Med. Pittsburgh, PA 15261 and Lilly Research Laboratories, Indianapolis, IN 46285.

The effects of LY-274614 (0.1-30 mg/kg i.v.) on bladder and external urethral sphincter (EUS) EMG activity were examined in the following preparations: 1) spinal cord intact rats (SCI), 2) chronic spinal cord (T_6-T_8) transected rats , (3) SCI rats with continuous intravesical infusion of 0.1% acetic acid (AA) to produce bladder irritation. Also examined were SCI rats treated with LY-274614 via intrathecal (i.t.) catheter at the L_6-S_1 segments. All animals were anesthetized with urethane (1.2 g/kg s.c.). In intact rats LY-274614 reduced the maximal amplitude of micturition contractions (MAC) and EUS activity in a dose dependent fashion. In chronic spinal cord transected rats the same doses had no effect on bladder activity but increased MAC at 0.3 and 1 mg/kg, and inhibited EUS activity at 10-30 mg/kg. Intrathecal administration of LY-274614 to intact rats, significantly reduced MAC and EUS activity in doses ranging from 0.12-30 ug. MAC recovered to 50% of control after 3 hrs following i.v. (30 mg/kg) or i.t. (6 ug) administration of LY-274614. AA infusion shifted the dose-response curve to the right for both bladder pressure and EUS EMG. The data suggest that excitatory amino acid (EAA) transmitter mechanisms at the level of the spinal cord are important in modulating bladder activity in the intact animal; but that these mechanisms do not contribute to bladder reflexes following chronic spinal cord transection.

59.5

ANALYSIS OF DENDRITIC END BRANCHES OF NEUROBIOTIN- AND HRP-INJECTED SACRAL PREGANGLIONIC NEURONS (PGN) IN THE CAT.

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21 PGN in the sacral parasympathetic nucleus of the cat were filled by intracellular injection of neurobiotin or HRP and were analyzed according to numbers of dendritic end branches (**DEB**) terminating in nine specific fields in the gray and white matter. Three groups of cells emerged: **Type I** (N=9) had an avg 17.5 DEB in 6.4 regions and had *key dendrites in lam I* and the lateral ventral horn. **Type IIa** (N=8) had an avg 14.2 DEB in 4.9 regions and *principal dendrites in the dorsal and lateral funiculus* (**DLF/LF**) but none in lam I. **Type IIb** (N=4) had an avg 12 DEB in 3.3 regions, no DEB in lam I or the DLF/LF. Cells in all three groups had similar numbers of stem dendrites (avg 4.9).

Although the dendritic trees exhibited a predominant transverse orientation, extending up to 1300-2200 μm mediolaterally and 2000-2700 μm dorsoventrally, further analysis revealed a significant rostrocaudal spread of dendrites of up to 700-1200 μm, significantly enlarging the sampling area of these cells.

We propose that these separate dendritic patterns exist because of the variety of afferent sources and unique control mechanisms required by functionally diverse groups of PGN. Our hypothesis, to be tested in further experiments, predicts that Type I PGN, with the major lamina I dendritic field, are especially dependent upon the visceral and somatic sensory input to this region and are likely to be involved in sexual function; Type IIa PGN which have many DLF/LF dendrites, but none in lamina I, are likely to be dependent upon bulbospinal pathways which is characteristic of bladder PGN.

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59.6

AN IN VITRO NEONATAL RAT PREPARATION FOR THE STUDY OF PERINEAL-BLADDER REFLEXES. L.Song,B.Schmidt and S.J.Shefchyk*. Depts. of Med. and Physiol., Univ. of Manitoba, Winnipeg, Canada R3E 0W3.

In neonatal rats, micturition is evoked by stimulation of the perineal skin (Kruse and deGroat, Am.J.Phys. 1990:258,R1508). In the present experiments, we describe this perineal-bladder reflex in an in vitro neonatal rat preparation.

Neonatal rats, 3 to 13 days old, were anaesthetized with ether and decerebrated. The brainstem and spinal cord dissection was performed in a chamber filled with cold Krebs solution bubbled with oxygen. The bladder, hindlimbs and perineal skin were left intact as were spinal nerves caudal to the L1 segment. One or two 27½ gauge needles were inserted into the dome of the bladder and connected to a pressure transducer and/or infusion syringe. Bladder reflexes were tested when the bath was warmed to 26°C. A small volume of Krebs solution containing red food colouring was slowly injected into the bladder. The perineum was stimulated by light, fast stroking of the skin with a cotton swab.

The perineal-bladder reflex could be elicited in all ages tested. Ten to twenty five seconds after the start of the stimulation, the bladder pressure increased and one could observe expulsion of fluid from the urethra. The peak pressure reached during stimulation was 5 to 9 mmHg depending on the age of the animal. When stimulation was stopped, the bladder pressure immediately returned to baseline. Sometimes stimulation evoked the bladder contractions which were subthreshold for evoking expulsion of fluid.

This preparation may be useful for further investigations examining physiological and pharmacological properties of perineal reflex pathways involved in micturition or sexual reflexes in the rat.

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59.7

PATCH CLAMP ANALYSIS OF AFFERENT AND EFFERENT NEURONS THAT INNERVATE THE URINARY BLADDER OF THE RAT.

N. Yoshimura* and W.C. de Groat, Dept. of Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Axonal tracing techniques were used in combination with patch clamp methods to examine the electrophysiological properties of afferent (AN) and efferent neurons (EN) innervating the urinary bladder (UB) of the adult rat. Fluorescent axonal tracers (fast blue or fluorogold) were injected into the UB 10-14 days prior to the removal of the L_6-S_1 dorsal root ganglia (DRG) and the major pelvic ganglia (MPG). Ganglia were dissociated with enzymatic-mechanical methods and individual dye-labelled cells were identified with fluorescent microscope. Bladder AN were small (mean; 27.6 ± 2.3 μm in size) and the majority (80%) exhibited TTX-resistant action potentials and Na^+currents that had high thresholds for activation (-10 to -30 mV). Bladder EN (mean; 28.5 ± 3.1 μm) had action potentials with high thresholds (-20 to -40 mV) that were TTX-sensitive. A transient outward current, typical of an A type K^+ current was activated by depolarizing pulses (10-15 mV) from holding potentials equivalent to the resting membrane potential (-60 to -70 mV). This outward current was detected in 75% of bladder AN but not in EN. ACh (10-100 μM) applied to EN produced an inward current, membrane depolarization and a prolonged burst of action potentials. A brief application of norepinephrine (1-10 μM) elicited a prolonged depolarization and an inward current but no firing in EN. It is concluded that: (1) axonal tracing with fluorescent dyes is useful for identifying specific populations of neurons for patch clamp studies, (2) bladder AN and EN are distinguished by different types of Na^+ and K^+channels and (3) the majority of bladder AN have high electrical thresholds for spike initiation which may reflect the large population of unmyelinated chemo-mechanosensitive afferents that innervate the bladder.

59.8

DIFFERENTIAL EFFECTS OF SPINAL CORD INJURY ON THE MORPHOLOGY OF BLADDER AFFERENT AND EFFERENT NEURONS.

M.N. Kruse*, S.L. Erdman, M. Tanowitz and W.C. de Groat Dept. of Pharmacology, Univ. of Pittsburgh, Pittsburgh PA 15261.

It has been reported that physical obstruction of the urethral outlet in adult rats results in bladder hypertrophy and an increase in size of dorsal root ganglion (DRG) and major pelvic ganglion (MPG) cells which innervate the bladder. Obstruction of the urethral outlet also occurs following spinal cord injury (SCI); this is a functional obstruction caused by simultaneous contraction of the bladder and urethral sphincter (bladder/sphincter dyssynergia). This study tested whether functional obstruction due to SCI also induces changes in bladder weight and bladder neuron size. The bladders of control or chronic spinal (4-9 weeks after transection at T8-T9) adult female Wistar rats were injected with either fluorogold (FG) or WGA-HRP under halothane anesthesia. Several days later, the MPGs and DRGs were removed, processed for either HRP or FG, and the areas of the labelled cells measured. Following SCI, there was a significant increase in bladder weight (400%) and DRG cell size (40%), but no significant change in MPG size. The increase in DRG cell size was observed with both tracers; however, the size of FG-labelled cells was ~30% less than the size of WGA-HRP cells. It was shown that both tracers are transported by the same population of neurons; therefore the observed difference is attributed to shrinkage due to the FG-dehydration process. The results indicate that functional and physical obstruction of the urethral outlet elicited similar morphologic changes in the bladder and DRG cells but that only physical obstruction increased the size of MPG cells. This suggests that in spinal injured animals, preganglionic neurons may influence MPG neurons to negate the effect of peripheral trophic substances released from the hypertrophied bladder. Supported by the American Paralysis Association and PVA Spinal Cord Research Foundation.

59.9

HYPERTROPHY OF NEURONS INNERVATING THE URINARY BLADDER OR COLON OF STZ-DIABETIC RATS

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Hypertrophy of smooth muscle occurs as a consequence of various stresses placed upon the organ. For example, partial obstruction of the urethra results in bladder hypertrophy. A concomitant observation was a substantial increase in the size of post-ganglionic neurons in the major pelvic ganglia (MPG). This finding suggested that urinary bladder hypertrophy observed in the STZ-diabetic rat might also be accompanied by changes in other neurons concerned with micturition or defecation. Using fluorescent dyes injected into the bladder or the colon, we have measured the sizes of various groups of neurons associated with these organs in control and STZ-diabetic rats. These include 1) post-ganglionic neurons in the MPG 2) post-ganglionic neurons in the inferior mesenteric ganglion, 3) dorsal root ganglion neurons 4) sympathetic chain ganglion neurons 5) preganglionic neurons in the sacral parasympathetic nucleus, 6) motor neurons in Onuf's nucleus innervating the external urethral sphincter. In addition we have measured neurons in some of these groups for rats which have been maintained on a 5% sucrose in water and restricted food diet. Only neurons which make direct contact with the bladder or the colon were hypertrophied (15-70%). We speculate that a trophic factor transported from the organ to the neuron is responsible for this effect.

59.10

NEUROANATOMICAL EVIDENCE FOR BRANCHING AXONS FROM THE RAT SACRAL CORD PROJECTING TO THE URINARY BLADDER AND URETHRA.

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Previous electrophysiological and functional data provided evidence for the existence of a population of preganglionic axons in the rat pelvic nerve which give rise to two peripheral branches: one providing an input to major pelvic ganglion cells innervating the urinary bladder (UB) and another one projecting distally to the wall of the urethra via the penile nerve (PeN). On the basis of these results a double retrograde axonal tracing technique has been used to ascertain the localization of cell bodies from which these branched axons arise. Fluorescent tracers Fast Blue (FB) and Diamidino Yellow (DY) were injected in postganglionic fibers to the UB or to the urethra (PeN), respectively. Microinjections resulted in the labeling of DY (12.1%), FB (15.6%) and DY-FB (5.7%) small (20-30 μm) neurons located in the dorsal root ganglia (DRG) corresponding to the last segments of the sacral spinal cord. These cells were observed in DRG ipsilateral to the site of injection (n=6), being absent in contralateral DRG or in ganglia taken from higher (lumbar) segments. Transection of the pelvic nerve (10 days before) ipsilateral to the site of tracers administration, resulted in the disappearance of double labeled neurons (n=4). DY, FB or DY-FB positive cell bodies were also found at the level of the last spinal cord segments (S2-S4) in the 'intermediolateral nucleus' and their estimated ratio (%) was 4:5:1. These anatomical data reinforce our previous functional findings and extend the knowledge on the neuroanatomy of the vesico-urethral inter-relationships.

59.11

EFFECT OF INTRATHECAL ADMINISTRATION OF THE NK-1 RECEPTOR ANTAGONIST GR 82,334, ON BLADDER MOTILITY IN ANESTHETIZED RATS

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It has been proposed that substance P mediates sensory transmission in the spinal cord, however the role of this tachykinin on vesical reflexes has not been determined yet.

Application of capsaicin (4 μg/50 μl) on the serosal surface of the urinary bladder triggers a supraspinal micturition reflex (SMR). GR 82,334 (1 nmol/rat, intrathecal, i.t.) strongly reduced reflex bladder contractions induced by capsaicin.

Infusion of saline evoked SMR. I.t. administration of GR 82,334 (up to 1 nmol/rat, i.t.) did not modify either the threshold for activation of the reflex or the amplitude of bladder contractions.

Perineal noxious pinching activated a somatovesical spinal reflex which was reduced by the NMDA antagonist 2-amino-5-phosphonovaleric acid (250 nmol/rat, i.t.). GR 82,334 (1 nmol/rat, i.t.) had no effect.

Intraurethral infusion of capsaicin (500 μg/600 μl) inhibited distension-induced SMR. Such inhibition was prevented by the 5-HT_{1A} agonist 8-OH-DPAT (330 μg/kg, i.v.) but not by GR 82,334 (1 nmol/rat, i.t.).

These results suggest that spinal NK-1 receptors mediate excitatory but not inhibitory bladder reflexes induced by activation of capsaicin-sensitive fibers. Other excitatory vesical reflexes, which are capsaicin-resistant, are not affected by GR 82,334.

59.12

FOS LABELLING FOLLOWING ELICITATION OF SEXUAL REFLEXES IN MALE RATS

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The proto-oncogene c-*fos* has been used as a neuroanatomical marker for functional activity. The urethrogenital (UG) reflex represents a reliable model of sexual reflexes in spinalized, urethane-anesthetized rats. After repetitive elictations of the UG reflex, activity-dependent maps of the lumbosacral cord were constructed from patterns of *fos*-like immunoreactivity with a polyclonal antibody to *fos* oncoproteins. These maps were compared to patterns of *fos* labelling in surgically prepared control animals in which UG reflexes were not elicted.

The patterns of *fos* labelling in experimental animals were different from that of controls. Scant *fos*-positive neurons were labelled in spinally intact controls or those control animals otherwise not subjected to repeated elicitations of the UG reflex. From L1-L3, labelled cells were found in the intermediolateral cell column and Lamina X, sites of the sympathetic preganglionic neurons of the hypogastric nerve. Lamina V-VII also contained *fos*-positive cells as did Lamina VIII of the medial ventral horn. From L4-S1, cells were localized in Laminae V-VII, in the region of the parasympathetic preganglionic neurons of the pelvic nerve. Neurons exhibiting *fos* labelling were also found in Lamina VIII, near the pudendal motoneurons of the dorsomedial nucleus, as well more laterally in the intermediate ventral gray and in Lamina X. From L1 to L6, numerous cells were found in Laminae I and II of the dorsal horn. Repetitive elicitation of the UG reflex therefore yields distinctive patterns of neurons with *fos* labelling which provide activity-dependent maps of the neural circuitry fundamental to spinal sexual reflexes.

59.13

THE URETHROGENITAL REFLEX EVOKED BY ELECTRICAL STIMULATION OF THE HYPOTHALAMUS AND MIDBRAIN PERIAQUEDUCTAL GREY

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The urethrogenital reflex (UG) is a reflex evoked in the anesthetized rat upon distention of the urethra. This reflex is thought to represent the neural concomitants of ejaculation. Previous work by our laboratory has demonstrated that the UG reflex is under tonic descending inhibition from neurons in the region of the nucleus paragigantocellularis (nPGi) in the ventral medulla. The medial preoptic region of the hypothalamus is known for its role in facilitating sexual reflexes and sexual behavior in awake animals. We examined the effects of electrical stimulation of hypothalamic and midbrain sites on the presence of the UG reflex.

Male rats were anesthetized with urethane and mounted in a stereotaxic frame. The UG reflex was evoked via saline perfusion and brief occlusion of the urethra. As previously reported the UG reflex cannot be evoked in the intact preparation. Bilateral electrical stimulation was performed using bipolar tungsten electrodes (100-400 μA, 0.2 msec, 50 Hz). Trains of stimuli, 1 sec on/off, were initiated and presence of the UG reflex examined. The UG reflex could be evoked upon stimulation of discrete regions of the hypothalamus or midbrain periaqueductal grey. Effective stimulation sites in the hypothalamus ran from the medial preoptic area through the dorsal hypothalamus at 0.5-1.75 mm lateral to the midline. In the midbrain, stimulation of the periaqueductal grey allowed the UG reflex to be elicited. These data indicate that brain regions shown to be important in regulating sexual behavior in awake animals also facilitate sexual reflexes in anesthetized animals.

59.14

CNS CELL GROUPS PROJECTING TO THE ISCHIOCAVERNOSUS MUSCLE TRANSNEURONALLY LABELLED WITH PSEUDORABIES VIRUS (PRV)

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Previously we reported spinal and brainstem neurons transneuronally labelled following injection of PRV into the penis. Those studies demonstrated labelled autonomic efferents and spinal interneurons in lumbosacral spinal cord regions known to be involved in pelvic function as well as brainstem neurons that project to the these areas. The present study was aimed at identifying CNS neurons controlling the ischiocavernosus muscle, which is involved in penile erection.

Rats were anesthetized with ketamine/xylazine and injected with 2 μl PRV (2-3 x 10^7 plaque forming units/ml) into the left ischiocavernosus muscle. Four days later rats were reanesthetized and perfused with 4% paraformaldehyde and spinal cord and brain removed. Sections of the spinal cord and brainstem were cut (30-50 μm) and processed with swine polyclonal antibody to PRV and visualized with avidin-biotin HRP complex. PRV labelled neurons were found in the dorsolateral nucleus (DLN) of L6/L5 ipsilateral to the injection site. Presumptive interneurons were consistently found in the lateral part of lamina V and VI and in lamina X of segments L5-S1. PRV labelled cells were also found in the ventral medulla, in the nPGi and A5.

These results demonstrate some overlap with the circuitry innervating the smooth muscle of the penis but also confirm significant differences in the underlying neuronal connectivity of these two systems.

59.15

ERECTION IN RATS MAINTAINED AFTER SECTION OF PELVIC AND HYPOGASTRIC NERVES.

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Five groups of males received two operations, one week apart, resulting in simultaneous or successive transection of the pelvic nerves (PNx), the hypogastric nerves (HgNx) or sham surgeries (SHAM). Four days after each surgery, males were tested for reflexive erections after two min of non-contact exposure to estrous females. Copulatory behavior was tested 5-7 days after the second surgery. Erectile function in both contexts was dramatically impaired by PNx, but at least 30% of males in each PNx group displayed ejaculatory patterns and had reflexive glans erections. HgNx males had more intense glans erections than did SHAM males, possibly due to removal of vasoconstrictor fibers. These data extend the findings of Lucio et al. (SfNS Abstr, **17**:1059) and confirm results (Sachs & Liu, J Urol, **146**:900) showing that transection of the cavernous nerves, the final common path of erectile fibers in the PN and HgN, does not preclude erection of the glans penis. Sexual excitation may promote sufficient inhibition of vasoconstrictor fibers in the motor pudendal nerve to allow partial erection after all known vasodilator fibers to the penile corpora have been transected. [Supported by HD-08933.]

59.16

BILATERAL INHIBITION BY SEGMENTAL AFFERENTS ON PUDENDAL MOTONEURON REFLEX DISCHARGES.

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In male rats, polysynaptic reflex discharges in pudendal motor axons and perineal muscles can be elicited by stimulating pudendal afferents. Natural or electrical stimulation of these afferents can also produce inhibitory effects. In this study, we investigated the bilateral characteristics of the segmental reflex inhibition produced by afferents in several nerves supplying the pelvic and perineal region. Mature male rats were anesthetized with urethane and acutely spinalized at T7-T8. Recording electrodes were placed on the ipsi- and contralateral motor branch (iMB, cMB) of the pudendal nerve. Stimulating electrodes were placed bilaterally on the dorsal nerve of the penis (iDNP, cDNP) and on the ipsilateral pelvic nerve (iPN), superficial perineal nerve (iSP), and motor branch of the pudendal (iMB).

Averaged reflex responses in iMB and cMB to paired electrical shocks (conditioning-test paradigm) to several two-nerve combinations were obtained. Stimulation of iDNP, cDNP, and iPN elicited reflex discharges in both MBs. An iDNP or cDNP conditioning stimulus produced significant bilateral inhibition of iDNP, cDNP, and iPN test stimuli with an ipsilateral predominance. An iPN conditioning stimulus however, only slightly inhibited evoked responses from iDNP, cDNP, and iPN. In addition, iPN evoked reflexes were significantly less vulnerable to high-frequency depression. Stimulation of iSP and iMB produced only slight bilateral inhibition of iDNP and cDNP evoked responses. Of the nerves studied, the DNP produced the most extensive bilateral inhibition. Supported by NS27511.

59.17

INHIBITION OF NITRIC OXIDE SYNTHASE IMPAIRS COPULATION AND GENITAL REFLEXES IN MALE RATS.

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The gas nitric oxide (NO) is an intercellular messenger that performs several functions, including promoting vasodilation and mediating glutamate stimulation of cGMP. The inhibitor of NO synthesis, N-nitro-arginine methyl ester (NAME) was administered systemically to male rats prior to testing their copulatory behavior and ex copula genital reflexes. In Experiment 1, NAME (50 mg/kg) profoundly inhibited copulation in a group of extremely proficient male rats. Mean number of ejaculations was reduced from 2.8 (vehicle) to 0.0 (NAME) ($p<.001$). Only two of 10 animals achieved vaginal intromissions during the 30 min NAME test.

In Experiment 2 the same dose of NAME was injected preceding genital reflex tests in restrained supine males. The mean number of erections following NAME injections was 1.65, compared to 15.75 following vehicle ($p<.01$). It is likely that a major factor in this reduction is impairment of vasodilation in the penile corpora.

Studies are currently underway in our lab to determine whether NO may also act in the brain to regulate genital reflexes and copulation.

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59.18

FUNCTIONAL AND HISTOCHEMICAL EVIDENCE FOR THE INVOLVEMENT OF NITRIC OXIDE IN REGULATION OF PENILE ERECTILE TISSUE.

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The present study explores the role of nitric oxide (NO) in vasodilation of the penis of the rat as it has been proposed that this compound is responsible for nonadrenergic-noncholinergic (NANC) neurotransmission in a variety of tissues. Field stimulation of precontracted strips of penile smooth muscle caused marked, transient relaxations which were inhibited by nitroarginine and n-monomethylarginine - compounds which interfere with the synthesis of NO. In some tissues, L-arginine, from which NO is synthesized, enhanced field stimulated relaxations. Penile neurons in the pelvic plexus, identified by the retrograde transport of fluorogold, were intensely positive for nicotinamide adenine dinucleotide phosphate (NADPH-diaphorase); an enzyme purported to be a nitric oxide synthase. These data lend support to the contention that nitric oxide is the NANC transmitter in penile parasympathetic nerves. Moreover, the presence of other populations of non-penile, NADPH + neurons in the pelvic plexus suggests that nitric oxide may be important in neurotransmission in other pelvic visceral tissues. Supported by NIH NS19839-09.

60.1

VISUALIZATION OF MAST CELL ACTIVATION BY ACTIVITY-DEPENDENT FLUORESCENT PROBE. J.Y. Wei*, V.L.W Go and L. Kruger#. Dept. of Medicine, Anatomy & Cell Biology# and Brain Research Institute, University of California Los Angeles, CA 90024.

Activity-dependent fluorescent probes have been used to visualize the endplates of neuromuscular junctions (Nature 314:357, 1985). The purpose of the current study was to determine whether sulforhodamine B (SFRM-B, Molecular Probes Inc) can be used to indicate the active state of peritoneal mast cells. Mast cells in rat peritoneal eluates (PECs) were harvested by peritoneal lavage and plated in tissue culture dishes. PECs were incubated with SFRM-B (200 μg/ml in rat saline) with or without secretogogic compound 48/80 (1 μg/ml). At various intervals, the dish was rinsed with saline and the fluorescence images were observed with a Leitz fluorescence microscope equipped with NPL Fluotar water immersion objectives and N2 cube. The images were captured with a sit camera, digitized, acquired and analyzed using Image-1 software. After 10 minutes of incubation, a varying number of granular bright red fluorescent spots can be located in individual mast cells, clearly distinguishable from background noise. The spots can be located throughout the cytoplasm. This characteristic image can only be seen in mast cell populations activated by compound 48/80 and correspond to bright field images of mast cell degranulation. These results verify activity-dependent uptake which may be quantitated. This probe has greater photostability and less leakage than acridine orange, its fluorescence persists if the preparation is stored overnight in the dark. Moreover, the dye has no obvious effect on the afferent discharge of mesenteric mechanoresponsive nerve terminals. This enables use of SFRM-B for the study of interactions between mesenteric afferent terminals and surrounding mast cells. (Supported by NIH grant NS28433 & 5685)

60.2

LONG-RANGE, IN VIVO STAINING OF WHISKER PRIMARY AFFERENTS WITH NEUROBIOTIN: COMPARISONS WITH HRP AND PHA-L. M.F. Jacquin, W.E. Renehan & P.M.E. Waite*. Anat./Neurobiol., St. Louis Univ. Sch. Med., St. Louis MO 63104; Henry Ford Hosp., Detroit, MI 48202; Anat., Univ. New South Wales, Sydney, Australia.

Conventional means for analyzing the morphology of identified primary afferents are limited by the short distances over which HRP and PHA-L move during the course of a recording session. We have encountered an alternative method that overcomes this limitation. Neurobiotin (NB, Vector) injections into rat trigeminal (V) primary afferents in the brainstem (N=56) provided rapid, long-range staining with recording and electrophoretic parameters that are traditionally used to eject HRP or PHA-L. When NB was injected into brainstem A-beta fibers responsive to whisker deflection, collaterals were darkly stained in each of the 4 V subnuclei and cervical dorsal horn. Labeled fibers were also seen in the V root and infraorbital nerve for a distance up to 15 mm from the injection site. Cell bodies in the ganglion were never labeled. Such long-range staining occurred within 4 hrs of tracer injection and bore no orderly relationship to survival time, but rather reflected the quality, duration and site of the injection. When HRP was injected into primary afferents under equivalent conditions, staining was limited to within 4 mm of the injection site. When PHA-L was injected into V ganglion cells, similar long-range staining occurred, but 1-2 wks of survival was required. Due to its rapid and robust transport, NB is therefore a convenient alternative to PHA-L for long-range staining of the projections of identified ganglion cells. Intracellular NB injection also produces rapid Golgi-like staining of fibers over much greater distances than HRP under equivalent staining parameters. DE07662, DE07734, NS29885, MRC.

60.3

JOINT RECEPTORS ANTEROGRADELY LABELLED WITH WGA-HRP. S.M. Madey, A.J. Wolff, R.A. Brand and K.J. Cole*. Orthopaedic Biomechanics Laboratory and Dept. of Exercise Science, University of Iowa, Iowa City, IA 52242.

We have used WGA-HRP to anterogradely label mechanoreceptors in joint tissue as a prelude to investigating the precise innervation patterns of the cruciate ligaments. This method may also provide a means to clarify distinctions among receptor types, and their distribution, in ligament and joint tissue.

Five to ten microliters of 2% WGA-HRP were pressure injected in the L5-S1 dorsal root ganglia of adult cats, anesthetized with IV Sodium Pentobarbital. After a survival time of 24-36 hours animals were sacrificed and joint tissue harvested for histochemical processing and analysis. Nearly complete labelling occurred in cell bodies in the dorsal root ganglia. Tibial nerve sections just distal to the knee showed dense labelling in about half of the axons, as would be expected for this mixed nerve. Receptors in the posterior capsule of the knee joint were densely labelled with reaction product. Using nomenclature for joint receptors introduced by Freeman & Wyke (J. Anat. 101:50, 1967), receptors in the posterior joint capsule resembled Type I and Type II. A third receptor type was consistently seen within the anterior cruciate ligament that could not be clearly distinguished from Type II or Type III. Additionally, nerve bundles and single axons were clearly visible and easily traced through the substance of the joint. Anterograde labelling of joint receptors with WGA-HRP provides a useful method for identifying neural elements in joint tissue, and will allow more accurate determination of the distribution of peripheral afferents to joint and ligament receptors. Supported by NIH grant AR40217.

60.4

USE OF RICIN TO ASSESS NEURONAL CONTRIBUTION TO TOTAL PROTEIN SYNTHESIS IN DORSAL ROOT GANGLIA. H-Z Tang and R Hammerschlag* Div. Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010

The ability of *ricinus communis agglutinin* (RCA_{120}), a potent protein synthesis inhibitor, to enter the retrograde transport system and cause degeneration of neuronal somata has been exploited previously to establish the origin of peripheral axons (*e.g. Science* 216:889, 1982). The present study utilizes this lectin to determine the neuronal contribution to the total newly-synthesized protein following incubation of a peripheral ganglion with a radiolabeled amino acid. The method was developed to determine whether the increase in fast axonal transport (FAxT) in hindlimb sensory neurons during tadpole metamorphosis (*Trans Amer Soc Neurochem* 21:116, 1990) was secondary to an up-regulation of neuronal protein synthesis within the lumbar dorsal root ganglia (DRG). Since the peripheral nerves of pre- and pro-metamorphic tadpoles are too small and fragile to permit intraneural injection of RCA_{120}, lumbar DRG and nerves were placed *in vitro* where the cut end of the nerve was made to swell by brief exposure to chilled distilled water and then placed in test solutions of ricin under hypotonic conditions. After 16 h incubation of the cut end in 2.5 mg/ml RCA_{120}, the decrease in incorporation of [^{35}S]met into DRG varied from 72 ± 12 % at stage V to 42 ± 8 % at stage XVIII. The ricin-treated preparations showed no evidence of FAxT, relative to contralateral controls, as assessed by accumulation of [^{35}S]protein at a ligature 6 mm from the DRG. Ricin inhibition of protein synthesis and FAxT was blocked by preincubation of the nerve trunk in 0.6 mM vinblastine, indicating that axonal transport is required for passage of the lectin to the neuronal cell bodies. *[Supported by HD 26956.]*

60.5

WHOLE NERVE CUFF ELECTRODE RECORDINGS IN HUMANS. T. Sinkjær*, M. Haugland, J. Haase. Department of Medical Informatics and Image Analysis, Aalborg University, Fredrik Bajersvej 7D, DK-9220 Aalborg and Department of Neurosurgery, Aalborg Hospital, DK-9000 Aalborg, DENMARK.

During restricted motor tasks, the microneurography technique has been able to provide a detailed picture of single afferent fibres in the peripheral nerves of human subjects. The drawbacks of this technique are that the population of units that one can sample is relatively small and that the recording electrode is easily dislodged if there is any significant movement of the surrounding tissue. These limitations can be circumvented when using nerve cuff electrodes that give a more global picture of the neural activity.

The aim of the present study was to explore the feasibility of adopting the nerve cuff electrode recording technique as a tool to investigate the cutaneous mechanoreceptive innervation in humans.

Whole nerve cuff electrodes were implanted on the sural nerve of three human subjects. The subjects went through a standard neurosurgical nerve-graft procedure and was in general anaesthesia during the experiment. All subjects gave their consents and the study was approved by the Local Ethical Committee.

The nerve signal recorded by the nerve cuff electrode contained clear slip-related information and information about the changes in the force applied perpendicularly to the skin. The activity recorded by the whole nerve cuff electrode corresponds to similar recordings in cats and monkeys, and to what would be expected from the summation of activity of the various types of mechanoreceptors that have been identified by microelectrode recordings in humans.

60.6

SENSORY NEURONS IN CULTURE ARE DIFFERENTIALLY RESPONSIVE TO BRADYKININ AND CAPSAICIN. C.L. Stucky[1],* S.A. Thayer[2] and V.S. Seybold[1]. [1]Dept. Cell Biol. and Neuroanatomy and [2]Pharmacol., Univ. of Minnesota, Minneapolis, MN 55455.

Bradykinin (BK) and capsaicin (CAP) are two chemical stimuli that are used to define nociceptive sensory neurons. We used primary cultures of rat dorsal root ganglion cells to determine whether receptors for these substances are present on different or overlapping populations of neurons. Cultures were prepared from 1 day neonatal rats; cells were cultured for 3-10 days. Increase in intracellular Ca^{++} was used as a bioassay for receptor activation. Changes in intracellular Ca^{++} were measured using the Ca^{++} sensitive dye, Indo-1. In the initial population of cells examined (N=40, small to medium in size), CAP (500 nM) and BK (100 nM) were selective stimuli for two distinct populations: approximately 50% of the cells were BK-/CAP+, 25% of the cells were BK+/CAP-, and 25% of the cells were BK-/CAP- but responded to 50 mM K^+. Among the CAP-sensitive cells, half exhibited an increase in intracellular Ca^{++} in response to cold and the other half were unresponsive. The mean increase in intracellular Ca^{++} in response to CAP was 2800 nM. The response to 50 mM K^+ was comparable to that evoked by CAP, although the responses to cold and BK were 25% and 50% as great, respectively. The increase in intracellular Ca^{++} in response to BK was not dependent on extracellular Ca^{++}; however, extracellular Ca^{++} was required for the response to CAP and cold. These data suggest that BK- and CAP-sensitive dorsal root ganglia neurons comprise two distinct populations of cells and a sub-population of CAP-sensitive neurons are responsive to cold. These studies were supported by NS17702 and T32DA07239.

60.7

PROTONS ENHANCE THE CAPSAICIN EVOKED INWARD CURRENT IN DORSAL ROOT GANGLION CELLS. M. Petersen* and R.H. LaMotte, Dept. of Anesthesiology, Yale Univ. School of Medicine, New Haven, CT 06510

Inflammation and ischemia may be accompanied by an endogenous release of algesic chemicals and an increase in the tissue proton concentration. Lowering the pH value of the external medium has been shown to enhance the responses of the peripheral terminals of certain nociceptive neurons (Steen et al. 1991). Our present purpose was to see if an increase in the concentration of protons modulates the responses to the algesic chemical, capsaicin, in dorsal root ganglion cells. Capsaicin is an exogenous substance that excites peripheral nerve endings of certain nociceptive primary afferents by opening a non specific cation channel. Under whole-cell voltage clamp conditions we investigated the effect of externally applied protons on the capsaicin evoked inward current in freshly dissociated dorsal root ganglion cells from adult rats. Low concentrations of capsaicin (200 nM to 300 nM) but not low concentrations of protons (pH 6.9 to 6.3) evoked an inward current in a subpopulation of cells. The amplitude of the capsaicin (pH 7.3) evoked inward current decreased with repeated applications of the drug but increased with increasing proton concentrations (pH 6.9 to 6.3). Increasing the acidification of the external medium from a control pH of 7.3 to a pH of 6.3 enhanced the capsaicin (300 nM) current by about seven times. Exposure of the cell to an acidic capsaicin solution (300 nM, pH 6.3) did not subsequently increase an evoked inward current by capsaicin (pH 7.3) nor lead to a proton (pH 6.3) evoked current where there had been none. Our results suggest that increased proton concentrations play an important modulatory role in the function of chemosensitive sensory neurons. Supported by P.H.S. Grant NS 14624.

60.8

REGULATION OF CUTANEOUS C-FIBER HEAT NOCICEPTORS BY NGF IN THE DEVELOPING RAT. By Lorne M. Mendell* & Gary R. Lewin, Dept. of Neurobiology & Behavior, SUNY, Stony Brook, New York, NY 11794.

Here we have asked if NGF is required for the normal physiological development of C-fiber afferents in rats. Animals were treated with NGF (2 μg/g) or anti-NGF (5 μl/g) from post-natal day 0-14 and 2-14, respectively. The latter treatment regime is known not to lead to sensory neuron death (Lewin et al. J. Neurosci. in press, 1992). Mature animals (5-15 weeks) were anaesthetized with urethane (1.25g/kg) and recordings made from single sural nerve C-fibers in thin filaments of dorsal roots. Units were identified by electrical stimulation of their presumptive receptive field in skin (Meyer et al., Brain Res. 561:252-261, 1991). They were subsequently characterized by their ability to respond to noxious mechanical, heat and cold stimuli. The principal finding was that the number of cutaneous C-fibers responding to noxious mechanical and heat stimuli (ie. C-MH fibers) was dependent on the amount of NGF available during development. Thus in control animals 30% (11/37 fibers) were C-MH whereas the proportion increased to 60% (25/42 fibers) in NGF treated animals and decreased to 10% (3/29) in anti-NGF treated animals. In the latter case a novel afferent type with a very low mechanical threshold (1.0 ± 0.5g compared to 15 ± 10g for control C-MH fibers) appeared to replace the C-MH fibers (34%, 10/29 fibers). Neonatal NGF treatment induced a long lasting mechanical hyperalgesia (Lewin et al. Soc Neurosci. Abst. 17: 546.3, 1991); however, this was not accompanied by a sensitization of C-MH to mechanical stimuli. These results suggest that the availability of NGF during development may regulate the number of C-fibers responding to noxious heat in the adult animal. Supported by NIH, NS 14899, NS 16996.

60.9

NGF INDUCED HEAT AND MECHANICAL HYPERALGESIA IN ADULT RATS. By Gary R. Lewin* & Lorne M. Mendell, Dept. of Neurobiology & Behavior, SUNY, Stony Brook, New York, NY 11794.

Administration of NGF to young animals can lead to the development of hyperalgesia (Lewin et al. Soc. Neurosci. Abst. 17: 546.3, 1991). Here we have investigated the effects of NGF on more mature animals. The behavior of animals to noxious mecahnical and heat stimuli was monitored in animals before and after between 1 and 4 systemic daily doses of NGF (1 μg/g I.P). After just one NGF dose a profound mechanical and heat hyperalgesia was apparent for at least 3 days. The threshold for foot withdrawal measured with von Frey hairs dropped from 162 ± 70g in the control period to 27 ± 16g 24 hours after the first NGF dose ($p<0.001$). The latency for foot withdrawal from a 49°C water bath dropped from 2.37 ± 0.4s to 1.48 ± 0.27s after NGF, $P<0.0001$. Mechanical hyperalgesia developed slowly after NGF treatment (latency 4-6 hours). After 4 daily injections of NGF the mechanical hyperalgesia was maintained and recordings were made from single Aδ nociceptors in these animals. Twenty single Aδ nociceptors were recorded and their mean mechanical threshold was 5.5 ± 3g, not significantly different from control fibers (4.3 ± 2.3g $P>0.9$, Kolmogorov Smirnov). However, heat hyperalgesia developed within 15 minutes of the NGF injection. Therefore, the heat hyperalgesia may be due to the sensitization of peripheral fibers to heat. In further experiments we investigated whether the heat hyperalgesia which develops after the induction of a CFA (complete freunds' adjuvant) monoarthritis was dependent on NGF. Anti-bodies to NGF (5 μl/g, s.c.) prevented the heat hyperalgesia that normally develops after CFA. Thus, in adult animals NGF production in injured peripheral tissues may be necessary for heat hyperalgesia to develop. Supported by NIH, NS 14899, NS16996.

60.10

REARRANGEMENT OF CGRP-IR FIBERS IN SKIN FOLLOWING NEONATAL ANTI-NGF TREATMENT. By James R. Tonra* Gary R. Lewin, Stephen B. McMahon*, & Lorne M. Mendell, Dept. of Neurobiology & Behavior, SUNY, Stony Brook, New York, NY 11794. *Dept. of Physiology, UMDS (St. Thomas'), London SE1 7EH.

Treatment of neonatal rats with anti-NGF from post-natal day (PND) 2-14 (5 μl/g, s.c) may force Aδ nociceptors to take on the phenotype of low threshold D-hair afferents (Lewin et al. J. Neurosci. in press, 1992). In adult animals Aδ nociceptor endings are thought to reside in the epidermal layer of skin (Kruger et al. J. Comp. Neurol. 198: 137-154, 1981). Furthermore, these afferents probably contain the neuropetide CGRP. We have used immunohistochemistry to localize CGRP-IR fibers and cells in skin and dorsal root ganglia (DRG) in adult (8-10 week) control or anti-NGF (PND 2-14) treated animals. The hairy skin innervated by the sural nerve was used. The epidermis of controls contained many CGRP-IR fibers (11.8 ± 6.4 fibers/section). Most of these fibers spanned its entire depth. In contrast, the epidermis from anti-NGF animals contained fewer CGRP-IR fibers (4.7 ± 2.3 fibers/section, significantly different from controls $p<0.001$). Most of these fibers only penetrated half way into the epidermis. The dermal plexus contained many CGRP-IR fibers and there appeared to be no quantitative or qualitative change after anti-NGF. We reconstructed the cell size distribution of CGRP-IR cells in the DRG. In control animals many of the smallest sensory neurons were CGRP-IR, however in anti-NGF treated animals most of these cells were not CGRP-IR. This change may be related to the decrease in the numbers of C-MH fibers after anti-NGF treatment (see Mendell & Lewin, Soc Neurosci. Abst. 1992). The results indicate that subsets of CGRP-IR sensory neurons are the targets of NGF during development. Supported by NIH, NS 14899, NS 16996.

60.11

Binding of [^{3}H]resiniferatoxin to capsaicin-sensitive sensory neurons: Regulation by nerve growth factor. I.F. James*, S.K. Hothi, J.J. Slack, S. Bevan, J. Donoghue, C.S.J. Walpole and J. Winter Sandoz Institute for Medical Research, Gower Place, London, WC1E 6BN.

Resiniferatoxin (RTX) acts on a subpopulation of primary afferent neurons via the same mechanism as capsaicin, but is at least 100 times more potent. We have measured high affinity binding sites for [^{3}H]RTX on membranes from adult rat dorsal root ganglia (DRG). The dissociation constant was 0.42 ± 0.07 nM, and the number of binding sites 54 ± 8 fmol/mg protein (means ± SEM, N=4). This confirms an earlier report from Szallasi and Blumberg (Brain Res. **524**, 106, 1990). Capsaicin and a series of capsaicin analogues competed for the RTX binding sites, phorbol esters did not compete. There were some anomalies in the competition assays. For example capsazepine (a competitive antagonist of capsaicin action) was a relatively poor competer for the RTX sites. The binding sites were found on membranes from DRG and spinal cord, not cerebellum, midbrain, cerebellar cortex, heart, skeletal muscle, or liver. Binding to DRG membranes from rats pretreated with capsaicin was reduced to 17 ± 7% (mean ± SEM, N=4) of binding to tissue from control animals. In primary cultures of DRG from adult rats, cells grown in the presence of nerve growth factor (NGF) expressed binding sites for [^{3}H]RTX, whereas binding to cells grown in the absence of NGF was undetectable. The properties, distribution, and regulation by NGF all suggest that the RTX binding sites correspond to the 'capsaicin receptor', but anomalies in the competition experiments suggest that capsaicin may act at more than one site.

60.12

FINE STRUCTURE OF B-HRP TRANSGANGLIONICALLY LABELLED AXONS AND TERMINALS IN LAMINA IIo OF THE ADULT RAT SPINAL CORD FOLLOWING PERIPHERAL AXOTOMY. **R. E. Coggeshall*, P. Shortland and C. J. Woolf. Departments of Anatomy and Neuroscience, University of Texas Medical Branch, Galveston, Texas, 77555-0843, USA, and Department of Anatomy and Developmental Biology, University College London, UK.**

Horseradish peroxidase conjugated to the B unit of cholera toxin (B-HRP) transganglionically labels the terminals of large myelinated afferents in the spinal cord (Robertson and Grant, Neuroscience, 14, 895-905, 1985). The labelled axons and terminals are prominent in laminae I and III and conspicuously absent in lamina II, as seen in the light microscope, in normal adult rats. If the sciatic or sural nerves are transected before the administration of the B-HRP, however, label is prominent in lamina II (Woolf et al., Nature, 355:75-85, 1992). One hypothesis to explain this finding is that large myelinated axons in lamina III expand their innervation territories into lamina II, particularly IIo, following axotomy. In accord with this, electron microscopic (EM) examination of the labelled elements in lamina IIo shows numerous labelled axons and presynaptic terminals at times ranging from 1 week to 8 months following the lesion.

60.13

PREPROENKEPHALIN AND PREPROTACHYKININ GENE EXPRESSION IN MONKEY SPINAL CORD AND SENSORY GANGLIA. S. Numan*, B.M. Davis, H.H. Traurig and K.B. Seroogy. Dept. of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536.

Few studies have examined perikaryal enkephalin and substance P localization in primate spinal cord or sensory ganglia. The standard protocol for chemically identifying neuronal somata usually employs immunocytochemistry in colchicine-treated animals. The lack of data in primates is most likely due the limited use of colchicine in these vertebrates. As an alternative approach, we used *in situ* hybridization of ^{35}S-labeled cRNA probes to examine the cellular expression of mRNAs for preproenkephalin (ppENK) and preprotachykinin (ppT) in spinal cord, spinal ganglia and trigeminal ganglia of cynomologus and squirrel monkey. In dorsal horn of cervical spinal cord, hybridization for the mRNAs was found in neurons in the superficial laminae as well as scattered throughout nucleus proprius. Labeled cells were also found adjacent to the central canal and sometimes in ventral horn motoneurons, although expression of ppT mRNA was rarely seen in the ventral horn. In cervical dorsal root ganglia (DRG), a major subset of the small, and a few of the large, neurons expressed ppT mRNA. DRG cells expressing ppENK were restricted to a minor subset (at least 4-fold less than the ppT mRNA-containing subpopulation) of small-sized neurons, although labeled cells were present in higher numbers than previously reported for perikaryal ENK immunostaining in rodent and cat DRG. In trigeminal ganglia, the pattern of ppT mRNA expression was similar to that found in DRG, but expression of ppENK mRNA was restricted to a few lightly-labeled neurons. These results demonstrate that primary sensory neurons and their target regions in dorsal horn of monkey express mRNAs for ppT and ppENK and suggest a role for tachykinins such as substance P and for the opioid ENK in primate somatosensory mechanisms. Moreover, a larger subset of DRG neurons express ppENK in monkey compared to other species.

60.14

5-HT$_3$ RECEPTOR BINDING IN THE DORSAL VAGAL COMPLEX AND OTHER NUCLEI OF THE HUMAN MEDULLA. D.C. Ohuoha, S.S. Wolf, J.E. Kleinman, and T.M. Hyde*. CBDB, IRP, NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

The distribution of 5-HT$_3$ receptors in peripheral tissue is well characterized. Much less is known about 5-HT$_3$ receptors in the central nervous system. Previous studies have identified 5-HT$_3$ binding sites in human dorsal motor nucleus of the vagus (DMN), spinal trigeminal nucleus, nucleus of the solitary tract (NTS), and the area postrema (AP). Binding in the AP has been contested by some investigators. In our study, sections from 3 levels of the caudal human medulla (n=7) were examined using the ligand [^{3}H]LY278584 in receptor autoradiography. We found distinct and consistent binding in the DMN and the commissural subnucleus of the NTS caudally, and the medial and intermediate subnuclei of the NTS and parts of the medial reticular formation more rostrally. Distinct binding in an arc separating the spinal trigeminal nucleus from its tract was apparent at caudal levels. Minimal binding was noted in the inferior olives. No definitive binding was noted in the area postrema. Our findings suggest that 5-HT$_3$ receptors may play a significant role in the neural network subserving visceral function.

60.15

ULTRASTRUCTURE OF PARATRIGEMINAL NEURONS PROJECTING TO THE PARABRACHIAL REGION: A RETROGRADE EM STUDY WITH WGA-HRP IN RAT. D.W. Saxon* and D.A. Hopkins. Department of Anatomy and Neurobiology, Dalhousie University, Halifax, Nova Scotia, CANADA B3H 4H7.

The paratrigeminal islands (PTI) in the dorsolateral medulla receive primary visceral and somatic afferent information, and have connections with a number of structures in the medulla, dorsolateral pons and thalamus. Previous ultrastructural analysis of the PTI (Saxon and Hopkins, '89) revealed two distinct populations of PTI neurons based on the number of axosomatic synapses. In order to identify efferent neurons in the PTI projecting to the parabrachial nucleus (PBN), WGA-HRP (1.0%, 0.01 - 0.03 μl) was injected stereotaxically into the parabrachial region. After 72 hours the medulla oblongata was processed for HRP histochemistry with ammonium molybdate stabilization followed by embedding for electron microscopy. Numerous neuronal somata throughout the PTI were labelled with HRP reaction product ipsilaterally, while a small number were labelled contralaterally. Retrogradely filled neurons were uniform in their morphological features and were characterized by their round or fusiform shape, high nucleus to cytoplasm ratio, sparse Nissl substance and paucity of axosomatic contacts. These neurons corresponded to a previously identified population of PTI neurons which contrasted with a second less numerous population of unlabelled neurons whose most prominent difference was the high number of axosomatic synapses. The present results show that a distinct population of PTI neurons conveys information, possibly convergent primary visceral and somatic information, to the PBN. Supported by MRC of Canada (Grant MT-7369).

60.16

CENTRAL PROJECTIONS OF THE TRIGEMINAL NERVE IN PIGEON. J.M. Wild* and H.P. Zeigler. Dept. of Anatomy, School of Medicine, Univ. of Auckland, Auckland, New Zealand and Dept. of Psychology, Hunter College, CUNY.

In birds, the trigeminal nerve innervates the beaks and periorbital regions, and in pigeon it is known to play a critical role in both the sensorimotor and motivational control of feeding behavior. In the present study we have used nerve injections of Cholera toxin B-chain conjugated to HRP (CTB-HRP) to trace the central projections of ophthalmic (V1), maxillary (V2) and mandibular (V3) branches, singly or in combination. CTB-HRP was visualized with TMB.

As in mammals, the entering root divides into ascending (TTA) and descending (TTD) tracts. TTA terminates partly within a *pars oralis* of nTTD, diffusely within a ventral part of the principal nucleus (PrVv), and densely within a compact, dorsal part of PrV (PrVd). V1 and V3 projections largely overlap within PrVd, although V3 projections alone occupy the caudal pole and V1 projections a rostro-dorsomedial component. V2 projections are much smaller and scattered, but there is a dense patch of terminations on the medial aspect of PrVd. Within the *pars interpolaris* and *pars caudalis* of nTTD, V3 projections are concentrated dorsomedially, V1 projections ventrolaterally, while V2 projections are scattered between. Within the spinal dorsal horn a medial (V3) to lateral (V1) organization of projections persists. All 3 branches project caudally to between C6 and C9, and all 3 have a small crossed component at caudal medullary and upper spinal levels. Projections from all 3 branches also contribute to a band of descending fibers which appears as the most lateral of several discrete fascicles of TTD (lTTD). lTTD terminates within the ventrolateral part of the external cuneate nucleus in the caudal medulla, and then as very dense "swirls" over cell bodies and mediolaterally extensive dendrites of neurons comprising lamina I of the dorsal horn of upper cervical segments. Both these regions of termination are Substance P-positive. There was no evidence of trigeminal projections to "non-trigeminal" regions (nucleus tractus solitarius, reticular formation, cerebellum) such as have been reported in cat and rat. [Supported by Grants MH-08366 & BNS-88-10722 to H.P.Z.]

SPINAL CORD

61.1

PRIMARY CULTURES OF POSTNATAL RAT SPINAL CORD NEURONS: IMMUNOCYTOCHEMICAL AND ELECTROPHYSIOLOGICAL STUDIES. L.J. Kehl*, G.I. Poliac and G.L. Wilcox. Graduate Program in Neuroscience and Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455, USA

The majority of work studying primary cultures of spinal cord neurons has been performed using fetal and neonatal tissue. We sought to compare characteristics between perinatal and more mature neurons. The objectives of these studies were to (1) culture rat postnatal spinal cord neurons and (2) examine their electrophysiological characteristics. Spinal cords from 10-15 day old Sprague Dawley rats were sectioned (300μm) on a vibratome and dissociated using pronase/thermolysin. Cells were plated onto glial monolayers and incubated (37°C, 10% CO_2) in DMEM with 10% fetal bovine serum. Neurons survived routinely for 1 to 2 weeks in culture. Immunostaining cultures from 15 day postnatal cord with an antibody directed against microtubule-associated protein (MAP2, courtesy I. Fischer), which is present specifically in neurons at 2 weeks postnatally, indicates that the cells are neurons. Patch clamp studies using the nystatin perforated patch technique were performed between 4 and 14 days after plating. Neurons maintained in culture for several days had voltage-activated currents (apparently including Na^+, K^+ and Ca^{++} components) supporting their neuronal nature. Some neurons were spontaneously active, showing apparent synaptic currents and spontaneous action potentials at depolarized membrane potentials; some were responsive to bath application of N-methyl-D-aspartate. These data demonstrate that viable neurons from postnatal rat spinal cord can be maintained in primary culture. [Supported by grants from NIDR (LJK) K15-DE-00225 and NIDA (GLW) R01-DA01933 & R01-DA04274].

61.2

GENETIC DIFFERENCES IN THE OCCURRENCE OF MECHANICAL HYPERSENSITIVITY (ALLODYNIA), MOTOR DEFICITS AND MORPHOLOGICAL DAMAGE AFTER SPINAL CORD ISCHEMIA IN RATS. **J.-X. Hao*,[1,2], Z. Wiesenfeld-Hallin[1], X.-J. Xu[1], H. Aldskogius[3] and Å. Seiger[2]. Depts. of [1]Clin. Neurophysiol., [2]Geriatric Medicine and [3]Anatomy, Karolinska Institute, [1,2]Huddinge and [3]Stockholm, Sweden.**

We have previously reported that transient spinal cord ischemia in Sprague-Dawley (SD) rats caused strong mechanical allodynia with minor motor deficits and morphological damage. We report here that the sensory, motor and histological outcome is strain-dependent in rats.

Transient spinal ischemia was induced by a photochemical reaction, leading to platelet aggregation, when a dye, Erythrosin B, injected i.v., is activated by an argon laser irradiating an exposed vertebra at mid-thoracic level. The vocalization threshold of the rats to mechanical stimulation was tested with calibrated von Frey hairs. Motor performance and morphological abnormalities were assessed.

The three strains of rats that were studied were spontaneous hypertensive (SHR), Wistar-Kyoto (WK) and SD. After induction of the ischemia, WK and SD, but not SHR, rats developed severe allodynia. In contrast, the rank order of the severity of motor deficits and extent of histological damage was SHR>WK>SD.

Genetic variability, which may involve a large number of factors of physiological singificance, can lead to various levels of predisposition for the development of pain and spinal cord damage after ischemia. Such factors may underly the wide variability in clinical outcome after ischemic CNS injury.

61.3

DISTRIBUTION OF NEUROKININ A DURING CONTROLLED SUPERFUSION OF THE RAT SPINAL CORD DORSUM.

H. BECK*, H. SCHRÖCK AND J. SANDKÜHLER. *Physiologische Institute der Universität Heidelberg, 6900 Heidelberg, F.R.G.*

Extrasynaptic transmission of information ("volume transmission") mediated by neuropeptides like Neurokinin A (NKA) may play an important role for neuronal plasticity in the spinal dorsal horn. Here we describe the spread of NKA in the spinal cord during controlled superfusion.

Experiments were performed on 7 rats deeply anesthetized with pentobarbital. A femoral artery and vein were cannulated to monitor blood pressure, heart rate and to sustain anesthesia by continuous infusion. The lumbar spinal cord was exposed by laminectomy and the dura mater reflected. For controlled superfusion a pool was formed on the cord dorsum with a specially prepared silicon rubber and filled with 10 μl of [125I]NKA (100 μM, 2 μCi) for periods of 15, 30 or 60 minutes. The blood pressure increased by 10 to 30 mm Hg during superfusions. At the end of the experiments the spinal cord was removed, frozen immediately, cut into 20 μm sections and exposed to X-ray film for autoradiography. Autoradiograms from the superfused spinal cord showed radiolabel up to a depth of 1.7 mm below the dorsal surface. In spinal cord sections taken from segments rostral or caudal to the pool, the radiolabel diminished rapidly. Thin-layer chromatography of tissue extract from superfused cord revealed a single major peak suggesting that no degradation of NKA took place. Little radioactivity was measured in blood and plasma and all tissues examined independent of superfusion time. Our results show that NKA - and probably other neuropeptides - diffuses well into the dorsal but not ventral horn of spinal cord during controlled superfusion of the cord dorsum.

Supported by a grant from the DFG (SA 435).

61.4

AUTORADIOGRAPHIC DISTRIBUTION AND CHARACTERIZATION OF NEUROPEPTIDE Y RECEPTORS IN RAT SPINAL CORD.

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Neuropeptide Y (NPY), an amidated 36-amino acid peptide, is widely distributed throughout the nervous system. In the spinal cord, particularly in the superficial layers of the dorsal horn, a rich system of NPY-like immunoreactivity and NPY receptor binding sites have been described. Recently, we also reported that NPY receptor binding sites localized in the superficial layers of the dorsal horn showed depletion following surgical and pharmacological manipulations (Brain Research, 574: 337-337, 1992). However, the NPY receptor type(s) present in the dorsal horn of the spinal cord has not been characterized so far. In the present study we have investigated this issue by determining the ability of PYY, pNPY, NPY_{13-36} and [Leu^{31}, Pro^{34}]-NPY to inhibit specific [^{125}I]PYY binding using quantitative receptor autoradiography. Specific [^{125}I]PYY binding is completely inhibited by PYY and pNPY whereas the fragment NPY_{13-36} (Y2) and the Y1 agonist [Leu^{31}, Pro^{34}]-NPY only partly competed for [^{125}I]PYY labelling even at 10^{-6}M. These results suggest that Y_1 and Y_2 receptor subtypes are likely present in the dorsal horn of the spinal cord. At present, the physiological significance of each receptor subtype is unknown but may relate to the modulation of spinal information. (Supported by MRCC).

61.5

IN VIVO ANALYSIS OF MONOAMINES IN INJURED SPINAL CORD USING MICRODIALYSIS

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Since the Osterholm's report of alteration of levels of catecholamines in injured spinal cord, several authors were unable to replicate his result. A reanalysis of the original finding by Osterholms laboratory in 1980 suggested the increase in the spinal cord norepinephrine after trauma was artifactual. We describe a method for *in vivo* analysis of biogenic amines in injured rabbit spinal cord using microdialysis and HPLC coupled with electrochemical detector.

New Zealand white rabbits were anesthetized with the Ketamine-Rompun (7:3 by volume, 1 ml/kg) and maintained under anesthesia using halothane:oxygen mixture for the duration of the experiment. Laminoctomy from T7-T10 was performed to expose the spinal cord. A 3mm tip microdialysis probe was inserted into the spinal cord in the midline at T9. The probe was perfused with artificial CSF at a rate of 1 μl/min. The dialysate was collected every 20 min and analyzed. After baseline levels of norepinephrine (NE) and dopamine (DA) were established, the exposed spinal cord at T9 was subjected to a 150 gm-cm force using the Allen weight drop method. A significant increase in extracellular NE, DA and 5-HT was evident during first 20 min after traumatic injury. During the second postinjury 20 min sampling interval, monoamine levels were still elevated, albiet to a lower extent than the 1st postinjury sampling period. The origin of NE, DA and 5-HT remains speculative. We postulate that the elevation of these biogenic amines was secondary to local release from stored vesicles at the injury site and responsible for the initiation of hemorrhagic necrosis process.

61.6

HALOTHANE REDUCTION OF AFFERENT INPUT TO THE SPINAL CORD IN DECEREBRATE, SPINALLY-TRANSECTED RATS.

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We have reported that pentobarbital and propofol produce a significant reduction in low-threshold (LT) receptive field (RF) size of some dorsal horn neurons in chronic cat experiments.[1,2] We have recently observed that halothane induced a reduction in LT RF size in acute anesthetized rat experiments. This reduction was maintained in the presence of reversible cold block of the spinal cord, suggesting a spinal site of action. In this IACUC approved study we investigated effects of halothane on LT RF size in decerebrate, spinally transected-animals to eliminate the effect of baseline anesthesia which was discontinued following decerebration and spinal cord transection. Blood pressure and PCO_2 were monitored as LT RFs were mapped. To date 10 LT neurons and 3 wide dynamic range (WDR) neurons have been studied. The mean baseline RF size of LT neurons was 218.0 ± 38.2 mm^2 (SE). The mean RF size was reduced by 66.4 ± 2.4% SE (65.6 ± 10.0mm^2 SE) and 97.2 ± 1.3% SE (5.81 ± 3.0mm^2 SE) by halothane to 0.5% and 1.0% respectively. Three WDR neurons also showed the same tendency. Receptive field size returned to baseline values with discontinuation of halothane administration. In 2 neurons, 50 µg of the $GABA_B$ antagonist, phaclofen, was administered intrathecally with no effect on the halothane induced reduction of RF size. These results suggest that, in the decerebrate, transected preparation, halothane alters LT afferent input to spinal dorsal horn neurons. This may contribute to anesthetic induced loss of sensation. It is unlikely that a $GABA_B$ system is involved in the effect of halothane on LT RF size. [1]Brain Res. 525:189-197, 1990. [2]Anesth. 73:A697, 1990. (Supported in part by NIH GM 29065.)

61.7

IMMUNOHISTOCHEMICAL LOCALIZATION OF CALRETININ IN RAT DORSAL ROOT GANGLIA AND SPINAL CORD.

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Calretinin (CR), a recently identified calcium-binding protein, is present in nervous tissue, including sensory pathways, where it may play an important role in regulation of cellular activity. Using immunocytochemistry, we examined the cellular localization of CR in dorsal root ganglia (DRG) and spinal cord (SC) of normal rats and after multiple unilateral dorsal root ganglionectomies. In the DRG, CR-like immunoreactivity (LI) was found in cell bodies and axons of a small population (≈10%) of medium to large sized neurons. In the SC, CR-LI was found in neurons and fibers in all laminae, except for neurons in the motorneuronal groups, and in the lateral spinal and lateral cervical nuclei. Dense fiber networks were also found in Clarke's column. The densest staining of both cells and fibers was in the superficial laminae, especially lamina II. CR-LI fibers were also observed in the fasciculi cuneatus and gracilis. Fasciculus gracilis exhibited the greatest number of labeled axons at the lumbosacral levels, but few labeled axons were found at the rostral thoracic and cervical levels. In contrast, the corticospinal tract at the base of the dorsal column was devoid of CR-LI. Unilateral multiple ganglionectomies resulted in a loss of CR-LI in the dorsal columns ipsilateral to the surgery, without any apparent decrease in the staining intensity in the spinal gray matter. Our observations demonstrate a unique distribution pattern of CR-LI compared to other calcium-binding proteins in the SC and suggest a role for CR in nociceptive and proprioceptive pathways.

61.8

PRIMARY AFFERENT TERMINALS IN BOTH DEEP AND SUPERFICIAL LAMINAE OF THE RAT DORSAL HORN ARE ENRICHED IN GLUTAMATE-LIKE IMMUNOREACTIVITY.

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The presence of glutamate-like immunoreactivity (Glu-LI) in primary afferent terminals (PATs) in the dorsal horn was quantitatively evaluated with the electron microscopic immunogold technique. PATs in laminae I and III-V were identified by peroxidase histochemistry following injections of choleragenoid-HRP into spinal ganglion Th 12 or 13. PATs in laminae II were identified by morphological criteria (dense sinusoid axon terminals, DSAs). The density of gold particles over PATs was compared to that over terminals with pleomorphic vesicles in laminae III-IV, that over large neuronal cell bodies in laminae III-V and to the average density of gold particles over lamina II and over laminae III-IV. One section through the dorsal horn in each of three rats were analyzed.

PATs in laminae I and III-V had similar labeling densities, whereas higher densities were present over DSAs. Expressed in percent of the average density of gold particles over laminae III-IV, the results were as follows: DSAs (n=20 in each section), 420%; PATs in laminae I (n=10) and III-V (III, n=20; IV, n=20; V, n=6-8), 265%; Large neuronal cell bodies in laminae III-V (n=5), 158%; Terminals with pleomorphic vesicles in laminae III-IV (n=20), 106%; Average density of gold particles over lamina II, 145%.

These results show that PATs in all dorsal horn laminae are enriched in Glu-LI, thus supporting a neurotransmitter role for glutamate in PATs throughout the dorsal horn.

61.9

NEUROPEPTIDES AND THEIR RECEPTORS IN THE RAT SPINAL CORD: EFFECTS OF SURGICAL AND CHEMICAL MANIPULATIONS. S. Kar* and R. Quirion. Douglas Hospital Research Center, Dept. of Psychiatry, McGill University, Canada H4H 1R3.

The site of the first synapse in pain pathways i.e., the dorsal horn of the spinal cord, is the main center for the integrative processing of nociceptive information. A functional interrelationship between neuropeptides of the primary afferent fibers and the spinal interneurones has been suggested in a variety of experiments. In the present study, following chemical (neonatal capsaicin treatment) and surgical (unilateral sciatic nerve section) manipulations, the possible alterations of immunoreactive Calcitonin gene-related peptide (CGRP), Substance P (Sub P), Enkephalin (ENK), Galanin (GAL) and Neurotensin (NT) and their corresponding receptor binding sites have been studied in rat spinal cord (Lumbar 4) emplyoing immunocytochemistry and quantitative *in vitro* receptor autoradiography respectively. The capsaicin treated rats, in comparison with controls, showed a depletion of CGRP, Sub P and GAL immunoreactivity but no alteration of ENK or NT immunoreactive fibers in the dorsal horn of the spinal cord. The autoradiographic distribution of receptor binding sites on the other hand, showed a relative increase of CGRP, neurokinin 1 (NK1) and GAL bindings, a decrease of μ opioid sites and no alteration of NT receptor sites in the dorsal horn of the capsaicin treated rats. Following section of the sciatic nerve, compared to contralateral side, a decrease of CGRP, Sub P, a slight increase of GAL but no visible alteration of either ENK or NT immunoreactive fibers was noticed in the superficial laminae of the spinal cord ipsilateral to nerve section. In keeping with alteration of the immunoreactive fibers, a corresponding increase of CGRP and NK1, a decrease of μ opioid and GAL and no alteration of NT receptor binding sites were noticed in the dorsal spinal cord ipsilateral to nerve section. These results taken together suggest that i) opioid regulates, in part, nociceptive information through a presynaptic inhibition of CGRP and Sub P containing primary afferent fibers ii) GAL which acts as an antinociceptive agent, especially after injury, possibly mediates its analgesic effects by acting on the second order neurones of the dorsal horn iii) NT is somewhat more circumscribed in its distribution and is not involved in direct regulation of nociception at the level of the spinal cord. (Supported by MRCC).

61.10

DEAFFERENTATION INDUCED INCREASE OF GALANIN IN THE RAT DORSAL HORN. A.A. El-Bohy*, K.E. Arsenault and C.C. LaMotte. Section of Neurological Surgery, Yale University School of Medicine, New Haven, CT. 06510.

In the spinal cord, intrathecal administration of galanin (GAL) increases the latency of the tail flick and hot plate tests, antagonizes the facilitatory effects of SP on the nociceptive reflex, and potentiates the spinal analgesic effect of morphine. In this study, we examined the light and ultrastructural changes in GAL immunoreactivity following sciatic deafferentation induced by injecting the sciatic nerve of anesthetized rats with proteolytic enzymes (10-20mg Pronase). The effects of this chemical deafferentation were examined in two animal groups, i.e., short term (10-13 days) and long term (4-6 months) after injection, using a computerized densitometry analysis system. In the short term group, we found a highly significant increase in GAL immunoreactivity on the pronase side. This pattern paralleled the distribution of the sciatic nerve, suggesting an association with the terminals of the sciatic nerve. In the long term group, the immunoreactivity on the pronase side was similar to that of the control side. At the ultrastructural level, GAL was localized in simple and glomerular terminals, with or without dense core vesicles, mainly in lamina I and II. On the pronase side, the label was seen in both degenerating and normal terminals as well as some dendrites, somata and nonmyelinated axons. These results suggest that the transient increase may be in part a response of the injured sciatic neurons but other neuronal elements may also be involved. (Supported by PVA Spinal Cord Research Foundation #880, the Eastern Paralyzed Veterans of America, and NIH Grant NS28876).

61.11

SPROUTING AND FUNCTIONAL PLASTICITY OF SPINAL PROJECTIONS OF REGENERATED PRIMARY AFFERENTS. H.R.Koerber*,[1], K. Mirnics[1], P.B.Brown[2] and L.M.Mendell[3]. Dept. of Neurobiol., Anat. & Cell Sci. Univ. of Pittsburgh[1], West Virginia Univ.[2] and SUNY-Stony Brook[3].

Tibial primary afferents were impaled with HRP-filled electrodes in the dorsal columns of anesthetized cats 9-17 mos. after transection and self union of the tibial nerve. After determining its adequate stimulus, each fiber was activated with single or pairs of current pulses via the microelectrode to elicit cord dorsum potentials (CDPs), and filled with HRP. The central projections of 13 Group I & II fibers were reconstructed from counter-stained sagittal sections. The longitudinal distribution of boutons was recorded and compared to the monosynaptic CDP amplitudes recorded at the four surface locations. The anatomical projections of 6 afferents resembled those of proprioceptors (most boutons ventral to lamina V); of these, 5 (4 group II and 1 group I) had reinnervated skin and 1 (group I) had reinnervated a subcutaneous structure. Unlike controls, 3 of these fibers elicited measurable CDPs which may be the result of numerous boutons in laminae IV & V supported by these fibers. The other 7 fibers had extensive projections in laminae III-V and elicited CDPs that were characteristic of cutaneous afferents; of these, 2 had reinnervated subcutaneous structures and 5, the skin. However, in some cases the longitudinal distribution of boutons did not match the CDP amplitude profile as closely as in controls. In addition, three of these fibers also had extensive longitudinal collaterals and boutons in laminae I & II and elicited CDPs characteristic of nociceptors in controls. This apparent sprouting of large myelinated fibers into superficial laminae confirms the finding of Woolf et al., Nature, '92 and also suggests that these projections remain functional after successful reinnervation of low threshold mechanoreceptors. These results suggest both anatomical and functional plasticity in the central projections of regenerated large myelinated primary afferents. (Supported by the NIH).

61.12

EFFECT OF PERIPHERAL NERVE CUT ON PEPTIDES IN DORSAL ROOT GANGLIA AND THE SPINAL CORD OF MONKEY WITH SPECIAL REFERENCE TO GALANIN. Z. Xu, G. Ju, R. Elde* and T. Hökfelt. Dept. of Neurobiology, Inst. of Neuroscience, Xian, 710032, PR. China and Dept. of Histology and Neurobiology, Karolinska Institute, 10401, Stockholm, Sweden

Five adult macaca underwent unilateral sciatic nerve transection. Three of them and one control animal were studied with immunofluoresence technique. The other two and one control animal were studied with in situ hybridization. Two weeks after peripheral axotomy the expression of peptides in dorsal root ganglia was changed for several peptides. There was a clear reduction in the number of CGRP, SP and SOM positive cell bodies. In contrast, there was a marked increase in galanin (GAL) positive cells. Thus, over 50% of all ganglion cells expressed this peptide after the nerve cut. However, the immunoreactivity and mRNA positivity were almost exclusively confined to small neurons. A few NPY, but no VIP/PHI positive cell bodies could be observed. With regard to fibers in the dorsal horn, there was a small reduction in CGRP- and SP-LI in the ipsilateral dorsal horn. A more pronounced decrease was observed for SOM-LI, where the strong immunoreactivity in fibers in outer lamina II virtually disappeared. Also NT, PHI and CCK-LI were distinctly reduced in the superficial layers of the ipsilateral dorsal horn. A dramatic increase in GAL-LI in the ipsilateral dorsal horn was observed, especially in the inner lamina II, where the GAL immunoreactive fibers formed distinct patches of densely packed varicosities after axotomy. In addition small, weakly GAL immunoreactive fibers could be observed in lamina III. Also the DYN-LI was increased in the ipsilateral dorsal horn with a more extensive distribution of fine fibers into deeper layers. Upregulation of GAL and CGRP in motoneurons on lesion side was also found. These results show, among others that a peripheral nerve lesion upregulates GAL in sensory neurons, not only as previously shown in rat but also in monkey. The analgesic effect of GAL already demonstrated in rat may therefore extend to primate.

PAIN MODULATION: HYPERALGESIA

62.1

ALTERATIONS IN C-FIBER RESPONSE TO MECHANICAL STIMULI IN THE STREPTOZOTOCIN-DIABETIC RAT. S.C. Ahlgren*, D.M. White, and J.D. Levine Departments of Anatomy, Medicine, and Oral and Maxillofacial Surgery and Division of Neuroscience, UCSF, San Francisco, CA 94143-0452.

Streptozotocin-diabetic rats (STZ-D) demonstrate hyperalgesia in behavioral tests of mechanical nociceptive threshold. This study examined the response to mechanical stimulation in C-fiber afferents in the saphenous nerve of STZ-D rats. There was no difference in the percentage of spontaneously active C-fibers found in 8 STZ-D rats (11.6 ± 1.0%) compared to 8 control (CON) rats (11.3 ± 0.7%) or in the average firing frequency of spontaneously active fibers (STZ: 87.9 ± 13.7 spikes/min, n=81; CON: 87.7 ± 13.8 spikes/min, n=79). The average mechanical threshold of C-fibers, measured with monofilament von Frey hairs (VFH), was also not significantly different in STZ-D and CON rats (STZ-D: 12.7 ± 4.7 g, n=99; CON: 11.2 ± 2.0 g, n=119). In addition, the average number of spikes elicited by a sustained (one minute) mechanical stimulus of low intensity (threshold VFH) was not different in STZ-D and CON C-fibers (STZ-D: 51.5 ± 9.0, n=35; CON: 52.1 ± 8.5 spikes/min, n=34). However, the application of a mechanical stimulus of high intensity (445 g) for one minute elicited a greater number of spikes in C-fibers from STZ-D rats (STZ-D: 149.7 ± 18.4 ; n=44 vs. CON: 84.7 ± 12.2spikes/min; n=45; t(87) = -2.762, p<0.01). This electrophysiological study suggests that changes in activity (firing frequency) to suprathreshold mechanical stimuli contribute to mchanical hyperalgesia in the STZ-D rat. (supported by NIH grant NS21647)

62.2

Parallels between joint nociceptor properties and pain report in subjects with pain in the TMJ. Loughner B., Cooper*, B. and Heft, M. W., Dept. of Oral and Maxillofacial Surgery, University of Florida, Gainesville, 32610.

Properties of afferents of the carrageenan inflamed (CI) temporomandibular joint (TMJ) were compared to pain report of patients complaining of pain in the TMJ. Recordings were made from the exposed trigeminal root ganglion of the goat, in response to dynamic and static jaw movement. Similar manipulations were made of the jaws of patients. Subjects rated pain using visual analogue scales. Nineteen afferents of the TMJ were characterized prior to and up to 3 hours following injection of 200 ul of carrageenan (CI; n=11) or saline (n=8). Prior to CI, afferents transduced dynamic (9/11 cases), and less frequently static stimuli (4/11 cases). Following CI, transduction of static events was observed in 9/11 cases and transduction of dynamic events 11/11 cases. Saline was without effect. Significant reduction of thresholds were also observed (11.2 +9.9 vs 5.1 +3.7N) and were significantly greater (t=2.2, df=a5, p<.04) than those observed in saline treated cases (14.1 + 15.0 vs 11.5+7.5N).

Six of 9 subjects that had pain on the day of testing reported increased pain on both static and dynamic movements. Consistent with the predominance of increased nociceptor static reactivity, jaw position (degrees of opening) was significantly related to pain report on the static but not on the dynamic test movement. Due to unpredictable voluntary and reflexive reactions to passive jaw movement, the force required to move the jaw was not proportional to jaw pain. Thresholds for pain (10.11 +4.99N and 10.75 +6.2 degrees) were in a range that approximated nociceptor physiology and fell well within the working range of the joint. It was concluded that enhanced static transduction in joint nociceptors could play a predominant role in signalling joint pain. Supported by NIDR DE8701.

62.3

SYMPATHETIC INNERVATION TO THE RAT TEMPOROMANDIBULAR JOINT. S.B. Milam, B. Hutchins, R.J. Hinton, and R.E. Dill*. Depts. of Oral Surgery & Biomedical Sciences, Baylor College of Dentistry, Dallas, TX 75246

Although the innervation of temporomandibular joint structures by branches of the trigeminal nerve has been well-documented, little is known of other sources of innervation (e.g., autonomics) to the joint. To investigate this, a methyl cellulose gel containing 4 % WGA-HRP was placed within the superior joint space unilaterally in growing rats weighing between 200-290 g. After a 24 hour survival period, the rats were killed and the superior cervical (SCG), trigeminal, pterygopalatine, and otic ganglia were harvested. Preliminary results of WGA-HRP histochemistry indicated that labelled neurons were present ipsilaterally in both the superior cervical and trigeminal ganglia. Numerical analysis revealed the percentage of labelled SCG cells to labelled trigeminal ganglion cells ranged between 44 % and 71 %. A small number of labelled cells was noted in the putative pterygopalatine and otic ganglia, but the identity of these ganglia apart from the trigeminal ganglion must be verified further before conclusions can be drawn. These data, although preliminary, suggest that sympathetic innervation to the temporomandibular joint is considerable, and may play a role in neurogenic induction of joint inflammation and pain.
(Supported by NIDR DE06982 and NEI EY06977).

62.4

ADRENERGIC EXCITATION OF CUTANEOUS NOCICEPTORS IN ADJUVANT-INDUCED ARTHRITIC RATS. J. Sato*, S. Suzuki, T. Iseki and T. Kumazawa. Dept. Neural Regulation, Res. Inst. Environ. Med., Nagoya Univ., Nagoya, 464-01 Japan.

To probe mechanisms underlying sympathetic-mediated pain sensations in some inflammatory diseases, we studied the effects of sympathetic activity and norepinephrine on cutaneous nociceptors (C-fiber polymodal receptors, CPMs) in adjuvant-induced arthritic rats. Polyarthritis was induced by intradermal injection into the tail of *M. butyricum* 0.6 mg/0.1 ml suspended in mineral oil. Two to 27 weeks after treatment, single nerve recording of CPMs was done using fine filaments dissecting from saphenous nerve. In arthritic rats but not in controls, many (70%) CPM units showed sustained ongoing discharges. In control animals, lumber sympathetic chain stimulation at 20 Hz for 30 sec (SS) or close arterial injection of norepinephrine 400 ng (NE) by itself did not excite any CPM units tested. On the other hand, SS and NE were excitatory for a subset (35-50%) of CPMs in arthritic rats. Our results suggested that the adrenergic-sensitive CPMs might have a important role in the mechanisms of pain sensations related to some inflammatory diseases.

62.5

HISTOLOGICAL AND ELECTROPHYSIOLOGICAL CHANGES INDUCED BY CARRAGEENAN IN RABBIT LUMBAR FACET JOINT CAPSULE. AC OZAKTAY*, JM CAVANAUGH, DC BLAGOEV, AI KING. Bioengineering Center, Wayne State University, Detroit, MI 48202.

The aim of this study was to investigate effects of carrageenan-induced inflammation of the lumbar facet joint capsule and surrounding muscle. **METHODS:** Rabbits (3-4 kg, Male) were anesthetized with Na pentobarbital, iv. An L5-L6 laminectomy and ventral ramus rhizotomy were performed. Recordings were made from split L6 dorsal roots. The L6-L7 joint capsule was searched with a glass probe for receptive fields. The units were characterized by their conduction velocities and mechanical thresholds. 0.1 ml of 2% Type II carrageenan (Sigma) in 0.9% NaCl was then injected into the receptive field. The threshold and unit activity were recorded at specific intervals for later computer analysis. At the end of the experiment the tissue was histologically examined with H&E staining. **RESULTS:** In most of the experiments the spontaneous background discharge rate increased in two phases. Based on average pooled multi-unit data from 8 experiments, the first phase was observed after the first 30 min by a large increase in discharge rate with 45 min duration. The discharge rate then decreased for 30 min. A second smaller increase was then observed with 60 min duration. Group III (n=17) units displayed a prolonged (90 min) first and a shortened (30 min) second phase based on averaged data. Three group IV units showed a first phase after the first 15 min with 40 min average duration. Thresholds of the characterized units ranged from 1.23 grams to glass rod and decreased with time. Unlike the normal joint capsule area, inflamed joints showed vasodilatation and edema in synovial capsule and additionally, leukocyte infiltration in the perivascular space within surrounding muscle tissue. Supported by NIH Grant NS-28994.

62.6

REACTIVE OXYGEN SPECIES DO NOT PLAY A MAJOR ROLE IN ACUTE HYPERALGESIA. M. Kress, B. Riedl and P.W. Reeh (SPON: European Neuroscience Association), Inst. f. Physiologie u. Biokybernetik, Universitätsstr.17, D-8520 Erlangen, F.R.G.

The aim of this study was to investigate the contribution of reactive oxygen species, which are massively produced in inflammation, to nociceptor excitation and sensitization.

Receptive fields of single C-fibers (n=78) were separately superfused with test solutions in a saphenous nerve skin in vitro preparation. Solutions containing hydrogen peroxide, pyrogallol (an autooxidzing agent producing superoxide anion), hydroxyl radical (as a reaction product from hydrogen peroxide and ferric EDTA) or nitric oxide at concentrations of up to 10^{-3}M were scarcely effective. 8/17 units were excited by stimulation with synthetic interstitial fluid of ph 6.1. Addition of 1 mM H_2O_2 did not further enhance responses. In contrast, a mixture of inflammatory mediators (bradykinin, histamine, serotonin, prostaglandin E_2 10^{-5}M, ph 7.4) in combination with 1mM H_2O_2 significantly increased the discharge frequency in 8/16 units as compared to the mediators alone. On average units were neither sensitized nor desensitized to mechanical stimuli although the variability of mechanical responsiveness had increased.

Extremely high concentrations of reactive oxygen species were needed to excite cutaneous unmyelinated nociceptors, if at all. This does, however not exclude their possible contribution to the development of chronic pain states as they are known to cause changes in lipids and proteins which may be of longer onset.
Supported by the DFG, grant Re-A3 in SFB 353.

62.7

CRAMPING PAIN AND DEEP HYPERALGESIA FOLLOWING INTRAMUSCULAR INJECTION OF CAPSAICIN. D.A. Simone*, G. Caputi, P. Marchettini and J.L. Ochoa. Division of Neuroscience Research in Psychiatry, Univ. of Minnesota, Minneapolis, MN, and Depts. of Neurology and Neurosurgery, Good Samaritan Hosp. and Oregon Health Sci. Univ., Portland, OR.

Neural mechanisms mediating muscle pain are poorly understood although this is a common symptom associated with various clinical syndromes. Since capsaicin (CAP) has been a useful tool to study mechanisms of cutaneous pain and hyperalgesia, we investigated whether CAP may serve as an experimental model for muscle pain as well.

Injections of vehicle (Tween saline) and (CAP) doses of 0.1, 1, 10 and 100 μg in 10 μl were given into the gastrocnemius soleus muscles of seven healthy volunteers. CAP produced immediate cramping pain which radiated around the injection site but was never referred to distant deep tissues or to skin. The magnitude and duration of pain increased monotonically as a function of CAP dose. The sensation of cramping pain from CAP was not due to muscle contractures as evidenced by electromyography. The quality of pain from CAP was witnessed as identical to that produced by intraneural electrical stimulation of small caliber muscle afferents. After injection of CAP, painful muscle tenderness to innocuous pressure developed in an area surrounding the injection. The area of tenderness increased with CAP dose. There was no evidence of hyperalgesia in the skin overlying the injection, confirming that the CAP injection was localized to muscle.

These data indicate that capsaicin may be a useful experimental model of muscle pain and deep hyperalgesia, and should provide valuable information regarding the neural mechanisms of muscle pain in correlative neurophysiological studies. Supported by NIH NS 24766 and 28747.

62.8

LASER EFFECTS ON MYELINATED AND NON-MYELINATED AXONS IN RAT PERONEAL NERVE. U. Wesselmann*, J. M. Kerns and W. Z. Rymer. Depts. of Physiology, Physical Medicine & Rehabilitation, Northwestern University Medical School, Chicago, IL 60611.

We have recently shown that Nd:YAG laser irradiation of rat peripheral nerve differentially impairs action potential transmission in small, slowly conducting sensory fibers as compared to large diameter afferents (Wesselmann et al., Physiol. Chem. Phys. & NMR, 23, 81-100, 1991). In addition, the number of small sensory neurons of the A-delta and C-fiber group labeled with HRP is significantly reduced after laser irradiation (Wesselmann et al., Exp. Neurol. 111, 251-262, 1991). In contrast the number of labeled large sensory neurons and motoneurons was not affected. To further evaluate this laser induced injury, we examined three distinct regions (site of laser irradiation, 10 mm proximal and 5mm distal) at the ultrastructural level by morphometric methods in the peroneal nerve (n=6). The contralateral side was sham-treated. Our results indicate that the number of myelinated and non-myelinated axons is not significantly altered at 7 days following laser irradiation. The diameter and the frequency distribution of the myelinated axons was unchanged. There were no consistent ultrastructural changes in the nerves associated with laser irradiation. This study demonstrates that functional alterations in laser irradiated nerves (nerve conduction velocity, HRP transport properties) are not necessarily accompanied by structural changes. Thus, Nd:YAG laser light might be a powerful tool to selectively alter functional properties in small, slowly conducting afferent fibers, without causing structural damage at the ultrastructural level at the site of irradiation.

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62.9

CHARACTERIZATION OF AN ARTHRITIS MODEL IN RAT:TEMPORAL CHANGES IN PAW WITHDRAWAL LATENCY AND DORSAL HORN GLU, SP, AND CGRP CONTENT. K.N. Westlund* and K.A. Sluka. Marine Biomedical Institute, UTMB, Galveston, TX 77555-0843.

An experimental knee joint arthritis was induced by intraarticular injection of 3% kaolin and 3% carrageenan in rats under short acting anesthesia. Side to side differences in paw withdrawal latency (PWL) to radiant heat stimuli and immunocytochemical staining for neurotransmitter/neuromodulator substances (GLU, SP, and CGRP) were determined before and at different time periods after induction of arthritis (4,8,12,24,48,72 hr and 1 wk). The PWL decreased on the inflamed side starting at 4 hr, demonstrating that heat hyperalgesia had developed, and returned to normal by 72 hr. SP content, determined by computer assisted quantitation, was decreased ipsilaterally at 4 hr (52%). However, by 8 hr SP increased (33%) and remained increased up to one week (24%). Immunoreactivity for CGRP was also elevated at 8 (32%) and 12 (46%) hrs, and 1 wk (25%). GLU content increased at 4 hr (35%) and returned to baseline by 72 hr. The time course changes in GLU content and PWL in the inflamed paw were parallel in the same animals indicating that GLU is involved in arthritic heat hyperalgesia. In 5 rats where hyperalgesia did not develop, there was no change in GLU or SP immunoreactivity, but CGRP increased in the dorsal horn region receiving knee joint afferents. These studies demonstrate that while primary afferents are activated in this arthritis model, more widespread dorsal horn changes in GLU and SP are necessary for the development of heat hyperalgesia. GLU, SP, and CGRP content changes follow different time courses during the development of arthritis and are therefore involved in differential processing of information from the periphery. The persistence of the rise in peptide content may serve a modulatory role in maintaining the increased responsiveness of dorsal horn neurons during arthritic hyperalgesia. (Supported by NS01445, NS11255, and Bristol-Myers Squibb Co.)

62.10

AMINO ACID RELEASE IN EXPERIMENTAL ARTHRITIS AND THE EFFECTS OF LIDOCAINE, CNQX, AND AP7 PRETREATMENT. K.A.Sluka* and K.N. Westlund. Marine Biomedical Institute, UTMB, Galveston, TX 77555.

Amino acid release in the dorsal horn of awake rats was examined by microdialysis during the development of arthritis induced by injection of 3% kaolin and 3% carrageenan into the knee joint. The following amino acids were measured by HPLC at baseline and for the first 8 hr of arthritis: ASP, GLU, ASN, GLN, SER, GLY, and TAU. Four groups of animals were examined: 1) injection of the knee joint with kaolin and carrageenan, 2) pretreatment of the spinal cord with lidocaine 12-24 hours before injection of kaolin and carrageenan, 3) and 4) pretreatment with CNQX or AP7 administered through the microdialysis fiber 1 hr before the injection of kaolin and carrageenan. An initial increase in all amino acids examined was observed on injection of the knee joint in arthritic rats (Group 1). Subsequently, there was a peak increase in ASP (184%) and GLU (188%) by 2.5-3.5 hr which persisted for 8 hr. While ASN showed no changes from control and GLN, SER, and GLY were variable. In lidocaine treated animals (Group 2), only an increase in ASP, SER, and GLY was observed on injection of the knee joint while no change was seen in ASP or GLU. There is a peak decrease in ASP (60%) between 5 and 6 hrs and GLU decreased to 71%. In the CNQX treated animals (Group 3) there was an increase of ASP, SER and TAU on injection of the knee joint. Increases in ASP and GLU seen normally after induction of arthritis, however were delayed by 2 hr. In the AP7 treated animals (Group 4), only an increase in TAU on injection of the knee joint was observed. ASP and GLU were not released on injection. After 4 hr only ASP increased with no change in GLU. This data indicates the induction of arthritis is accompanied by an increased release of excitatory amino acids ASP and GLU which are important in the generation of acute arthritic hyperalgesia. This pattern of release is altered by pretreatment of the spinal cord/dorsal roots with lidocaine, CNQX, and AP7.

62.11

A BILATERAL INCREASE IN NADPH-DIAPHORASE ACTIVITY IN THE RAT LUMBAR SPINAL CORD FOLLOWING UNILATERAL HINDPAW INFLAMMATION. R.J. Traub[1], A. Solodkin[2] and G.F. Gebhart[1]. Depts. of Pharmacology[1], Anatomy and Neurology[2], University of Iowa, Iowa City, IA 52242.

Inflammation involves metabolic changes in the periphery and the CNS. Hindpaw inflammation results in the upregulation of *c-fos* and dynorphin in spinal dorsal horn neurons somatotopically appropriate to the site of injury. In this context it was of interest to determine if there are similar changes in enzymatic activity (e.g. NADPH-diaphorase) in spinal neurons as a result of hindpaw inflammation.

Male Sprague-Dawley rats (300-400 g) were given an intraplantar injection of 3 or 6 mg (100-200 µl) carrageenan. Survival times ranged from 1-24 hrs. Following perfusion with 4% paraformaldehyde, 40 µm sections through the L4-L5 spinal segments were histochemically reacted (60 min, 37°C) with ß-NADPH and nitroblue tetrazolium.

In controls the greatest number of stained neurons were observed in the superficial dorsal horn, including lamina III, with lesser amounts in the neck of the dorsal horn and around the central canal. Varicose axons were scattered throughout the gray matter, but were densest in the superficial dorsal horn and were present in the dorsal and ventral bundles around the central canal. Unilateral hindpaw inflammation resulted in a *bilateral* increase in: 1) the number of labeled neurons in the dorsal horn; 2) the number of very darkly stained neurons in laminae IIi-III; 3) the density of axons and varicosities throughout the gray matter. These increases were observed equally ipsi- and contra-lateral to the inflamed hindpaw.

These results suggest the inflammation process modulates NADPH-diaphorase activity within the spinal cord. Since previous studies had shown it coexists with certain peptides and is a putative NO synthase, it could be of interest to determine the neurochemical identity of NADPH-diaphorase stained elements under these conditions. Supported by DA 02879 to GFG.

62.12

REGULATION OF C-FOS AND JUN B mRNA TRANSLATION IN RAT SPINAL NEURONS FOLLOWING CUTANEOUS FORMALIN INJECTION OR UV-B IRRADIATION: INFORMATION STORAGE AT THE mRNA LEVEL ?

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Noxious inflammatory stimulation was applied to rat hindpaws by either unilateral plantar injection of dilute formalin (5% in tyrode) or by cutaneous UV-B irradiation (10,7 J/cm^2). Levels of c-fos (formalin) and junB (UV) transcripts and encoded proteins in the lumbar spinal cord were evaluated by slot-blot analysis and immunocytochemistry, respectively.

Compared to vehicle-injected controls, c-fos mRNA was increased in the ipsilateral and (to a minor degree) the contralateral side of the spinal cord 2 h following formalin injection, whereas intense c-FOS immunoreactivity was completely lateralized. In the UV-inflammation model, the significant increase in junB mRNA 6 h after UV-B exposure was not reflected by a corresponding change at the protein level.

We have reported on the potentiated synthesis of c-FOS in spinal neurons following unilateral formalin injection when preceded by an identical stimulation applied to the contralateral paw [Leah et al., Eur. J. Neurophysiol. 415, Suppl.1, R90]. This could now be explained by induction of c-fos expression in contralateral neurons by the first stimulus without subsequent translation. Comparison of formalin and UV related data suggest that translational efficiency may depend on electrical activity in nociceptive afferents.

Supported by Deutsche Forschungsgemeinschaft.

62.13

CHRONIC PAIN CAUSES AN INCREASE IN AMINO ACID RELEASE IN THE MIDBRAIN PERIAQUEDUCTAL GRAY. A MICRODIALYSIS STUDY. W.M. Renno*, F.G. Williams, J. Hautman, and A.J. Beitz. Department of Pathobiology, University of Minnesota, 1988 Fitch Ave. St. Paul, MN 55108.

It has been well documented that systemic administration of opiates or direct injection of opioid peptides into the PAG produces a profound antinociception which is thought to be associated with inhibition of neuronal activity in the PAG. This inhibitory effect has been postulated to result from opiate inhibition of GABAergic neurons in the PAG. Whether this opioid-GABAergic system is affected in chronic pain states has not been investigated. Microdialysis was chosen to perform direct and dynamic studies of amino acid concentrations in the PAG in control rats and in animals subjected to acute and chronic inflammation caused by injection of 120 ul of complete freund's adjuvent (CFA) into the hindpaw. We also examined the effects of acute and chronic pain on amino acid transmitter release from the PAG following depolarization with 75 uM veratridine. Veratridine induced release of aspartate and glutamate in rats treated with CFA 24 hrs prior to dialysis was double that of the control group treated with mineral oil 24 hrs prior to dialysis. GABA release was significantly increased in the group treated with CFA 7 days before dialysis. The increase GABA release was twice that of control groups or the 24 hrs CFA treated group. Based on data from other experiments we believe that this increase in the veratridine induced GABA release may be due to an increased opiate inhibition of GABA resulting from the induction of chronic pain. In sum these data indicate that acute pain (24hrs) causes a net increase in the release of glutamate and aspartate in the PAG, while chronic pain (7days) results in a net increase in GABA in PAG dialysates. This work was supported by NIH grants DA06687, DE06682, NS28016.

62.14

CORTICAL TONIC FACILITATORY INFLUENCE ON THALAMIC AND DORSAL HORN RESPONSES EVOKED BY SURAL N.STIMULATION AND CARRAGENINE IN RATS. I.Omaña-Zapata*, F.Pellicer, M.Condés-Lara. Instituto Mexicano de Psiquiatría, 14370 México, D.F.

Cortical action on thalamic centralis lateralis nucleus (Cl) and dorsal horn cells was studied during electrical suprathreshold sural nerve stimulation (SN) before and after carragenine (CAR) hindpaw injection. Cortical activity was transiently and reversible suppressed by the cortical spreading depression (CSD). Simultaneous recordings were obtained at prefrontal cortex, Cl and lumbo-sacral spinal cord (SC) level. The CSD did not modify the SC responses to SN stimulation in 25(93%) cells and decreased the frequency in 2(7%) cells. After CAR, 12(36%) SC cells increased their spontaneous activity and presented bursting discharges. Nineteen (58%) SC cells were recorded during 3 CSD, 14(43%) cells did not modify their spontaneous or evoked responses; five (15%) cells decreased their activity (in around 56%) after 124 ± 33 min. of CAR. In 24(89%) Cl cells, the CSD blocked their responses to SN stimulation. After CAR, 8(53%) Cl cells enhanced their activity; and 12 (80%) Cl cells, the CSD suppressed the CAR evoked activity. These results show that CAR enhanced the basal activity of SC and Cl. The delayed CSD-evoked suppression of activity at SC level suggests a development of a descending cortical facilitatory control. The blockade of this control diminishes the nociceptive effect upon the SC activity evoked by SN stimulation and CAR irritation.

63.1

LIGHT REGULATION OF A CALCIUM/CALMODULIN-DEPENDENT PROTEIN PHOSPHATASE IN THE *LIMULUS* LATERAL EYE. D.Z. Ellis-Ibidapo and S.C. Edwards* Dept. of Biology, Inst. for Biomolecular Sciences, University of South Florida, Tampa, FL 33620.

Calcium has been shown to play an integral role in the photoresponse of the photoreceptors in both the vertebrate and invertebrate organisms. For example, in photoreceptors from the horseshoe crab, *LIMULUS*, increasing Ca^{2+} levels mediate the state of light and dark-adaptation. In these cells, light, via Ca^{2+} activates a calcium/calmodulin (Ca/CaM)-dependent protein kinase which increases the phosphorylation of a 46kD protein (46A). Our previous results (Herrera et al., 1992) suggested that the protein phosphatase (PrP) responsible for the dephosphorylation of 46A is a type 1, whose activity is regulated by calcineurin, (PrP 2B, a Ca/CaM-dependent PrP). Using a 32P-labelled peptide fragment of the regulatory subunit of cAMP-dependent protein kinase as a substrate, (Blumenthal et al., 1986) we have identified calcineurin-like (CaN) activity in homogenates of *LIMULUS* visual and nonvisual neuronal tissues. Comparison of activities present in preparations containing nonphotoreceptor neuronal tissue, photoreceptor cell bodies and their axons demonstrate that Ca/CaM PrP activity is highest in the brain, lateral optic nerve and lateral eye respectively and that these activities are present in membrane as well as soluble fractions. We have shown that this activity is decreased by heparin, protamine and interestingly, okadaic acid (OKA 1,000 to 1 nM). This latter property is significant for it suggests that the *LIMULUS* Ca/CAM PrP is different from mammalian CaN which is not inhibited at low concentrations. Most importantly, light regulates the Ca/CaM PrP activity in the lateral eye. While there is no significant difference in basal PrP activity for long-term dark or light-adapted preparations, Ca^{2+} produced an increase in Ca/CaM PrP activity only in long-term light adapted cells.

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63.2

COMPUTATIONAL ANALYSIS OF THE *LIMULUS* RETINA: SIMULATION OF VISUALLY-GUIDED BEHAVIOR

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We have developed a computational model of the *Limulus* retina to investigate the neural representation of the animal's visual world. The model is based closely on the anatomical and physiological properties of the eye and its optics. The visual inputs are transformed via the optical properties of individual corneal lenses to determine the effective photon fluxes that excite the neural units (ommatidia) comprising the retinal network. Each ommatidium is simulated by a compartmental model of its spike-generating neuron that has variable conductances corresponding to light-sensitive excitatory inputs, firing of action potentials, and inhibitory inputs arising from both its own spike activity and that of neighboring neurons. We describe a computationally efficient implementation of the model on a parallel computer (Connection Machine, Model CM-5).

We use the model to investigate the neural representation of real scenes. Specifically, we simulate the spatial and temporal neural code that the eye sends to the brain in response to visual inputs that evoke known behaviors. We have shown that *Limulus* sights targets at distances between 0.4 and 1.0 meters dependent on the target size and contrast, but independent of time of day. Both day and night, *Limulus* turn towards objects that subtend as few as 4 ommatidia. We compute the neural responses to targets of various sizes and contrast moving at various speeds and relate these to visually-guided behavior. We also investigate the sensitivity of the neural code to changes in the anatomical and physiological state of the eye.

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63.3

LINEAR AND NON-LINEAR CODING OF CONTRAST RESPONSES IN FLY PHOTORECEPTORS.

M. Juusola, M. Järvilehto, E. Kouvalainen and M. Weckström*. Depts. of Physiology and Zoology, University of Oulu, SF-90220 Oulu, Finland.

In photoreceptors both long-lasting light stimuli (background) and changes thereof (contrasts) modulate the cells' membrane potential. We have recorded intracellular voltage responses to positive and negative contrast steps of varied duration superimposed on a varied background. The contrast responses are surprisingly linearly dependent on the contrast if the stimulus is of small duration (≤2 ms) or the background is low (<100 quanta/s). With longer lasting contrast steps or with higher backgrounds the responses to positive contrast (intensity increment) are increasingly smaller than the responses to negative contrast (intensity decrement). These photoreceptor properties are caused partly by kinetic properties of voltage-dependent K^+-channels (Weckström et al. J. Physiol.440:635-57, 1991), but the light-current (recorded with single electrode voltage clamp) rectifies into the same direction as well.

63.4

EXPRESSION OF VOLTAGE-GATED SODIUM CHANNEL-LIKE IMMUNOREACTIVITY IN THE CAT AND MONKEY RETINA.

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Polyclonal and monoclonal antibodies, generated to the α subunit of the voltage-gated sodium channel (αVGNaC), were employed to assess the cell types containing these channels in the mature cat and monkey retina. Immunoblot analyses of retinal proteins revealed that these antibodies labeled a band in the 260 KD region which is the expected molecular weight of the αVGNaC. In both the cat and monkey several types of retinal cells were immunolabeled. With the polyclonal antibodies immunoreactivity was observed in ganglion cells, optic axons, horizontal cells, and cones. A smaller number of cells, presumed to be bipolar neurons, were labeled in the inner nuclear layer (INL). With the monoclonal antibody there was staining in the fiber layer, relatively weak labeling of ganglion cell somata, and a continuous band of immunoreactive cell bodies situated in the inner half of INL. By immunolabeling isolated cells dissociated from the cat retina, it was possible to demonstrate unequivocally that bipolar cells were in fact labeled with the antibodies to αVGNaC. The differences in the labeling observed between the polyclonal and monoclonal antibodies was interpreted as reflecting the presence of different α subunits in the mammalian retina. Collectively, our findings suggest that αVGNaCs are expressed on a more diverse population of retinal cells than expected on the basis of previous physiological and immunohistochemical studies. (Supported by NIH and NMSS)

63.5

MEASUREMENT OF NEURONAL IMPEDANCE IN FREQUENCY DOMAIN WITH WHITE NOISE MODULATED CURRENT INJECTION USING SINGLE ELECTRODE TECHNIQUE.

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The determination of membrane properties of small neurons is difficult because only one intracellular electrode can be used. We have developed a system where, while recording intracellularly current is injected into the cell using discontinuous (switched) technique. The magnitude of the current (0.05-0.5 nA peak-to-peak) is modulated pseudorandomly. The neuron's input impedance is then calculated, via FFT, as the ratio of cross-power spectrum (of input current and output voltage) and input power spectrum. By using time-domain averaged responses to the same pseudorandom sequence we can estimate the linearity of the membrane input-output relation with the coherence.

The described technique can be used to determine the input impedance of invertebrate photoreceptors, and both invertebrate and vertebrate visual interneurons, large monopolar neurons and horizontal cells, respectively.

63.6

TYPE I NADPH-DIAPHORASE (NADPHd) REACTIVE RETINAL NEURONS SHOW FOS-LIKE IMMUNOREACTIVITY (FOS-IR) AFTER LIGHT EXPOSURE.

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Nitric oxide synthase (NOS) generates a readily diffusible messenger molecule, nitric oxide, and is responsible for the neuronal NADPHd histochemical reaction. In the rabbit retina, two populations of cells in the inner nuclear layer (INL) are NADPHd reactive: larger, more densely staining cells termed Type I; and more numerous and smaller cells termed Type II. The Type I cells have long processes and are presumably involved in long-distance interactions within the retina. To examine whether these NADPHd reactive cells are stimulated by light, retinas of dark-adapted rabbits that were exposed to flashing light for 1h and sacrificed 1h later were immunostained for Fos proteins and double labelled for NADPHd. Dark-adapted, control retinas had no Fos-IR, whereas light induced Fos-IR in a minority of cells in the ganglion cell layer and INL, as reported previously (Sagar and Sharp, Mol. Brain Res., 1990). In the INL 25-30% of Fos-IR cells were NADPHd reactive, 70-80% of the Type I and 10% of the Type II NADPHd reactive cells showed Fos-IR. The results indicate that NOS-containing cells are activated by flashing light in the rabbit retina.

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63.7

GABA ACTIVATES L-TYPE Ca CHANNELS IN THE EMBRYONIC CHICK RETINA.

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GABA is present before synapse formation in the chick retina (Hokoç et al. '90). The action of GABA was studied by measuring intracellular Ca^{++} concentration with Fura-2 in the neural retina of embryonic day 3 chicks.

Puff-application of 100 μM-GABA increased the Ca^{++} concentration. This response was blocked by 10 μM-bicuculline and mimicked by muscimol but not by baclofen. The GABA response was abolished in Ca-free medium or in 2 mM-Co, suggesting the involvement of Ca channels. Depolarization with high-K solution also induced the Ca^{++} increase. This Ca response was completely blocked by 10 μM-nifedipine and enhanced by 5 μM-Bay K 8644, showing the characteristics of L-type Ca channels. The Ca response to GABA showed the same pharmacological properties as the L-type Ca channels.

We conclude that GABA activates L-type Ca channels via $GABA_A$ receptors. The depolarization may be caused by efflux of Cl^-. GABA can regulate the intracellular Ca^{++} concentration through L-type Ca channels during the early period of neurogenesis in the chick retina.

63.8

DOPAMINE AND PHORBOL ESTER-INDUCED POTASSIUM EFFLUX IN THE CHICK RETINA: EFFECTS OF MELATONIN.

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Potassium efflux (assessed as $^{86}Rb^+$ efflux) was studied in retinal suspensions from 1 to 17-day-old posthatched chicken. Dopamine (DA) increased K^+ efflux rate 1.5 fold. This effect was dose-dependent (EC_{50} 22 μM), was mimicked by the D1-selective agonist SKF-38393 and reversed by the D1-selective antagonist SCH 23390. The DA action was independent of adenylyl cyclase (AC) activation, as the membrane permeable cAMP analogs showed no potency. Moreover, DA did not affect cAMP accumulation in retinal suspensions. The protein kinase C (PKC) activator, 4β-phorbol 12-myristate 13-acetate (4β-PMA) also stimulated retinal K^+ efflux in a dose-dependent manner (EC_{50} 4 nM). This effect was mimicked by 4β-phorbol 12,13-didecanoate but not by the inactive isomer 4α-PMA. When added together, DA and 4β-PMA behaved in an additive manner suggesting a PKC-independent action for DA. Moreover, DA did not stimulate phosphoinositide hydrolysis, a well-known pathway to generate diacylglycerol, an endogenous activator of PKC. DA also inhibited retinal Na/K-ATPase (assessed as ouabain-sensitive $^{86}Rb^+$ influx). However, the DA-elicited K^+ efflux was largely independent on this inhibition. As melatonin (MEL) is known to interact with DA in the retina, the effects of MEL on K^+ efflux were studied. MEL affected neither basal nor DA nor 4β-PMA-stimulated K^+ efflux. To conclude, DA stimulates K^+ efflux in the chick retina via a D1-receptor mediated action independently on AC and PKC. MEL does not seem to modulate this action of DA.

63.9

RETINA-SPECIFIC BOVINE cDNAs ENCODING HOMOLOGS OF THE *norpA* AND *ninaA* PROTEINS OF *DROSOPHILA*.

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The *norpA* and *ninaA* genes encode, respectively, a photoreceptor-specific phospholipase C (PI-PLC) and cyclophilin, which are important in phototransduction and rhodopsin folding, respectively. Mutations in the *norpA* gene also cause photoreceptor degeneration in *Drosophila*. We looked for evidence of retina-specific bovine homologs of these proteins by screening a bovine retinal cDNA library under reduced stringency conditions using probes derived from *norpA* and *ninaA* sequences. Among the positively hybridizing clones isolated, we concentrated on two: one isolated with the *norpA* probe and the other isolated with the *ninaA* probe. Sequence analysis showed that the proteins deduced from the two cDNA clones showed strong homology to the *norpA* (PI-PLC) and *ninaA* (cyclophilin) proteins, respectively. In Northern analysis, the *norpA*-like bovine cDNA recognized a 7.4 kb transcript and the *ninaA*-like cDNA, a 3.1 kb transcript. Both these transcripts were present in blots of retinas but not of any other tissue tested. Results of tissue *in situ* hybridizations suggest that both transcripts are localized in photoreceptors as well as retinal neurons of the inner nuclear and ganglion cell layers.

63.10

STEREOSELECTIVE HYDROLYSIS OF RETINYL ESTERS BY CHICKEN NEURORETINA AND RETINAL PIGMENT EPITHELIUM.

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The distribution of retinyl esters in the chicken neuroretina and retinal pigment epithelium (RPE) has been reported to be unusual in comparison to other vertebrate species (Rodriguez & Tsin, 1989, *Am. J. Physiol.* 256, R255-R258). In the present study, retinyl ester hydrolase (REH) activities in neuroretina and RPE tissue homogenates and their subcellular fractions were partially characterized using [^{3}H]-11-*cis* and all-*trans* retinyl palmitate as substrates. Ester hydrolysis in both tissues was predominantly stereoselective for the all-*trans* isomer and was heat-labile. The rate of hydrolysis was protein, time, pH, and substrate dependent. Maximum hydrolysis occurred at pH 5.0 and in the mitochondrial/lysosomal fractions (63% of total) of both tissue preparations. However, neuroretina homogenates exhibited a significantly higher rate of hydrolysis for all-*trans* retinyl palmitate (V_{max} = 111 *p*mol/min/mg in neuroretina vs. 71 *p*mol/min/mg in RPE) but little difference in substrate affinity (K_m = 5 *u*M in neuroretina vs. 4.5 *u*M in RPE). Since REH activity toward the 11-*cis* isomer was significantly lower in both tissues (60 *p*mol/min/mg for neuroretina, 27 *p*mol/min/mg for RPE) than that observed for the all-*trans* isomer, and a comparable substrate affinity (5.8 *u*M for neuroretina, 2.5 *u*M for RPE), this would suggest that the all-*trans* retinyl ester in the chicken retina may play an important role in the visual cycle.

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63.11

Immunolocalization of basic fibroblast growth factor (bFGF) in the rodent outer retina demonstrated with an anti-rodent bFGF antibody

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Despite the fact that bFGF has been isolated from the retina, its retinal localization remains controversial. To establish the precise cellular distribution of bFGF in the C57BL/6J mouse and Sprague-Dawley rat retinas, we have used anti-bovine (AA 1-24, AA 1-15), anti-human (whole molecule) and anti-rat (AA 1-23) bFGF antibodies in immunocytochemical studies. Immunolocalization with these antibodies revealed two distinctly different retinal staining patterns. With antibodies against bovine or human bFGF, some ganglion cells, cells in the inner portion of INL and developing horizontal cells were stained as previously reported in the rat retina. Using the anti-rat bFGF antibody, in the adult mouse, heavy labeling was observed at the level of the photoreceptor outer segment; whereas in the adult rat, Muller's cells and RPE were intensely labeled. In the P5-10 mouse and P5 rat, the staining patterns were virtually identical, with bFGF immunolabeling localized to the outer retina, in the cytoplasm and nuclei of developing photoreceptors and in cells located in the outer portion of INL. From P1 until P5, no difference was observed in the staining intensity between *rd* and normal mice. From P6 to P10, the *rd* retinas were more intensely labeled in those cells described above than the normal retinas at equivalent ages. bFGF immunolabeling with anti-rat bFGF antibody could be blocked by preabsorption in rat and mouse retinas with rat bFGF antigen (AA 1-23), and blocked in developing but not in adult mouse retinas with mouse bFGF antigens (AA 1-25, AA 12-25). bFGF labeling with anti-bovine antibody could not be blocked when preabsorbed with the rodent bFGF antigens; and the staining with anti-rat antibody was not blocked by preabsorption with bovine bFGF antigen (AA 1-24) in the rat, but was blocked in the mouse retina. These results indicate that minor species differences in a short segment of the bFGF molecule used to generate these antibodies may result in major differences in apparent bFGF immunolocalization.

63.12

IDENTIFICATION OF GAMMA-AMINOBUTYRIC ACID-A (GABA-A) RECEPTOR SUBUNIT TRANSCRIPTS IN RODENT RETINAE.

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Cloning studies have identified six alpha subunits, three beta subunits, three gamma subunits and one delta subunit in rodent brains and two rho subunits in human retina. The biochemical, pharmacological and physiological properties of a given GABA-A receptor seems to be dependent upon the constituent subunit classes of that receptor. Vertebrate retina has GABA as well as GABA-A and GABA-B receptors. As a prelude to the localization of different GABA-A receptor subunit classes in mouse and rat retinae, we have studied the expression of different subunit classes by reverse transcription and polymerase chain reaction (PCR) using rat primers for alpha 1-6, beta 1-3, gamma 1-3 and delta and human primers for rho 1. Our results suggest that alpha 1, beta 1 and 3 and rho 1 mRNA are more prevalent than that of other subunits studied. Alpha 4-6, gamma 1 and gamma 2 long transcripts were not detectable after 35 cycles of PCR. This suggests the expression in retina of a relatively distinct collection of GABA-A subunit mRNAs when compared with the other regions of the CNS. Analysis of the cellular distribution of GABA-A subunit mRNAs may permit the formulation of models relating subunit composition with physiological responses in identified subtypes of retinal neurons.

63.13

EFFECTS OF IDPN ON THE STRUCTURE AND FUNCTION OF THE RAT VISUAL SYSTEM. S. Barone Jr.*[1], D. Herr[2], K. Crofton[2]. [1]METI, RTP, NC 27711; [2]NTD, USEPA, RTP, NC 27709.

Short-term repeated administration of 3,3'-iminodipropionitrile (IDPN) results in a complex neurobehavioral syndrome (cf Cadet, 1989 *Neurosci Biobehav Rev* 13:39). Adult male Long-Evans rats received IDPN (400 mg/kg i.p.) on 3 consecutive days and were sacrificed 24 hours after the first dose, 24 hours after the third dose, 3 days after the third dose, 1, 2, 5, or 10 weeks (wks) after the third dose for histological analysis of the eye. IDPN intoxication resulted in opacification of the cornea (3 days) and detachment of the retina (1 week). The corneal changes were transient and were not overtly apparent at 2 wks post-treatment while retinal reattachment took place between 5 and 10 wks. However, early necrosis in the inner nuclear layer (INL) was seen before retinal detachment (3 days post-dosing) followed by progressive retinal degeneration (5 and 10 wks). Retinal detachment was less severe at 10 wks. A dose-response study (0, 100, 200, 400 mg/kg x 3 days) of visual electrophysiology and histology was performed 2 wks post-treatment. Decreased flash evoked potential (FEP) peak N_{30} and N_{56} amplitudes and increased latencies of peaks P_{21} and P_{46} were observed in a dose-related manner, with significant decrements at the highest dose. Results indicate that non-neural structures in the eye recover in a time-dependant manner, while neurodegeneration of the visual retina is progressive, even when the retina has reattached. At 2 wks after dosing, retinal detachment in the 400 mg/kg dose group and degeneration in the INL of the 200 and 400 mg/kg dose groups was associated with decreased amplitudes and increased latencies of electrophysiological responses to visual stimulation.

63.14

POSITIVE AND NEGATIVE COMPONENTS OF THE DARK-ADAPTED CAT ERG L.J. Frishman*, J.G. Robson[1], and L. Du, College of Optometry, Univ. of Houston, Houston, TX 77204; [1]Physiological Lab, Cambridge, UK (CB2 3EG)

Component potentials generated by different retinal processes sum algebraically to produce the electroretinogram (ERG). We used brief (<1 msec) ganzfeld flashes to re-examine the components of the vitreal ERG in dark-adapted anesthetized cats. We conclude that the ERG reflects activity of several processes whose responses are linearly related to intensity at low intensities, but then saturate at different higher intensities. At the lowest intensities the significant ERG components are the negative scotopic threshold response (STR) and a positive potential which (unlike PII) saturates at a lower intensity than STR. Below 2-4 quanta/deg^2 (incident at the cornea) either this positive potential is the larger (the ERG reaching 1-2 μV positive) or the two potentials cancel, making the STR appear to have a threshold. At higher intensities the positive potential stops increasing, and the STR then dominates and increases linearly with intensity up to about 1/3 of its maximum amplitude (of roughly 40 μV). At intensities about 10 times greater, the negative (STR) peak saturates rather abruptly to remain constant up to intensities at least another 100 times higher, and PII emerges as an obvious b-wave. After blocking the small positive and negative (STR) components with 2.5-8.5 μMols of intravitreal NMDLA, the b-wave was evident at intensities 10 times lower than normal, its amplitude was greater than normal, and it was proportional to intensity up to about 1/5 of its maximum amplitude of 1500 μV. This suggests that normally there is significant mutual cancellation of STR and PII over a wide range of intensities, so that amplitudes of STR and PII are greater than indicated by the positive and negative peaks in the ERG and the underlying STR saturates less abruptly. Intraretinal recordings of field potentials showed that only in peripheral retina were onset latencies fast enough to match the ERG components evoked by weak stimuli. Supported by NIH grant EY06671.

63.15

CIRCADIAN MODULATION OF STRUCTURE IN THE JAPANESE QUAIL RETINA

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Retinomotor movements are regulated by light and an endogenous clock in lower vertebrates. Circadian interactions of dopamine and melatonin control cone myoid movement in *Xenopus* (1). Dopamine and its agonists and antagonists modulate structural changes in fish retina (2). Physiological studies in the Japanese quail have shown a circadian rhythm in rod-cone dominance (3 &4).

Here we report that a circadian clock controls retinomotor movements in the quail. Illumination in the day or night has no detectable effect. Processes of the retinal pigment epithelium (RPE) migrate towards the outer limiting membrane (OLM) during the subjective day, surrounding rod outer segments (ROS) and partially surrounding cone outer segments (COS). At night, the RPE retracts, exposing the COS and ROS. Immunocytochemical staining for tyrosine hydroxylase locates dopaminergic cells in the amacrine layer. Intravitreal injections of dopamine agonists and antagonists do not disrupt the structural rhythms. Structural and functional rhythms do not appear correlated. Circadian control of retinomotor movements and RPE migration may be involved in metabolic stability rather than changes in sensitivity.

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63.16

CIRCADIAN MODULATION OF RETINAL FUNCTION IN THE JAPANESE QUAIL: ROLE OF DOPAMINE

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Rod-cone dominance exhibits a circadian rhythm in the Japanese quail retina. In constant darkness (DD), retinal sensitivity (ERG b-wave) is high at night and and low during the day; and spectral sensitivity shifts from rod dominance at night to cone dominance during the day (1). Melatonin and dopamine appear to interact to regulate circadian physiology in the vertebrate retina (2). We hypothesize that dopamine and melatonin mediate the circadian rod-cone shift in the quail retina.

Dopamine antagonists (haloperidol, sulpiride) injected in the vitreous during the day shift the retina to the sensitive, rod-dominated nighttime state. A dopamine agonist (quinpirole) injected at night has the opposite effect. Destruction of dopaminergic cells with 6-OH dopamine leaves the retina in the rod dominated state regardless of time of day. Dopamine, thus, appears to mediate cone dominance. Retinal dopamine synthesis is high during the day and low at night in DD as indicated by DOPA levels following inhibition of DOPA decarboxylase activity *in situ*. Retinal melatonin synthesis is low during the day, and high at night in DD as measured by tryptophan hydroxylase activity *in situ* and melatonin levels (3).

The counterphase circadian rhythms of dopamine and melatonin suggest that dopamine mediates cone dominance during the day, and melatonin mediates rod dominance at night.

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63.17

CHARACTERISTICS OF LIGHT-EVOKED ALKALINIZATIONS IN CAT RETINA. F. Yamamoto* and Y. Honda. Dept. of Ophthalmology, Kyoto University School of Medicine, Kyoto, 606 Japan.

Measurements of $[H^+]_o$ by intraretinal ion-selective microelectrodes showed that pH outside dark-adapted rods was relatively acidic in the dark, with maximum acidity (pH 7.04) in the outer nuclear layer (Yamamoto, F. and Steinberg, R.H., Soc. Neurosci. Abstr. 15:206, 1989). Illumination, which was at rod saturation level, produced intraretinal alkalinization that was largest (up to 0.2 pH units) in outer nuclear layer (Borgula, G.A. and Steinberg, R.H., Invest. Ophthalmol. Vis. Sci. Suppl. 25:289, 1984). We studied the effects of various light-stimuli on light-evoked alkalinizations using double-barrel H^+-selective microelectrodes in the intact cat eye, *in vivo*. Stronger lights above rod saturation level made the recovery after light-offset very slow. Short and strong light produced alkalinization as well as the long flash with the intensity of rod saturation level. And the stimuli-summation of short flashes induced alkalinizations as well as the long continuous lights. These results suggests that the light-evoked alkalinizations might be concerned with the light adaptation of rods photoreceptors.

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63.18

TRANSGENIC MODIFICATION OF THE MOUSE RETINA. M.A.McCall*, R.G. Gregg., and L.R. Stanford. The Waisman Center and the Department of Comparative Biosciences. University of Wisconsin, Madison, WI 53706

Genetic ablation has been used to selectively eliminate individual cell populations in a number of somatic organs and tissues. In this study, we have attempted to determine if this technique can be used to eliminate a specific neuronal population, and might thereby prove to be a useful tool in defining the role of these populations in the function of a neural circuit or system. A fusion gene, consisting of the human rhodopsin promoter linked to an attenuated diphtheria toxin gene, was injected into the male pronucleus of fertilized mouse ova, which were then transplanted into pseudopregnant recipients. The animals born from this procedure were then tested, using Southern Blot Analysis, to identify those that had incorporated the fusion gene into their genome. These mice were then used to establish a colony of transgenic animals. Because the transcription of the diphtheria toxin gene is controlled by the rhodopsin promotor, the rod photoreceptors should be selectively eliminated in those animals that inherit the transgene. To determine the functional consequences of the fusion gene, we recorded the electroretinogram from 4 transgenic animals and found that electrical activity could not be evoked from the retinae of any of the affected transgenic animals. Subsequent light microscopic examination of the retinae of these animals showed that both the photoreceptor outer segments and the outer nuclear layer were absent; the remaining retinal layers appeared to be unaffected. These results indicate that the incorporation of a fusion gene can cause the selective elimination of a retinal cell class, and that the effects of this manipulation can be demonstrated both morphologically and physiologically. These results also suggest that this strategy might be used to eliminate other selected elements from neural circuits or systems, possibly providing a very direct method for assessing the contribution of specific neuronal populations to neural processing. Supported by NIH Grant EY 04977 and NSF Grant BNS-9015226.

63.19

ENDOGENOUS ADENOSINE DILATES RETINAL ARTERIOLES IN THE NEWBORN PIGLET. J.M. Gidday*, T.M. Lanius, A.R. Shah, and T.S. Park. Department of Neurosurgery, Washington University School of Medicine, St. Louis, MO 63110.

In an effort to test the hypothesis that the purine metabolite adenosine is a metabolic regulator of retinal blood flow (RBF), the present study was undertaken to determine the effect of increased endogenous adenosine concentrations on retinal arteriolar tone. Newborn pigs (<4 d old) were anesthetized (isoflurane) and paralyzed (pancuronium). The iris was dilated (1% tropicamide), and the cornea and lens were excised and replaced with a glass coverslip. The diameter of a retinal arteriole (55-95 μm) was measured on-line at 310x using an image processor. A 20 μl aliquot of 10 μM iodotubercidin, an inhibitor of adenosine kinase, or 5 μM NBTI, an adenosine transport inhibitor, directly microsuffused onto the abluminal surface of the vessel via a 30 ga intravitreal catheter, increased arteriolar diameter by 24±3% and 30±4%, respectively. Administration of 50 μM DPMX, an A2-selective adenosine receptor antagonist, concomitant with NBTI completely blocked the dilatory response. Thus, resistance vessels of the retina possess A2 adenosine receptors that, when activated by increases in endogenous adenosine concentrations, induce retinal arteriolar vasodilation. These findings are consistent with the participation of adenosine in the metabolic regulation of RBF in the newborn.

SUBCORTICAL VISUAL PATHWAYS: LGN

64.1

A Model of a Thalamic Cell Incorporating Voltage-Clamp Data on Ionic Conductances. D.K. Smetters* Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Thalamic neurons contain an unusually large variety of voltage- and time-dependent conductances, causing them to respond differently to the same pattern of input depending on the past activity of the cell. To understand how thalamic cells respond to sensory input, it is necessary to understand how these currents contribute to their response to current injection, synaptic stimulation, and modulatory neurotransmitters. The complex interaction between these currents confounds *a priori* understanding of their roles from examination of current- and voltage-clamp recordings. We have therefore constructed a detailed computational model of a thalamic relay cell, based on voltage clamp data obtained from thalamic slices and isolated relay cells. We find that our model reproduces most of the current-clamp behavior of thalamic cells, including tonic and burst modes, rebound low-threshold spikes after hyperpolarization, oscillatory behavior, and extremely long delays to fast spike firing with just-threshold depolarization.

Analysis of the model provides insight into the role of various voltage-dependent conductances in the response of these cells. We are particularly interested in the role of potassium currents in regulation of firing mode, as some of these currents are modulated by a number of neurotransmitters (McCormick and Prince, 1987,1988; McCormick and Williamson 1991), and they have been the subject of several recent voltage-clamp studies (Huguenard et. al. 1991; McCormick 1991). Simulation results lead us to predict that a fast, calcium-activated potassium current similar to Ic (Adams et. al. 1986) can explain the amplitude and time course of the fast after-hyperpolarization, a prediction supported by patch clamp data from cultured rat thalamic cells (Esguerra and Smetters, unpublished). Simulations also suggest that the kinetics of the low-threshold calcium current, in combination with a voltage-independent potassium current are sufficient to produce the long delay to low-threshold spike seen after small hyperpolarization, without requiring involvement of additional voltage-dependent potassium currents. The model also leads us to suggest that voltage-dependent conductances play a vital role in the subthreshold behavior of thalamic cells.

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64.2

Relay and Burst Response Modes in the LGN Vary with the Alertness of the Cat. Stephen Lehmkuhle*, John A. Baro, and William Guido†. School of Optometry, University of Missouri-St. Louis, St. Louis, MO 63121 and †Dept. of Neurobiology, SUNY, Stony Brook, NY 11794

Relay cells of cat LGN can respond to visual stimuli in one of two modes: relay and burst. The burst mode is due to the activation of Low Threshold (LT) Ca^{++} spikes only when the cell is hyperpolarized. Because membrane potential fluctuates with behavioral state, we wished to determine the extent to which response mode would vary with level of alertness. Visual responses were recorded extracellularly in an awake behaving cat, and separated into tonic and LT burst components. LT bursts were recognized as bursts of action potentials with interspike intervals $\leq$ 4 msec that are preceded by a silent period of $\geq$ 100 msec.

The behavioral state of the cat was classified as alert or drowsy by monitoring the EEG activity of striate cortex. The animal was classified as drowsy when the ratio of the power of the synchronized (4-9Hz) versus desynchronized (10-20 Hz) EEG activity was greater than 5 and the RMS value exceeded a preset value. Spike arrival times were calculated during both pre- and post-stimulus periods, and separated into LT burst and tonic discharge categories.

We found that LGN cells respond in both relay and burst mode during alert and drowsy states. However, LT burst activity was more prevalent during the drowsy state than the alert state. During the drowsy state, LT burst activity increased and tonic activity decreased. During the alert state, LT burst activity decreased and tonic activity increased. This trend occurred for both baseline and stimulus driven activity. One implication is that relay and burst mode may have further significance for sensory processing, perhaps alerting visual cortex to a change in behavioral state. (Supported by NSF BNS-8819706)

64.3

NITRIC OXIDE CONTROLS OSCILLATORY ACTIVITY IN THE THALAMUS. **H.-C. Pape*. Abt. Neurophysiol., Ruhr-Univ., D-W-4630 Bochum, Germany.**

The enzymes for the synthesis of nitric oxide (NO), a possible regulatory molecule in the central nervous system, are specifically co-localized with mesopontine cholinergic neurons. Since these neurons control thalamocortical activity during various states of the sleep/waking cycle, we evaluated the possible modulatory role of NO in the thalamus. Local application to relay neurons of the guinea pig and cat dorsal lateral and medial geniculate nuclei maintained in vitro, of the NO-donating drug 3-morpholinosydnonimine (SIN1; 0.5-2 mM) resulted in a decrease (30 %) in resting input resistance (49 MΩ) associated with a 1-3 mV depolarization from resting potential (-63 mV) in 89 % of the neurons (n=56). Three sets of experiments indicated that this response resulted from the generation of NO: i) The NO-donor nitroprusside (NP; 0.2-5 mM) elicited a very similar response, and the effects of SIN1 and NP were mutually occlusive. ii) The SIN1-derivative molsidomin (10 mM), which requires cellular enzymes generally not present in situ to generate NO, was ineffective. iii) Prior extracellular application of the NO-scavenger hemoglobin (100 μM) prevented the action of SIN1. Furthermore, 8-bromo-cyclicGMP (1 mM, local) imitated the action of SIN1, and a near-maximal effect of 8-bromo-cyclicGMP occluded responses to SIN1, suggesting an involvement of the cyclicGMP system. Voltage-clamp experiments demonstrated that the response to NO resulted from a positive shift in activation by +5 mV of the hyperpolarization-activated cation current, I_h.

The influence of NO dampened rhythmic-oscillatory burst-activity of thalamocortical neurones, but it did not affect the generation of tonic sequences of fast spikes. These findings may indicate a diffusible intercellular signalling mechanism different from that of classical neurotransmitters for controlling the functional state of the thalamocortical network, regardless of any direct connection through synapses.

64.4

MODULATION OF RETINO-GENICULATE TRANSMISSION THROUGH ACTIVATION OF ß-ADRENOCEPTORS IN THE CAT. K. Funke, H.-C. Pape and U.T. Eysel. Dept. of Neurophysiology, Faculty of Medicine, Ruhr-University Bochum, W-4630 Bochum, Germany. **(SPON: European Neuroscience Association)**

The ascending noradrenergic pathway is one candidate for state-dependent modulation of retino-geniculate transmission. Quite non-uniform results were obtained with micro-iontophoresis of noradrenaline in the lateral geniculate nucleus and stimulation of the coeruleo-geniculate pathway in rat and cat. In order to investigate the effect of selective stimulation of adrenoceptors, we micro-iontophoretically applied the ß-adrenoceptor agonist isoprenaline (ISO) during simultaneous recordings of lateral geniculate relay cells in the anaesthetized (N_2O/O_2, 70/30%; halothane, 0.3%; or pentobarbitone 2-4 mg/kg·h), paralysed (alcuronium chloride 0.15 mg/kg·h) cat. ISO effects on spontaneous discharge patterns and visual processing were studied and related to the global state of the animal, as derived from the pattern of electroencephalographic (EEG) activity. We found the following ISO effects: *i)* the occurrence of rhythmic or non-rhythmic burst discharges during highly synchronized, δ-like EEG was significantly suppressed, whereas tonic single spike activity was significantly less affected, irrespective of the pattern of EEG; *ii)* receptive field center and center-surround responses were not significantly altered when studied during less synchronized, lower amplitude/higher frequency EEG, but we found a significant facilitation of the sustained spot response, when this response component was attenuated during synchronized EEG; *iii)* late phases (100-400 ms) of binocular and long-range lateral inhibition were significantly reduced, and this effect was similar to the strong reduction of the late inhibitory phase when the EEG changed from a highly synchronized to a less synchronized pattern. Long-lasting inhibitory responses elicit burst discharges during highly synchronized states of the EEG and may also impair retino-geniculate transmission. Our results provide direct *in vivo* evidence that ß-adrenoceptor activation attenuates long-lasting inhibitory mechanisms to enable the faithful processing and transmission of visual signals.

64.5

ULTRASTRUCTURAL STUDIES OF AFFERENTS TO THE LATERAL GENICULATE NUCLEUS. S. Feig, D. Van Lieshout, J.Harting*, Anatomy Dept., Univ. of Wis. Med. Sch. , Madison, WI 53706

We have used EM-autoradiagraphy to analyze the morphology and synaptic relationships of six afferents to the lateral geniculate of Galago crassicatatus.

Retinal terminals contain round vesicles, pale mitochondria, and make asymmetrical synapses (RLP's). Terminals arising from cells in area 17, the superior colliculus (SC) and the parabigeminal nucleus (PG) contain round vesicles, dark mitochondria and make asymmetrical synapses (RSD's). The RSD's associated with these three projections exhibit no obvious morphological differences . Terminals arising from the pretectum (PT) and thalamic reticular nucleus (TRN) contain pleomorphic vesicles, dark mitochondria and make symmetrical synapses (F1's).

RLP's within the parvi- and magnocellular layers target juxtasomatic regions of geniculate neurons. They share these regions with F profiles; some of the F1 variety arise from the PT and TRN.

The juxtasomatic regions of neurons within the small-celled koniocellular layers are relatively free of retinal terminals . Instead these regions are almost exclusively innervated by F profiles; some of the F1 terminals originate from the the PT and TRN. RLP's within the koniocellular layers innervate intermediate and small sized dendrites, where they occasionally lie adjacent to terminals arising from the SC and the PG.

Labeled corticogeniculate terminals target intermediate to small sized dendrites in all layers and have never been observed adjacent to RLP's.

Labeled PT terminals are presynaptic to F2 profiles, and therefore account for at least some of the F1 to F2 synapses. In contrast, labeled TRN profiles have never been observed presynaptic to F2 profiles.

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64.6

MECHANISMS OF FREQUENCY DEPENDENT FACILITATION OF CORTICOTHALAMIC EPSPs M. von Krosigk* and D.A. McCormick. Yale Univ. Med. Sch. New Haven, CT

Repetitive activation of corticothalamic inputs to thalamic relay cells is known to result in marked frequency dependent potentiation. Here, we investigate the cellular basis of this potentiation and contrast it with the properties of retinal inputs using an *in vitro* slice preparation of the guinea-pig dorsal lateral geniculate nucleus (LGNd).

Delivery of a train of electrical stimuli to corticothalamic inputs to LGNd relay cells resulted in a sequence of EPSPs exhibiting marked, frequency-dependent facilitation, while delivery of similar stimulus trains to optic tract did not. Two mechanisms were found: an increase in neurotransmitter release and GABAergic disinhibition. The frequency-dependent facilitation of corticothalamic inputs persisted after the block of GABAergic inhibitory synaptic transmission and was associated with an increase in both NMDA and non-NMDA receptor mediated components, suggesting that this facilitation is mediated by, at least in part, an increase in neurotransmitter release. Examination of $GABA_A$ and $GABA_B$-mediated IPSPs revealed that these synaptic potentials show depression during direct and repetitive activation, suggesting that an additional mechanism in the facilitation of corticothalamic inputs may be GABAergic dis-inhibition.

These results indicate that frequency dependent facilitation of corticothalamic inputs occurs both through an increase in transmitter release as well as through disinhibition.

64.7

THE HISTAMINERGIC INNERVATION OF THE LATERAL GENICULATE COMPLEX. D.J. Uhlrich*, K.A. Manning & T.P. Pienkowski. Department of Anatomy, University of Wisconsin, Madison, WI 53706.

Histamine (HA) receptors are present in the lateral geniculate nucleus (LGN), and HA appears to play a role in determining the physiological response characteristics of LGN relay neurons *in vitro*. However, the anatomy of the histaminergic innervation of the LGN is unclear. In tissue from 3 cats fixed with 1% paraformaldehyde and 4% carbodiimide and processed for immunohistochemistry, we examined the histaminergic projection to the LGN using an antibody to HA.

HA-reactive neurons, now collectively referred to as the tuberomammillary nucleus, were restricted to the hypothalamus. All laminae of the LGN and adjacent nuclei contained fibers with well-labeled *en passant* swellings of varied size (0.5-2.0 μm) and little branching. Label was dense in regions involved in the retinal W cell pathway, in particular, the ventral lateral geniculate nucleus (vLGN). Parvocellular C laminae of the LGN, the perigeniculate nucleus, and portions of the medial interlaminar nucleus showed intermediate amounts of label. The geniculate A laminae had only sparse labeling. Average varicosity size was greatest in the vLGN. Electron microscopic examination in lamina A revealed HA profiles of unusual morphology. Swellings contained round or elliptical vesicles, many extremely large (diam 20-200 nm, avg 70 nm). Thus far, intriguing appositions are common, but no clear synapses are seen, despite serial reconstruction. HA profiles are not associated with any particular geniculate profile or element. These results support the idea that HA works nonsynaptically as a neuromodulator in the LGN, affecting relay cell responses as a function of behavioral state.

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64.8

RESPONSES OF CELLS IN RABBIT LATERAL GENICULATE NUCLEUS TO DRIFTING SINUSOIDAL GRATINGS. S. Molotchnikoff* and V. Durand. Dépt de biologie, Université de Montréal, C.P. 6128, succ. A, Montréal (Québec) Canada H3C 3J7

Last year we reported an analysis of cortical cell responses to sinusoidal gratings in rabbits. Similarly to cats, rabbit's simple cells respond with a modulatory pattern while complex neuron discharges in a non-modulatory fashion. In the present investigation, we have recorded from the lateral geniculate nucleus with the aim to compare responses of the latter with responses of cortical cells to gratings. Single cell activity was recorded in anesthetized and paralyzed rabbits. Receptive fields were identified as concentric (ON-OFF) and uniform with the help of appropriate stimuli. Ninety-five per cent (N = 37) of geniculate neurons respond to gratings (≈60-65% in cortex). As in the cortex the majority are low-pass (LP) in spatial domain and band-pass (BP) in temporal domain. All proportions between LP, BP, and High Pass cells are equivalent at both levels. The most striking results is the absence of non-modulated discharges to gratings of the geniculate units; indeed all cell responded by a modulation of their evoked firing. Thus in rabbits, the non-modulatory pattern which typically characterizes complex cells, is established at the level of the striate cortex.

Supp. CRSNG.

64.9

IN THE DORSAL LATERAL GENICULATE BODY OF RATS TWO TYPES OF RECEPTORS SEEM TO MEDIATE THE ACTION OF CHOLECYSTOKININ. H. Davidowa, D. Albrecht, H.-J. Gabriel and U. Zippel. Inst. of Physiology, Charité, Humboldt-Univ., Berlin O - 1040, FRG. (SPON: European Neuroscience Association).

Various transmitters gate the transmission of signals within thalamic relay nuclei. The possible role of CCK has been studied in rats anesthetized with urethane. CCK-8S or Boc CCK 4 (0.05 to 1 mM, pH 7.8) as well as CCK A - or B - antagonists were applied iontophoretically. Background and light evoked activity in about one third out of 190 neurons were facilitated, in one sixed inhibited by CCK-8S. The amount of activity change depended on the current (20 to 80 nA). Boc CCK 4 had similar effects, but not so strong as CCK-8S. The effects could partly be blocked by A- antagonists, partly by B- antagonists. There was no relation of the direction of change to the blocking action of A- or B- antagonists. Cells influenced in their activity by CCK were not uniformly distributed within the dLGB.

The results indicate that modulations of geniculate firing caused by CCK are receptor mediated, and that in the dLGB CCK A- as well as B- receptors seem to be present.

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64.10

THE TEMPORAL PROPERTIES OF RECEPTIVE FIELDS IN THE LGN: A COMPARISON OF KITTENS AND CATS. D.Cai, G.C.DeAngelis , R.D. Freeman and T.E.Cohn*. Group in Vision Science, School of Optometry, University of California, Berkeley, CA 94720.

Receptive field properties of the neurons in the lateral geniculate nucleus (LGN) change during normal postnatal development (Mitchell and Timney, 1984). Although changes in spatial properties are well documented, relatively little is known about temporal aspects of receptive field structure during normal development.

We have studied temporal aspects of receptive field structure of LGN neurons in anesthetized and paralyzed adult cats and 4 week old kittens. Orientation, spatial frequency and temporal frequency tuning curves are obtained using drifting sinusoidal gratings. Cells are classified as X or Y based on a linearity test with counterphase gratings. Subsequently, a version of the reverse correlation technique (Jones and Palmer, 1987) is used to obtain a spatiotemporal receptive field profile for each neuron.

Temporal frequency tuning curves from 4-week old kittens show pronounced attenuation at high frequencies, as compared to those from adults. The mean optimal temporal frequency increases from approximately 3 Hz for kittens to approximately 7 Hz for cats. The mean high cut-off frequency increases from approximately 8 Hz for kittens to approximately 16 Hz for cats. Using the reverse correlation technique, we have analyzed various parameters of the temporal response. Response latency, defined as the reverse correlation delay which produces a maximal response profile, is, on average, 35 ms msec for cells from adults and 60 ms for cells from 4-week old kittens. While most adult LGN neurons show a clearly defined biphasic temporal response, neurons in the LGN of kittens often show a monophasic temporal response under the same conditions of stimulation. No peak latency difference is found between on-center neurons and off-center neurons during development.

Overall, these results demonstrate that the temporal characteristics of receptive fields in the LGN continue to develop considerably beyond 4 weeks of age. Similar findings have been reported for neurons in the striate cortex (DeAngelis et al, 1991, Invest. Ophthalmol. Visual Sci. Suppl. 32:1253). (EY01175)

64.11

STATE-DEPENDENT MODULATION OF THE TEMPORAL TRANSFER PROPERTIES OF CAT LATERAL GENICULATE NEURONS.

P. Mukherjee* and E. Kaplan. Rockefeller University, New York, NY 10021

The activity of the lateral geniculate nucleus (LGN) is affected by changes in arousal during the sleep-wake cycle. Here we report state-dependent effects on the dynamics of cat LGN relay cells associated with the occurrence of low-threshold bursts, which are known to be generated by activation of the T-type Ca^{2+} channel.

Spikes and retinal S-potentials were simultaneously recorded from single LGN neurons of anaesthetized and paralyzed cats in response to drifting sinusoidal gratings varying in temporal frequency (TF). Transfer functions were obtained by computing the amplitude ratio and phase difference of the fundamental Fourier components of the LGN response and the retinal response at each TF. Bursts were identified from spike trains as clusters of action potentials with interspike intervals ≤4 ms. The electroencephalogram (EEG) was recorded over area 17.

In states of low arousal, characterized by slow-wave EEG activity, LGN relay cells had markedly bandpass temporal transfer functions, with peak transmission between 2-8 Hz. The LGN response phase led that of the retina at low TF, a difference that declined with increasing TF until the LGN response lagged the retinal response at TF > 6 Hz. Neurons in this state frequently fired bursts. However, under higher arousal levels (marked by low-amplitude high-frequency EEG), the same relay cells displayed a flat temporal transfer function with little phase difference between the LGN and retinal responses. In this state, relay cells showed little or no bursting activity. These results demonstrate that the LGN is a temporal filter of retinal information whose dynamics can be modulated by changes in arousal. A neural model of LGN relay cells, incorporating quantitative T-channel kinetics, suggests that this temporal tuning can be explained by a hyperpolarization of these neurons. This alteration in membrane potential may be regulated by brain-stem centers projecting to the LGN. *Supported by EY-4888 & EY-1428*

64.12

Lagged cell responses are more variable in latency than are nonlagged responses in cat LGN cells. S.M. Sherman*, W. Guido, and S.-M. Lu. Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

Lagged responses have been identified for LGN cells of cats, and these differ from more conventional nonlagged responses on a number of criteria. Most distinctions have been made on the basis of histograms from averaged responses to flashing spots. Among other differences, lagged responses as gleaned from these histograms are slower, less sharply defined, and generally poorer. It is not clear whether these slower and poorer lagged responses are present in individual stimulus/response cycles, or whether they are partly an artifact of the averaging process. To answer this question, we recorded lagged and nonlagged responses to flashed spots from a sample of LGN cells in cats and analyzed these on a trial-by-trial basis. We identified the beginning of the response epoch in each trial as the first interspike interval ≤10 msec (or, for some cells, ≤20 msec). As expected, we found that the lagged responses displayed a significantly longer latency for the initial response than did nonlagged ones; however, the variation in this latency among trials was much greater for the lagged responses. Thus any averaged histogram constructed from these responses would artificially smear lagged responses more than nonlagged ones. To control for this, we removed variability in latency among individual trials of both response types by shifting the timing of all responses to each trial by the temporal difference between the first response of each trial (as defined above) and the average first response latency of all trials. Such a latency transform has little effect on average histograms of nonlagged responses, because these are already well synchronized. However, it dramatically alters lagged histograms, making these responses appear to be much more brisk. In fact, many of the qualitative differences between lagged and nonlagged histograms disappear with this procedure. We conclude that an important difference between nonlagged and lagged responses is the greater variability of the latter on a trial-by-trial basis. (Supported by USPHS grants EY03038 and EY06082).

64.13

Retinal Y Axons Contact Interneurons in the Lateral Geniculate Nucleus of the Cat. M. E. Bickford*, S. C. Van Horn, and S. M. Sherman. Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

It is well established that GABAergic interneurons in the cat's lateral geniculate nucleus (LGN) participate in the modulation of retinogeniculate transmission along the X pathway. However, there has been some controversy concerning the involvement of LGN interneurons in the Y pathway. We investigated this with the electron microscope by anatomically examining the postsynaptic targets of retinogeniculate Y axons intracellularly labeled with HRP. Tissue containing parts of two labeled axons (one innervating lamina A and the other, lamina A1) was serially sectioned, and selected grids were stained for the presence of GABA, using postembedding immunocytochemical techniques. We obtained two lines of evidence that Y retinal axons contact interneurons. First, 31 of the 95 retinal synaptic contacts examined thus far (32%) were made onto profiles that contained vesicles. Reconstructions identified these profiles as small swellings, some of which were identified as GABA-positive. Since the dendritic appendages of interneurons are the only known source of GABAergic, vesicle-containing profiles that receive synaptic input, we interpret this as evidence that some of the output of Y retinal axons is onto the appendages of interneurons. Second, reconstructions revealed that some dendrites that receive Y retinal input give rise to swellings that contain vesicles. We interpret this as evidence that Y retinal axons can also contact the dendritic shafts of interneurons. These results suggest that although the majority of the synaptic contacts of Y retinal axons are made directly onto geniculocortical relay cells, a portion of their synaptic output is directed to interneurons, providing a possible anatomical substrate for feedforward inhibition in the Y pathway. (Supported by USPHS grant EY03604.)

64.14

PROGRESSIVE CHANGE IN CALCIUM BINDING PROTEINS IN MONKEY LATERAL GENICULATE NUCLEUS AFTER MONOCULAR ENUCLEATION. C. Gutierrez and C.G. Cusick*. Neurosci. Prog. and Anat. Dept., Tulane Univ. Med. Sch., New Orleans, LA 70112.

In the lateral geniculate nucleus of monkeys, neurons in magno- and parvicellular layers contain strong parvalbumin immunoreactivity (PV-ir) and little calbindin immunoreactivity (CB-ir). To examine the effects of sensory deafferentation on the distribution of these proteins, PV-ir and CB-ir were localized histochemically in 6 adult macaque monkeys 1-7 mos. after monocular enucleation. In each case, PV-ir and CB-ir in fibers decreased within the deafferented parvi- and magnocellular layers. Whereas the numbers of PV-reactive cells did not differ significantly in the deafferented and intact layers, CB positive cells increased in the deafferented layers. At 1-3 months after injury, CB-ir in deafferented cells was very light. At 4-7 months, numbers of CB positive cells increased further (up to 14-fold that of the intact layers and over half the PV cells in the deafferented layers). CB-ir in cells also increased in density, and at 7 months neuropil staining was strong in the deafferented layers. The results suggest that some neurons that normally express only PV co-localize CB in response to deafferentation, and "new" expression of calbindin may increase over several months. Supported by NEI-EY08906.

64.15

SPATIAL FILTERING PROPERTIES OF LGN X-CELLS IN CATS. H. Cheng, Y.M.Chino*, E.L. Smith III, K. Yoshida, and J. Hamamoto, College of Optometry, University of Houston, Houston, TX 77204

Whether spatial filtering properties of X-LGN neurons differ from their retinal inputs is still a matter of debate. We re-examined this issue by directly comparing LGN responses and their simultaneously recorded retinal input, S-potentials (SP). We found: 1) the bandwidths of spatial frequency response functions were narrower in many LGN cells compared to their SPs. 2) In all but two cells, the transfer ratio (defined as the firing rate of LGN cells divided by that of its SPs) was reduced at low spatial frequencies compared to that for the cell's peak or for high spatial frequencies. 3) Transfer ratios in contrast response functions were lowest with low spatial frequency stimuli in the majority of neurons. 4) Differences in contrast thresholds and contrast gains between LGN responses and SPs were greater for the lower spatial frequency range.

Comparisons of receptive field center-surround mechanisms indicated that both the center (Rc) and surround (Rs) sizes are virtually the same for both LGN cells and their retinal inputs. However, the center sensitivity (Kc) was much lower in LGN neurons relative to that of their SPs, whereas the surround sensitivity (Ks) was similar in LGN and SP responses. Our data support the idea that the decreased responses of LGN neurons for low spatial frequency gratings is primarily due to lower sensitivity in the receptive field center mechanism of LGN neurons relative to their retinal inputs rather than increased surround strengths.

64.16

SPONTANEOUS AND EVOKED GABAERGIC SYNAPTIC CURRENTS IN THALAMO-CORTICAL NEURONES OF THE RAT LATERAL GENICULATE NUCLEUS. N. LERESCHE* and A. GUYON. Dept. de Neurochimie-Anatomie, Institut des Neurosciences, Univ. Paris VI, 9 quai St Bernard, Paris 75005, France.

Thalamo-cortical neurones were identified on morphological criteria in slices of the rat dorsal lateral geniculate nucleus and whole cell currents were recorded using the patch clamp technique. Postsynaptic currents occurring spontaneously, or elicited by extracellular stimulation in the vicinity of the recorded neurone, were analyzed. Spontaneous postsynaptic currents were observed in every recorded neurone. At a holding potential of -60 mV, and with a high internal Cl^-, the currents were inward and had amplitudes ranging from less than 10 pA to 425 pA. All the spontaneous currents were blocked by 10 μM bicuculline indicating that they were due to the activation of postsynaptic $GABA_A$ receptors. The 10-90% rise time of these spontaneous GABAergic currents was 0.86±0.19 ms. Their time course of decay could be fitted to an exponential function with one or two time constants of, respectively, 18.19±3.02 ms (mean±s.d.) or 4.47±0.77 and 33.27±3.74 ms. Both the rise and decay time course were independent of the current amplitude. This activity was frequently organized in bursts lasting 1.8 to 4.3 sec.

Stimulus-evoked postsynaptic currents were also recorded. The stimulus-evoked GABAergic currents, recorded under experimental conditions similar to the spontaneous currents, had a 10-90% rise time of 1.93±0.54 ms. Their time course of decay could be fitted to an exponential function with one or two time constants of, respectively, 24.42 ms or 10.26±2.46 and 49.30±10.98 ms. The difference in the time course between spontaneous and evoked GABAergic currents, recorded in the same neurone, suggests that these responses may arise from synapses having different locations.

64.17

GLYCINERGIC MODULATION OF THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR PLAYS A CRITICAL ROLE IN RETINOGENICULATE TRANSMISSION TO NON-LAGGED X AND Y CELLS. H.E. Jones*, R.H. Levy, A.M. Sillito and P.C. Murphy. Dept. of Visual Science, Inst. of Ophthalmology, Judd St., London WC1H 9QS, UK.

There is now strong evidence to support the view that NMDA and non-NMDA receptors are involved in the transfer of the visual input from both X and Y retinal ganglion cell afferents to lagged and non-lagged relay cells in the feline dorsal lateral geniculate nucleus (dLGN). Blockade of NMDA receptors effectively eliminates the visual response of dLGN cells. In vitro work shows that activation of the NMDA receptor is subject to allosteric facilitation by glycine acting at a strychnine insensitive site within the NMDA receptor complex. We have examined the extent to which the visual response in the dLGN is influenced by modulation from the strychnine insensitive glycine site in vivo. We examined the effect of iontophoretic application of 2 non-competitive antagonists for the glycine site, 7-chloro-kynurenate (7-CK) and 1-hydroxy-3-amino-2-pyrrolidone (HA-966), on the responses of non-lagged X and Y cells recorded in the dLGN of anaesthetized, paralysed cats. Seven barrelled glass micropipettes were used for recording and iontophoretic application of drugs. The visual stimulus comprised a spot of light flashed within the receptive field centre. Iontophoretic application of either 7-CK or HA-966 greatly reduced and in some cases blocked visual responses at ejection current levels of the antagonists compatible with a selective action on the responses to NMDA with respect to agonists acting at non-NMDA receptors. These data provide further support for the view that NMDA receptors are involved in the transfer of retinal input to non-lagged dLGN cells, and demonstrate that factors influencing the glycinergic modulation of the NMDA receptor are capable of exerting a profound influence on sensory processing at the thalamic relay in the visual system.

64.18

POTENTIATION AND DEPRESSION OF THE STRENGTH OF THE TRANSFER OF THE RETINAL INPUT IN THE DORSAL LATERAL GENICULATE NUCLEUS (dLGN). A.M. Sillito* and H.E. Jones. Dept. of Visual Science, Inst. of Ophthalmology, Judd St., London WC1H 9QS, UK.

There is now strong evidence to support the view that both NMDA receptors and non-NMDA receptors are involved in the transfer of information at retinogeniculate synapses. Taking note of the evidence indicating that synapses utilising excitatory amino acid receptors can exhibit long term potentiation (LTP) and long term depression (LTD), we have explored the possibility that it might be possible to modify the strength of the transfer of information at the retinogeniculate synapse. We have recorded from single cells in the A laminae of the dLGN of anaesthetized, paralysed, adult cats with seven barrelled glass micropipettes. Receptive fields were mapped and then stimulated with a small flashing spot located within the receptive field centre. The spot was generated either as an increase in illumination above background (on-centre cells) or a decrease below (off-centre cells). We then explored the effect of pairing the stimulus with either iontophoretic application of NMDA or GABA. Responses were tested systematically at intervals starting five minutes after termination of the pairing procedure. In some, but not all the cells so far studied, the drug pairing resulted in respectively, an enhancement (NMDA) or depression (GABA) of visual responsiveness with respect to control response magnitudes. We also observed response enhancements after visual responses were paired with iontophoretic application of quisqualate which acts at AMPA and metabotropic receptors. The sustained component of the visual response immediately after the onset transient appeared to be particularly susceptible to these procedures. The data suggest the capacity for a long term change in the gain of the transfer of visual information at the retinogeniculate synapse that is not simply linked to the fast dynamics of visual processing or state dependent changes imposed by the extrinsic modulatory systems.

64.19

CALCYPHOCINE-IMMUNOREACTIVITY IN THE DORSAL LATERAL GENICULATE NUCLEUS OF THE CAT. F. Vandesande, H. Demeulemeester, W. Van Duffel, G.A. Orban, J.J. Vanderhaeghen*, and C.W. Heizmann. Lab. Neuroendocrinol., Lab. Neuro- and Psychophysiol., Catholic Univ. Leuven, Lab. Neuropathol. and Neuropep. Res., U.L.B., Brussels, Belgium, Dep. Pediatrics, Univ. Zürich, Switzerland.

In this study, we investigated wheter the GABAergic neurons in the dLGN of the cat can be subdivided by their content of the calcium binding proteins: calcyphocine (CF), calbindin (Cal) and parvalbumin (PV). Here we report only the results of the A laminae.

In the A laminae about 72% of the neuronal population was CF-IR. Of the CF-IR cells, about 52% contained Cal and 12% contained PV, while about 70% of the Cal and 40% of the PV-IR cells were CF-IR. Areal measurements of the CF-IR neurons in the A laminae revealed a mean soma size of 221 μm^2, with soma sizes ranging from 50 to 700 μm^2. Studies with WGA-Biotin retrogradely transported from the visual cortex showed that in the A laminae about 58% of the CF-IR cells and 32% of the Cal-IR cells were retrogradely labeled. Retrogradely labeled PV-IR cells were absent.

These and previous results (Demeulemeester H. et al., Exp. Brain Res., 83: 513, 1991) indicate that at least four different IR geniculate cell populations can be identified: (1) cells (+) for PV and Cal; (2) cells (+) for PV and (-) for Cal; (3) cells (-) for PV and (+) for Cal; (4) cells (-) for PV and Cal. The results with WGA-Biotin indicate that populations (1) and (2) are interneurons, and (3) and (4) are relay cells. Each of these populations can again be subdivided by the presence or absence of CF.

64.20

EFFECTS OF ACETYLCHOLINE ON THE VISUAL RESPONSE OF LAGGED CELLS IN THE LATERAL GENICULATE NUCLEUS OF THE CAT. E. Hartveit* and P. Heggelund. Dept. of Neurophysiol., Univ. of Oslo, Norway.

Relay cells in the cat LGN can be divided into lagged and nonlagged cells based on their visual response properties. Lagged cells are initially suppressed by a flashing light spot and they have longer latency to the visual response than nonlagged cells. There is evidence that the initial suppression is mediated by intrageniculate GABAergic inhibition (Heggelund & Hartveit, J. Neurophysiol., 1990, 63, 1347). The retino-geniculate transmission can be strongly influenced by input from the peribrachial region (PBR) of the brain stem. The cholinergic afferents seem to be particularly important. The response of lagged cells is markedly influenced by electrical PBR stimulation, but the initial suppression is maintained. We have extended these previous findings by examining the direct effects of acetylcholine (ACh) on the visual response of lagged cells.

The response of single cells to a flashing light spot was recorded before, during and after iontophoretic application of ACh. The spot approximately filled the receptive field centre. For comparison some of the cells were also tested by application of the GABA-A antagonist bicucullinemethchloride (BMC).

During the application of ACh there was an increase of both the spontaneous activity and the visually driven response. The latency to onset and half-rise of the response could be reduced. However, the visually evoked initial suppression was resistant to ACh. In contrast, during BMC application the initial suppression was abolished and a short latency initial transient response component occurred.

We conclude that the effects of iontophoretic application of ACh on the visual response of lagged cells were markedly similar to the effects of electrical PBR stimulation. The lagged/nonlagged distinction as well as the characteristic response properties of the lagged cells were maintained also during the direct ACh application.

SUBCORTICAL VISUAL PATHWAYS: RETINA AND COLLICULUS

65.1

PARALLEL PROCESSING IN THE RETINOFUGAL PROJECTION OF SYRIAN HAMSTERS. Sonal Jhaveri* and Gerald E. Schneider, Dept. of Brain & Cognitive Sciences, Mass. Inst. of Technology, Cambridge, MA 02139

The lateral geniculate body (LGB) of anesthetized adult hamsters was exposed via a lateral approach. Under direct visual guidance, the retrograde cell tracers Fluorogold and rhodamine-labeled microspheres, were pressure-injected, one each in topographically equivalent parts of the dorsal (LGBd) or ventral (LGBv) nuclei of the LGB. After survivals of 1-4 weeks, animals were perfused with 4% paraformaldehyde. The brains were cut and examined for localizing the injection sites, and wholemounts of the ipsilateral and contralateral retinae were analysed with a UV filter and RITC filter for detecting the Fluorogold and the microsphere labeling, respectively.

The overwhelming majority of retrogradely labeled retinal ganglion cells (RGCs) in the contralateral retina had either one or the other label; very few cells were double-labeled. Moreover, RGCs back-labeled from the ipsilateral LGBv comprised a predominantly uniform population of medium-sized cells which were distinct from cells projecting to the ipsilateral LGBd. Preliminary experiments combining injections of one retrograde tracer in LGBv and another in the superior colliculus (SC) indicate that the LGBv and SC receive innervation from many of the same RGCs.

Our results demonstrate that separate populations of RGCs innervate the LGBd and LGBv and suggest that the LGv and SC share more by way of visual input than do the two nuclei of the LGB. These observations support earlier (unpublished) data from our laboratory showing that many optic tract terminals in the LGBv form a fine plexus bearing numerous tiny beads; these terminals are quite distinct from RGC terminals in the LGBd (Erzurumlu et al., Br. Res., 1988).

SUPPPORT: NIH Grants EY05504, EY00126 and EY02621.

65.2

TRANSPORT OF CALCIUM GREEN DEXTRAN VISUALIZED VIA CONFOCAL MICROSCOPY IN THE VISUAL SYSTEM OF THE RAT. D.M. O'Malley*, S.M. Lu, W. Guido and P.R. Adams. Howard Hughes Medical Institute and Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY, 11794.

Fluorescent dextrans can be transported in both the retrograde and anterograde fashion (Nance, 1991). A new calcium indicating dye, calcium green, is available bound to 10,000 molecular-weight dextrans (Molecular Probes). We were interested in whether calcium green dextran (CGD) could be transported and subsequently used as a calcium activity indicator. First, the responsiveness of CGD was tested and found to behave similarly to non-conjugated calcium green; it's fluorescence in an intracellular-like medium increased 7 fold as free [Ca^{++}] was raised from 5 nM to 2 µM.

To determine whether the dye could be axonally transported, we injected 1-2 µl of a 20% solution of CGD in distilled water into the visual cortex of young rats. After survival periods of 3 to 7 days, the rats were sacrificed and 300 µm thick coronal slices of brain were cut on a vibroslicer. The slices were perfused with oxygenated artificial CSF in a chamber mounted on the stage of a Biorad MRC 600 confocal microscope (inverted configuration). Labelling was observed in cortical areas surrounding the injection site as well as in contralateral cortical areas, apparently via callosal projections. Labelled axons and cells were present in the ipsilateral lateral geniculate nucleus (LGN) but not in adjacent thalamic nuclei. Some labelled axons could be followed for several hundreds of microns. Occasional cells in the LGN were labelled well enough to follow a number of their dendrites for 50 to 100 µm.

To further support that CGD was axonally transported, we injected 1-2 µl of CGD into the eye (vitreous) of young rats. After 3-4 days, extensive fields of punctate labelling were observed within the LGN. This labelling often took the form of rings, suggesting the presence of nerve terminals surrounding cell bodies. The degree and extent of labelling suggest that CGD may have use as a retrograde and anterograde tracer. We are currently testing whether transported dye remains responsive to Ca^{++}.

65.3

RETINAL PROJECTION TO THE MESOPONTINE CENTRAL GRAY IN THE RAT. H. Shen, T. Honda, and K. Semba*., Dept. of Anatomy, Dalhousie Univ., Halifax, N.S. B3H 4H7 Canada.

During the course of a study on the central connections of the retina, we have identified a retinofugal projection to the central gray at the pontomesencephalic junction in the rat. The projection was confirmed with both anterograde and retrograde tracing techniques. Following intravitreal injections of choleratoxin subunit B, immunohistochemically visualized varicose afferent axons were seen in a ventrolateral region of the mesopontine central gray. The labelling was predominantly contralateral. Many of these labelled axons were intermingled with serotonin-immunoreactive neurons in lateral aspects of the dorsal raphe nucleus. Some fibers were also found further laterally, beyond the boundary of the dorsal raphe nucleus but within the central gray. Following injections of the retrograde tracer fluorogold into the mesopontine central gray area (without contamination of previously known targets of retinal projections), ganglion cells in the retina were labelled. Electron microscopic examination of axonal labelling in the central gray is currently underway. These data provide evidence for the existence of a direct projection from the retina to the central gray at the level of pontomesencephalic junction. The direct retinal projection to the mesopontine central gray, including the dorsal raphe nucleus, may have a role in sensorimotor coordination, and/or regulation of circadian rhythms and sleep and wakefulness.

65.4

RETINAL PROJECTION IN THE RAT BRAIN AS DEMONSTRATED BY CHOLERA TOXIN FOR AN ANTEROGRADE TRACER. Y. Matsumoto[1]* and M. Kawata[2]. Dept. of Ophthalmol.[1], Dept. of Anatomy[2], Kyoto Pref. Univ. of Med., Kawaramachi Hirokoji Kamigyo-ku, Kyoto 602, Japan.

Retinal central projection pathways have been extensively studied with the aid of degenerative methods and anterograde transport techniques of tracers such as horseradish peroxidase and fluorescent dyes. We now report new findings of rat retinal projections in the brain by using more sensitive tracers of cholera toxin B-subunit (CTB) in conjunction with immunohistochemistry.

Fifteen adult male rat unilaterally received intravitreal injection of 0.1% CTB. Thirty-six hours later, these animals were perfused with phosphate-buffered saline and a fixative, and their brain sections were prepared in the coronal and sagittal plane at 20μm on a cryostat. These sections were incubated with anti-CTB serum for 3 days at 4°C. Then, immunohistochemical procedure was performed with avidin-biotin-peroxidase complex methods.

The immunoreactive products were clearly identified as thin winding fibers with various-sized varicosities or fine smooth fibers. Terminals of retinal axons were observed in the following regions which have not been reported so far to receive retinal afferents: both sides of the ventromedial hypothalamic nucleus, lateral dorsal thalamic nucleus, visual tegmental relay zone; contralateral side of the vertical limb of the diagonal band nucleus, lateral habenular nucleus, superior geniculate nucleus, anterior pretectal nucleus, periaqueductal gray; ipsilateral side of the anterior dorsal thalamus, lateral posterior thalamic nucleus and inferior colliculus. The data described above suggest that the brain contains substantial systems of projection fiber from the retina.

65.5

MEDIAN STRIP OF OVERLAP FOR ALPHA CELL DECUSSATION IN PIGMENTED RABBIT RETINA. B. Lia* & P. Kuthan. Dept. Ophthalmology, RJ-10, University of Washington, Seattle WA 98195

Provis & Watson (81) found large-diameter (≥20μ) retinal ganglion cells with contralateral projections throughout the rabbit's temporal crescent. These might be presumed to include alpha cells. In the cat, crossed-projecting alpha cells are found in the temporal hemiretina, but mainly within a limited zone near the vertical meridian (Illing & Wässle '81). We wondered whether a qualitative difference exists between these species for alpha cell decussation. Therefore, we examined the decussation pattern of morphologically-identified alpha cells in Dutch belted rabbits. Projection laterality was determined by retrograde labeling following injection of green fluorescent latex microspheres (Molecular Probes) into one optic tract and red into the other. In superfused wholemount preparations, the temporal crescent was first delineated by the median edge of uncrossed labeling. Along sampling transects at various distances parallel to this uncrossed median edge, cells with large somata were injected with carboxyfluorescein to reveal dendritic structure. Ganglion cell classes were distinguished by morphological criteria (Peichl et al. '87; Pu & Amthor '90). Initial findings confirm that large cells with crossed projections can be labeled throughout the temporal crescent. However, the great majority of these did not prove to be of alpha cell morphology. Rather most were of either On or On-Off direction-selective morphology. Those few alpha cells within the temporal crescent with crossed projections were found mainly in a narrow zone along the uncrossed median edge and not throughout the temporal crescent. The median edge for crossed alpha cells is situated only somewhat temporal to their uncrossed median edge. Thus, it appears that alpha cells in the rabbit decussate in a pattern quite similar to that in the cat.

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65.6

PERIOD FOR REORGANIZATION OF RETINO-GENICULATE PROJECTIONS FOLLOWING ABLATION OF AREAS 17 & 18 IN THE IMMATURE CAT. H. E. Pearson*, S. G. Lomber, B. R. Payne and P. Cornwell. Temple University School of Medicine, Philadelphia, PA 19140 and Boston University School of Medicine, Boston, MA 02118.

In the cat, ablation of visual cortical areas 17 & 18 within two days of birth is characterized by a reduction in retinal projections to the A-laminae and the maintenance of projections to the C-complex of the dorsal lateral geniculate nucleus (dLGN). Ablation of the same areas in adult cats is characterized by a modest increase in retinal projection density in each layer as the nucleus atrophies. The purpose of the present study was to define the transitional period between newborn-like and adult-like reorganization of retino-geniculate projections following visual cortex ablation (VCA). Areas 17 & 18 were removed from cats between the day of birth (P1) and P30 and from cats 6mo or older. Retinal projections were quantified following the anterograde transport of tritiated amino acids injected into an eye at least 6mo after the visual cortex ablation. As visual cortex was removed in increasingly older kittens (P1->P30) there was a progressively smaller reduction in retinal projection density in the dLGN A-laminae. Retinal projections to the A-laminae in P30 VCA cats were indistinguishable from those in adult cats that incurred visual cortex ablation. In a second group of cats, retinal projections were examined 1, 2 or 3mo after incurring VCA on P1. In all of these cats, the pattern and density of retinal projections were identical to those in P1 VCA cats which were examined in adulthood. These results show: 1) that retinal projections to dLGN can be modified by VCA only during the first postnatal month; and 2) that such reorganizations induced by P1 VCA occur during the first postnatal month. (Supported by NIMH #44647, NINDS #25196, and the Whitaker Health Sciences Fund.)

65.7

ANTEROGRADELY LABELED RETINOCOLLICULAR PROJECTIONS IN THE CAT EXHIBIT HIGH LEVELS OF GLUTAMATE IMMUNOREACTIVITY. Ljubomir Jojich and Roberta G. Pourcho*. Wayne State Univ., Detroit, MI.

Glutamate-like immunoreactivity has been reported previously in the perikarya of essentially all ganglion cells in the cat retina. This immunoreactivity may represent metabolic pools as well as transmitter glutamate. In order to determine whether elevated levels of glutamate are also present at the terminals of these cells, anterograde labeling was combined with postembed immunostaining of the superior colliculus.

Retinocollicular projections were identified by their content of anterogradely transported WGA-HRP which was visualized with DAB followed gold-substituted silver enhancement. Glutamate immunoreactivity was quantified from post-embed immunogold staining. The mean density levels of gold particles in retinal terminals were 3.2-3.9 times higher in retinal fibers and terminals than in postsynaptic elements, many of which are known to be GABAergic. Parallel sections that were processed for GABA-IR supported the postsynaptic and interneuronal localization of GABA.

The positive identification of retinal terminals by use of anterograde labeling and the high concentration of immunoreactivity in these terminals are consistent with a neurotransmitter role for glutamate in retinocollicular projections of ganglion cells.

65.8

TRANSECTION OF SUPERFICIAL OPTIC TRACT (SOT) IN HAMSTERS SPARES ANTI-PREDATOR BEHAVIOR BUT ELIMINATES ORIENTING TO VISUAL STIMULI. **Gerald E.Schneider*, Sonal Jhaveri & Amanda S. Battisti, Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.**

Transection of the superficial axons of the SOT at the level of the LGBd spares internal optic tract (IOT) fibers, which extend through the LGB. Deepest IOT axons project to the olivary pretectal nucleus and superficially in the superficial gray layer (SGS) of the SC (Schneider & Jhaveri,'83). Would selective elimination of the SOT have different consequences for the two types of SC-dependent visually elicited behavior, orienting and anti-predator responses (Merker, MIT thesis,'80; rev. Dean et al.,TINS,'89)?

We made unilateral SOT transections in 6 adult hamsters at the level of the LGBd. Control animals had the exposure alone. Testing was done 1-2 times/wk for more than 9 wk, at least 2 hr before the onset of the dark cycle for orienting, and less than 2 hr for anti-predator tests. During a 10 min test, a seed stuck onto a 1x1x2 cm piece of black rubber on the end of a wire handle was presented about 6 in from the head, in all parts of the field. Turning responses were recorded on diagrams of the visual field. Anti-predator behavior was studied while the animal foraged for seeds on a raised platform: the black rubber object was thrust into the temporal visual field about a meter from the head, and any change in behavior (e.g. freezing, running) was noted. The presentation was repeated on the other side.

For experimental animals with right SOT cuts, freezing, but no orienting responses, occurred for stimulus presentations in the upper temporal field of the left eye. Both types of responses were normal for the (unperturbed) visual field of the right eye. The data, with earlier findings, indicate that anti-predator behavior is subserved by a population of retinal axons terminating in the superficial part of SGS; these axons are not sufficient for orienting.

SUPPORT: NIH grants EY00126 (for behavior), and EY05504 (for anatomy).

65.9

NMDA AND NON-NMDA EXCITATORY AMINO ACID (EAA) RECEPTORS AND VISUAL RESPONSES OF CAT SUPERIOR COLLICULUS (SC) NEURONES. K.E.Binns and T.E.Salt*. Institute of Ophthalmology, University of London, London WC1H 9QS, UK.

EAAs such as L-glutamate may be neurotransmitters of retino-fugal and other visual pathways. We have examined the possible role(s) of NMDA and AMPA/kainate receptors in the responses to visual stimuli of neurones located in the superficial SC. Multibarrel pipettes were used to perform single-neurone recordings and iontophoresis of EAAs and their antagonists in cats anaesthetised with Halothane/Nitrous Oxide/Oxygen.

The antagonist CNQX was found to antagonise responses to iontophoretically applied kainate or AMPA on 14 neurones. Such currents of CNQX were found to antagonise neuronal responses to visual stimuli (drifting bars or gratings, or flashing spots) to 59% of their control values. The NMDA receptor antagonist CPP was applied onto 33 neurones. On 25 of these, visual responses were reduced to 57% of control values. On the remaining eight neurones, CPP either had no effect or potentiated visual responses, even though the antagonist blocked responses to NMDA on the same neurones.

These results indicate that EAA receptors of both the NMDA and kainate/AMPA receptor type may be involved in the mediation of responses of superficial SC neurones to visual stimuli, although the relative contribution of these receptor types may vary between neurones.

65.10

EFFECTS OF ANGIOTENSIN II (AII) ON SYNAPTIC POTENTIALS IN THE SUPERIOR COLLICULUS (SC) OF HAMSTER. R.D.Mooney*, M.-Y. Shi, Y. Zhang, S.V. Savage and R.W. Rhoades. Dept. of Anatomy. Medical College of Ohio, Toledo, OH 43699

The superficial layers of the SC have a high density of AII receptors, the function of which is yet unknown. *In vivo* results which we report in this meeting indicate that many SC neurons are influenced by AII and that the receptors mediating these effects are either postsynaptic or common to both retinotectal and corticotectal afferents. To test whether AII had postsynaptic effects, we made intracellular recordings from superficial SC neurons *in vitro* using an SC slice preparation to which the entire optic tract (OT) remained attached. To date we have tested 21 neurons, the average resting potential of which was -68.5 $\pm$ 9.1 mV (mean $\pm$ s.d.) and the average input resistance was 129.4 $\pm$ 58.5 MΩ. Electrical stimulation of the OT (500 μA, 75 μs) produced EPSPs with an average amplitude of 8.4 $\pm$ 1.3 mV. Bath-application of AII (10 μM) decreased the EPSP amplitude in 17 cells (81%) by at least 30% and increased the EPSP in one neuron. For the whole sample, AII reduced the EPSP amplitude to 60.4% of control (paired t-test t=8.1, $p<.001$). Application of AII produced comparatively little change in membrane properties of the neurons: input resistance decreased by only 4% on average and the resting potential increased by 3% to -70.5 mV. Multiple regression analysis showed that changes in EPSP amplitude were not significantly correlated with changes in either membrane potential or input resistance ($r^2=0.15$, $p>0.1$). The lack of a clear postsynaptic effect of AII suggests that it may be affecting some aspect of retinotectal and perhaps also corticotectal synaptic transmission. EY04170 and EY08015.

65.11

EFFECTS OF ANGIOTENSIN-II (AII) ON ACTIVITY OF VISUAL NEURONS IN THE SUPERIOR COLLICULUS OF THE HAMSTER. Y. Zhang*, R.D. Mooney and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

The superficial layers of the rodent superior colliculus (SC) contain AII receptors in relatively high density, but we have no information about how this hormone may affect the responses of collicular neurons. In this study, extracellular single unit recording and receptive field mapping techniques were used to assay the effects of AII upon the responses of SC cells *in vivo*. The visual responses of 77 visually responsive SC cells were quantitatively evaluated before, during, and usually after micropressure ejection of AII (10 μM); 71% of these neurons had their visual responses reduced by at least 30% by AII and in 13%, responses were reduced by >90%. The effects of AII upon responses evoked by electrical stimulation of either optic chiasm (OX) or visual cortex (CTX) were also evaluated. AII significantly reduced responses to OX stimulation in 50% of 101 cells tested and to CTX stimulation in 55% of 42 cells. The correlation in response decrements during AII application for 35 cells tested with both OX and CTX was highly significant (linear regression $r^2=.685$; $p<.0001$) indicating that the effect of AII was common to both pathways. We evaluated the ability of the AII-1 receptor antagonist DUP753 to block the effects of AII in 7 cells. When AII alone was applied, the responses of these neurons were reduced an average ($\pm$ s.d.) of 62.7 $\pm$ 28%. During concurrent ejection of AII and DUP753, the reduction was only 9.5 $\pm$ 18.4% (paired t-test t=6.6, $p<.001$). These results indicate that AII has strong suppressive effects upon the responses of visual SC neurons and that these effects might be mediated by AII-1 receptors. EY04170 and EY08015

65.12

EFFECTS OF SEROTONIN (5-HT) ON SYNAPTIC POTENTIALS OF NEURONS IN THE SUPERIOR COLLICULUS (SC) OF THE HAMSTER. M.Y. Shi*, R.D. Mooney, S.V.Savage, Y. Zhang, and R.W. Rhoades, Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

The results of previous studies from our laboratory concerned with the effects of 5-HT in the SC *in vivo* suggested that this amine influenced the responses of superficial layer neurons by both pre- (i.e. on retinotectal axons) and postsynaptic mechanisms. To better evaluate the relative contributions of these mechanisms, we developed an *in vitro* slice of the SC with the entire optic tract (OT) attached and recorded synaptic responses intracellularly to electrical stimulation of the OT near the chiasm. To date, we have tested 47 SC neurons. These cells had an average resting potential of -64.1 $\pm$ 6.3 mV (mean $\pm$ s.d.) and input resistance of 135.1 $\pm$ 56.6 MΩ. Electrical stimulation of the OT (500 μA, 75 μs) produced EPSPs that averaged 7.8 $\pm$ 2.3 mV. Bath application of 5-HT (100 μM) reduced the EPSP amplitude by at least 30% in 42 neurons (89%) and by at least 50% in 72% of the sample. The average reduction was 59 $\pm$ 25% (paired t-test, t=-14.8, $p<.01$). In contrast, the average input resistance of the 47 neurons was reduced by only 5.8% and membrane potential by only 2%. Changes in EPSP amplitude were not significantly correlated with those in input resistance or membrane potential ($r^2=.001$, $p>.36$). The profound effect of 5-HT on EPSP amplitude in the absence of any distinct change in the membrane properties of the postsynaptic neurons suggests a presynaptic site of action on retinotectal fibers. However, this does not rule out the possibility that 5-HT may also act postsynaptically on the receptor and/or channels related to the transmitter employed by retinal axons. EY04170 and EY08015.

65.13

EFFECTS OF SEROTONIN (5-HT) UPON RETINOTECTAL, CORTICOTECTAL, AND GLUTAMATE-INDUCED ACTIVITY IN THE SUPERIOR COLLICULUS OF THE HAMSTER. X. Huang*, R.D. Mooney and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

There is evidence that 5-HT_{1B} receptors are located on retinotectal axons and that other 5-HT receptor subtypes may also be present in the superior colliculus (SC). We used iontophoresis of 5-HT and single cell recording *in vivo* to test whether the effects of 5-HT on SC neurons were specific to optic inputs or shared by other pathways. Two strategies were used. For neurons activated by electrical stimulation of both the optic chiasm (OX) and visual cortex (CTX), the effects of 5-HT upon these responses were compared. For other cells, we compared the effects of 5-HT on visual responses and upon glutamate-evoked activity during synaptic blockade induced by continuous Mg^{2+} iontophoresis. Activity evoked by OX stimulation was suppressed by at least 30% in 96% of the cells tested (n=25) and by >90% in 64%; CTX-driven activity was reduced to these levels in 40% and 24% of the neurons, respectively (Wilcoxon matched pairs test: z=4.18, $p<.0002$). 5-HT suppressed visual activity (>30%) in 97% of the cells tested (n=40) as compared to only 35% of neurons for glutamate-induced activity (z=4.8, $p<.0001$). There was no correlation between the degree of response reduction for the two types of stimulation in either experiment. This suggests that even though 5-HT did suppress responses to CTX stimulation and glutamate-induced activity, the mechanism underlying these actions may be different and generally less potent than that affecting OX-evoked responses. EY04170 and EY08015.

65.14

THE ULTRASTRUCTURAL ORGANIZATION OF PROFILES LABELED BY ANTIBODIES TO GAMMA-AMINOBUTYRIC ACID (GABA) IN THE SUPERIOR COLLICULUS OF THE RABBIT. R.R. Mize*, R.H. Whitworth[1], B. Nunes-Cardozo[2], and J.J.L. van der Want[2], [1]Dept. of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112, and [2]The Netherlands Ophthalmic Research Institute, P.O. Box 12141, 1100AC, Amsterdam, Netherlands.

At least three types of GABAergic profile have been identified in the superior colliculus (SC) of the cat and monkey. It is not known whether these classes are found in other species. We therefore have studied the organization of GABAergic profiles in the SC of the rabbit. The ultrastructure of profiles labeled by an antibody to GABA was examined with electron microscope post-embedding immunocyto-chemistry using a secondary antibody conjugated to 10-15 nm gold particles that permits the unobstructed visualization of profile morphology.

Three distinct types of profile labeled by the GABA antibody were found in the rabbit SC. One type of putative presynaptic dendrite (PSD) was of large-calibre and had small, discrete clusters of pleomorphic vesicles at sites of synaptic contact with other, conventional dendrites. These PSDs received few synaptic contacts and none from axon terminals of retinal origin. A second type of putative PSD contained pleomorphic vesicles scattered throughout the cytoplasm. These PSDs were found within retinal glomeruli and frequently received input from retinal terminals. In addition, a number of putative axon terminals with more flattened synaptic vesicles were labeled by GABA. These profiles varied in morphology, but had a higher gold particle density than putative PSD profiles. Quantitative analysis of gold particle density revealed a consistent pattern of labeling over different profile types, even when the particle density was quite low. Our results suggest that there are three separate types of GABAergic synapse in the SC of rabbit as is the case in cat and monkey. These synapse types must represent a phylogenetically conserved organization common to many mammals. Supported by USPHS grant EY-02973.

65.15

SUPERIOR COLLICULUS NEURONS THAT PROJECT TO THE INFERIOR OLIVE ARE RELATED TO THE CHOLINERGIC PATCHES IN THE INTERMEDIATE GRAY LAYER OF THE CAT. C.J. Jeon* and R.R. Mize[2]
[1]Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis TN 38163 and [2]Dept. of Anatomy, Louisiana State Univ. Medical Center, New Orleans, LA 70112.

We have previously shown that superior colliculus (SC) neurons which project through the tecto-ponto-bulbar pathway to the cuneiform region of the midbrain tegmentum form discrete cell clusters. These clusters overlap the patches of cholinergic fibers found in the intermediate gray layer (IGL) of the cat SC. In this study, we have examined the relationship of these cholinergic fiber patches to other neuron projection systems in SC, including those that project to the medial accessory nucleus of the inferior olive (IO) and to the dorsolateral pontine gray nucleus (PGN). These neurons were labeled retrogradely with horseradish peroxidase (HRP). Some sections were counterstained with choline acetyltransferase (ChAT) immunocytochemistry. When injections were centered within the dorsal portion of the medial accessory nucleus of IO, two tiers of HRP labeled cell clusters were found in the contralateral SC. One tier was found in the upper IGL and the other in the intermediate white and deep gray layers. These IO projection neurons were mostly small to medium in size (11.4-32.9 μm in average dimater). Three to six clusters were found in each tier. In sections combining HRP and anti-ChAT labeling, the IO projection cells in the upper IGL clusters were distributed just above or surrounding the ChAT immunoreactive patches. Only a few HRP labeled cells were localized within the ChAT patches, although some cells outside the patches had dendrites extending into the patches. Neurons projecting to the PGN did not form any obvious clusters and were scattered both within and outside the ChAT patches. To conclude, the IO projection neurons in the upper IGL must be tightly coupled to cholinergic afferents in this structure. Supported by EY-02973.

65.16

THE SYNAPTIC ORGANIZATION OF THE RABBIT PRETECTO-COLLICULAR SYSTEM. AN ULTRASTRUCTURAL DEMONSTRATION OF GABAERGIC AND NONGABAERGIC PROJECTION NEURONS. B. Nunes Cardozo[1], J.J.L. van der Want[1] and R.R. Mize[2]. P.A. Apkarian* . [1]Dept. of Morphology, The Netherlands Ophthalmic Res. Inst., P.O. Box 12141, 1100AC Amsterdam, The Netherlands and [2]Dept. of Anatomy and Neuroscience Center, Louisiana State University, New Orleans, LA 70112.

Neurons in the pretectal Nucleus of the Optic Tract (NOT) which project to the superior colliculus (SC) have been studied using the retrograde transport of Cholera toxin subunit B conjugated to 7 nm gold particles (CTB-AU). Postembedding immunocytochemistry using an antiserum against GABA was applied on the same material to determine whether some or all of these cells contain GABA. A series of small pressure injections of CTB-AU were placed in the superficial layers of SC in a rostral-caudal sequence. Retrogradely labeled cell bodies in the NOT were visualized with silver enhancement of the gold particles. Retrogradely labeled neurons were found throughout the NOT, with slightly increased numbers in the rostral portion of the nucleus. The cells had large to medium sized cell bodies with electron lucent cytoplasm, extensive Golgi apparatus, and stacks of endoplasmic reticulum. Moderate GABA immunoreactivity was observed in some but not all of these labeled neurons. NOT neurons projecting to SC received retinal axon terminals (R) and from synaptic terminals with flattened vesicles thought to be of axonal origin (F profiles) and profiles with pleomorphic vesicles thought to be of dendritic origin (P profiles). These inputs were found on both the cell bodies and dendrites of these cells. Many of the F and P profiles showed GABA immunoreactivity. The GABAergic projection from the NOT to SC in rabbit is similar to that to that previously reported in cat and supports the idea that GABAergic neurons in NOT exert an inhibitory control over SC neurons, some of which may be involved in the relation of eye movements. Supported by USPHS Grant EY-02973.

65.17

OSCILLATORY PROPERTIES OF NEURONS IN A NEWLY DEFINED REGION OF THE PRETECTAL AREA Rowshanak Hashemiyoon and John K. Chapin*, Dept. of Physiology/Biophysics, Hahnemann University, Phila., PA 19102.

The pretectal area is typically subdivided into three regions (medial, posterior, and anterior) in which only a few nuclei are well-described. Here we provide evidence suggesting the existence of a previously undefined, physiologically distinct nucleus in the medial-most portion of the pretectal area of the rat. This nucleus is almond shaped, measuring about 1.5mm mediolaterally and 1.0mm dorsoventrally at its center. Neurophysiological recordings in this area are obtained either through single, drivable microelectrodes used in mapping studies in the pentobarbital anaesthetized rat, or through chronically implanted bundles of microwire electrodes allowing simultaneous recording of multi-single neurons. The most striking characteristic of the neurons in this area is their synchronous oscillatory behavior which is observed under both anaesthetized and awake conditions. These oscillations are exhibited most strongly in the dark. Increasing luminosity of light stimulation of the whole visual field of the contralateral eye causes a slight decrease in frequency and marked tonic decrease in the intensity of the oscillation. Some neurons in the lateral part of this region exhibit phasic excitatory responses to light stimulation of circumscribed parts of the visual field. The frequency of oscillation depends strongly on anaesthetic state: under deep anaesthesia the frequency occurs at approximately 10 Hz, and bifurcates to 20Hz in light anaesthesia and normal wakefulness. Furthermore, under urethane anaesthesia some neurons in the lateral part of this region exhibit 40 Hz oscillations. Finally, analysis of the dynamic properties of these oscillations indicates that they may exhibit fractal (chaotic) properties. To conclude, the wide-field luminance sensitive oscillatory properties of this subnucleus may play a role in visual processing in the pretectal area. *Supported by grants NS23722, AFOSR-90-0266, and AA06965 to JKC.*

65.18

IMMUNOCHEMICAL HETEROGENEITY IN THE TECTO-LP PATHWAY OF THE RAT. R.D. Lane*, D.M. Allan, C.A. Bennett-Clarke, R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, Ohio 43699.

The projection from the rat's superior colliculus to the posterior lateral nucleus (LP) of the thalamus includes neurons which are adenosine deaminase (ADA) positive (Miguel-Hidalgo et al., Brain Res. 476:189, '89). In the stratum opticum of the colliculus, the ADA neurons are primarily wide-field vertical cells. We have recently identified a population of calbindin immunoreactive neurons of similar shape which were likewise located in the stratum opticum. To determine if these two populations overlapped and whether the calbindin immunoreactive neurons projected to the LP, the fluorescent retrograde tracer, true blue was injected in the LP of rats which were subsequently processed for immunofluorescent localization of ADA and calbindin. The expression of ADA and calbindin among the true blue labeled tecto-LP cells was: 64.4 % calbindin, 1.1 % ADA, 14.6 % both calbindin and ADA. On the bases of the distribution of the ADA and calbindin terminal fields in the LP as well as the phenotypic expression of the two markers by the tecto-LP cells, two major tecto-LP subdivisions were defined. The ADA-calbindin positive subdivision, which projects to the rostral-medial portion of the LP, and the strictly calbindin subdivision, which appears to project to a broader area within LP. These results suggest that the rat is more similar to the cat (Abramson and Chalupa, J. Comp. Neuro. 271:397, '88, Hutsler and Chalupa, J. Comp. Neuro. 312:379, '91) than previously though in possessing multiple subdivisions of the tecto-LP pathway. Support: HL36573, EY04170, EY08015.

65.19

DIFFERENTIAL PROJECTIONS TO TECTAL LAMINAE FROM THE PERIHYPOGLOSSAL NUCLEI IN THE CAT. S. Higo*, T.Matsuyama , J.Kawano , and S.Kawamura, Div. of Morphological Neural Science, Kumamoto Univ. Graduate Sch. of Medical Sciences, Kumamoto 860, JAPAN.

Efferent projections of the perihypoglossal nuclei to the superior colliculus (SC) were examined with WGA-HRP method. Following WGA-HRP injection into the hypoglossal complex including the nuclei prepositus hypoglossi(PH) and intercalatus(INT), orthogradely labeled fibers were traced to SC via the reticular formation after crossing the midline. In SC, two terminal zones were found with marked contralateral predominance: one is in the superficial(laminae I and III-2), and the other in the deep(lamina IV)layers. SC injection restricted to the deep layers(laminae IV-VI)led to retrogradely labeled cells only in the caudal PH. By contrast, the injection confined to the superficial layers(laminae I and III-2) produced labeled cells exclusively in the rostral INT. These findings suggest that PH is related to the oculomotor function, whereas INT may participate in the visual sensory function, since the superficial layers of SC receive inputs from only visual sensory structures such as the retina and the visual cortex, and give rise to ascending projections.

66.1

MORPHO-FUNCTIONAL ORGANIZATION OF PERISTRIATE CORTEX IN *DIDELPHIS MARSUPIALIS*. S. Martinich*, C.E. Rocha-Miranda, M.A. Marrocos and M.G.P. Rosa. Instituto de Biofísica, UFRJ, Rio de Janeiro, Brazil.

Didelphis exhibits many general features of the basal mammalian stock in neural and other systems. Thus, it could be an appropriate model for studies of cortical evolution. We have examined the visual cortex of *Didelphis marsupialis* intending to formulate some general principles of cortical organization.

Multiunitary recordings in the peristriate cortex (PS) indicate that a single visual map specular to V1 exists adjacent to ST, markedly anisotropic and with large receptive fields. PS appears anatomically heterogeneous: patterns of commissural connections, myelin density and cytochrome-oxidase activity reveal periodical bands running rostro-caudally. Besides, three consecutive fields of patchy labelling are obtained in topographically related portions of PS after single WGA-HRP injections in ST, the two more caudal, possibly corresponding to V2 and V3, formed by strings of 3-4 foci. PS-tectal neurons also distribute in consecutive fields. The intervals between foci from the strings of ST-PS connections are much smaller than those between commissural bands, while a commissural belt parallel to the ST-PS border separates the two caudal fields from the rostral one. A postero-lateral PS region adjacent to ST and to the auditory cortex is identified as a separate area by the hodology. The more sensitive tracing with fluorescent dyes injected in PS and ST reveals a much higher degree of dispersion in intra as well as interhemispheric (homo and heterotopic) connections between these regions, but still with rough topographic correspondences.

From these results we suggest that the visual processing in the opossum, as in more specialized mammals, is segregated beyond ST in several cortical fields, some of them organized in a modular fashion. Moreover, the complex requirements of image analysis may impose on a species with few cortical areas more widespread ipsi and contralateral projections than those reported for other mammals.

66.2

FUNCTIONAL SEGREGATION BETWEEN THE LATERAL SUPRASYLVIAN AREAS AND AREAS 7/21A IN THE CAT: PERMANENT DEFICITS FOLLOWING LESIONS K. Krüger*, A. Groh, W. Kiefer Institute for Neuroinformatics, Dept. of Theoretical Biology, Ruhr-University Bochum, ND 04, Universitätsstr. 150, D-4630 Bochum, Germany

The behavioural functions of the suprasylvian cortex in the cat were analysed in combination with bilateral lesions of parts of the lateral suprasylvian areas (LSA, lesion 1), area 7 and area 21a (2) and a combination of lesions (1) and (2). We studied the contribution to pattern recognition with detection experiments and the contribution to depth perception, with the cats binocularly or monocularly, in a 2AFC procedure on a jumping stand. Both test series were performed before and after the lesion.

Our results revealed clear functional segregation between the LSA on one hand and areas 7 and 21 a on the other hand. Lesion (1) led to significant deficits in all test situations which were dependent on a relative velocity of moving patterns against the background. Binocular and monocular depth perception was impaired by more or less same degree. Lesion (2) led to modest but significant deficits of more or less same degree in nearly all test situations. A smaller lesion yielded significant deficits only when the background was moved and at the same time a low relative velocity occurred. The monocular depth perception was nearly unaffected, the binocular one was impaired by a factor of about 4. Lesion (3) roughly provoked a combination of the effects of the lesions alone in both test series.

We conclude that the LSA are involved in pattern recognition whenever it is associated with motion in combination with object-background interactions suggesting an involvement in the alnalysis of object- and self-induced motion. Interpretation of the results after lesion (2) is based on the conjecture that the mechanism of vergence movements or of binocular fusion might be impaired.

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66.3

NEURONS IN V1, V2, V3, AND V4 ENCODE INFORMATION ABOUT COLOR AND PATTERN OF CURRENT AND REMEMBERED STIMULI. J.W. McClurkin*, M–N. Chee–Orts, and L.M. Optican. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

Responses of neurons in four visual cortical areas (identified by receptive field size and electrode location) were recorded in a monkey trained to choose one of three parafoveal stimuli based on their color or pattern. The discrimination depended on a foveal cue that was turned off 67 msec before the stimuli appeared. The cue was one of 6 colored squares or 6 black & white patterns. The stimuli consisted of all 36 combinations of those 6 colors and 6 patterns.

The response of the neuron was modulated by the pattern and color of the stimulus on the receptive field, and by the pattern or color of the preceding cue. The relative amounts of information about pattern and color transmitted by a neuron depended on the discrimination required. When pattern was the cue, 10 of 33 neurons transmitted significantly more information about pattern than about color. When color was the cue, 5 of 33 neurons transmitted significantly more information about color than about pattern ($P < 0.05$). The other neurons transmitted the same amount of information about pattern and color.

Information developed continuously, but not uniformly, throughout the time-course of neuronal responses. A large amount of information was encoded in the initial 50 msec of the response. Most neurons also encoded a large amount of information in a second 50 msec interval, beginning 20-30 msec after the first. Information about pattern also developed more quickly than that about color in each area.

These results show that neurons in V1-V4 carry information about the color and pattern of both current and remembered stimuli. Furthermore, the relative amounts of information carried by a neuron depend upon the behavioral task. Finally, the development of information over time in different areas suggests that temporally modulated waves of activity may form a code for visual information (next abstract).

66.4

NEURONS IN PRIMATE V1, V2, V3, AND V4 SHARE THE SAME TEMPORAL CODES FOR COLOR AND PATTERN. J.A. Zarbock, J.W. McClurkin, and L.M. Optican*. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

In a discrimination task, neuronal responses were modulated by the color and pattern of both the current stimulus and the preceding cue (previous abstract). The response to each stimulus could be represented as the product of two waveforms, one for color and one for pattern. Feature-specific waveforms for each color and each pattern were isolated from the neuronal responses by a neural net. The product of these feature waveforms predicted the neuronal responses to stimuli with color and pattern combinations not used to train the neural net.

Feature waveforms were often similar for all neurons within a cortical area. To compare these waveforms across cortical areas, all the responses from neurons within each area were pooled. Waveforms encoding pattern were strikingly similar across all areas, irrespective of the behavioral task. Waveforms encoding color differed between cortical areas, depending on the behavioral task. The color waveforms V1 and V4 were different, and showed no task dependence. The color waveforms in V2 and V3 were different from those in V1 and V4 when pattern was the cue, but were identical to those in V1 when color was the cue.

Neuronal responses could also be decomposed into waveforms for the pattern and color of remembered cues, even though the cues did not fall on the receptive field of the neuron. The waveforms for the pattern and color of the cue were similar to those of the receptive field stimuli.

These results suggest that neurons convey information about compound visual features by multiplexing feature-specific messages together. Furthermore, the cue-dependence of color waveforms suggests that visual processing in V2-V3 can be altered to help achieve the objectives of different discrimination tasks. Finally, the invariance of pattern waveforms suggests that, at least for these stimuli, the processing of pattern information is completed in V1.

66.5

SELECTIVITY FOR FEATURES BEYOND ORIENTATION, COLOR, SIZE, AND SIMPLE TEXTURE IN THE PRESTRIATE AREAS V2 AND V4. E. Kobatake* and K. Tanaka. RIKEN, Japan

To uncover principles of representation of object's image, we have studied the stimulus selectivity of single cells in the latter areas of the ventral visual pathway, TE and TEO. Here, we went back to V4 and V2, to know to what extent and what kinds of integration takes place in these former areas. Recordings were made from anesthetized, immobilized monkeys (*Macaca fuscata, n=2*). Responsiveness of each cell was examined to both simple stimuli (bars, spots, and simple textures) and complex objects. If a cell responded maximally to a particular object, we determined the critical feature by reducing the complexity of the object's image. The responses to the best simple stimulus and to the best complex stimulus (the critical feature if identified) were then compared.

The majority of V4 cells (88% of 82 cells) responded to certain simple stimuli. No complex stimulus that might activate a cell more strongly could be found in 3/4 of these V4 cells (65% of the total), but for the remaining quarter (23%), response to the best complex stimulus was significantly larger than that to the best simple stimulus. For example, one cell responded to a purple disk, but a tail view of an eggplant was much more effective. By simplifying the image of the eggplant, we determined the critical feature as white concentric rings on a grey disk base. The remaining 12% of the V4 cells responded only to a particular complex feature but not to any simple stimuli. All but 2 out of 41 cells recorded from V2 were activated maximally by some simple stimuli. One of the 2 cells responded maximally to a tapering bar, which was reduced from a tip of pencil. Though many orientation and width of bars were carefully tested, the response to the best bar was still only 65% of that to the tapering bar ($p<0.05$).

We conclude that integration of object-information takes place in V4 and probably even in V2. Although it is difficult to quantify the complexity of the critical features, it appears that the complex critical features in V2 and V4 were simpler than those in TEO and TE.

66.6

COLOR SPACE TUNING OF MACAQUE AREA V4 NEURONS: CARDINAL DIRECTIONS ARE NOT PREFERRED. Karl Zipser, Robert P. Dolan and Peter H. Schiller*. M.I.T. Dept. of Brain and Cognitive Sciences, Cambridge MA

The color space tuning of neurons in macaque LGN is clustered along two cardinal directions, but area V1 neurons are tuned to intermediate directions as well (Lennie et al, 1984,1990). Psychophysical experiments suggest that color perception is coded along the cardinal directions of color space, and it is of interest to investigate whether neurons at high levels of color processing reconstruct this cardinal direction specificity. In this work, tuning in the isoluminant color plane was tested for single units in area V4 of the behaving macaque, using color gratings on isoluminant backgrounds. Only 37% of units responded preferentially to cardinal direction stimuli over non-cardinal directions tested, approximately chance for this study. These results provide no evidence for preferential cardinal direction tuning in V4.

As a control for responses to luminance artifacts present in the stimuli, units which responded well to low luminance contrast were not included in the sample. A small fraction of units responded best to color gratings in spatial orientations orthogonal to preferred orientations for black and white gratings.

66.7

ATTENTIONAL MODULATION OF RESPONSES IN AREA V4 OF THE MACAQUE. S.J. Luck*, L. Chelazzi, S.A. Hillyard, and R. Desimone. Lab. of Neuropsychology, NIMH, Bethesda, MD 20892 and Dept. of Neurosciences, UCSD, La Jolla, CA 92093.

A previous study of V4 cells found that attending to one stimulus within the receptive field and ignoring another can cause the response to the ignored stimulus to be suppressed beginning 30 msec after response onset. We have now studied V4 cells in a new paradigm modeled after tasks that have demonstrated attentional modulation of evoked potentials in humans using wider separation of attended and ignored stimuli. In this task, sequences of several brief (50 msec) stimuli were presented asynchronously at two locations in the visual field, and the monkey was required to detect a target square among nontarget rectangles in the sequence at one of the locations (i.e., the attended one), ignoring stimuli at the other. Of 28 cells recorded in one rhesus monkey, 16 showed significantly smaller responses to nontargets when they were ignored compared to the same stimuli when attended, consistent with prior studies. However, the suppression began much later than in earlier studies, beginning approximately 100 msec after response onset, and was found when both attended and ignored stimuli were located within the receptive field as well as when one set was inside and one outside. The results suggest a new source of late attentional modulation of V4 cells, operating over a wide spatial range. Supported in part by the Human Frontier Science Program Organization.

66.8

ELECTROPHYSIOLOGICAL STUDIES OF COLOR PROCESSING IN HUMAN VISUAL CORTEX. T. Allison*, A. Begleiter, E. Roessler, G. McCarthy, A. Nobre and D. Spencer. Neuropsychology Laboratory, VA Medical Center, West Haven, CT 06516, and Dept. of Neurology and Section of Neurosurgery, Yale University School of Medicine, New Haven, CT 06510.

Clinical evidence in humans and single-unit studies in monkeys suggest that a region of prestriate cortex is specialized for the processing of wavelength information. The human homolog of monkey V4 is thought to be located in the lingual and fusiform gyri lateral and inferior to striate cortex. Here we present the first electrophysiological evidence that this region responds preferentially to color information.

Thirteen patients were studied with electrodes (either subdural electrodes on surface cortex including the lingual and fusiform gyri, or depth probes in the occipital pole superior to the calcarine sulcus) chronically implanted for localization of epileptogenic foci. A 1-sec red or blue "adaptation" stimulus was followed by a red or blue "test" stimulus. We predicted that visual evoked potentials (VEPs) elicited by the test stimulus would be larger when preceded by an adaptation stimulus of the different color than when preceded by the same color.

About 50% of all recording sites on presumptive V4 showed a statistically significant color effect. Other areas had smaller percentages of sites showing a significant color effect: superior occipital pole, 30%; striate cortex, 11%; inferior temporal gyrus, 6%; inferior non-V4 cortex, 5%; all other sites, 6%.

Stimulation (5 sec trains of 50 Hz, 0.1 msec duration constant current pulses, 1-10 mA intensity) of some V4 locations elicited negative (desaturation of color) or positive (evocation of color) effects in the contralateral half field, usually crossing the horizontal meridian. By contrast, stimulation of striate cortex evoked quadrantic retinotopic sensations devoid of color change.

Supported by the Veterans Administration and by NIMH grant MH-05286.

66.9

NEURAL ENSEMBLE MEASUREMENT OF STIMULUS SELECTIVITY IN INFERIOR TEMPORAL CORTEX. P. M. Gochin*, M. Colombo, G. A. Dorfman, G. L. Gerstein and C. G. Gross, Dept. of Psychology, Princeton University, Princeton NJ 08544.

Inferior temporal cortex (area TE) in the primate appears to represent complex visual stimuli with some type of ensemble code. Thus, characterization of single unit properties is an insufficient approach to measurement of stimulus discrimination capacity. A method for measuring the stimulus discrimination capacity of neural populations was applied to small groups of neurons recorded in posterior area TE of monkeys performing a delayed matching-to-sample task. The results show that the shapes tested were well distinguished by a neural population code. The number of neurons and stimuli, type of stimuli, and measurement interval all influence ensemble discriminative capacity. Evaluation of the delay period activity shows that considerable information about stimulus identity persists once the stimulus is no longer visible, but that there is a significant degradation after only 1/2 sec. This leaves some question as to whether the post-stimulus activity in this region of TE is actually part of a short-term memory mechanism. Furthermore, there appeared to be little influence of persisting neural activity from the sample stimuli on the response to the matching stimuli. Thus, the primary function of posterior TE appears to be the representation of shape for objects currently in view.

66.10

THE HETEROGENEITY OF ADJACENT NEURONS IN INFERIOR TEMPORAL CORTEX. T.J.Gawne*, E.N.Eskandar†, and B.J.Richmond. Lab of Neuropsychology, National Institute of Mental Health, Bethesda, MD 20892. †HHMI.

There are three possibilities for encoding information in a small area of cortex: 1.Neurons could have identical characteristics, thus conveying redundant information, 2. neurons could give different responses to the same stimuli, thus conveying independent information, or 3. neurons could cooperate with each other to encode more information jointly than they do separately, i.e. synergistically.

We isolated the signals from 28 pairs of neurons from a single microelectrode, and separated the responses into signal (average response to each stimulus) and noise (deviation from the average). Using linear regression, a mean of only 18.7% of the magnitude of the signal carried by one neuron could be predicted from the magnitude of the signal of the other neuron, and only 22.0% could be predicted by including the temporal modulation of the signal. For the noise, the figures were 5.5% and 6.3% respectively, even less than for the signal.

When we computed the information carried about a stimulus by a neuronal response for each neuron separately and for both considered jointly, we found that adjacent neurons carried information that was an average of only 25.6% away from pure independence, similar to the results seen with linear regression. There was no evidence for strongly synergistic codes.

If adjacent neurons had identical characteristics, and shared the same set of inputs, then the noise on the outputs of these neurons would be highly correlated, and averaging the outputs would not reduce the signal to noise ratio. We propose that the independence of adjacent neurons both provides a rich description of many stimulus properties, and minimizes the amount of correlated noise, in a local group of neurons. These two principles may be a major constraint on the organization of inferior temporal, and possibly all, cortex.

66.11

EFFECTS OF SCOPOLAMINE ON A WORKING MEMORY TASK AND ACTIVITY OF NEURONS IN INFERIOR TEMPORAL (IT) CORTEX. E.K. Miller* and R. Desimone. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Scopolamine, a muscarinic antagonist, has been shown to impair performance in memory tasks. However, the site of action is not known. IT cortex is critical for normal memory and we have reported that responses of IT neurons carry information about stimuli held in memory, even when other stimuli intervene in the retention interval. We therefore tested the behavioral and physiological effects of scopolamine in the same task. The task was delayed matching to sample, with 0-4 intervening items between the sample and final matching stimulus. Two rhesus monkeys received systemic injections of either 10 ug/kg scopolamine or saline. Scopolamine impaired performance of the task with 0 intervening items, but the impairment was not exacerbated with increasing number of intervening items (and delays). In contrast to the striking behavioral deficit, there was little or no effect on either the stimulus selectivity of IT neurons or the mnemonic modulation of their responses. These results suggest that the site of action of scopolamine is not IT cortex or that scopolamine disrupted task performance by interfering with a non-mnemonic mechanism.

66.12

VISUAL RESPONSE PROPERTIES OF NEURONS IN THE TEMPORAL POLE OF MONKEYS. K. Nakamura, K. Matsumoto, A. Mikami* and K. Kubota. Dept. of Neurophysiology, Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

The visual response properties of neurons in the monkey temporal pole (area TG or area 36) were studied in monkeys (Macaca mulatta) by having the monkeys perform a visual discrimination task and a visual fixation task. Neurons (n=121) responded well to complex visual stimuli (photographs of human faces, monkeys, food, etc.). However, since simple stimuli (a red circle, a green square, a white bar, etc.) elicited either no or little response (n=24), we were unable to determine properties of their receptive fields with conventional methods using moving or stationary bar stimuli on a display (24 deg height, 34 deg width). Onset latencies of responses ranged from 100 to 300 ms (mean±SD, 163±43 ms). In most neurons, only a few stimuli elicited large responses. There was no noticeable effect on the stimulus-selective properties when the color of the visual stimulus was removed. Furthermore, even when only a portion of stimuli (for example, either a right or left half) was presented, the neuronal responses persisted. These findings suggest that the temporal pole neurons may be associated with cognitive process rather than with the extraction of some simple feature of visual stimuli, such as color or shape.

66.13

FOCAL SPATIAL ATTENTION SUPPRESSES RESPONSES OF VISUAL NEURONS IN MONKEY POSTERIOR PARIETAL CORTEX.

M.A. Steinmetz*, C.E. Connor and K.M. MacLeod, Bard Laboratories of Neurophysiology, Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205

We tested the effects of focal spatial attention on the responses of visual neurons in cortical area PG of monkeys performing a spatial match to sample task. Animals were trained to fixate a central target light while directing their attention to a transiently cued spatial location and to respond by releasing a behavioral key when a stimulus appeared at the cued location. In each trial, 1-5 stimuli were presented in random order at fixed locations in a 80° dia. grid centered on the fixation point. The effect of varying the locus of attention was examined by comparing receptive field maps generated with the animal's attention directed toward different grid locations.

Neural responses to stimuli presented at a given location were weakest when the animal's attention was directed toward that location. This suppressive effect was usually confined to one grid location, but occasionally extended over larger regions of the receptive field. These results may explain the "foveal sparing" observed when parietal visual neurons are studied using fixation target dimming tasks, and may reflect a role for PVNs in reorienting attention towards novel stimuli appearing outside the current locus of attention. Supported by NIH RO1 EY09129.

66.14

INTENDED MOVEMENT ACTIVITY OF AREA LIP NEURONS IN DELAYED DOUBLE SACCADE TASKS.

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The lateral intraparietal area (LIP) of the macaque's posterior parietal cortex contains neurons that maintain elevated activity during a delay before a saccade to the remembered location of a visual target (memory saccade task). This directionally tuned "memory" activity could encode the location of the sensory stimulus or the plan to shift gaze towards it. These alternatives can be distinguished by examining the memory activity while the monkey prepares to make two saccades. Further studies of these neurons in a delayed double saccade paradigm support the hypothesis that the memory activity of most LIP neurons reflects the monkey's plan to make the next intended saccade.

Two targets are flashed briefly while the monkey maintains fixation; after a delay the fixation spot is extinguished and two saccades are made in sequence in darkness to the targets' remembered locations. Most LIP neurons are active in the delay period if the first movement is in the preferred direction. They are much less active if a stimulus appears in the receptive field but the impending saccade is in a nonpreferred direction, and least active if the second stimulus appears in the receptive field but neither saccade is in the preferred direction. In the case where only the second saccade is in the preferred direction, most LIP neurons fire in the period between the two saccades. This activity almost invariably appears tens or hundreds of milliseconds after the start of the first movement, consistent with the sequence of motor plans in the monkey's behavior.

Many LIP cells are inhibited before memory saccades in the direction opposite their preferred one. We found that this inhibition also appears in the delayed double saccade task, almost exclusively when the next saccade is in the antipreferred direction. Thus LIP neurons maintain their directional tuning in the delay before two saccades, and their activity in general predicts the direction of the next saccade to be made.

66.15

A COMPETITIVE LEARNING MODEL OF THE NEURONS IN AREA 7A.

G. Ganis* and K. Zhang. Dept. of Cognitive Science, UCSD, La Jolla, CA 92093-0515.

A backpropagation model of how neurons in area 7a represent visual space in head coordinates was put forward in Zipser and Andersen (1988). Such a model was trained to transform retinal coordinates into head centered coordinates by combining retinal and eye position information. After training, the receptive field and spatial gain field of the hidden units were similar to those of 7a neurons. Similar results were obtained by Mazzoni et al. (1990) by using reinforcement learning. Both models have to use error signals for training the network. The objective of our study was to see whether it is possible to obtain similar results by using a biologically plausible hebbian-type learning. The procedure allows to control what the hebbian learning mechanism can learn by controlling the statistical structure of the input, which is similar to the 'errorless' competitive learning procedure discussed by Rumelhart and Zipser (1985). Such a control is achieved by adding a 'teaching' signal that is highly correlated with the features that have to be encoded by the weights. In our model, such teaching signal is provided by units tuned to head position like the ones which have been found in the hippocampus of primates (Rolls et al. 1991). The head position signal can induce a classification of the retinal position and eye position pairs according to a head-centered coordinate system. We found that the visual receptive fields obtained by keeping eye position constant often were very similar to the ones obtained by using backpropagation. We also found that the eye position signal often had a planar dependence on the fixation point (planar spatial gain fields). Finally, we examined the role of the teaching signal (head direction). We found that the planar spatial gain fields developed even without any teaching signal.

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66.16

RESPONSES OF INFERIOR TEMPORAL AND HIPPOCAMPAL NEURONS DURING AUDITORY-VISUAL AND VISUAL-VISUAL DELAYED MATCHING-TO-SAMPLE IN MONKEYS.

M. Colombo* and C. G. Gross, Dept. of Psychology, Princeton University, Princeton, NJ, 08540.

We recorded from 143 IT neurons and 67 H neurons in two monkeys trained to perform both AV and VV DMS tasks with a 5 sec delay. The auditory stimuli were a high frequency tone and a burst of white noise and the visual stimuli were a 4-lobed pattern and a picture of a monkey face.

More H units (38.7%) responded during the delay than IT units (23.8%). In IT more of the delay units exhibited selective responding, that is, responses in the delay following one stimulus and not the other, whereas in H a greater number of delay units fired nonselectively. The mean absolute spike rate difference from baseline for the IT delay units was 1.75 spikes/sec, compared to 4.56 spikes/sec in H. In examining the correlation between performance (50-75% vs 75-100%) and the occurence of delay units, we found a 220% increase in the number of delay units in the higher performance bracket in IT compared to a 30% increase in H.

The significance of these results for claims of electrophysiological correlates of visual memory will be discussed.

66.17

VISUAL RESPONSE PROPERTIES OF SINGLE NEURONS IN POSTERIOR CINGULATE CORTEX OF RHESUS MONKEY.

S.Y. Musil*, C.R. Olson, and M.E. Goldberg. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892, and Department of Anatomy, University of Maryland at Baltimore, Baltimore, MD 21201.

Recent experiments in this laboratory have demonstrated that neurons in posterior cingulate cortex (CGp) of the rhesus monkey carry signals related to the amplitude and direction of saccadic eye movements and to the orbital angle of the eye. They are also sensitive to visual influences as indicated by the fact that eye-movement-related activity is less robust in darkness than in visible surroundings.

We have now investigated the visual response properties of neurons in CGp. We have found that CGp neurons can be driven by large textured stimuli although they do not respond phasically to the presentation of small (0.2°) dim spots even when they are targets for eye movements or shifts of attention. We tested neurons with a variety of visual stimuli varying in size, brightness and spatial frequency. Neurons responded phasically to visual stimuli at a level that increased monotonically with size and brightness. Response strength remained constant across large variations of spatial frequency as long as total luminance was held constant. Neurons responded to the onset or offset of the stimulus regardless of whether it was static or moving and the mean latency of visual responses was 68ms. Response strength depended only to a limited degree on visual-field location. In approximately half of visual neurons, responses could be elicited by presenting a strong stimulus at any location in the visual field.

We conclude that the size and brightness of visual stimuli, rather than pattern, movement or location, are the primary determinants of visual response strength in posterior cingulate cortex.

67.1

RESPONSES OF SINGLE UNITS IN THE LSO-CIRCUIT TO AMPLITUDE MODULATED SOUNDS. P.X. Joris* and T.C.T. Yin. Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI. 53706

Binaural cells of the lateral superior olive (LSO) are sensitive to interaural time differences (ITDs) of amplitude modulated (AM) sounds. They receive excitation from the ipsilateral ear through spherical bushy cells (SBCs) and inhibition from the contralateral ear from globular bushy cells (GBCs) relayed through an inhibitory neuron in the medial nucleus of the trapezoid body (MNTB). We compared responses to AM stimuli, in terms of synchronization R as modulation frequency fm and SPL were varied, from the neurons relaying input to the LSO, from LSO cells themselves, and from auditory nerve (AN) fibers.

Cells providing the contralateral input to the LSO showed stronger envelope phase-locking than AN fibers and SBCs of comparable CF. For both SBCs, GBCs, and MNTB cells the decline in R with SPL parallels that of AN fibers. Also the cutoff frequencies of the modulation transfer functions (MTFs) cover the same range as AN fibers. Group delays obtained from the slope of cumulated phase-fm functions were orderly with CF and cell type.

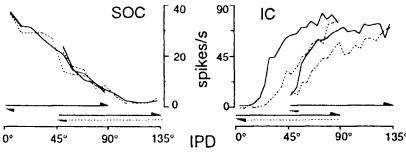
LSO cell responses to ipsilateral AM signals had R values that exceeded those of SBCs. When binaurally stimulated, with modulation of the signal in the contralateral ear only, LSO responses had higher R values than MNTB or GBC responses. On average, envelope synchronization of LSO resonses was less dependent on SPL than was the case for cells of the input pathways. For any given LSO cell the maximal R values and MTF cutoff frequencies for contra- and ipsilateral modulation were similar.

These results suggest that the anatomical specializations in this circuit may have evolved to enable interaural time comparisons.

67.2

RESPONSES TO TIME-VARYING INTERAURAL PHASE DISPARITY IN GERBIL SUPERIOR OLIVE: EVIDENCE FOR HIERARCHICAL PROCESSING M.W. Spitzer* and M.N. Semple. Dept. of Anatomy & Neurobiology, University of California, Irvine, CA 92717.

Interaural phase disparity (IPD) of low frequency tones is encoded by superior olivary complex (SOC) neurons, which provide a major source of binaural input to the inferior colliculus (IC). For most IC units, the response associated with a given IPD depends on the direction and range of IPD modulation (right), indicating sensitivity to temporal stimulus features (Spitzer & Semple '90 Soc. Neurosci. Abst. 16: 299.17). Responses to time-varying IPD stimuli were recorded from single units in SOC of anesthetized gerbils. Recording sites were localized histologically with respect to SOC subnuclei. For most SOC units, responses to IPD modulations are symmetric for both directions and contiguous for overlapping ranges (left), indicating insensitivity to temporal stimulus features. These data suggest a hierarchy in which initial encoding in SOC yields a faithful representation of instantaneous IPD. Higher order processing in IC generates sensitivity to changing IPD (Supported by NIDCD grant DC 00364).



67.3

FREE-FIELD STATIC AND DYNAMIC SOUND SELECTIVITY OF NEURONS IN PRIMARY AUDITORY CORTEX OF THE CAT. P. Poirier*, H. Jiang, M. Ptito, F. Leporé, J.-P. Guillemot. Univ. de Montréal and Univ. du Québec, Montréal, Qc, H3C-3J7.

Electrophysiological studies using free-field or dichotic stimuli in the primary auditory cortex of cats have revealed the existence of neurons sensitive to sound-sources in space and to moving stimuli. Similarly, behavioral-ablation studies in the cat suggest that the integrity of primary auditory cortex (A1) is essential for coding contralateral and frontal sound-sources. We report here free-field analyses of the azimuthal selectivity using fixed-sound-sources and stimuli simulating movement in different directions. Azimuthal space representation was obtained from single units in A1 of anesthetized and paralyzed cats. Stimuli, consisting of broad band noise bursts were delivered randomly in a double-wall quasianechoic chamber via 16 loudspeakers each separated by approximately 10°. In the static condition, most cells responded to contralateral hemifield stimulation. However, some cells were strictly ipsilateral. Moreover, two types of omnidirectional cells were found: some responded maximally at all static positions; others showed a clear inhibition in the center of the frontal plane. In the dynamic condition, most cells preserved their azimuthal selectivities. However, a minority of cells responded only to the onset of the dynamic stimulus independent ly of their static azimuthal selectivity. In addition, some cells preferred auditory motion in only one direction (either toward the contralateral or the ipsilateral azimuthal postions). *(supported by FCAR and CRSNG).*

67.4

NONLINEAR RESPONSES OF SINGLE UNITS IN THE BARN OWL INFERIOR COLLICULUS TO CONTINUOUSLY VARYING INTERAURAL PHASE AND INTENSITY DIFFERENCES. J. Wang* and A. Moiseff. Dept. of Physiology and Neurobiology, Univ. of Connecticut, Storrs, CT 06269.

Sound stimulus delivered through earphones with changing interaural phase and intensity disparities (IPDs and IIDs) mimics to some degree a moving sound source. We recorded single units extracellularly in the central nucleus of the inferior colliculus of anesthetized barn owls in response to continuously varying IPDs generated by a binaural beat (with a few Hertz difference between left and right ears) and continuously varying IIDs generated by a biphasic ramp, as well as to static IPDs and IIDs.

These units commonly demonstrated clear left-right asymmetry to binaural beat stimuli in which a unit showed higher overall sensitivity either when the left frequency was higher or when the right frequency was higher. These units also showed progressively higher overall firing rates as the beat frequency was increased progressively. The results suggest possible involvement of the neurons in encoding direction (left vs right) and velocity of moving acoustic sources. Responses of these direction-sensitive units showed the following significantly different features in comparison with their responses to static IPDs. First, the IPD tuning curves to dynamic stimuli were much sharper than those to static stimuli (the sharpness defined as IPD bandwidth at 50% maximum discharge rate). Secondly, dynamic stimulus also introduced a large shift in best IPD (the IPD at the maximum firing rate) with respect to the best IPD under static stimulus conditions. Finally, the maximum discharge rates within an IPD cycle (from -180 to 180 degrees) under dynamic stimulus conditions were higher than under static conditions. In response to continuously varying IIDs, these units each showed a preference to one of the four IID-varying modes (increasing or decreasing IID from left to midline, increasing or decreasing IID from right to midline). All these nonlinear response properties suggest that these neurons are well suited for processing relative changes in IPDs and IIDs thus they could encode direction and velocity of a moving acoustic source crossing a restricted spatial location.

This study was supported by NIH NIDCD DC00277 to AM.

67.5

NEURAL CORRELATES OF INTERAURAL TIME PROCESSING IN THE AUDITORY EVOKED POTENTIALS OF THE BARN OWL. O. Calvo and A. Moiseff*. Dept. of Physiology and Neurobiology, Univ. of Connecticut, Storrs, CT 06269

Studies have shown that the barn owl uses interaural time difference (ITD) to localize sounds in azimuth. The present study investigated the neural activity correlated with ITD processing using the brainstem auditory evoked potential (BAEP). In the owl, nucleus laminaris is the first structure where ITD processing takes place. We studied the changes in the short latency complex (first 2 to 6 msec) of the BAEP which is correlated with n. laminaris activity.

Barn owls were anesthetized with ketamine hydrochloride (4mg/kg). Digitized signals (one cycle sound wave) from 5 to 8KHz with ITD ranging from 0 to 1000 µsec were delivered through earphones. Averaged BAEPs were recorded in response to monaural (left and right), binaural and spontaneous stimuli, respectively. The sum of the left and right monaural responses was subtracted from the binaural response. The binaural interaction revealed by this calculation was identified as a waveform peak that had a consistent shape.

The latency of this binaural component increased by as much as 1 msec as the ITD increased from 0 to 1000 µsec. The longest latencies were observed in response to ITD >400 µsec. The amplitude of the component decreased as ITD increased. Maximum amplitudes were observed in response to ITD <200 µsec. Finally, the duration of this component decreased as much as 50% as ITD was increased from 0 to 1000 µsec. These results indicate that binaural interaction that occurs at biologically relevant ITDs (i.e., 0-200 µsec) can be distinguished from interactions at non-biological ITDs based on BAEPs. This technique provides a non-invasive method of assessing ITD processing.

This study was supported by NIH NIDCD DC00277 to AM; OC is sponsored by LASPAU.

67.6

FORWARD MASKING PROPERTIES OF NEURONS IN THE DORSAL COCHLEAR NUCLEUS. [1]J.A. Kaltenbach*, [2]R.J. Meleca, and [1]P. Falzarano
[1]Dept. of Audiol., [2]Dept. of Otolaryngol., Wayne State Univ., Detroit, MI 48201

Forward masking is defined psychoacoustically as a reduction in the audibility of a sound (a probe) which results when that sound is preceded by another sound (a masker). When two sounds occur close together in time the degree of forward masking can provide some measure of the temporal resolution of the auditory system. Previous studies indicate for example, that the response to a sound can be suppressed for periods of up to 250 milliseconds following a masking sound. However, the degree of masking is dependent on both the delay interval as well as the degree of spectral overlap between the masker and the probe. In an effort to describe these spectral and temporal aspects of forward masking neurophysiologically we tested the response of neurons in the DCN of hamsters to probe tones as a function of both the frequency of and time delay following a masker tone. The probe tone was a 10-20 msec tone burst set at CF while the masker was a 30 msec tone varied between 3 and 32 kHz. Both the probe and masker were held constant at 70-80 dB; for each masker frequency the delay between masker and probe was varied between 0 and 500 msecs. The response to the probe was plotted as a bar whose height was proportioned to the number of impulses counted during the period of the probe tone stimulus. The results indicate that the DCN displays a variety of forward masking effects. In some cases the response to a probe is suppressed (i.e. bar heights = 0) over a frequency range which diminishes systematically with increasing delay reaching a well-defined peak at some specific frequency. In others, the masking effect appears as a more circumscribed patch of suppression within the frequency vs. delay space, and many began after 20-30 msecs following masker offset. In either case the frequency at which the response is suppressed over the widest range of delays corresponds to the CF of the masker, and this appears to be independent of the probe frequency, provided the latter falls within the unit's response area. For most neurons the response to the probe can be suppressed out to 250 msecs, although a few can be suppressed out to 500 msecs. Much of this masking appears to be centrally mediated since the bandwidth of suppression commonly exceeds the bandwidth of the neurons response area.

67.7

SPECTRAL INTEGRATION IN TYPE IV UNITS OF DORSAL COCHLEAR NUCLEUS (DCN): LINEAR AND NON-LINEAR MECHANISMS. I. Nelken, E.D. Young, and B.J. May*. Center for Hearing Sciences, Johns Hopkins Sch. of Med., Baltimore, MD 21205.

Type IV units in DCN are suppressed by tones, but excited by wide band noise. This fact suggests that nonlinear spectral integration mechanisms are in operation in the DCN. We studied these mechanisms using a variety of stimulus combinations, including single tones, pairs of tones, noise bands, pairs of noise bands, and band-reject noise. Our goal was to understand how the inhibitory response to narrow band stimuli becomes excitatory with wide band stimuli.

At low signal level (<10-20 dB re threshold), type IV units sum stimulus energy across frequency in an approximately linear fashion. Narrow noise bands produce the same response as equal-energy tones and the response to a wide band noise can be constructed by adding the response to a band-reject noise and the response to a complementary noise band. At higher signal level, nonlinear mechanisms become important and these equivalencies no longer hold. The nonlinearity is demonstrated by facilitatory responses to two-tone stimuli, in which a unit is inhibited less by a two-tone stimulus than by each tone individually. Responses to noise bands show the same effect in that high-level noise bands inhibit units less than equal-energy tones.

These data are used to estimate parameters of linear and non-linear models of energy summation from which predictions are made of responses to other complex stimuli. These results form a basis for the study of complex auditory neurons which promises to provide predictive models of neurons' input/output characteristics.

Supported by grants from NIDCD and a Fulbright Scholarship.

67.8

FM PROCESSING IN CAT PRIMARY AUDITORY CORTEX (AI): TIMING OF NEURONAL DISCHARGES. P. Heil*, R. Rajan and D.R.F. Irvine. Dept. of Psychology, Monash University, Clayton, Vic 3168, Australia.

Neurons in cat AI are sensitive to the rate of frequency change (RCF) of frequency-modulated (FM) sweeps. As sweeps of different RCF also differ in their long-term frequency spectra, it is conceivable that spectral rather than temporal sensitivity might underlie the RCF sensitivity of many neurons in AI. To solve this issue the timing of discharges to FM sweeps was analyzed in AI of barbiturate-anesthetized cats. Sweeps had a fixed modulation range that included the entire excitatory frequency response area (EFRA) of the neuron.

In each case (N=350), plots of response latencies to sweeps of different linear RCF for a given direction of modulation versus sweep duration were optimally fitted by a straight line, intersecting the ordinate at a value near the neuron's response latency for high-level characteristic-frequency (CF) tones. Thus, discharges of a given neuron to unidirectional sweeps of different RCF were initiated at the same instantaneous frequency (effective F_i; derived from the slopes of the linear regressions). In contrast, effective F_is were always lower for upward than for downward sweeps, although such sweeps had identical long-term spectra. Discharges were evoked only when the frequency was modulated towards CF and not when modulated away from it, and were initiated before the modulation reached CF. Also, effective F_is were unaltered by changes in modulation range, as long as the beginning and ending frequencies fell outside the neuron's EFRA.

These data are incompatible with the idea that sensitivity to the long-term frequency spectra of FM signals might underlie RCF sensitivity in AI. Further, and in contrast to FM rate and directional sensitivity, the timing of FM discharges of neurons in AI largely reflect properties of auditory-nerve fibers (Sinex, D.G. and Geisler, C.D. Hearing Res.4:127-148, 1981). *Supported by the NHMRC of Australia and a Feodor-Lynen scholarship of the Alexander von Humboldt-Stiftung to P.H.*

67.9

RESPONSES OF SINGLE UNITS IN THE DORSAL NUCLEUS OF THE LATERAL LEMNISCUS AND PARALEMNISCAL ZONE OF AN ECHOLOCATING BAT. E. Covey*. Department of Neurobiology, Duke University, Durham N.C. 27710

Connectional evidence suggests that the dorsal nucleus of the lateral lemniscus (DNLL) and the paralemniscal zone (PL) play a role in binaural analysis. Responses of neurons in DNLL and PL were characterized in the echolocating bat *Eptesicus fuscus*, by presenting sounds monaurally or binaurally to awake animals, and recording responses of single neurons. Binaural responses were not evenly distributed within DNLL. Caudally, 86% of neurons tested were binaural; rostrally, 69% were monaural. This difference suggests that the rostral area is a separate functional subdivision, termed here the dorsal paralemniscal zone (DPL). Many binaural neurons in DNLL had complex response functions with inhibition at some interaural sound level differences and facilitation at others, suggesting restricted spatial receptive fields. In DNLL, DPL and PL, neurons with similar best frequencies were clustered together in a mosaic pattern rather than forming a precise tonotopic map. Discharge patterns in DNLL included phasic, sustained, and chopper types; in DPL, most units were phasic. In PL, the most common discharge pattern was the sustained type. When DNLL neurons were tested with pairs of tones, 26% showed a facilitated response to the second tone, with maximal facilitation at specific delays. Supported by NIH grant DC-00607.

67.10

NEURAL PROCESSING BY THE COCHLEAR NUCLEUS. J. Colombo, R.D. Frisina*. Otolaryngology Div., Univ. of Rochester Med. Ctr., Rochester, NY 14642-8629.

A crucial first step to auditory processing is to extract signals from background noise. One method is to improve signal-to-noise ratios in higher auditory centers as compared to the auditory nerve. Two mechanisms by which some cochlear nucleus (CN) unit types improve signal gain are: 1) by decreasing a unit's average firing rate in the presence of noise as compared with quiet and 2) by increasing a unit's synchrony in the presence of noise as compared with quiet. It is hypothesized that these mechanisms coexist in cells which receive inhibitory inputs, are unrelated, and are a function of different features of CN cells. The average-rate mechanism may be due to auditory spectral processing and thus a function of off-best frequency (BF) inhibitory input. The synchrony mechanism may be due to auditory temporal processing and thus a function of dendritic and post-synaptic membrane parameters. These mechanisms are being investigated by studying the physiology and anatomy of the CN unit types which receive inhibitory inputs, and by simulating their responses in computer models designed to emulate the neurons. [Work supported by Veda Inc., RICHS, and NIH-NIDCD-R29 DC00408.]

67.11

ROLE OF VENTRAL COCHLEAR NUCLEUS IN EYEBLINK CONDITIONING TO AN ACOUSTIC CS IN CONSCIOUS CATS. X.F. Wang,* C.D. Woody and E. Gruen. UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024.

Recordings made from a total of 393 units of the ventral cochlear nucleus showed short latency responses characterized by brief increases in discharge to 70 db click and hiss stimuli. After blink conditioning produced by forward pairing of click as CS with glabella tap and hypothalamic electrical stimulation (570-10ms ISI, see Hirano et al., Br. Res. 1987), followed 2.4 s by hiss as DS, the magnitude of response to both CS and DS increased. After pseudoconditioning, with the UCS preceding the CS, even greater increases occurred.

The results indicate that adaptations occur which promote transmission of auditory signals by neurons of the ventral cochlear nucleus. Although the behavior changed after conditioning to support discriminative elicitation of a blink CR by the forward paired acoustic stimulus, the activity at the ventral cochlear nucleus changed with sensitization and did not depend as the behavior on the order of pairing. In contrast the activity of neurons of the dorsal cochlear nucleus depended on the order of pairing and changed in such a way as to support discrimination of the CS from the DS.

(Supported by The Deafness Research Foundation.)

67.12

DYNAMIC FREQUENCY TUNING OF CAT AUDITORY CORTICAL NEURONS. H.R. Dinse* and C.E. Schreiner. Dept. Neuroinformatic, Theoretical Biology, Ruhr-Univ. Bochum, RUB, D-4630 Bochum and Keck Center for Integrative Neuroscience, UCSF, San Francisco, CA 94143.

We investigated the time dependence of frequency tuning in AI of Pentobarbiturate anesthetized cats. Auditory stimuli consisted of tone bursts of 50 to 200 ms duration and were presented via calibrated headphones. Data analysis was based on PSTHs. Tuning curves (frequency-intensity plots) were constructed on the basis of presentations of at least 10 frequencies around the CF at 10 different sound levels. Time dependence was analysed by time-slice technique, i.e. by computing sequences of frequency-intensity plots for successive time steps. In addition, frequency-time plots were computed for neuron responses recorded at given sound levels. Very similar to what was described for the visual system (Dinse et al. (1990), CINS 1:199), the sequences of tuning curves revealed substantial evidence for a profound time dependence of frequency tuning in auditory cortex. Sharpness of tuning increased over time and the overall shape of the tuning characteristics changed. In the frequency-time plots, frequency transitions from low to high frequencies, or vice versa became apparent. The time scale of these dynamics was in the range of 30 to 50 ms, 3 to 5 times faster than in the visual system. The results are discussed in respect to possible benefits of dynamically based processing of inherently time-variant signals. In addition, comparison between both modalities offer a unique way of differentiating modality specific modes of processing from those that are biased by constraints of cortical architecture and cortical processing principles.

Supported by the DFG Di 334/4-1 and by a Naval Research Grant (to C.S.)

67.13

IN VITRO ANALYSIS OF SYNAPTIC INTEGRATION IN THE MEDIAL SUPERIOR OLIVE. B. Grothe* and D. H. Sanes. Center for Neural Science, New York Univ., New York, NY 10003.

The medial superior olive (MSO) is known to be involved in processing interaural time differences, largely due to the coincidence-detection of excitatory inputs from each ear. However, there is now strong anatomical evidence for inhibitory afferents to MSO neurons in all investigated mammals and a clear inhibitory influence on response patterns in the mustache bat's MSO (Grothe et al., PNAS; 1992). Therefore, we investigated the synaptic integration *in vitro*, using a gerbil *(Meriones unguiculatus)* brain slice preparation (Sanes, J Neurosci 10; 3494, 1990). Electrical stimulation of either afferent pathway produced an excitatory response that was usually sufficient to evoke an action potential. The latency of synaptically evoked action potentals was level dependent and decreased up to 1 ms with increasing stimulus intensity. The ability to synaptically evoke an action potential during the refractory period was also level dependent. Although these response characteristics can be explained by excitatory interactions, there were clear inhibitory potentials evoked by both ipsilateral and contralateral stimulation. As monolateral stimulus intensity was increased, the inhibitory influences became more prominent, and finally blocked the evoked action potential. In addition, action potentials and excitatory postsynaptic potentials could be reversibly blocked by delivering the inhibitory transmitter glycine. Since these level dependent effects not only influence the probability of an action potential occuring, but also determine the timing of the MSO output, these results have implications for the processing of interaural time differences at higher levels. (Supported by NIH DC00540 and Deutsche Forschungsgemeinschaft)

67.14

CHANGES IN TONOTOPIC ORGANIZATION OF AUDITORY CORTEX (AI) OF C57BL/6J (C57) MICE ASSOCIATED WITH SENSORINEURAL HEARING LOSS J. F. Willott*, L. M. Aitkin, and S. L. McFadden. Northern Illinois Univ., DeKalb, IL 60115.

AI was physiologically mapped in anesthetized C57 mice by measuring thresholds for tone-evoked multiple-unit activity. Adult C57 mice exhibit sensorineural hearing loss beginning with very high frequencies (>40 kHz) by 2-3 mo. and progressing to lower frequencies (>20 kHz by 6 mo.). One-mo.-old mice exhibited tonotopic organization with high frequencies represented dorsocaudally, low frequencies rostroventrally, and middle frequencies (10-20 kHz) in between. As high frequency sensitivity was lost, characteristic frequencies (CFs) in the 10-13 kHz range became more numerous. In 2- and 3-mo.-olds these were largely confined to the "middle" region of AI; in 6- and 12-mo.-olds, many units in the dorsal region now had CFs in this range. Because of the "proliferation" of responsiveness to middle frequency tones (for which age-related threshold shifts were minimal), the mean CF threshold for AI units showed little change despite severe loss of sensitivity for high frequencies.

67.15

SINGLE TRIAL AUDITORY MAGNETIC EVOKED FIELDS. J. Stocker, M. Reite*, P. Teale. Neuromagnetism Laboratory, Dept. of Psychiatry, Univ. of Colo. Health Sciences Center, Denver, CO 80262.

We recorded left anterior (from near the ingoing extrema) auditory evoked fields (EF) and simultaneous C_z evoked potentials (EP) from 7 normal subjects using a 7 channel gradiometer. Stimuli were 20 sec duration 1 kHz tone pips delivered at an ISI ranging from 1100 to 2000 msec. Individual single MEG trials were examined for obvious visual evidence of the 100 msec latency M100 component in that trial, and categorized as yes (obvious single trial response at M100 latency) or no (no apparent response in single trial). Approximately 25 to 50% of single MEG trials contained obvious evoked responses in each subject. No apparent latency variation was noted. No EEG trials contained obvious N100 responses.

Averages of 25 MEG single trials containing obvious responses produced M100 components with amplitudes ranging from -408 to -730 femptoTesla (fT). Averages of 25 MEG trials containing no apparent responses produced same latency M100 components with mean amplitudes ranging from -36 to -292 fT. Paired t-tests showed the two groups to differ at $p<.001$. Averages of the corresponding two $N=25$ EEG trials recorded simultaneously produced N100 components whose amplitudes were the same, and independent of M100 amplitude. The two groups did not differ by paired t-tests.

These findings suggest MEG recordings contain fundamentally different information about temporal lobe auditory processing than do vertex recorded auditory EPs. Supported by USPHS MH47476.

67.16

INTRACELLULAR RECORDINGS FROM OCTOPUS CELLS IN COCHLEAR NUCLEI OF MICE IN SLICES. N.L. Golding, D. Robertson, R.E. Wickesberg, D. Oertel*. Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI 53711.

Octopus cells of the posteroventral cochlear nucleus carry acoustic information from the cochlear nuclei to the lemniscal nuclei forming one of the parallel pathways out of the cochlear nuclear complex.

We made intracellular recordings from identified octopus cells in slice preparations of the mouse cochlear nuclei. Results from cells injected intracellularly with biocytin revealed large dendrites that typically were oriented perpendicular to the path of auditory nerve fibers. These cells had large axons that could be followed in some cases into the stria of Monokow. Eight of the ten axons that were traced into the stria of Monokow had collateral branches. Of these, five had collaterals in the octopus cell area, four in the granule cell lamina and one in the deep layer of the dorsal cochlear nucleus. Shocks of variable strength to the auditory nerve root evoked EPSPs that were finely graded in amplitude and probably result from the convergent input from many auditory nerve fibers. EPSPs were monosynaptic and brief, with latencies of 0.7 ± 0.1 msec (mean ± SD) and durations of 1.2 ± 0.4 msec (mean ± SD). Inhibitory synaptic inputs were not observed. Input resistances did not exceed 25 MΩ and action potentials recorded in the cell body were small.

The dendritic orientation, the brief and finely graded EPSPs, and the intrinsic electrical properties suggest a role in detecting the coincidence of auditory nerve inputs, and accounts for the precisely timed action potentials at the onset of acoustic stimuli.

67.17

MECHANISMS UNDERLYING THE RESPONSE OF NEURONS IN THE CHICK N. MAGNOCELLULARIS TO STIMULUS TRAINS. Reyes, A.D., Spain, W.J*., and Rubel, E.W Bloedel Hearing Research Center, University of Washington, Seattle, WA. 98195 and The Seattle VA Medical Center.

Neurons of the Nucleus Magnocellularis (NM) relay information about the frequency and timing of auditory stimulus from the VIIIth nerve to higher centers in the chick auditory system. We are interested in the membrane properties of NM neurons that underlie this process.

Whole cell recordings were performed in transverse slices (50 - 70μm, 37°C) of the chick brainstem containing NM. Patch electrodes were guided onto the cells with the aid of Nomarski optics. Trains of brief (0.1 - 0.4ms), suprathreshold current pulses were injected into the cell at 50 to 500 Hz to mimic the arrival of synaptic current from VIIIth nerve afferents.

The ability of NM neurons to generate action potentials at the stimulus frequency was assessed by dividing the number of evoked action potentials by the number of current pulses in the train. The ratio of action potentials to current pulses decreased to less than 1 at stimulus rates greater than 200 Hz (ratio = 0.5 at 500 Hz). The action potentials were not randomly distributed throughout the stimulus train but occurred in regularly spaced clusters. Substitution of $MnCl_2$ for $CaCl_2$ in the extracellular solution (0 Ca solution) reduced the ratio of action potentials to current pulses to about 0.3 at all stimulus frequencies. In addition, the clusters of action potentials were more widely spaced. The spikes appeared to remain phaselocked to the current pulses. There was no change the input conductance and in action potential amplitude, duration, threshold, and afterhyperpolarization.

In voltage clamp, depolarizing steps from -60 mV evoked a fast activated, potassium current (threshold about -55 mV). Perfusion with the 0 Ca solution blocked a persistent component of the potassium current with an activation threshold at about -40 mV.

These data suggest that a Ca^{2+} dependent K^+ current may be important for sustaining discharge rate of action potentials during rapid VIIIth nerve volleys.

67.18

RESPONSE PROPERTIES OF SINGLE NEURONS IN THE VENTRAL COCHLEAR NUCLEUS OF AN ECHOLOCATING BAT: COMPARISON WITH HIGHER LEVELS. S. S. Haplea, J. H. Casseday* and E. Covey, Department of Neurobiology, Duke University, Durham, NC 27710.

Neurons in the ventral nuclei of the lateral lemniscus (VNLL) and the inferior colliculus (IC) of the big brown bat, *Eptesicus fuscus*, exhibit specializations for analysis of temporal patterns and for fine frequency discrimination. As a first step in determining the origin of these specializations, we recorded from single neurons throughout the anteroventral cochlear nucleus (AVCN) and the posteroventral cochlear nucleus (PVCN) of this species. All neurons in AVCN and PVCN had V-shaped tuning curves. PVCN neurons were more broadly tuned to frequency (average Q10dB of 6.4) than were AVCN neurons (average Q10dB of 10.1); ventral cochlear nucleus (VCN) neurons with phasic discharge patterns were more broadly tuned to frequency (average Q10dB of 4.3) than all other response classes. No level-tolerant filters of the type seen in the IC were present, and no neurons in AVCN or PVCN were as broadly tuned as those in parts of the VNLL. These data indicate that the sharpening or broadening of tuning curves seen at higher auditory levels does not originate exclusively in AVCN or PVCN.

For virtually all neurons in AVCN and PVCN, latency varied as a function of sound frequency or amplitude. However, some neurons in PVCN resembled neurons in the columnar division of VNLL (VNLLc) in that they were more broadly tuned than other neurons in the cochlear nucleus, had phasic discharge patterns, had little or no spontaneous activity, and at best frequency, the variability of response latency was small (<0.15 msec). However, their average latency varied as a function of frequency or amplitude. In contrast, the latency of VNLLc neurons remained constant over large variations in frequency or amplitude. These data suggest that phasic neurons in PVCN may be an input stage from which temporal resolution is increased in the lateral lemniscus. Research supported by NIH grants DC-00607 and DC-00287.

67.19

TEMPORAL AND SPECTRAL CONTRIBUTIONS TO DELAY SENSITIVITY IN AN FM BAT. W. G. Paschal* and D. Wong. Dept. of Anatomy, Indiana Univ. Sch. of Med., Indianapolis, IN. 46202

Delay-sensitive neurons in the auditory cortex of *Myotis lucifugus* exhibit facilitative responses to pulse-echo pairs at specific delays. In a previous study, the essential echo quarter (EEQ) was determined by dividing the echo (60 kHz in 4 ms) into four equal quarters (15 kHz in 1 ms). These quarters were presented at the same temporal relationships as found when the entire echo was presented at best delay. This study examined how the delay between pulse and EEQ and how the echo frequencies affected facilitation. When presenting the EEQ in the temporal position of the first quarter (or best delay), a decrease in half of the maximum response was observed. Non-EEQs presented in the temporal position of the EEQ did not evoke facilitation. These results demonstrate that not only are specific delays but also that the frequencies found within EEQs were both important for facilitation. By narrowing the FM sweeps of EEQs, the exact frequencies sufficient for maximum facilitation were found to average 8 kHz. These results may provide insight into the temporal and spectral contributions of delay sensitivity.

67.20

NEURAL MECHANISM OF DELAY SHIFT WITH AMPLITUDE VARIATION IN DELAY-SENSITIVE CORTICAL NEURONS OF *Myotis lucifugus*. H. Teng* and D. Wong, Dept. of Anatomy, Indiana University School of Medicine, Indianapolis, IN 46202

A previous study showed that delay-sensitive cortical neurons were tuned to best echo delay (BD) and best facilitation amplitude (FA) combination. Neurons were categorized into three classes according to how amplitude variation affected the BD. Over the entire range of FAs, *delay-shift* neurons systematically changed their BD, whereas *delay-tuned* neurons had a constant BD. *Limited delay-shift* neurons had a constant BD in a limited FA range, but shifted their BD at the upper and/or lower FAs. This study examined the neural mechanism underlying the BD shift.

Neurons shifted to shorter BD when pulse and echo amplitudes approximated each other, and to longer BDs at relatively high pulse FA or low echo FA. A delay shift often corresponded to a change in response latency which in turn corresponded to the amplitude used. This suggests that the amplitudes in pulse-echo pairs determine delay-tuning of individual neurons. The fact that facilitation latencies to the pulse-echo pairs correlated to latencies to individual sounds of the pair indicates that the synchronization of pulse and echo excitation is the mechanism of facilitation at the BD shift. This study provides further evidence of parallel amplitude channels in the auditory system of this FM bat.

BRAIN METABOLISM AND BLOOD FLOW I

68.1

EFFECTS OF FLUOROCITRATE AND CHEMICAL HYPOXIA ON ASTROCYTE FUNCTION IN VITRO. R.A. Swanson* and S.H. Graham. Dept. of Neurology, Univ. of California San Francisco and V.A.M.C., San Francisco, CA 94121.

Fluorocitrate (FC) and fluoroacetate (FA) inhibit the Krebs cycle enzyme aconitase and thus inhibit oxidative metabolism. These agents have been used *in vivo* to inhibit glial metabolism. We examined the effects of these agents and of the mitochondrial poisons sodium azide (AZ), antimycin A (AA), and 2,4 dinitrophenol (2,4DNP) on glutamate uptake and glutamine production in primary rat cortical astrocyte cultures. After 3 hours pre-incubation with these agents ^{3}H glutamate uptake was determined by measuring ^{3}H accumulation during 7 minutes incubation with 20uM unlabeled glutamate in Hank's solution, pH 7.2, 37C. In 5 trials, 25mM AZ, 10ug/ml AA, and 1mM 2,4DNP each reduced uptake by 50-70%, while 25mM FA and 1mM FC each reduced uptake by 10-25%.

Glutamine accumulation in the media was determined by HPLC after incubating cultures for 6 or 24 hours with the inhibitors plus 20uM glutamate. In 2 experiments 25mM AZ and 10ug/ml AA each reduced glutamine production by 75%, while 25mM FA and 1mM FC had no effect. No cell death resulted from incubation with the inhibitors for 48 hours. These findings suggest that (1) astrocytes *in vitro* can survive by glycolysis alone; (2) these astrocyte functions are only partially inhibited by blockade of oxidative metabolism; and (3) FC and FA cause a relatively weak inpairment of these astrocyte functions *in vitro*.

68.2

CHANGES OF CEREBRAL MICROVASCULAR HEMODYNAMICS BY ISOVOLEMIC HEMODILUTION. Shinn-Zong Lin*, Div. Neurosurgery, NDMC & TSGH, Taipei, Taiwan, ROC.

Changes of local cerebral microvascular hemodynamics by isovolemic hemodilution were investigated in adult Wistar-Kyoto rats. Local cerebral blood flow (LCBF), and microvascular red blood cell (M-RBC) and plasma (M-P) volumes in 14 brain structures were measured using 14C-IAP, 55Fe-labeled RBC's, and 14C-inulin, respectively. The results showed that in the control group the cerebral microvascular hematocrits (mHct) were 26-40% with mean of 35%, which was 69% of the mean systemic hematocrit (sHct = 51%); mean transit time of blood (Tb) through cerebral microvessels were 0.63-1.82 sec with mean of 0.94 sec; transit time of red cells (Tr) were 0.45-1.17 sec with mean 0.64 sec; and transit time of plasma (Tp) were 0.8-2.5 sec with mean 1.26 sec. In the hemodilution group, the mean mHct was 28%, which was 88% of the sHct (32%); LCBF's of the 14 areas were about 60% higher than those of the control animals; Tb was 0.62 sec (66% of the control group); Tr was 0.54 sec (84% of the control); Tp was 0.66 sec (52% of the control). These results indicate that isovolemic hemodilution results in a marked increase in the plasma (not RBC) flow velocity in cerebral microvessels, by which LCBF is increased. The increase in the plasma flow velocity may lower the extraction fraction of glucose and increase the clearance rate of waste products in the brain. Therefor, hemodilution may be feneficial for cerebral ischemia.

68.3

INITIAL CONCENTRATION OF BRAIN FREE GLUCOSE DETERMINES RATE OF ISCHEMIC GLUCOSE DISAPPEARANCE FOLLOWING RAPID BRAIN EXTRACTION IN RATS. T.A. Tishler, B.E. Stoller, J.L. Williams, M.D. Jarvik*, and W.H. Oldendorf. Neuroscience Laboratory, Veterans Administration Medical Center, West L. A., Los Angeles, CA 90073.

Six groups of male Sprague-Dawley rats were studied for brain free glucose concentrations present 5 sec. and 30 sec. after bisecting-decapitation. Group 1 was a control group and was untreated prior to decapitation. Group 2 was fasted prior to decapitation, but was otherwise untreated (the other five groups received food *ad libitum*). Group 3 received i.p. dextrose, 5% in water, 15 min. prior to decapitation. Group 4 received i.v. phloretin, 150 mg/kg, 4 min. prior to decapitation. Groups 5 and 6 received pentobarbital, 50 mg/kg i.p., 10 minutes before bisecting-decapitation. In order to maintain a normal body temperature of 37°C group 5 was externally warmed. Group 6 was not externally warmed and was studied at a hypothermic body temperature of 34°C.

At time of sacrifice, the rat head is simultaneously bisected and decapitated. Details of the method have been described (Oldendorf & Stoller, 1991; Oldendorf, Stoller, Tishler & Williams, 1992). Each half of the in situ brain is aspirated under -250 Hg pressure into a modified small plastic syringe (3 ml) and then extruded through a needle (20 g.) as a fine strand into a multidose sealed vial containing a relatively large volume (10 ml) of 2 M urea at 95°C. This extrusion into the 2 M urea takes place 5 seconds (first brain half) or 30 seconds (second brain half) after bisecting-decapitation. After cooling, sonication, and centrifugation of the brain homogenate, the supernatants are measured enzymatically for brain free glucose concentrations. An ischemic glucose disappearance rate was calculated from each pair of brain halves for each rat.

Results indicate the ischemic glucose disappearance rate is proportional to the concentration of glucose present in brain at time of bisecting-decapitation.

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68.4

CORTICAL CEREBRAL BLOOD FLOW (CBF) IS STEREOSELECTIVELY ENHANCED BY (+)2-METHYLPIPERIDINE: A MODULATOR OF CENTRAL NEURONAL NICOTINIC RECEPTORS J.L. Raszkiewicz*, J.W. Turek, and S.P. Arneric. Neuroscience, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064-3500

This laboratory has shown that activation of basal forebrain (BF) neurons elicits marked increases in cortical CBF which are mediated in part by neuronal nicotinic receptor interactions (Linville & Arneric, *Neurobiol. Aging* 12:503, 1991). Previous work suggested that (+)2-methylpiperidine [(+)2-MP] is a putative positive allosteric modulator of brain receptors labelled by [^{3}H]nicotine (Sloan et al., *Life Sci.* 37:1367, 1985), since it increased the specific binding of [^{3}H]nicotine. This study sought to determine whether the nicotinically mediated increases in cortical CBF can be further enhanced by a positive allosteric modulator of brain nicotinic receptors.

Male Sprague-Dawley rats (3-5 months) were anesthetized (urethane 1.5 g/kg, s.c.), paralyzed (d-tubocurarine, 0.6 mg/kg, i.m.), and arterial blood gases controlled. Changes in microvascular perfusion were monitored in the fronto-parietal cortex (1.0-2.5 mm rostral: 2.0-3.0 mm lateral to bregma) using laser-doppler flowmetry. The BF received unilateral electrical microstimulation (100 μA ; 2 msec pulses; 10 sec. trains) at 12.5, 25 and 50 Hz. Dose response curves were generated following i.v. administration of (+)2-MP or (-)2-MP [0.01 - 10 mg/kg] and compared to saline control. (+)2-MP enhanced both the resting (100 ± 10 to 146 ± 28 % control) and BF-elicited increases in cortical CBF (@ 25 Hz :153 ± 11 to 222 ± 23 % of resting CBF) while modestly increasing mean arterial pressure (MAP) from 76 ± 3 to 92 ± 7 mmHg at 1.0 mg/kg (N=5). In contrast, (-)2-MP appeared to diminish resting CBF, BF-elicited increases in cortical CBF and MAP (N=3).

CONCLUSION: Nicotinic transmission linked to BF neurons regulating cortical CBF is stereoselectively enhanced by (+)2-MP.

68.5

ENDOTHELIN-INDUCED VASOCONSTRICTION IN BRAIN SLICES: INHIBITION OF ACUTE AND TONIC CONSTRICTION. O. Sagher, W. Szeto, X-Q. Zhang, N.F. Kassell and K.S. Lee*. Dept of Neurosurgery. Univ. of Virginia, Charlottesville VA 22908.

Cerebral vessels were studied in submerged, *in vitro*, brain slices using computerized videomicroscopy. Artificial cerebrospinal fluid (ACSF) was superfused continuously over the slices during these experiments and drugs were applied by addition to the ACSF. Endothelin-1 (ET-1) was more potent than endothelin-3 (ET-3) in constricting the cerebral vessels, suggesting the participation of an ETA type receptor. Vasoconstriction by ET-1 was characterized by a large, acute phase of constriction followed by a smaller, tonic phase. Pretreatment with the kinase inhibitor, H7, or the lipase inhibitor, bromophenacyl bromide, inhibited both phases of constriction in a dose-dependent manner. Submerged, *in vitro*, brain slices represent a useful model system for studying the mechanisms of cerebrovascular regulation in pial and penetrating microvessels. The participation of kinase and lipase systems in endothelin-induced cerebrovascular constriction will be discussed.

68.6

ACTION OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE-38 ON PIGLET CEREBRAL MICROCIRCULATION. S. Tong, H. Parfenova, M.Shibata, W.M. Armstead, J.B. Schweitzer[*,a] and C. W. Leffler. Laboratory for Research in Neonatal Physiology, Departments of Physiology & Biophysics, and Pathology[a], University of Tennessee, Memphis, Tennessee.

The purpose of this study was to determine the effects of a synthetic pituitary adenylate cyclase activating polypeptide containing 38 amino acids (PACAP-38) on newborn piglet cerebral microcirculation. a-chloralose-anesthetized newborn piglets(1-5 days old) were equipped with closed cranial windows and diameter of pial arterioles were recorded. Cortical periarachnoid cerebrospinal fluid (PCSF) samples were collected for measurement of cyclic AMP by radioimmunoassay. PACAP-38 was applied topically for 10 min periods. cAMP levels in PCSF were 1.7 ± 0.5, 3.7 ± 1.0, 3.9 ± 0.9 and 4.8 ± 1.2 fold of the baseline levels at 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6}M of PACAP-38, respectively. PACAP-38 produced dose-dependent dilation of pial arterioles. The maximum increases of pial arteriolar diameter were $7 \pm 2\%$, $23 \pm 7\%$, $31 \pm 8\%$ and $36 \pm 6\%$ at 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} M, respectively. The dilation developed slowly reaching a maximum at 7-8 min. Therefore, the vasodilation and increases in cAMP caused by PACAP-38 correlate well. PACAP is a potent dilator of the newborn piglet cerebral circulation and the location of this peptide around cerebral vessels suggests a possible role in control of cerebrovascular tone.

68.7

A METHOD FOR MEASUREMENT OF BLOOD FLOW IN SMALL DURAL ARTERIES- EFFECTS OF TRIGEMINAL STIMULATION AND ANTI-MIGRAINE DRUGS. G.A.Lambert,* J.Michalicek and V.Gordon. Institute of Neurological Sciences, University of New South Wales, Sydney, Australia.

The pain of migraine and cluster headache is associated with changes in blood vessel diameter, blood flow and vessel-wall properties, but it is not known whether the vascular changes arise from the pain or cause it. It is possible that ant idromic release of vasoactive peptides from the trigeminal nerve produces the vascular effects. We have found that electrical stimulation of the trigeminal ganglion produces changes in carotid and cortical blood flow. Both responses are mediated via a reflex pathway traversing the greater superficial petrosal nerve, rendering an antidromic mechanism unlikely. However, the cortical microvasculature has little trigeminal innervation and may not contribute to migraine pain. We have therefore extended these studies to measure flow in the middle meningeal artery (mma), a small dural artery richly innervated by trigeminal sensory fibres and a possible source of pain in migraine. Cats were anesthetised with chloralose and electromagnetic flow probes placed around the common carotid arteries. Stimulating electrodes were placed in each trigeminal ganglion (Vg). One trigeminal root was sectioned through a tunnel in the petrous temporal bone. Each mma was exposed, kept surrounded by warm saline and optically isolated from the underlying cortex. Meningeal blood flow was measured using infrared photoplethysgmography, using fibre optic probes and a custom-designed detector circuit. Inhalation of CO_2 produced increased signal from the detector circuit and carotid occlusion produced a decrease, confirming that the system gives a measure of blood flow. Stimulation of Vg increased carotid and meningeal flow, ipsilateral to the stimulus site only on the side with an intact trigeminal root. Intravenous injection of dihydroergotamine (DHE, 40 µg/kg) and sumatriptan (80 µg/kg) produced complex changes in carotid and meningeal flow, but no appreciable or prolonged vasoconstriction. Neither drug significantly affected responses to trigeminal stimulation. We conclude that, although the mma is innervated with nerves containing vasoactive peptides, activation of these nerves produces little change in caliber of or flow in the artery.

68.8

KETANSERIN BLOCKS THE INHIBITION OF CHLORIDE EFFLUX FROM CHOROID PLEXUS INDUCED BY SEROTONIN OR ERGOTAMINE. C. Johanson*, P. Budhiraja and D. Palm. Dept. Clin. Neurosci., Program in Neurosurgery, Brown U. & R.I. Hospital, Providence, RI 02902.

Intraventricularly-administered serotonin decreases CSF secretion rate by a mechanism poorly understood. Because chloride (Cl) transport by choroid plexus (CP) is integral to CSF formation, we tested the hypothesis that serotonin inhibits Cl efflux from choroidal epithelium. Lateral ventricle CP was excised from adult rats and loaded with ^{36}Cl in artificial CSF (aCSF). The time course of efflux of ^{36}Cl from CP was measured at 37° C. Cl efflux was determined as a rate coefficient, k (sec-1). The k value for controls was $0.0251 \pm .002$. Serotonin in aCSF at a concentration of 0.1 µM did not alter Cl efflux, but at 1 and 10 µM it decreased the k value for Cl by 18 and 21%, respectively. Ergotamine, like serotonin, induces a potent agonistic response when it binds to the 5-HT1c receptor in CP. At 10 µM, ergotamine reduced Cl efflux rate by 24%. When the CP was preincubated with ketanserin (10 µM), neither serotonin nor ergotamine (10 µM) was able to reduce Cl efflux. The antagonist ketanserin alone in aCSF (10 µM) did not significantly alter the k value for Cl. As standard reference inhibitors, acetazolamide (0.1 mM), DIDS (disulfonic stilbene, 0.1 mM) and nialamide (1 mM) decreased Cl efflux from CP by 40%, 44% and 21%, respectively. We conclude that serotonin can alter Cl release from in vitro CP, and that this inhibitory effect is mediated by way of the 5HT1c receptor.
(Supported by NIH NS 27601 and by funds from R.I. Hospital)

68.9

EFFECT OF ANGIOTENSIN II ON BLOOD FLOW TO THE CHOROID PLEXUSES OF THE RAT. A. Chodobski,* J. Szmydynger-Chodobska, and C.E. Johanson. Dept. Clin. Neurosci., Program Neurosurg., Brown Univ. & RI Hosp., Providence, RI 02902

Blood flow to the choroid plexuses of the lateral (LCPBF), third (IIICPBF), and fourth (IVCPBF) ventricles was measured in male Sprague-Dawley rats anesthetized with pentobarbital sodium (50 mg kg^{-1}, ip) and artificially ventilated. ^{123}I-*N*-isopropyl-*p*-iodoamphetamine (IMP) was used as a tracer with assumption that IMP acts in the brain as a "chemical microsphere". Animals were infused iv with IMP at a constant rate for 30 sec to deliver ~50 µCi of IMP per rat. Reference blood samples were withdrawn using withdrawal pump. Rats were killed by decapitation. Plexuses were removed separately and weighed on electronic balance before gamma-counting. Angiotensin II (AII) was infused iv for 10 min before blood flow measurement at the following rates: 10, 30, 50, 100, and 300 ng kg^{-1} min^{-1}. Under control conditions, LCPBF was 3.19 ± 0.07 ml g^{-1} min^{-1}. This value was lower ($P<0.01$) than IIICPBF and IVCPBF (3.90 ± 0.17 and 3.95 ± 0.11 ml g^{-1} min^{-1}, respectively). LCPBF decreased 15% and 23% ($P<0.01$) during AII infusion at rates of 30 and 50 ng kg^{-1} min^{-1}, respectively. IVCPBF decreased 14% ($P<0.05$) during AII infusion at a rate of 30 ng kg^{-1} min^{-1}. IIICPBF was not affected by any dose of AII used. It is concluded that AII at moderate doses lowers both LCPBF and IVCPBF. The lack of changes of blood flow to the choroid plexuses with higher AII doses suggests a complex interaction of the peptide with these vascular beds. Supported by NIH Grant NS 27601.

68.10

THE ROLE OF VASOPRESSIN AND THE SYMPATHETIC NERVOUS SYSTEM IN MEDIATING THE INHIBITORY EFFECT OF ANGIOTENSIN II ON CSF FORMATION IN THE RAT. J. Szmydynger-Chodobska,* A. Chodobski, and C.E. Johanson. Dept. Clin. Neurosci., Program Neurosurg., Brown Univ. & RI Hosp., Providence, RI 02902

We have previously shown both in the rabbit and the rat that angiotensin II (AII) inhibits cerebrospinal fluid (CSF) production when administered at low doses into the cerebroventricular system. Centrally released AII both activates the sympathetic nervous system and increases vasopressin secretion. The present study was aimed at elucidating the role played by the above factors in mediating the effect of AII on CSF formation. The experiments were performed on rats anesthetized with pentobarbital sodium (50 mg/kg, ip) and artificially ventilated. CSF formation rate was measured using the ventriculocisternal perfusion method with blue dextran 2000 as an indicator substance. The cerebroventricular system was perfused with artificial CSF at a rate of 5 µl/min through cannulas introduced into both lateral ventricles, and CSF samples were collected from the cisterna magna. Control CSF production was 3.42 ± 0.08 µl/min. Central administration of AII at a rate of 6.3 pg/min decreased CSF production to 2.70 ± 0.15µl/min ($P<0.01$). When animals were pretreated with V_1-vasopressinergic receptor antagonist [$d(CH_2)_5$Tyr(Me)AVP, 10 µg kg^{-1} h^{-1}], the inhibitory effect of AII on CSF formation was abolished (3.78 ± 0.30 µl/min). Pretreatment with adrenergic receptor antagonists, prazosin (1 mg kg^{-1} h^{-1}), phentolamine (2 mg kg^{-1} h^{-1}) or propranolol (1 mg kg^{-1} h^{-1}), did not affect the response of CSF production to AII. All blockers themselves had no effect on CSF formation. It is concluded that the inhibitory effect of centrally released AII on CSF formation can be mediated by increased secretion of vasopressin. Supported by NIH Grant NS 27601.

68.11

PENTOBARBITAL ALTERS PLASMA AND RED CELL FLOW THROUGH CEREBRAL PARENCHYMAL MICROVESSELS. L. Wei*, T. Otsuka, D. Bereczki, V. Acuff, K. Pettigrew, C. Patlak, and J.Fenstermacher. Dept. Neurolological Surgery, SUNY/Stony Brook, Stony Brook, NY 11794-8122.

Pentobarbital (Ptb) lowers local cerebral blood flow, glucose utilization, and glucose influx throughout the brain and may alter the distribution of plasma and red cells through local parenchymal microvascular systems. This postulation was tested by measuring the distribution volumes of ^{55}Fe-labeled red cells (Vr) and ^{125}I-albumin (Vp) in parenchymal microvessels of the rat brain and combining Vr and Vp to yield blood volumes (Vb) and microvessels hematocrits (mHct). Tissue radioactivity was assessed by quantitative autoradiography. Ptb treatment increased Vr in most brain areas, decreased Vp in some, and raised mHct in most areas; Vb was, however, only changed slightly. Ptb treatment, thus, appears to lower the flow velocities of red cell (Fr) and albumin (Fp) through parenchymal microvessels, makes these velocities similar (in control rats: Fr > Fp), and does not decrease the number of perfused microvessels.

68.12

OPIATE MECHANISMS IN THE REGULATION OF CEREBROCORTICAL BLOOD FLOW DURING HYPERCAPNIC STRESS. N.T. Sandor, I. Horvath, E. Dora, G. Kunos* and A.C. McLaughlin NIAAA, Rockville, MD 20852.

The mechanisms underlying the increases in cerebral blood flow (CBF) and cerebral oxygen consumption ($CMRO_2$) in hypercapnia are still poorly understood. It is generally accepted that intracellular acidosis can be an important factor evoking increased CBF in hypercapnia and central neuronal mechanisms also play a role in regulating the cerebral circulation. The observation that the sympathoadrenal system is activated by hypercapnia suggests that adrenal medulla-derived substances could be involved in regulating the response of cerebral circulation and metabolism to CO_2.

We investigated the possible role of adrenal medulla-derived opioid peptides in cerebrocortical hypercapnic hyperemia in two groups of ketamine anesthetized rats: intact rats treated with different opiate antagonists and chronic adrenal demedullated animals

CBF was meaured in the cerebral cortex by the Kety-Schmidt technique. $CMRO_2$ was calculated from the product of CBF and the arterial/venous difference of oxygen.

In intact animals naloxone and the selective ∂-receptor antagonist, naltrindole, but not the μ antagonist, ß-funaltrexamine, reduced the hypercapnic increase of CBF and $CMRO_2$ by approximately 50%. Adrenal demedullation also decreased the hypercapnic CBF and $CMRO_2$ by the same value.

These observations suggest that endogenous opioid peptides, most likely adrenal medulla-derived enkephalins, mediate, at least in part, the increases in CBF and $CMRO_2$ during hypercapnia, via activation of ∂-opiate receptors.

68.13

DEVELOPMENTAL CHANGES IN IDENTIFIED CEREBRAL ARTERIOLES AND VENULES IN INDIVIDUAL MICE AT TWO AGES

G.D. Foltz, C.M. Rovainen* and T.A. Woolsey. Depts. Cell Biology & Neurological Surgery, Washington Univ. Sch. Med., St. Louis, MO 63110

Blood vessels over the left somatosensory barrel cortex in single animals were followed from 1 day after birth (P1) to adult (P30) by *in vivo* videomicroscopy through cranial windows. Mice were anesthetized with 1-2 mg/gm urethane IP or Metofane. FITC-dextran (200 mg/kg) was injected intracardially or intravenously. Surface vessels were imaged through cranial windows with an epiflourescence microscope and SIT camera at all ages. On P1 vessels were scanned through the transparent, intact dura after which the skull flap over the craniotomy was closed and the scalp sutured. At P8-P30 the same mice were rescanned through enlarged closed cranial windows. Individual vessels were identified at both ages for direct comparison in video montages. In P1 mice surface venules lack prominent deep sources and are small, irregular, and extensively connected with the primordial plexus of pial capillaries. As the cerebral cortex grows, large smooth-walled venules and distal venular branches are molded from the pial capillary plexus. The pattern of the middle cerebral artery and its major branches changes little. Arterial segments lengthen with cortical expansion. Substantial remodeling of collaterals in arteriolar loops through growth and regression occurs as flow shifts to supply the brain parenchyma. The pial capillaries so prominent at birth slowly regress by P14 and are absent in the adult. Velocities of flow through the same vessels can be measured during serial *in vivo* video recordings. This approach provides direct observations of dramatic growth and remodeling of cerebral blood vessels as the cortex develops.

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68.14

ANOXIA-RESISTANT TURTLE BRAIN HAS HIGH ASCORBATE LEVELS WITH REGIONAL AND SEASONAL DEPENDENCE.

M.A. Pérez-Pinzón*, E. Lee and M.E. Rice. Dept. Physiology and Biophysics, NYU Medical Center, New York, NY 10016.

Oxygen free radicals have been suggested to cause neuronal degeneration following an ischemic insult. One factor may be that the antioxidant defense system of mammalian brain is compromised during ischemia, when a decrease in the concentration of key antioxidants such as ascorbic acid occurs. In contrast, anoxia-resistant turtle brain maintains ascorbic acid levels for many hours during anoxia (Rice and Cammack, Neuroscience Let 132:141, 1991). Preliminary studies suggested that ascorbate levels in turtle brain might be higher than mammalian tissues, but with seasonal variability. To confirm this, we have mapped ascorbate content of turtle brain in summer and winter. Ascorbate was determined using reversed phase HPLC with an electrochemical detector set at +0.7 V vs Ag/AgCl. Turtle brain ascorbate levels in either season were significantly higher than those found in mammalian brain. Higher levels were found in the summer- and warm-acclimated turtles. The highest levels were found in olfactory bulb, cortex and dorsal ventricular ridge (DVR) (mean value 5.33 μmol/g). Optic tectum and cerebellum showed intermediate values (mean values 3.37 and 4.06 μmol/g, respectively). Lowest values were found in brain stem and spinal cord (mean value 2.03 μmol/g). A significant 30% decline in ascorbate levels occurred in olfactory bulb, cortex, DVR, optic tectum and cerebellum in winter-acclimated turtles, while no difference was observed in brain stem and spinal cord. These results point toward a metabolic regulation of brain ascorbic acid levels in turtle. **Funded by NIH grant NS-28480.**

68.15

ADENOSINE PARTICIPATES IN THE MEDIATION OF PIAL ARTERIOLAR DILATION INDUCED BY HYPOTENSIVE HYPOGLYCEMIA IN THE NEWBORN PIG. T.S. Park*, V.J. Ruth, A.R. Shah, E.R. Gonzales, J.M. Gidday. Department of Neurosurgery, St. Louis Children's Hospital, Washington University School of Medicine, St. Louis, MO 63110.

Studies in our laboratory and others have documented autoregulatory dilations of pial arterioles and increases in CBF in the newborn piglet in response to either systemic hypotension (HT) or hypoglycemia (HG). The present study was aimed at elucidating the role of the adenosine in mediating the hyperemic response to the combined condition of HT/HG. Anesthetized newborn piglets (n=7) were outfitted with closed cranial windows and the changes in diameter of two sizes of pial arterioles were monitored by videomicroscopy in response to HG (insulin; 25 U/kg i.v.) and subsequent HT to MABP = 30 mmHg. In 10 other piglets, the adenosine receptor antagonist 8-sulphophenyl-theophylline (8-SPT; 10μM) was superfused through the window prior to HG. Results are shown as % increase (mean±SEM) in baseline diameters:

	25-50 μm Diameter		*50-100 μm Diameter*	
	Control	8-SPT	Control	8-SPT
HG	53±8	26±7 #	26±4	15±5
HT/HG	81±7 *	51±6 #*	46±5 *	31±4 #*

[# = p <0.05 vs control group; * = p<0.05 vs HG alone]
Our results indicate that adenosine is involved in mediating the dilatory responses to both HG, and HT/HG.

68.16

SINGLE INWARD RECTIFIER CHANNELS IN RAT CEREBROVASCULAR SMOOTH MUSCLE CELLS. EFFECT OF HALOTHANE. H. Yan, D.A. Mathers* and E. Puil. Departments of Physiology and Pharmacology, University of British Columbia, Vancouver, B.C., V6T 1W5.

The effect of halothane on single inward rectifier channels was investigated in isolated inside-out patches of cerebrovascular smooth muscle cells (CVSMs) derived from the cerebral arteries of adult Wistar rats (200-250 g). CVSMs were enzymatically dispersed from the basilar, middle, posterior communicating, and posterior cerebral arteries and maintained in vitro for 2-4 days prior to use.

Isolated inside-out patch clamp recordings were made at room temperature (21-23 °C) using a List EPC-5 amplifier. Patch electrodes contained external solution (in mM): 140 KCl, 3 EGTA, 0.36 $CaCl_2$ and 10 HEPES. The cytoplasmic membrane face was bathed in a saline containing (in mM): 140 KCl, 3 EGTA, 3.1 $CaCl_2$ and 10 HEPES.

Inward single channel currents were ellicited by hyperpolarizing pulses from a holding potential of -30 mV. This channel had a mean conductance of 82pS and a mean open time of 0.7 msec in symmetrical 140 mM KCl solutions. At a holding potential of -90 mV, halothane (3-5% carrier gas, 1 L/min. compressed air) applied internally in 4 patches produced a 40% reduction in mean open probability of these inward rectifier channels.

68.17

ATTENUATION OF THE CBF-RESPONSE TO SENSORY STIMULATION BY SYSTEMIC ADMINISTRATION OF THE NO-SYNTHASE-INHIBITOR Nω-NITRO-L-ARGININE (L-NNA). U.Lindauer, A.Villringer, U.Dirnagl*. Dept.of Neurology, University of Munich, 8000 Munich 70, F.R.G.

We studied the role of the endothelium derived relaxing factor nitric oxide (NO) in the coupling of CBF to local demand. Wistar rats were anesthetized with Halothane in O_2/N_2O, tracheotomized, and artificially ventilated. Systemic arterial pressure (SAP), endexspiratory pCO_2, and body temperature were continuously monitored. The whisker barrel cortex was exposed by thinning the parietal bone and rCBF was continuously monitored through the translucent bone with Laser-Doppler flowmetry. Anesthesia was then switched to i.v. Chloralose. The whiskers were stimulated by deflection (3/s) for 1 minute. 10 mg/KG of L-NNA were injected intravenously. 10 min and 1 h after injection, whisker stimulation was repeated at the NO-blockade induced hypertensive SAP and with SAP reduced to baseline values by exposure of the lower body of the animal to hypobaric pressure. Results see table below. Normalization of the hypertensive SAP did not change the stimulation responses. NO-blockade led to rhythmic CBF-fluctuations (6-8/min). The extreme hypertension disrupted autoregulation in 3 animals after 15 minutes, and no 1 h stimulation was feasible. We conclude that blockade of the endothelial NO-synthase is not sufficient to abolish the CBF response to stimulation. Time dependent blockade of the brain parenchymal NO-synthase may further attenuate but not abolish the response.

	$\Delta rCBF_{stim}$ (%±SD)	SAP (mmHg±SD)	CBF (%±SD)
Baseline	17.9 ± 4.3 (n=5)	116 ± 17 (n=5)	100 (n=5)
10 min	12.0 ± 3.2 (n=5)	170 ± 6 (n=5)	98 ± 14 (n=5)
1 h	8.5 ± 0.7 (n=2)	154 ± 11 (n=5)	79 ± 6 (n=2)

68.18

EARLY HYPOPERFUSION OCCURS DURING SPREADING DEPRESSION IF NITRIC OXIDE SYNTHESIS IS INHIBITED. R.B. Duckrow* and D.C. Beard. Department of Neurology, The University of Connecticut Health Center, Farmington, CT 06030.

Nitric oxide may be an endothelium-derived relaxing factor which mediates cerebral blood flow (CBF) responses to neurotransmitters or carbon dioxide. The role of nitric oxide production in mediating CBF changes associated with spreading cortical depression (SCD) was assessed by inhibiting nitric oxide synthetase with L-nitroso-arginine-methy-ester (L-NAME) and inducing cortical spreading depression in awake rats. Rats were prepared for measurement of regional CBF while awake and restrained by a plaster hip-cast. After recovering from halothane/nitrous oxide anesthesia for one hour, SCD was induced by passing direct current through a bipolar electrode previously implanted in the lateral frontal cortex. CBF was measured using [14C]isopropyliodoamphetamine and quantitative auto-radiography. L-NAME, 30 mg/kg i.v., was given 15 minutes before induction of SCD and CBF was measured 90 seconds later. CBF was compared to rats given L-NAME without induction of SCD and to rats with SCD without treatment with L-NAME. In control rats and rats treated with L-NAME, SCD was accompanied by the usual phases of early brief hyperperfusion and delayed prolonged hypoperfusion. However, an additional brief phase of hypoperfusion (75% flow reduction) appeared before the early hyperperfusion phase in L-NAME-treated rats. This early hypoperfusion should coincide with the depolarization phase of SCD. This suggests that vasoconstriction caused by elevated K+ is normally balanced by simultaneous EDRF-mediated vasodilation involving nitric oxide production. (PHS NS24109)

68.19

CSF METABOLITES IN RELATION TO SEVERITY AND REVERSIBILITY OF NEUROLOGICAL DYSFUNCTION IN EXPERIMENTAL PORTAL-SYSTEMIC ENCEPHALOPATHY (PSE). G. Therrien*, J. Butterworth, C. Rose and R.F. Butterworth, Neuroscience Research Unit, André-Viallet Clin. Res. Centre, Hôpital Saint-Luc (University of Montreal), Montreal, Quebec, Canada H2X 3J4.

Previous studies suggest that PSE results from neurotoxic effects of ammonia and/or alterations of brain amino acids. In order to assess these possibilities, CSF was obtained using a cisterna magna catheter technique and analyzed for content of ammonia, lactate and amino acids at various stages during the development of (and recovery from) PSE. PSE was induced in portacaval shunted rats by administration of ammonium acetate which led to a reproducible neurological syndrome (loss of reflexes, stupor, coma, followed by recovery). Early PSE was associated with 2-3 fold increases of CSF tyrosine, tryptophan and phenylalanine. Further deterioration of neurological status was closely correlated with increased CSF ammonia, alanine and lactate, all of which returned to normal values following normalization of neurological status. No significant alterations of neurological status nor of CSF lactate or alanine were observed in sham-operated animals nor in shunted animals receiving equimolar amounts of sodium acetate (vehicle). No further increases of tyrosine, phenylalanine or tryptophan were observed at coma stages of encephalopathy. CSF content of GABA, was not altered at any stage during the development of PSE. These findings do not support a pathogenetic role for GABA nor monoaminergic dysfunction (resulting from increased availability of precursor amino acids) in late stage PSE. On the other hand, the observation of increased ammonia and of concomitantly increased lactate and alanine in CSF suggest decreased oxidation of pyruvate in the brains of these animals, implying that alterations of cellular energy metabolism and pH could be involved in the pathogenesis of severe PSE. Monitoring of CSF lactate or of brain lactate by ^{1}H-NMR could offer a useful means of evaluation of PSE in humans (Funded by MRC Canada)

68.20

INCORPORATION OF [^{3}H]ARACHIDONIC ACID INTO BRAIN PHOSPHOLIPIDS AND RELATIVE SPECIFIC ACTIVITY OF BRAIN PRECURSOR POOLS. D. Purdon, K. Washizaki, Q.R. Smith, J.J. DeGeorge, N.M. Appel* and S.I. Rapoport, Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892 and Food and Drug Administration, Rockville, MD 20857.

Our aim is to model the incorporation of fatty acid (FA) from plasma into brain phospholipid (PL) including turnover of precurser pools, i.e., free FA and acyl-Coenzyme A (AcCoA). [^{3}H]Arachidonic acid ([^{3}H]AA) was infused into pentobarbital-anesthetized rats to obtain a high blood level (50 μCi/ml). At varying times the infusion was stopped and animals killed by microwave irradiation. Brains were excised and PL extracted using a modified Folch procedure. PL, free FA and neutral lipid (NL) were purified from the lower organic phase by thin layer chromatography. AcCoA was isolated from the upper aqueous phase using a Sep-Pak and reversed phase HPLC. Brain FA and the FA component of AcCoA were analyzed as methyl esters by gas chromatography; comparison with associated radioactivity allowed determination of specific activities (SA). [^{3}H]AA incorporation into brain PL and NL was linear over the time course of the experiment (1 - 10 min) with most of the tracer being found in phosphatidylinositol and phosphatidylcholine. The SA of precursor pools (brain free FA and AcCoA) increased rapidly to a steady state ($t_{1/2}$ <1 min) with brain SA's being considerably lower than in plasma (<10 %). These results suggest that turnover of AA in AA-containing phospholipids is more rapid than previously assumed and that plasma unacylated AA makes only a relatively minor contribution compared to other sources of this FA such as plasma triglyceride and brain recycled AA.

68.21

FATTY ACID SYNTHETASE (FAS) IN FETAL RAT BRAIN AND LIVER DURING PLACENTAL ISCHEMIA. Z.Binienda, R.R. Holson, C.S. Kim*, T. Flynn, W. Slikker, Jr. and R.J. Feuers. NCTR/FDA, Jefferson, AR 72079 and CFSAN/FDA, Washington, DC 20204.

The effect of uterine vascular clamping on fetal brain and liver FAS activity was studied in nineteen Sprague Dawley rats in order to identify early and sensitive biomarkers of fetal hypoxia. Ischemic conditions were produced by ligation of uterine vessels at the ovarian and cervical end of one uterine horn for up to 20 min under halothane anesthesia. Fetuses of the other horn served as controls. Sham operated dams controlled for surgical stress. Fetuses were rapidly frozen in liquid nitrogen at the end of the ligation. Brains and livers were then transferred into dishes placed on dry ice, dissected, and stored at -80°C. FAS and total brain free fatty acids (FFA) were determined in tissue homogenates using a miniature fast analyzer or by gas chromatography. FAS activity rose in fetuses from the control horn (+36%,p=0.055) but did not change in the hypoxic horn (compared to shams). Compared to the fetuses from the control horn, FAS activity was lower in hypoxic fetuses (-34.4%,p=0.015). FFA rose in fetuses of both horns. The response of liver FAS to surgery and hypoxia was similar to brain FAS. The association between brain and liver FAS activity and brain FFA concentration may serve as a biomarker of hypoxia; however, the role of surgical stress has to be identified.

68.22

ALTERATIONS IN BRAIN GLUCOSE TRANSPORT PROTEINS, GLUT1 AND GLUT3, FOLLOWING HYPOXIC-ISCHEMIC INJURY IN THE IMMATURE RAT. S.J. Vannucci*, L.B. Willing, and R.C. Vannucci. Dept Pediatrics, Penn State Univ, Hershey, PA 17033.

Alterations in cerebral energy metabolism and glucose utilization due to perinatal hypoxia-ischemia (H-I) persist into the recovery period. The initial step in cerebral glucose utilization is the transport of glucose across the blood-brain barrier (BBB) and transport into neurons and glia. Two glucose transporter isoforms have been identified in brain: GLUT1 is concentrated in the BBB; GLUT3 is in neurons. The purpose of this study was to correlate known changes in cerebral glucose utilization with changes in GLUT1 and GLUT3 in an experimental model of perinatal H-I brain damage. 7 d postnatal rats were subjected to unilateral common carotid artery ligation followed by hypoxia (8% O_2); brains were removed following 24 h of recovery. Samples were removed from the middle of each cerebral hemisphere for Western blot analysis of GLUT1 and GLUT3. GLUT1 was increased by 49.5±11.1% ($p<.05$) in the ipsilateral hemispheres of brains with moderate to severe damage. GLUT1 in the contralateral (undamaged) hemisphere did not differ from control. Ipsilateral GLUT3 tended to be lower (89.1±10% of control) in these animals; contralateral GLUT3 was slightly increased (111±14% of control). These changes may account for our previous observation of increased brain glucose but decreased utilization in the damaged hemisphere and increased utilization in the contralateral undamaged hemisphere.

69.1

NIGROSTRIATAL ABERRATIONS INDUCED BY WEAVER GENE ARE PRESENT AT BIRTH. *B. Ghetti* and L. C. Triarhou*. Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

The weaver mutation (*wv*) leads to a loss of mesencephalic dopamine cells in the adult. By breeding female *wv/wv* mice with either +/+ or *wv/wv* males (which are occasionally fertile), we obtained, respectively, obligatory weaver heterozygotes and homozygotes, in order to study the effects of the mutation on the nigrostriatal system at birth. The genotype of the animals studied was further confirmed by examination of the cerebellar histology. Using tyrosine hydroxylase immunocytochemistry, a quasi-normal substantia nigra is seen in both genotypes; nonetheless, the characteristic deficit of dopaminergic dendrites previously described in adult carriers and homozygotes is evident at birth. In semi-thin sections of homozygous weaver midbrain embedded in Epon, extensive and diffuse cellular death is seen both in the ventral and dorsal mesencephalon. Moreover, a striking number of neurons are observed to undergo dark degeneration in the weaver striatum, just beneath the subependymal plate. These results indicate that the weaver effects on the nigrostriatal system are already taking place at birth, which parallels in temporal terms what is known for the cerebellum. Since midbrain dopamine cell loss further progresses in adult life, we conclude that the mesotelencephalic pathology is a combined developmental and degenerative disorder. (Supported by USPHS RO1-NS14426 and PO1-NS27613).

69.2

GENETIC AND PHYSICAL APPROACHES TOWARD THE ISOLATION OF THE MOUSE MUTANT GENE *weaver*. Anne E. Mjaatvedt* and Roger H. Reeves, Developmental Genetics Laboratory, Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Weaver (wv) is a mouse cerebellar mutant whose phenotype is characterized by a failure of granule cell migration and is detectable as early as the day of birth (P0). The gene has been mapped to distal mouse chromosome 16, but its product is not known. We are using the phenotype and chromosomal location to isolate *wv*. *wv* was previously mapped 1cM proximal to *Ets-2* on Chr 16 using a C57Bl/6 *wv/wv* X (C57Bl/6 *wv/wv* X MOLD/Rk) backcross. We mapped the *Ets-2* related gene, *Erg* to a position 0.94 cM proximal to *Ets-2* on more than 400 progeny of an intersubspecific backcross , and 1 cM distal to *wv* on the *wv* backcross, indicating the probable gene order, CEN--*wv-Erg-Ets-2* on Chr 16. Since 1 cM on this backcross is equivalent to less than 2Mb, chromosome walking using yeast artificial chromosomes (YACs) presents a viable strategy for isolating the *wv* gene. We obtained 2 YACs with 450kb inserts that hybridized with mouse *Ets-2*. These YACs were shown to contain *Erg* as well. The ends of one YAC have been isolated using retrofitted vectors (Hermanson,G.G. et al., '91) and will be used to screen other YAC libraries for overlapping YACs that may contain the *wv* locus. We have also isolated several cerebellum specific, mouse chr.16 specific genomic clones by screening a reduced somatic cell hybrid library with cDNA made from P0 cerebellum. These clones are currently being mapped and characterized. Using the complementary strategies of positional cloning and biological information, we hope to isolate the *wv* gene. Supported by PHS grants F32 HDO7501(AEM) and R01 HG00405(RHR).

69.3

BASIS OF GRANULE CELL LOSS IN THE MURINE CEREBELLAR MUTATION, MEANDER TAIL. K.M. Hamre* and D.Goldowitz, Dept. of Anat. & Neurobiol., Univ. of Tenn., Memphis, TN 38104

The autosomal recessive murine mutation, meander tail (gene symbol=*mea*), results in the near-total demise of a highly specific cerebellar population, anterior lobe granule cells. We are seeking to identify the underlying biological basis of *mea* gene action. Shimada and Langman (Am. J. Anat. 129: 247-260, 1970) have demonstrated that treating neonatal mice with a thymidine analog and mitotic poison, 5-fluorodeoxyuridine (FUdR), resulted in varying degrees of granule cell loss, particularly in the anterior lobe. Thus, we hypothesized that the *mea* gene works to target anterior lobe granule cells specifically by inhibiting their cell cycle. To test this hypothesis we carried out two sets of experiments. The first experiments used FUdR at varying embryonic and neonatal times to determine which injection regimen most closely mimicked the *mea* phenotype. Mice were injected with FUdR either embryonically (E) or postnatally (P) on E17 alone, E17+E18, E17+ P0, P0 alone, or P1+P2. The phenotype of mice exposed to FUdR on E17+P0 most resembled the *mea* mutation. The second experiments used BrdU (5-bromo-2'-deoxyuridine) to label mitotically active granule cells and to determine if alterations in the cell cycle exist in *mea* mice. P0 heterozygous and homozygous mice were injected with BrdU and allowed to survive for 1 hour. Tissue sections were processed for BrdU immunocytochemistry and labeled and unlabeled cells were counted in both the anterior and posterior lobes. The results demonstrated that while there were far fewer cells in the EGL in the anterior lobe in homozygous mice as compared to heterozygous mice, the labeling index in *mea*/*mea* mice is the same or higher than in controls. These results demonstrate that *mea* and control granule cells have equivalent S-phase dynamics and that inhibition of mitosis is unlikely to be an important factor in the the massive loss of *mea* granule cells. Thus, while FUdR treatment and the *mea* gene affect the same population of cells, the underlying mechanisms likely differ between these two developmental perturbations. Support: USPHS GR NS07323.

69.4

SUBSETS OF *MDX* MICE PURKINJE CELLS LACK IMMUNOREACTIVITY FOR CALCIUM BINDING PROTEINS D-28K CALBINDIN AND PARVALBUMIN. F. Rossi, D. Minciacchi, M. Sassoè Pognetto, F. Tempia and P. Strata*, Dept. of Human Anatomy and Physiology, I-10125 Turin, Italy

It has been recently shown that dystrophin, the protein product of the gene responsible for Duchenne muscular dystrophy, is normally localized at postsysnaptic densities of cerebellar Purkinje cells (PC) in the mouse. Although dystrophin is not present in *mdx* mutant mouse PCs (Lidov et al., 1990, Nature 348:725), no morphological or functional alteration has been reported, which could be referred to the lack of this protein. We are investigating the structural and functional features of PCs in *mdx* mice in order to clarify the role of dystrophin in the central nervous system. The cerebellum of young adult *mdx* mice, aged 30 to 60 days, appears to be fully developed with no abnormalities in foliation and cytoarchitectonics. By contrast, immunocytochemical staining for D-28k calbindin and parvalbumin, which normally stain the whole population of PCs, reveals that subsets of these neurones are not labelled in *mdx* mice. Immunonegative neurones are grouped in clusters with sharp boundaries, distributed throughout the whole cerebellum, giving a patchy appearance to the labelling of the cerebellar cortex. The lack of immunoreactivity for these calcium binding proteins suggests that an abnormal calcium homeostasis, which has been proposed to play a central role in the pathogenesis of Duchenne muscular dystrophy, is also present in a subpopulation of *mdx* PCs, and may affect their functional properties. Ultrastructural investigations are now in progress, aimed at determinining possible abnormalities in the synaptic investment of *mdx* mice PCs, which may be related to an altered calcium homeostasis. (Supported by Telethon)

69.5

CO-LOCALIZATION OF TYROSINE HYDROXYLASE AND ZEBRIN IMMUNOREACTIVITIES IN PURKINJE CELLS OF TOTTERING AND TOTTERING-LEANER COMPOUND HETEROZYGOUS MUTANT MICE.

L.C. Abbott* and J. A. Heckroth. University of Illinois, Dept. of Vet. Biosci., Urbana, IL 61801 and Indiana University School of Medicine, Terre Haute Center for Med. Ed., Terre Haute, IN 47809.

Previously we reported that TH-immunoreactive (IR) Purkinje cells in adult tottering (*tg/tg*) and tottering-leaner compound heterozygote (*tg/tg^{la}*) mice are organized into rostrocaudal stripes through all lobules of the cerebellar vermis and hemispheres (Heckroth, J.A. and L.C. Abbott, Neurosci. Abst. 17:159). This pattern of TH expression in the mutant cerebella bears close similarity to the reported pattern of expression for the Zebrin molecule in the cerebella of normal mice. In the present study we report, using two color immunohistochemistry, that the rostrocaudal stripes of TH immunoreactivity correspond with the rostrocaudal stripes of Zebrin immunoreactivity in both *tg/tg* and *tg/tg^{la}* adult mice. The majority of Purkinje cells in the mutant mice that express TH-IR also express Zebrin-IR. However, there are a few cells in the rostrocaudal stripes that appear to express only TH or Zebrin immunoreactivity, but not both. Abnormal Purkinje cell function may be involved in production of the motor system dysfunction exhibited by these mutant mice. The relationship between abnormal gene expression in sagittal stripes of Purkinje cells and the behavioral ataxia and intermittent movement disorder observed in *tg/tg* and *tg/tg^{la}* mutants remains to be determined. (Supported by BRSG Funds to L. C. Abbott (ILLB-44-1230) and J. A. Heckroth (S-507-RR5371).)

69.6

ROSTRAL CEREBELLAR MALFORMATION, A TRANSGENIC INSERTIONAL MUTATION: GENETIC AND HISTOLOGICAL ANALYSIS. L.A. Rund*, R.T. Bronson, B.B. Boyer and L.P. Kozak University of Illinois, Urbana, IL 61801 and The Jackson Laboratory, Bar Harbor, ME 04609.

We have identified a transgenic insertional mutation which is allelic to rostral cerebellar malformation (*rcm*), a spontaneous cerebellar mutation in the mouse mapping to Chromosome 3. Mice homozygous for the transgenic insertional mutation, *rcm^{1g}* have a tipsy, uncoordinated walk and a structurally altered cerebellum. At the gross level the anterior folia are reduced in size while the posterior folia appear largely unaffected by the mutation. In the adult cerebellum, disruptions in the laminar structure are evidenced by irregularities in the Purkinje cell monolayer, ectopic displacement of the granule cells into the molecular layer and protrusions of molecular layer into the internal granule cell layer. During postnatal development aggregates of external granule cells migrate across the molecular layer, through the Purkinjé cell layer and into the internal granule cell layer. Labelling of cells in the S-phase of the cell cycle with bromodeoxyuridine showed that granule cells within the aberrant aggregates as well as cells which had already migrated into the internal granule cell layer were dividing. These characteristics of the abnormally migrating cells in the *rcm^{1g}* mutation suggest features of folia development, as if multiple regions in the external granule cell layer have received signals to form folia. Accordingly, one interpretation of the data is that the *rcm* gene is involved in signalling folia formation and that the mutant phenotype is a genetic form of cerebellar micropolygyria.

Making use of the molecular tag provided by the mitochondrial uncoupling protein transgene, we have cloned an 8 kb piece of DNA flanking the transgene insertion site. Using an interspecific backcross panel this clone mapped to the distal portion of Chromosome 3, linked to *Amy-1* and *Mpmv-9*. This is the same region to which the spontaneous *rcm* mutation maps. This insertional mutation provides an excellent opportunity to identify a gene or genes controlling cerebellar development.

69.7

RETINAL PIGMENT EPITHELIAL CELL PROLIFERATION RATES IN VITILIGO MUTANT MICE. M. Ruiz, M. Tang and R.L. Sidman*. Division of Neurogenetics, N.E. Regional Primate Research Center, Harvard Medical School, Southborough, MA 01772-9102.

In vitiligo (vit/vit) mutant mice, the retinal photoreceptor cells begin a life-long progressive degeneration beginning at about postnatal day 60 (P60). However, pigment epithelial (PE) cells appear both functionally (Kosaras, et al., this issue) and histologically (Smirnakis, et al., ARVO, 1991) abnormal much earlier. The present study examines the proliferation behavior of retinal PE cells. Cells in mitosis were selectively stained with R3 antibody (gift of U. Drager) and rhodamine-labelled secondary antibody in Carnoy-fixed PE whole mounts from wild-type (+/+) and vit/vit mice on the C57BL/6J strain background at 5 time points: P0, 2, 4 , 6 and 8. For +/+ mice the total counts for R3-labeled PE cells per whole mount were 94, 82, 102, 54 and 2, respectively. Corresponding counts for vit/vit mice were 60, 282, 329, 212 and 8, thus suggesting a shift in the amplitude and timing of peak cell proliferation postnatally in the mutant PE. The rates of DNA synthesis in PE cells were studied by injecting +/+ and vit/vit mice at P0 with 50 mg/kg of bromodeoxyuridine (BrdU) intraperitoneally one hour before sacrifice. PE whole mounts were stained with fluorescein-conjugated anti-BrdU primary antibody. Total number of labelled cells were 816 in +/+ and 1170 in vit/vit, thus resulting in a DNA synthesis to mitosis ratio of 8:1 for +/+ mice and 20:1 for vit/vit. This ratio distortion and the difference in mitotic cell number between mutant and control mice as measured with the R3 antibody, suggest a gene-controlled abnormality in cell cycle kinetics in vit/vit mutant mice.

Supported by NIH Grant EY06631.

69.8

PHAGOSOMES IN RETINAL PIGMENT EPITHELIAL CELLS OF VITILIGO MUTANT MICE. B. Kosaras*, S.W. Smirnakis, M. Tang and R.L. Sidman. Division of Neurogenetics, N.E. Regional Primate Research Center, Harvard Medical School, Southborough, MA 01772-9102.

The recessive vitiligo mutation maps to chromosome 6, close to or allelic with microphthalmia (Tang, et al., in press). The vit/vit pigment epithelium (PE) is abnormal before birth. Photoreceptor cells differentiate normally, but after postnatal day 60 (P60) their outer segments break up and form lamellar debris between PE and neural retina. Photoreceptor cells degenerate progressively for the next year or more. We ask here whether functional interactions between PE and neural retina are already affected at P23 and P36, when photoreceptor cells are still intact. Wild-type (+/+) and vit/vit mice on the C57BL/6J strain were perfused with aldehyde fixative at 3 time points in the diurnal cycle: in weak dawn light, 1.5 hr after room lights turned on, and 10.5 hr later, just before lights went off. We counted phagosomes in ~37 PE cells per specimen in electron micrographs. At P23 +/+ mice averaged 3.3 phagosomes/cell profile at the first two time points, and decrease to 1.9/cell at the third while vit/vit mice averaged 0.5 phagosomes/cell profile at the first two time points and 0.07/cell at the third. P36 +/+ mice showed about 2/3s as many phagosomes as the younger +/+ control. In contrast, phagocytic activity in vit/vit mice was greater at P36 than P23 (x=1.1 /cell in the first two time points, and x=0.75 /cell at the 3rd time). Also, the distribution of phagosomes differed with genotype. In +/+ mice the apical region of PE cells consistently contained more phagosomes than the basal region, while in vit/vit mice the apical zone always had fewer phagosomes than the basal part. The results indicate a functional impairment of vit/vit PE cells prior to photoreceptor degeneration. Supported by NIH Grant EY06631.

69.9

COCHLEAR DISORDER ASSOCIATED WITH MELANOCYTE ANOMALY IN MICE WITH A TRANSGENIC MUTATION. M.Tachibana[1]*, Y.Hara[2], D.Vyas[3], C.Hodgkinson[3], J.Fex[1], K.Grundfast[1] and H.Arnheiter[3]. [1]Lab. of Molecular Biology, NIDCD [2]Lab. of Neurochemistry and [3]Lab. of Viral and Molecular Pathogenesis, NINDS, NIH, Bethesda, MD 20892

We have generated eight lines of transgenic mice containing mouse vasopressin-beta-galactosidase fusion constructs. One of these lines, *VGA-9*, harbors approximately 50 transgene copies at a single chromosomal site. When bred to transgene homozygosity, mice of this line showed a complete loss of skin pigmentation, microphthalmia, and cochlear abnormalities. The vascular stria of the cochlea was thin and deficient in melanin pigment which is normally produced by intermediate cells, *i.e.* by neural crest-derived melanocytes. The marginal cells of the stria were thin and lacked characteristic basal infoldings. Degeneration of outer hair cells was also observed in homozygous mice, but this alteration was likely secondary to the strial abnormalities. In contrast to homozygous *VGA-9* mice, heterozygous *VGA-9* mice were pigmented and had no anatomical alterations in either eye or cochlea. Since the integrated transgene provides a marker for cloning an endogeneous gene necessary for normal pigmentation and proper development of the inner ear, the transgene line *VGA-9* may become valuable for the study of the molecular genetics of inner ear disorders associated with pigment abnormalities in both mice and humans.

69.10

TIMING OF NEURODEGENERATION IN THE MOTOR NEURON DEGENERATION (MND) MOUSE. A.Messer*, J.Plummer, M.C.MacMillen and W.N.Frankel. Wadsworth Center for Laboratories and Research, N.Y. State Dept. of Health and Dept. of Biomed. Sci., Sch. of Public Health, SUNY, P.O. Box 509, Albany, NY 12201-0509; and The Jackson Laboratory, Bar Harbor, ME.

The mouse mutant *Motor neuron degeneration* (*Mnd*) displays an adult-onset progressive degeneration of upper and lower motoneurons, with mild symptoms recognizable at 6 months, leading to spastic paralysis and premature death at 10 -12 months on the C57Bl/6 (B6) background. When *Mnd* was outcrossed to the AKR background, 56-63 of 160 affected (*Mnd/Mnd*) F2 progeny of the outcross/intercross showed mild symptoms by 4.5 - 5 months, and death by 7 months. This accelerated timing effect seems to be strain-specific, since it is not seen in outcrosses to 3 other strains. The gene(s) must be segregating independently of the *Mnd* gene itself, since only 35 - 39% of the *Mnd/Mnd* mice show early onset. We have eliminated the *Fv* viremia locus, and are mapping the AKR gene(s) responsible.

Even on the later-onset B6 background, abnormally-accumulating lipofuscin-like material can be seen in spinal cord as early as the first month. The material is present in both increasing numbers of cells, and in increasing amounts within individual cells, as the animals age. The level of pathological involvement well before the onset of clear clinical symptoms suggests that the degenerative process is an extremely gradual and protracted one.

The interaction of the timing gene(s), which we hypothesize affects the substrate on which the *Mnd* gene is acting, with the early steps in the degenerative process, should provide a model for delayed neuronal degeneration. (Supported by the ALS Association.)

69.11

AGE-RELATED CHANGES IN CHOLINERGIC AND GABAERGIC PARAMETERS IN THE BRAIN OF THE SAM P/8 SENESCENCE ACCELERATED MOUSE V. Reddy, J.F. Flood, J.E. Morley and R. Strong. Departments of Pharmacological and Physiological Science and Internal Medicine, St. Louis University School of Medicine, and GRECC, St. Louis VA Medical Center, St. Louis, MO, 63125

Senescence accelerated prone mouse strains (SAM-P) and resistant strains (SAM-R) were established by Takeda and his colleagues at Kyoto University in the early 1980's. Based on longevity curves and phenotypic changes characteristic of aging, SAM-P strains show an inherited, early onset, accelerated advancement of senescence. Moreover, early onset deficits in learning and memory were reported that are not accounted for by age-differences in sensorimotor capabilities. The early onset, irreversible, severe deficits of learning and memory in the SAM-P/8 mice suggest that this may be a useful animal model of human dementia. We therefore performed experiments to determine if biochemical alterations occur in the SAM-P/8 mice similar to those that occur in Alzheimer's disease.

We measured ChAT activity in the cortex of SAM-P/8 mice 4, 8 and 16 months of age and in hippocampal regions of SAM P/8 mice aged 2, 8, and 12 months. ChAT activity was decreased by 33% at 8 months of age and 51% by 16 months of age in the cortex, and 50% in the hippocampus and septal area of SAM-P/8 mice at 12 months. SAM R/1 mice showed no alterations in these brain areas with age. The neurochemical specificity of these alterations is indicated by the fact that GAD activity, measured in portions of the homogenate used to measure ChAT, was not altered in any brain region examined, except in the septal area, where it increased. These findings raise the possibility that the SAM-P/8 mice might be a useful animal model for at least some aspects of human dementia.

69.12

EFFECT OF CHRONIC TREATMENT WITH TFMPP, A PUTATIVE 5-HT_1-RECEPTOR AGONIST, ON FEEDING BEHAVIOR AND WEIGHT GAIN, PLASMA INSULIN LEVELS AND HYPOTHALAMIC NEUROPEPTIDE mRNA EXPRESSION IN OBESE ZUCKER RATS. J. Rouru U. Pesonen K. Isaksson R. Huupponen and M. Koulu*. Department of Pharmacology and Clinical Pharmacology, University of Turku, SF-20520 Turku, Finland.

Obese Zucker rat is a model of genetic obesity characterized by hyperphagia, hyperinsulinemia and insulin resistance. The present investigation was performed to study long-term effects of the putative 5-HT_1-receptor agonist trifluoromethylphenylpiperazine (TFMPP) on food intake and weight gain in obese Zucker rats. The influence of chronic TFMPP on expression of hypothalamic appetite regulating neuropeptide mRNAs was also investigated using *in situ* hybridization. In addition, plasma insulin and glucose levels, and brain 5-HT_{1C}- and 5-HT_2-receptor densities were analyzed. TFMPP (4 mg/kg/day s.c.) significantly reduced food intake during the first seven days, but this effect declined to a non-significant level after 14 and 28 days. Reduced food intake was associated with decreased body weight gains during first and second weeks of treatment, but no effect was found thereafter. Plasma insulin concentration was lowered by TFMPP treatment without impairment of glucose homeostasis. TFMPP treatment did not alter expression of CRF mRNA in the paraventricular nucleus or NPY mRNA in the arcuate nucleus. Chronic TFMPP administration significantly reduced densities of 5-HT_2-receptors in the claustrum, cingulate and parietal cortex, but not 5-HT_{1C}-receptor density in the choroid plexus. It is concluded that the anorectic and weight gain lowering effect of TFMPP deteriorates during long-term treatment in obese Zucker rats. Chronic TFMPP treatment reduces hyperinsulinemia, the potential therapeutic usefulness of which in the treatment of hyperinsulinemic states should be investigated.

69.13

HYPOTHALAMIC NEUROPEPTIDE EXPRESSION AFTER FOOD RESTRICTION IN ZUCKER RATS: EVIDENCE OF PERSISTENT NEUROPEPTIDE Y GENE ACTIVATION. U. Pesonen*, R. Huupponen, J. Rouru, and M. Koulu. Department of Pharmacology, University of Turku, SF-20520 Turku, Finland

Obese Zucker rat is a model of genetic obesity characterized by hyperphagia, hyperinsulinemia and other endocrine abnormalities. In order to elucidate pathogenetic mechanisms contributing to disturbed feeding behavior in these animals, the effect of food-restriction (50 % reduction in individual 24 h food intake for 2 weeks) on three hypothalamic neuropeptides involved in the control of feeding behavior was studied in lean and obese Zucker rats. The levels of preproneuropeptide Y (preproNPY), preprocorticotropin releasing factor (preproCRF) and preprosomatostatin (preproSOM) mRNAs were determined using *in situ* hybridization technic. In addition, plasma insulin and corticosterone concentrations were analyzed. Food restriction significantly increased the expression of preproNPY mRNA in the arcuate nucleus in both Zucker phenotypes, while the expressions of preproCRF mRNA in the paraventricular nucleus (PVN) and preproSOM mRNA in the periventricular nucleus (PeV) were not altered. The basal expression of preproNPY mRNA was significantly greater in obese control animals than in lean control animals. Food restriction lowered plasma insulin levels, but did not change plasma corticosterone levels. It is concluded that food restriction activates NPY gene transcription in the arcuate nucleus of lean and obese Zucker rats. The results provide evidence that orexigenic NPY plays a role in the adaptation altered feeding status and reduced caloric intake, and that the enhancement of NPY expression is similar both in lean and obese Zucker rats.

69.14

NEAR MICROSCOPIC MR IMAGING IN SITU OF BRAIN OF PHENYLALANINE HYDROXYLASE DEFICIENT MICE AT 9.1 TESLA. S. Kornguth*, A. Shedlovsky, M. Anderson, J Markley. Univ. of Wisconsin, Madison, WI 53705.

Shedlovsky and colleagues have isolated a mouse strain deficient in phenylalanine hydroxylase ($PAH^{HPH5.1}$), a model for PKU. PKU is associated with mental retardation, hypomyelination and other anatomical and physiological abnormalities. We have successfully imaged the brain of mice and rats in situ at a resolution of 90 X 90 microns in the X,Y plane and 120 microns in the Z plane. The brains of mice that were homozygous for the deficiency, and of control littermates (heterozygous), were imaged *in situ* using proton spin density imaging parameters (TE 28 ms; TR 3040 ms). The resolution obtained was 400 X 400 microns in the X,Y, plane and 400 microns in the Z plane. The mice were raised on a normal mouse diet and imaged at 3.5, 4.5, 5.5, 6.5, 7.5 weeks postnatum. Following imaging, the brains were removed, fixed, sectioned and stained with cresyl violet. The magnetic resonance images, gross structure and histological appearance of the brains from the homozygous animals and control littermates will be compared. The histology of the mutant mice will be compared with that observed in the brain of a 28 year old patient who died with untreated PKU.

69.15

TREATMENT OF W256 TUMORS IN IMMUNOCOMPETENT RATS USING HERPES SIMPLEX VIRUS MUTANTS. **J.Tjuvajev[1], R.G.Blasberg[1], J.Berk[1], J.B.Posner[1,*], S.D.Rabkin[2], D.W.Pfaff[3] and M.G.Kaplitt[3]. [1]Dept. of Neurology, and [2]Program in Molecular Biology, Memorial-Sloan Kettering Cancer Center, New York, NY 10021; [3]Laboratory of Neurobiology and Behavior, Rockefeller Univ., New York, NY 10021**

Previous studies have indicated that cytolysis by wild-type herpes simplex virus (HSV) can attenuate tumor growth in rats, while HSV mutants have been shown to be effective therapeutic agents against a human tumor grown in nude mice. We have employed two HSV mutants, a thymidine kinase deficient virus (TK-) and a ribonucleotide reductase mutant (RR-). Both mutants efficiently killed 7/7 human tumor cell lines in tissue culture. The TK- HSV also killed the rat RG2 glioma and W256 carcinoma lines as efficiently as the human lines, while the rat C6 glioma was inefficiently killed. For further study, we selected the TK- virus and the W256 line, which is a tumor commonly used as a model of metastatic brain disease. Under conditions of rapid cell growth in tissue culture, the wild-type and TK- HSV infections were similar . In resting cells, however, the TK- was compromised while the wild-type was not. Thus, the proliferative activity of the tumor at the time or site of viral injection may influence efficacy. W256 tumors growing subcutaneously in male Sprague-Dawley rats were then treated with intratumoral injections of TK- HSV, while controls received equal volumes of vehicle. 4/9 experimental tumors completely regressed, with no recurrence after 6 months, while tumor growth in the remaining animals was significantly attenuated with respect to matched controls. HSV mutants are, therefore, potentially viable therapeutic agents in immunocompetent animals.

EPILEPSY: HUMAN STUDIES AND ANIMAL MODELS I

70.1

ABERRANT MOSSY FIBER-CA2 CONNECTIONS IN HUMAN TEMPORAL LOBE EPILEPSY (TLE). A. Williamson*, G. M. Shepherd and D. D. Spencer. Sects of Neurosurg. and Neurobiol. , Yale Univ. Med. Sch., New Haven, CT 06510.

Medial temporal lobe sclerosis (MTS) is the most common form of medically intractable human TLE. A number of anatomical changes occur in the hippocampi of these patients including sprouting of the mossy fibers into the inner molecular layer and up to an 80% loss of specific populations of hilar interneurons and of pyramidal cells in CA1 and CA3. The pyramidal cells of CA2 and the dentate granule cells are relatively spared. These changes do not occur in the hippocampi of a comparison group of patients with TLE produced by extrahippocampal tumors (TTLE). Seizure activity can propagate from the MTS hippocampi even though the primary output paths (CA1 and CA3) have been destroyed. We hypothesize that the sprouted mossy fibers contact CA2 cells in an aberrant fashion, thus providing a route by which seizure activity can leave the hippocampus.

Slices were prepared from resected hippocampi of both types of patients and intracellular recordings were made from CA2 pyramidal cells. Cells were filled with neurobiotin to confirm their morphology and location. There were no significant differences in the membrane properties between these two groups. There were, however, a number of differences in the synaptic properties. In TTLE CA2 cells, stimulation of the Schaffer collaterals in stratum radiatum of CA3 produced an EPSP followed by a biphasic IPSP in all cells studied (n=7). In these cells, the amplitudes of the EPSPs varied directly with membrane potential. In the MTS CA2 cells, however, EPSPs were not followed by IPSPs and their amplitudes did not vary significantly with the membrane potential. Even though IPSPs were not present in the sclerotic cells, bursts of action potentials could not be evoked with excitatory synaptic stimulation.

The connectivity of granule cells to CA2 pyramidal cells was examined by stimulating different sites in the dentate gyrus. In 9/10 MTS cells EPSPs or action potentials could be produced by stimulation of the mossy fibers either in the hilus or in the granule cell layer. By contrast, in TTLE tissue, cells could only be driven by stimulation of the Schaffer collaterals in CA3. These data suggest that the mossy fiber sprouting into CA2 in sclerotic hippocampi may provide the route for a seizure to generalize.

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70.2

HEAT-INDUCED SEIZURES IN RATS: ANATOMICAL AND FUNCTIONAL REORGANIZATION IN THE BRAIN. N. C. de Lanerolle*, W. Jiang, and D. D. Spencer. Section of Neurosurgery, Yale Univ. Sch. of Med., New Haven, CT. 06510

Febrile seizures occur in 3 - 4% of children between 6 months and 5 years of age. About 2% of them progress to epilepsy, especially temproral lobe epilepsy (TLE) in later years. Previous data show that there is a greater degree of neuronal damage in patients with a history of febrile seizures. To study the effect of febrile seizures on pathogenesis in the brain Sprague-Dawley male rat pups (22 days) were exposed for 4 min to warm water (45.0°C) every 4 days. Most rat pups had a seizure on the first exposure when the animals core body temperature rose from approximately 38.0 to 44.0°C. The behavioral seizure of these rats varied from facial clonus, head nodding and body jerks to forelimb clonus, rear limb clonus and falling over in full blown tonic clonic seizures. The behavioral seizure lasted from 10 sec to 6 min (median duration 3 min). This was followed by a postictal quiescent period ranging from 10 min to about 1 hour. Epidural EEG recording during a seizure revealed an EEG pattern typical of a seizure, with a post seizure suppression of activity. In rats exposed to 12 such seizures over a period of about 2 to 3 months the Timm stain revealed sprouting of mossy fibers into the inner molecular layer of dentate gyrus. Sprouting was strongest at the tip and angle of the blades of the dentate granule cell layer. After 24-seizures there was increased sprouting. Intracellular recording from 24-seizure rats showed evidence of hyperexcitable granule cells and CA1 neurons. When 12-seizure rats were reexposed to warm water after a period of 6 months since the 12th seizure, many displayed prolonged (10 min) tonic-clonic seizures followed by a long (2 - 5hr) period of status epilepticus (tooth-chattering). Silver staining for degenerating neurons revealed massive cell loss in the amygdala, pyriform cortex, ventral hippocampus and mediodorsal thalamus. These findings suggests that alteration of neuronal structure and function in this model resemble those seen in TLE associated with childhood febrile seizures. [NS27081 & NS06208].

70.3

ULTRASTRUCTURAL ANALYSIS OF PEPTIDE SYSTEMS IN THE DENTATE GYRUS OF HIPPOCAMPI FROM PATIENTS WITH EPILEPSY. M. F. Philips*, D. D. Spencer, and N. C. de Lanerolle. Section of Neurosurgery, Yale Univ. Sch. of Med., New Haven, CT. 06510.

Light microscopic studies of immunostained fascia dentata (FD) of hippocampi surgically removed from two groups of patients with medically intractable temporal lobe epilepsy revealed organizational differences with respect to the somatostatin, neuropeptide Y, and substance P like-immunoreactive (SLI, NPYLI, and SPLI respectively) systems [de Lanerolle et al, Brain Res., 495: 387-395, 1989]. One group with extrahippocampal temporal lobe tumors (TTLE) demonstrated SLI, NPYLI, and SPLI interneurons in the hilus and peptide specific patterns of immunoreactive fibers in the molecular layer (ML). The other group with no apparent temporal lobe tumors (CTLE) revealed a loss of SLI, NPYLI, and SPLI hilar interneurons and concomitant changes in the fiber-like staining patterns in the ML.

The immunoreactive fiber staining in the FD of the TTLE patients was ultrastructurally identified and compared to that in the FD of the CTLE group. In the TTLE, SLI dendrites were found ramifying from the hilus up through the inner molecular layer (IML). The CTLE FD showed a significant loss of SLI dendrites in the hilus and the IML. SLI terminals, restricted to the outer molecular layer (OML) in the TTLE group, were found throughout the entire ML.

In the TTLE group, NPYLI terminals were most concentrated in the OML, but were found throughout all layers of the FD. Few NPYLI dendrites were found in the ML. Large axon-terminal complexes traversed the OML and the IML and often contacted several dendrites at once. The CTLE FD had more of these terminals in the IML.

In the TTLE group the SPLI fibers in the ML are mostly terminal in nature. They are concentrated mainly in the OML and in a bandlike distribution on either side of the granule cell layer (GCL). These small terminals synapse on proximal dendrites of granule cells in the IML. In the CTLE FD the SPLI fiber band on the hilar side of the GCL disappears and there are large SPLI terminals studding the proximal dendrites of granule cells. Large SPLI terminals were also found synapsing on granule cell somata. The synaptic reorganization of these peptide-immunoreactive systems may provide a neural substrate for hyperexcitability in the hippocampus. [Supported by NS27081 and the Howard Hughes Medical Student Research Training Fellowship]

70.4

ELECTRON MICROSCOPIC VISUALIZATION OF HIPPOCAMPAL MOSSY FIBER BOUTONS IN THE DENTATE MOLECULAR LAYER AFTER STATUS EPILEPTICUS. M.M. Okazaki*, D.A. Evenson and J.V. Nadler, Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

In response to the neuronal degeneration associated with status epilepticus, hippocampal mossy fibers develop recurrent collaterals that invade the dentate molecular layer. Mossy fiber collaterals have been suggested to form an aberrant recurrent excitatory circuit by making synapses on dentate granule cells. This hypothesis was tested by labeling both mossy fibers and granule cells with biocytin. Status epilepticus was produced by treating adult male rats with 325 mg/kg pilocarpine preceded by 1 mg/kg methscopolamine. At least 4 months later, transverse slices of the caudal hippocampus were prepared and biocytin was ionophoretically applied to stratum lucidum of area CA3b. After allowing 3 h for retrograde transport, the slices were fixed and cut into 50 μm-thick sections, biocytin was visualized with avidin/biotinylated HRP/diaminobenzidine and thin sections were prepared for electron microscopy.

Each thin section contained a few labeled dentate granule cell bodies and dendrites. In sections from rats that developed recurrent mossy fibers, but not in sections from control rats, labeled boutons were observed in the dentate molecular layer. Labeled boutons were located mainly in the inner third of the molecular layer, but a few were located farther from the cell body layer. Most were seen to be engaged in synaptic contact with one or more dendrites. A few were contacting labeled dendrites. These results suggest that recurrent mossy fiber collaterals do form synapses and, at least in some cases, innervate granule cells. (Supported by NIH grant NS 17771.)

70.5

HYPEREXCITABILITY OF THE DENTATE GYRUS IN SEIZURE-SENSITIVE MONGOLIAN GERBILS. P.S. Buckmaster[1]* and P.A. Schwartzkroin[1,2], Depts. of Physiology and Biophysics[1] and Neurological Surgery[2], School of Medicine, University of Washington, Seattle, WA 98195.

Previous studies have revealed anatomical differences in the hippocampi of seizure-resistant (SR) and seizure-sensitive (SS) Mongolian gerbils. SS gerbils have more GABAergic neurons and more GABAergic punctae contacting inhibitory basket cells in the dentate gyrus than SR gerbils. Investigators have hypothesized that inhibition of basket cells in the dentate gyrus of SS gerbils causes disinhibition of granule cells and results in hyperexcitability (Peterson et al., 1985, *Brain Res.* 340: 384-389). To test this hypothesis we asked, Do SS gerbils have hyperexcitable dentate gyri? Adult gerbils were classified as SS or SR by their response to a novel environment (plastic basket). Animals that seized within 1 min over the 3 testing periods were designated SS; animals that never seized were considered SR. A paired-pulse stimulation paradigm was used to assess excitability of the dentate gyrus. Animals were anesthetized (1.25 gm/kg urethane, i.p.), and recording (glass pipette, 5-15 Mohms, 2 M NaCl) and stimulating (concentric bipolar, 0.25 mm diameter) electrodes were placed stereotaxically in the dentate gyrus and the perforant path, respectively. The ratio of the second population spike amplitude to the first population spike, at stimulus intervals from 10-100 ms, was measured. SS and SR gerbils both showed strong paired-pulse inhibition (ratio < 1) at 10-20 ms stimulus intervals and paired-pulse facilitation (ratio > 1) at 40-80 ms stimulus intervals. However, paired-pulse facilitation was greater in SS gerbils (e.g., mean ratios at 60 ms stimulus interval: SS = 3.75, SR = 1.59) and persisted to longer stimulus intervals (i.e., greater than 80 ms) than in SR gerbils. These results confirm the hypothesis that SS gerbils have hyperexcitable dentate gyri and are consistent with the hypothesis of increased inhibition of basket cells.

70.6

Characterization of monosynaptic GABAergic IPSPs in hippocampal CA1 pyramidal cells from kainic acid treated rats. S. Williams*, P. Vachon and J.-C. Lacaille. Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

The present experiments assessed whether disconnection of GABAergic synapses from interneurons to CA1 pyramidal cells contributed to the hyperexcitability in kainate (KA) lesioned hippocampus. Hippocampal slices were obtained from adult rats 2-4 weeks after bilateral intraventricular injection of KA (0.65 µg in 1 µl saline, pH 7.4) and from sham-treated or control rats. Intracellular recordings were obtained from 47 pyramidal cells of KA-treated animals in areas of slices displaying hyperexcitable field potentials. Following str. radiatum stimulation (0.05 ms, 25-350 µA), 22 cells were hyperexcitable (≥ 2 action potentials) and the remaining cells (n=25) displayed single action potentials (non-hyperexcitable) as control cells (n=20). A long duration, voltage-sensitive component was associated with subthreshold EPSPs in a majority of hyperexcitable (12/15) and non-hyperexcitable (3/5) but not in control cells (1/10). When excitatory synaptic transmission was blocked with NMDA and non-NMDA antagonists (40 µM AP5 and 20 µM CNQX, respectively), stimulation of str. radiatum elicited biphasic monosynaptic IPSPs in all hyperexcitable (n=9), non-hyperexcitable (n=9) and control cells (n=8). Monosynaptic IPSPs were not different in terms of amplitude, latency, E_{rev} or conductance changes between cells in KA-treated and control groups. In hyperexcitable cells, the early component of monosynaptic IPSPs was reduced by the $GABA_A$ antagonist bicuculline (100-200 µM; n=7). The late component was reduced by the $GABA_B$ antagonist 2-OH-saclofen (2 mM; n=3). Comparable results were observed in non-hyperexcitable cells (n=4) from KA-treated animals and in control cells (n=5). In conclusion, $GABA_A$ and $GABA_B$ synapses appear intact and functional in hyperexcitable CA1 pyramidal cells of KA-lesioned hippocampus.

Supported by the Medical Research Council of Canada and Savoy Foundation.

70.7

IN VIVO HYPOXIA CAUSES HYPEREXCITABILITY IN SLICES OF IMMATURE RAT HIPPOCAMPUS AND NEOCORTEX. F.E.Jensen* and C.E. Stafstrom. Dept. of Neurology, Children's Hospital and Harvard Medical School, Boston, MA 02115.

In vivo, hypoxia is epileptogenic in immature rats (P5-17), but not in adults (*Ann. Neurol.*, 1991;29:629-637). To further examine this age-dependent difference in hypoxia-induced excitability, we prepared brain slices from P10 (n=11) and adult (P60) (n=4) rats immediately after exposure to hypoxia (4% O_2) *in vivo*. Extracellular activity was recorded from hippocampal (n=28) and neocortical slices (n=28) from hypoxic rats. Slices from age matched normoxic rats (controls) were maintained in the same chamber for comparison. In CA1, responses evoked by Schaffer collateral stimulation in slices from hypoxic pups frequently revealed polyphasic field potentials that were not seen in controls at either age or in slices from hypoxic adults. In neocortical layers II/III, the duration of responses to white matter stimulation were prolonged (48-75 msec) compared to controls (28-60 msec) at P10. Polyphasic responses were more complex and prolonged in both layers II/III and V in P10 slices from hypoxic rats compared to controls. In addition, long latency field potentials with onset > 80 msec from the stimulus were observed in neocortex in some of the slices from hypoxic P10 rats. In contrast, neocortical recordings from layers II/III and V in slices from hypoxic adults were usually similar to control slices, although prolongation of the evoked response was occasionally observed.

These results suggest that slices from immature rats which have undergone hypoxic seizures *in vivo* are hyperexcitable, and may prove useful as a model system for the mechanisms of seizure ontogenesis in perinatal hypoxia.

70.8

NMDA RECEPTOR IS INVOLVED IN EXCITATORY SYNAPTIC TRANSMISSION IN THE SUPERFICIAL/MIDDLE LAYERS OF THE EPILEPTOGENIC HUMAN NEOCORTEX. Hwa GGC*, Mattia D, Avoli M. MNI, McGill Univ., Montreal, Que., Canada.

Intracellular recordings were made from layers II-IV of human neocortical slices resected from the temporal (n = 18), frontal (n = 3) and occipital (n = 1) regions of 22 epileptic patients. The neurons (n = 61) discharged in a regular-spiking fashion and displayed an average resting Vm of -74mV, input resistance of 30MΩ, action potential amplitude of 92mV. Following synaptic stimulation at subthreshold intensity, the neurons responded with a depolarizing postsynaptic potential (PSP) with fixed latency to onset (0.8-4 ms). At suprathreshold intensities, a single action potential was observed in 95% of the population while an all-or-none bursting discharge was recorded in the remaining 5%. Vm analysis of the PSP evoked by low intensity stimulation revealed an excitatory PSP (EPSP) that increased in size with depolarization and decreased in size with hyperpolarization. This type of anomalous voltage behavior could also be mimicked by an intrinsic response evoked by a brief pulse of depolarizing current. With higher intensity stimulation, inhibitory PSPs were activated and could impose a shunting effect on the EPSP at depolarized Vm. Pharmacological experiments showed that the NMDA antagonist CPP (5 μM) could greatly reduce or block the peak amplitude of the EPSP evoked by low intensity stimulation and attenuated the late phase of the bursting discharge. These findings demonstrate that NMDA receptors are operant in the epileptogenic human neocortex and appear to play an important role in the excitatory interaction between neurons. The presence of an all-or-none bursting discharge in some neurons suggests that this interaction might be up-regulated, possibly through the activation of NMDA receptors.

70.9

CHANGES IN GLUTAMATE RECEPTOR BINDING AND EXCITATORY FIELD RESPONSES IN THE DENTATE GYRUS OF EPILEPTIC PATIENTS. P. McGonigle, A. Dai, M.J. O'Connor and L.M. Masukawa*. Depts. of Pharmacology, Surgery and Neurology, University of Pennsylvania and Depts. of Neurology and Surgery, Graduate Hospital, Philadelphia, PA.

Although the role of glutamate receptor activation in excitation of the hippocampus is accepted, the contribution of changes in receptor density and responsiveness to electrophysiological abnormalities observed in temporal lobe epilepsy is not well understood. It has been reported that the density of NMDA receptors in the dentate gyrus of patients who have undergone temporal lobectomies either increases (McDonald et al., 1991; Geddes et al., 1990) or does not change (Hosford et al., 1991). We also observed no significant difference in the binding of [3H]-MK-801 to NMDA receptors in the dentate gyrus of epileptic patients (n=28) compared to post-mortem controls (n=6). However, we observed that the relationship between the ratio of the early component of the field EPSP (non-NMDA receptor activation) to the longer duration EPSP (NMDA receptor activation) of dentate granule cells (n=5) and a measure of hilar anatomic abnormality (cell density) approached significance (p=.07). We did not observe a significant correlation between the binding of [3H]-MK-801 to NMDA receptors or [3H]-CNQX to quisqualate receptors in the molecular layer of the dentate gyrus or the hilus with hilar cell density. These results suggest that changes in the electrophysiology measured in this study are not due to alterations in the overall NMDA or quisqualate receptor density. Receptor changes in discrete subregions maybe responsible for the differences in physiological responses that were observed. (Supported by GM 34781, NS 23077 and the Pew Charitable Trusts)

70.10

PRESENCE OF PAIRED-PULSE FACILITATION AND NON-GABA-A PAIRED-PULSE INHIBITION REVEALED BY MODERATE TO LOW INTENSITY STIMULATION IN THE DENTATE GYRUS OF EPILEPTIC PATIENTS. K. Uruno, M.J. O'Connor and L.M. Masukawa. Depts. of Neurology and Surgery, Graduate Hospital and Univ. of Pennsylvania, Philadelphia, PA.

Paired-pulse stimulation of dentate granule cells via perforant path input in brain slices from temporal lobe epileptic patients produces inhibition at 20 msec interstimulus intervals at moderate stimulation intensities (40-50% of maximum intensity). The magnitude of the paired-pulse inhibition (PPI) varied from complete to 10% inhibition. In the presence of 15-20 uM bicuculline, the PPI was reduced but in some cases was not affected. Because of the presence of a prolonged EPSP and accompanying population spikes, the PPI after bicuculline was initially attributed to a reduction of action potential generation by voltage-dependent membrane currents. To eliminate membrane depolarization that occurs during bicuculline disinhibition, we measured PPI during low intensity stimulation which reduced the likelihood of NMDA receptor mediated conductances and multiple spiking or during APV (20 uM). Low intensity stimulation in bicuculline led to paired-pulse facilitation (PPF) which suggested that the PPI before bicuculline was mediated by GABA-A receptors. During APV at moderate stimuli intensities PPI was not substantially eliminated although multiple spiking and prolonged EPSPs were reduced indicating that the PPI was not due to an increase in membrane conductance. The results suggest the presence of a non-GABA-A mediated PPI that is activated at moderate or higher stimulus intensities. (Supported by NS-23077)

70.11

INCREASED EXCITABILTY IN THE EPILEPTOGENIC HUMAN TEMPORAL LOBE: A CURRENT STRENGTH ANALYSIS. S.U. KHAN,*C.L. WILSON, E.J. BEHNKE. Dept. of Anatomy and Cell Biology, Dept. of Neurology, and Brain Res. Inst. UCLA School of Medicine, Los Angeles, CA 90024.

In 25 depth electrode patients with medically intractable unilateral temporal lobe epilepsy, the excitability of hippocampal pathways was investigated by stimulating them with single pulses of ascending current strength from thereshold to maximal response. The measure of excitability was defined as "c50" the current required to evoke a response 50% of maximal amplitude. Hippocampal sites investigated included anterior hippocampus, middle hippocampus, entorhinal cortex, presubiculum, posterior hippocampal gyrus and middle hippocampal gyrus. The pathways joining these structures include perforant path, retrohippocampal path and hippocampal association path. Sites were classified as epileptogenic (EPG) or non-epileptogenic (NEG) according to whether they were on the side where the patient's spontaneous seizures always started or never started, respectively .

The mean c50 level for 35 EPG hippocampal sites was 2.60 mA (SEM=0.12) while c50 for 63 NEG sites was 3.22 mA (SEM=0.13). In a two way analysis of epilepsy vs. current, the difference in excitability between EPG and NEG pathways was significant at the $p<.002$ level ($df=1$, $F=10.1$) Across the three EPG hippocampal pathways studied, c50 was always lower than in NEG, but there was no significant difference between pathways.

The lower current strength required to evoke 50% response demonstrates greater excitability of the EPG hippocampal principal neurons. Supported by NIH grant NS02808.

70.12

THE INVOLVEMENT OF GABAERGIC TRANSMISSION WITHIN THE POSTERIOR THALAMIC NUCLEI (PO) IN REGULATING SEIZURES IN RATS. S. G. Xu, D. S. Garant, E. F. Sperber and S. L. Moshé. Departments of Neurology, Neuroscience and Pediatrics, Albert Einstein College of Medicine, Bronx, NY 10461.

There is evidence indicating that specific thalamic nuclei may be involved in the expression and control of seizures. In the present study, the function of the posterior thalamic nuclei (PO) in the regulation of seizures was investigated in adult rats. Several GABAergic agents were bilaterally infused into the PO via implanted cannulae. The GABA-elevating agent, γ-vinyl-GABA (GVG, 20 μg) significantly suppressed clonic and tonic seizures induced by flurothyl. Muscimol (a $GABA_A$ agonist, 100 ng) also inhibited clonic and tonic seizures, whereas bicuculline (a $GABA_A$ antagonist, 100 ng) facilitated these seizures. Baclofen (a $GABA_B$ agonist, 200 ng) was less effective at suppressing clonic seizures, and did not alter the susceptibility to tonic seizures. The findings suggest that GABAergic neurons in the PO may be involved in seizure control. The γ-aminobutyric acid (GABA) system in the substantia nigra pars reticulata (SNR) also plays a key role in the control of seizures. Based on anatomical evidence for the nigral GABAergic projections, direct or indirect, to the thalamic nuclei including the PO, we hypothesize that the GABAergic pathway from the SNR to the PO may be involved in the control of seizures.

70.13

EFFECTS OF SEPTO-HIPPOCAMPAL PROJECTIONS ON EXPERIMENTAL GENERALIZED SEIZURES. J.W. Miller*, G.M. Turner and B.C. Gray. Dept. of Neurology and Neurological Surgery (Neurology), Washington Univ. Sch. of Med., St. Louis, MO 63110.

Epileptic seizures occur less often during wakefulness or paradoxical sleep, two conditions during which septo-hippocampal theta activity is seen. In preliminary studies of this relationship, we have determined effects of different, *in vivo*, experimental manipulations in the medial septal nucleus of the rat on hippocampal activity and seizures induced by systemic pentylenetetrazol.

When myoclonic and facial-forelimb seizures were induced by 50 mg/kg intraperitoneal pentylenetetrazol, injections of 1 to 2.5 μg of the cholinergic agonist carbachol in the medial septal nucleus led to a cessation of behavioral seizures and EEG spiking within 15 to 30 seconds, accompanied by the appearance of hippocampal theta activity. Bipolar electrical stimulation of the medial septal nucleus at frequencies of 4-8 hertz produced similar anticonvulsant actions and also induced rhythmic hippocampal activity at the stimulus rate. Electrolytic lesions of the medial septal nucleus resulted in the chronic disappearance of hippocampal theta activity and lowered the thresholds of myoclonic and facial forelimb seizures induced by timed intravenous infusion of pentylenetetrazol, although tonic-clonic seizures were not affected.

Regulation of the physiological state of the hippocampus via septo-hippocampal projections is likely to be a critical mechanism by which arousal state influences the probability of seizure occurrence. *Supported by NIH Grant NS14834.*

70.14

CHANGES IN EXTRACELLULAR SPACE DO NOT MEDIATE STIMULUS TRAIN INDUCED EPILEPTOGENESIS IN HIPPOCAMPAL SLICES. J. Jing, S. Clark, W.A. Wilson, P.G. Aitken*, Dept. Cell Biology, Duke Med. Center, and VA Med. Center, Durham, NC 27710

Electrographic seizures (EGS) can be induced in hippocampal slices by repeated delivery of stimulus trains to the CA3 region (Science, 245:648-651, 1989). We tested the hypothesis that a decrease in extracellular space (Interstitial Volume Fraction, ISVF) underlies this model of epileptogenesis. Transverse hippocampal slices 0.60 mm thick from 14-22 day old rats were maintained at 32.8°C in an interface chamber. EGS were provoked by stimulating Schaffer collaterals with trains (60Hz, 2 sec, intensity for maximal population spike) every 10 minutes while recording extracellular responses in CA3 or CA1. EGS of 16-35 sec duration occurred after 1-3 trains. ISVF was determined using the method of Nicholson and Phillips (J. Physiol., 321:225-257, 1981) which measures diffusion of tetramethyl ammonium (TMA) through extracellular space from a point source to a TMA-sensitive electrode .060-.150 mm distant. ISVF was measured in st. radiatum or st. pyramidale of CA3 or CA1 before, and then 2 and 7 minutes after each train during, the induction of EGS (to a maximum of 10 trains). Control slices were maintained identically without delivery of stimulus trains, with ISVF measurements taken at equivalent time points. Control slices showed no ISVF change during 2-2.5 hour experiments. Experimental slices showed an average 14% decrease in ISVF (range -0.3 to 26.9); this change, however, was not correlated with the development of EGS. Full-blown EGS could occur without any ISVF change. These data suggest that while modest changes in ISVF may result from seizure activity, persistent ISVF change is not a major mechanism in this model of epileptogenesis.

70.15

INCREASED EXCITATORY RESPONSES IN GENETICALLY EPILEPSY-PRONE RAT HIPPOCAMPUS. T.L. Pencek*, S. Verma and M. Moran Neurosurgery Laboratory, Southern Illinois University School of Medicine, Springfield, IL 62702.

The genetically epilepsy-prone rat (GEPR) is a well known model for audiogenic seizures of brain stem origin. We wanted to see if there were differences in hippocampal excitability, using the CA_3 to CA_1 pathway for our extra investigation. We recently reported that a marked increase in paired pulse facilitation occurs in the GEPR hippocampus CA_1 pyramidal cell layer, compared to normal Sprague Dawley rats. This is amplified in low K solutions. We have now attempted to separate the excitatory and inhibitory components of hippocampal hyperexcitability. Bicuculline produced a marked increase in paired pulse facilitation in GEPRs. The amount of facilitation in normal rats in a Bicuculline solution resembled that in GEPRs without Bicuculline. This suggests that reduced gamma amino-butyric acid-mediated inhibition may contribute to abnormal GEPR responses. Our results in low K+ solutions show a two-fold increase in facilitation suggesting the involvement of a K+ channel in the enhanced excitation. Intracellular recording from CA_1 pyramidal cells in low K+ medium show an increase in long after hyperpolarization (LAHP) potential. Preliminary studies in GEPR rats showed that the LAHP is greater than in the normal rats. The spike amplitude increases markedly in low K+ and a higher current is required to produce action potentials. Further hippocampus research in GEPRs may provide information on the genetic mechanisms of epilepsy in pyramidal cells.

70.16

EXCITATORY INTERACTIONS OF BURSTING CELLS IN HUMAN MESIAL TEMPORAL LOBE. B. COLDER*, R.C. FRYSINGER, C.L. WILSON, M.F. LEVESQUE, AND R.M. HARPER, Dept. of Anatomy and Cell Biology, Dept. of Neurology, Div. of Neurological Surgery, and Brain Res. Inst. UCLA School of Medicine, Los Angeles, CA 90024.

Epileptogenisis in human mesial temporal structures is associated with cell loss and changes in electrophysiological function of remaining neurons. We examined the interactive properties of simultaneously recorded spike trains from amygdala, hippocampus, and associated cortical areas to investigate the extent of neuronal intercorrelations as a function of single cell discharge properties. We analyzed recordings of spontaneous activity from epilepsy patients undergoing chronic depth electrode implantation for the diagnosis of focal origion of temporal lobe epilepsy. Individual neurons from multi-unit spike trains were digitally separated and cells were sorted into "bursting" and "non-bursting" on the basis of the auto-correlograms. Cells that displayed an increased probability of discharge near the origin of an auto-correlation histogram were designated as bursting cells. The nature and degree of cell-to-cell interactions were determined by cross-correlation histograms. Cells that fired in bursts were more likely to show an increase in synchronous firing with other cells, whereas non-bursters were more likely to form cross-correlations that showed a decreased probability of discharge of the dependent cell. The amygdala showed a much larger proportion of bursting cells than other areas studied. We speculate that bursting mesial temporal neurons may form physiological networks that play a significant role in the discharge synchrony characteristic of epileptogenesis. Supported by NS 02808

70.17

LOCAL INTERACTIONS OF CARDIAC MODULATED NEURONS OF HUMAN HIPPOCAMPUS AND AMYGDALA. R.C. Frysinger*, B. Colder, M.F. Levesque and R.M. Harper. Brain Res. Inst., Div. of Neurosurgery, and Dept. of Anatomy & Cell Biology, UCLA Sch. of Med., Los Angeles, CA. 90024

Single-cell activity recorded from hippocampus and amygdala in patients with epilepsy is reduced in structures ipsilateral to the seizure focus. The number of cells with discharge modulated by the cardiac cycle, however, is relatively unchanged, suggesting that this type of cell may be less vulnerable to epileptogenic changes. We have investigated the degree and type of interaction of cardiac modulated cells with other cells recorded from the same local area to determine the extent to which these cells participate in local networks. Unit recordings were taken from bundles of 9 microwires implanted in amygdala, hippocampus and overlying cortical areas in patients undergoing presurgical seizure monitoring. Up to 8 channels of unit activity from each bundle were simultaneously digitized, and individual spike trains were digitally discriminated off-line for analysis of local interaction. Analysis of 66 cells revealed 9 with significant cardiac modulation and 19 with some form of local interaction. Within this sample, cells with cardiac modulation did not interact with non-cardiac modulated cells. These findings suggest that cardiac modulated cells represent a relatively discrete population within mesial temporal structures, and the relative lack of local interactions may help protect such cells from seizure-related damage. Supported by NS 02808.

70.18

MINIATURE AND EVOKED INHIBITORY POSTSYNAPTIC CURRENTS IN DENTATE GRANULE CELLS OF HUMAN AND RAT HIPPOCAMPAL SLICES. Masako Isokawa* and Michel F. Levesque. Brain Research Institute and Dept. of Neurosurgery, University of California, Los Angeles, CA 90024-1761.

Whole-cell patch-clamp techniques were used to record inhibitory postsynaptic currents (IPSCs) from dentate granule cells (DGCs) in slices. In rats, hippocampal slices were prepared from normal male Sprague-Dawley (50-100g). In humans, the hippocampal tissue was obtained from epileptic patients who underwent a surgical treatment for intractable seizures. The perforant path stimulation evoked inward postsynaptic currents (PSCs) at a holding potential (Vm) close to the resting. PSCs were biphasic at Vm=-20; EPSCs as an early inward component and IPSCs as a late outward component with a reversal potential near -40 mV (CsF electrode). At Vm=0, near the reversal potential for EPSCs, IPSCs were measured as an outward current with the amplitude of 90-100 pA. These findings were similar in both rats and human DGCs. However, when high frequency stimulation (100Hz for 3 sec) was administered, they responded to it in a different fashion. High frequency stimulation temporarily, but significantly, increased the IPSC amplitude in normal rat DGCs (for 10-20 ms at Vm=0). This increase was blocked by 50 μM APV. In human epileptic DGCs, the IPSC amplitude, measured at Vm=0, was considerably reduced (more than 1/3 of the original) after the 100 Hz stimulation. Spontaneously-occurring miniature IPSCs (mIPSCs) were observed in both rat and human DGCs. Their amplitude varied from 10-100 pA and some of them were as large as evoked IPSCs (Vm=0). They persisted in TTX (1μM) but were blocked in BMI (10μM). DGG (1 mM) and APV (50 μM) did not abolish mIPSCs. However, they altered the frequency of mIPSCs, especially increasing the occurrence of high amplitude mIPSCs in human epileptic DGCs. Supported by NIH Grant NS02808.

70.19

INTRINSIC AND SYNAPTIC PROPERTIES OF RAT SUBICULAR NEURONS. D MATTIA, GGC HWA, M AVOLI, P GLOOR*. MNI, McGill Univ., Montreal, Que., Canada.

The subiculum is a region through which most of the hippocampal output is routed. Because of its strategic location, it could play an important role in the spread of epileptiform activity from and within the limbic system. In this study, intracellular and extracellular recordings were made from the subicular region of rat brain slices maintained in vitro. Analysis of the current-voltage relationship revealed membrane inward rectifications in both depolarizing and hyperpolarizing direction. Following the injection of positive current pulses, single action potentials were followed by a fast afterhyperpolarization (AHP), a depolarizing afterpotential and a medium AHP. A slow AHP was discernible after repetitive firing. Synaptic stimulation from the alveus elicited excitatory postsynaptic potentials and doublets of action potentials intracellularly, and double population spikes extracellularly. Hyperpolarizing inhibitory postsynaptic potentials were not seen. Pharmacological experiments with excitatory amino acid antagonists indicate that the double population spikes were blocked by CNQX (3-5 μM), but remained insensitive to CPP (10-20 μM). Our findings demonstrate that the intrinsic membrane properties of subicular neurons are similar to those of other central neurons. Moreover, the excitatory connection between the hippocampus and the subiculum was primarily mediated by non-NMDA receptors. Supported by MRC of Canada, Savoy Foundation, FOREP and Heart and Stroke Foundation.

71.1

LASTING CHANGES IN NEUROPEPTIDE Y (NPY) RELEASE IN THE RAT HIPPOCAMPUS INDUCED BY ELECTRICAL KINDLING OR KAINIC ACID (KA). A. Vezzani*, M. Rizzi, A. Monno, A. Galli and R. Samanin. Lab. of Neuropharmacology, Mario Negri Institute for Pharmacological Research, Milan, Italy.

NPY release was measured in rat dorsal hippocampal slices during electrical kindling of the hippocampus or one month after KA (10 mg/kg, i.p.). Spontaneous release was increased bilaterally 1.5 times on average ($p<0.05$) 48 h after preconvulsive stage 2 kindling compared to shams (26.2±5.2 fmol/ml every 10 min). 25,50 and 100 mM KCl increased NPY release in shams in a Ca^{2+}-dependent manner (2±0.2, 4.4±0.3 and 6.1±0.9 times baseline, $p<0.01$). A larger increase (3.0±0.3, 4.1±0.75 and 1.7±0.07 times sham values at 25,50 and 100 mM KCl $p<0.05$ and 0.01) was found bilaterally at stages 2 and 5. No differences occurred 48 h after a single afterdischarge. An average two-fold increase in the 50 mM KCl-stimulated NPY release ($p<0.05$) was found in the dentate gyrus of rats with behavioral seizures after KA compared to controls. These changes may have interesting functional consequences on hippocampal epileptogenesis.

71.2

TRH mRNA AND FOS-LIKE IMMUNOREACTIVITY ARE CO-LOCALIZED IN LIMBIC STRUCTURES FOLLOWING AMYGDALA KINDLED SEIZURES. Jeffrey B. Rosen*, Jamie Abramowitz, and Robert M. Post. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

Previous studies have shown that both the expression of the immediate-early gene, *c-fos*, its protein product, Fos, and TRH mRNA are transiently increased in the same limbic structures following kindled seizures. To further elucidate this association, the present study examined whether TRH mRNA and Fos-like immunoreactivity (Fos-LI) are co-localized in the same cells following amygdala kindled seizures.

Rats were kindled by once daily electrical stimulation of the amygdala. Five hours following the last stage 5 seizure, the rats were fixed and the brains removed and sliced. Sections were sequentially labelled with an oligonucleotide probe for TRH mRNA and an antibody for Fos and Fos-related antigens.

Following kindling, increases in TRH mRNA and Fos-LI were primarily found in the pyriform, entorhinal and perirhinal cortices and the dentate gyrus. The mean percent of Fos-LI labelled cells with TRH mRNA was 62% in the pyriform, 70% in the entorhinal and 80% in the perirhinal. Intense TRH mRNA and Fos-LI labelling were found in the dentate gyrus, but were too dense to measure. Control rats had very low levels of both TRH mRNA and Fos-LI. This study demonstrates that increases in both TRH mRNA and Fos-LI following kindling are highly co-localized and suggests that Fos may act as a transcription factor for TRH.

71.3

PERSISTENT ENHANCEMENT OF CALCIUM CURRENTS AFTER KINDLING EPILEPTOGENESIS IN THE HIPPOCAMPUS OF THE RAT. W.J.Wadman* and M.Vreugdenhil Exp. Zoology, Univ. of Amsterdam Kruislaan 320 1098 SM Amsterdam, The Netherlands.

Daily application of a tetanic stimulation (50 Hz, 2 s) to afferent fibres induces in the projection area an epileptic focus characterized by an increased excitability and afterdischarges, ultimately leading to generalized tonic, clonic convulsions (kindling model of epileptogenesis). Stimulation was daily applied through electrodes implanted in the Schaffer collaterals of the hippocampus of the rat, epileptogenesis was monitored through a second bundle straddling the pyramidal cell layer in area CA1. Experimental and implanted control groups consisted of at least 7 animals. Kindling was continued until six generalized convulsions (Racine class 5). Rats were sacrificed and neurons were enzymatically (trypsin) and mechanically dissociated from the focal area. Calcium currents were measured under voltage-clamp in the whole-cell patch configuration. Previously we showed that 24 hours after the last seizure the slow inactivating (SI, time constant 75 ms) and the non-inactivating (NI) component of the calcium current (activated by a depolarization to 0 mV from a 3 s prepulse at -120 mV) were enhanced by 36% and 39% in amplitude, without changes in kinetics or voltage dependency. The fast inactivating component (15 ms) did not change. We now show that this enhancement is persistent at six weeks after the last kindling stimulation (28% and 42% for SI and NI) which relates it to the kindled state instead of being an aftereffect of the epileptiform discharge. From double pulse protocols and repetitive activation of calcium currents we concluded that changes in calcium dependent inactivation can not explain our findings.

Potassium currents were investigated with the same techniques. The non-inactivating delayed rectifier was not changed after kindling. Measured 24 hours after kindling the A-current showed a transient enhancement in amplitude (62% higher than control) which disappeared at six weeks after kindling.

The kindled state of increased excitability.is therefore specifically associated with a persistent enhancement of slow calcium current.

71.4

EFFECTS OF PARTIAL HIPPOCAMPAL KINDLING IN VIVO ON INTRACELLULARLY RECORDED PAIRED-PULSE RESPONSES AND MEMBRANE PROPERTIES OF HIPPOCAMPAL CA1 NEURONS IN VITRO. D. Zhao, Xiao-Wen Fu* and L. Stan Leung. Depts. Physiology and Clin. Neurol. Sci., Univ. Western Ontario, London, Ontario N6A 5A5 Canada.

Kindled rats received 15 afterdischarges (ADs) evoked hourly, 5 times a day, by 1-sec 100Hz stimulus trains delivered to one hippocampal CA1. Control rats received the same number of pulses at a similar intensity but at 0.17 Hz. One-2 days or 21-23 days after the last stimulation, hippocampal slices were obtained from the rats, with the experimenter blind to the previous history of stimulation. Paired-pulses at 10-200ms interpulse intervals (IPIs) were delivered to the str. radiatum near CA3, and intracellular responses were recorded from the CA1 neurons. Previous studies in our laboratory indicate that the population excitatory postsynaptic potentials (EPSPs) and population spike in CA1 were facilitated by partial kindling. This study confirms the facilitation of paired-pulse EPSPs with intracellular recordings. The intracellular EPSP paired-pulse index (EPI= slope of 2nd EPSP/ slope of 1st EPSP) was significantly larger in 'kindled' than 'control' neurons, on either day 1 or day 21 after kindling ($P<0.001$, $F(df=1,22$ or $28) > 13$). Whether the first pulse evoked a spike or not did not affect the EPI of kindled or control neurons, suggesting that the afterhyperpolarization (AHP) did not affect the EPI. Membrane properties that were not affected by kindling included the resting membrane potential, threshold, height and duration of the action potential, the fast AHP amplitude , input resistance and the EPSP threshold. In other rats, bath perfusion of bicuculline methiodide (25-50 μM) and perfusion of 8mM $[K^+]_o$ significantly increased the intracellular EPI. Since paired-pulse facilitation of the EPSP is probably presynaptically mediated, kindling, bicuculline and 8mM $[K^+]_o$ may all suppress presynaptic GABA-A inhibition. (Supported by NSERC).

71.5

A POSSIBLE ROLE FOR THE HORMONES OF THE HPA AXIS IN THE DEVELOPMENT OF KINDLED SEIZURES. G. K. Weiss, M. Fernandez, N. Castillo, T. Hoffman, A. Ratner* and K. Lucero. University of New Mexico School of Medicine, Department of Physiology, Albuquerque, New Mexico.

We are investigating the possibility that hormones of the HPA axis (CRF, ACTH, CORT) may influence the development of kindled seizures. It is known that the HPA axis is activated during kindled seizures. Is there a feedback effect of these hormones on the development of kindled seizures? Previous experiments investigating the effect of adrenalectomy on the kindling procedure have been inconclusive. We now report that adrenalectomy decreases the relatively rapid kindling rate when kindling is done in the evening (circadian cort high); increases the relatively slow rate of kindling when it is done in the morning (circadian cort low) and has no effect on mid-day kindling rate. Other experiments using metyrapone to block CORT production produced the same results. Studies in which dexamethasone was injected prior to kindling increased the rate in both the morning and evening kindled rats. We also kindled Lewis rats at the evening time. They have been shown to lack both a circadian and stress response of the HPA axis. They required 16 kindling stimulations to progress through stages 1-2 while the controls (Fisher-344) took 6.8. Is the difference related to the absence of a HPA axis response? Studies are in progress to further investigate this possibility, and determine which hormones of the HPA axis may play a role in the development of kindled seizures. (Supported by NIH Grants RR08139 & NS23262)

71.6

EFFECTS OF AMINOPHOSPHONOVALERIC ACID (APV) ON CARBACHOL-INDUCED KINDLING IN THE RAT AMYGDALA; A PRELIMINARY REPORT. **D.Saucier* and D.P. Cain, Department of Psychology, Univ. of Western Ontario, London, Ontario, CANADA N6A 5C2.**

The effects of APV, a competitive NMDA antagonist (7.5 µg in 0.5 µl) on carbachol-induced (5nM in 0.5 µl) kindling were studied using chemitrodes implanted in the right basolateral amygdala (BLAM) of male rats. An electrode was implanted in the contralateral structure for recording purposes. APV was injected (using an infusion pump) into the BLAM 15 minutes prior to the injection of the carbachol. Control rats were given either injections of saline 15 minutes prior to carbachol injections or carbachol injections only, into the BLAM. Injections were given every 48 hours. The effects of the injections were measured both behaviourally and electrographically. Kindled seizures occurred in the APV + carbachol group, although the overall duration of epileptiform spikes was significantly shorter relative to controls. Behavioural convulsions appeared similar in the two groups. These data suggest that activity of local NMDA receptors is not essential for carbachol kindling. Supported by an NSERC grant to DPC.

71.7

LAMINAR PROFILE OF $[K^+]_o$ INCREASES AND EPILEPTIFORM ACTIVITIES IN NEOCORTICAL SLICES FROM PTZ-KINDLED RATS. D.Saar, E. Barkai, and M.J. Gutnick,* Dept. Physiology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beersheva, Israel.

In a model of chronic epilepsy - kindling induced by systemic pentylenetetrazole - neocortical slice neurons at different subpial depths responded differently during evoked interictal events. Cells in deep layers generated a paroxysmal depolarization shift (DS) with a reversal potential around 0 mV, similar to neurons at all depths in acute, picrotoxin-induced hypersynchrony. However, in superficial neurons E_{DS} was around -50 mV; it gradually shifted in the depolarizing direction during low frequency (.2-1 Hz) stimulation. In recordings with ion-sensitive microelectrodes, largest increases in $[K^+]_o$ during single paroxysmal events were recorded in deeper layers of kindled slices, as opposed to superficial layers of picrotoxin-treated slices. In both the chronic and the acute models, the rise in $[K^+]_o$ began after the onset of the paroxysmal wave, and was correlated with the later part of the DS. In both models, bath application of APV (20 μM) caused a decrease in the duration of the DS, and marked attenuation of the associated rise in $[K^+]_o$.

Drop application of glutamate in normal neocortical slices bathed in TTX, evoked CNQX-resistant, APV-sensitive focal increases in $[K^+]_o$ which were greatest in superficial layers. By contrast, in slices from kindled rats the $[K^+]_o$ rises were greatest in deep layers.

These data indicate that this form of kindling entails a long-term functional change in synaptic organization of the local neocortical circuitry; they are consistent with the hypothesis that laminar redistribution of NMDA-type receptors is involved.

Supported by a grant from the DFG (SFB 194)

71.8

LONG LASTING CHANGES IN TRANSMISSION FOLLOWING REPEATED PERFORANT PATH STIMULATION DEPENDS ON TEST PROTOCOL. N.W. Milgram*, M. Khurgel[1] G.O. Ivy[1] A. Hartawidjojo[1], and R.J. Racine.[2] Univ. of Toronto, Scarb., ON M1C 1A4[1] and McMaster Univ., Hamilton, ON L8S 1B9[2].

Recurrent seizures induced either chemically or by repeated electrical stimulation produce a long-lasting *increase* in inhibition generated in dentate gyrus (DG) by stimulation of the perforant path (PP). This finding has been reported from freely moving and from anesthetized animals, as well as in brain slices. A long-lasting *decrease* in inhibition has been reported following sustained electrical stimulation of PP in urethane anesthetized animals. In this study, the measure of inhibition was based on a protocol for recording *frequency dependent inhibition* (FTP) which develops during the delivery of a train of pulse pairs (2Hz).

In an attempt to account for these different long-term changes, we studied paired pulse inhibition produced by sustained PP stimulation in rats which were chronically prepared with electrodes in PP and DG. Baseline measures of paired pulse inhibition and facilitation at a range of interpulse intervals were taken in both the freely moving state, and under urethane. Additionally, we obtained baseline measures of FTP during delivery of 2 Hz trains of pulse-pairs at interpulse intervals of both 40 and 60 msec. Following recovery from the stimulation treatment, the animals were studied at regular intervals over a 2 month period. No consistent changes were noted in paired pulse inhibition at short inter pulse intervals during this period, but every animal showed a *decrease* in paired pulse facilitation. At the end of this period, the animals were again anesthetized with urethane, and were retested for FTP. This test revealed consistent evidence of inhibitory loss at a 60 msec, but not at a 40 msec interpulse interval. Additionally, there was an increased frequency suppression of the response to the first pulse of the pulse pair. The results therefore provide evidence that frequency dependent suppression is distinct from inhibition produced by presentation of a single pulse.

71.9

SITE-SPECIFICITY OF KINDLING ANTAGONISM IN RATS. *M. E. Corcoran*, T. H. Gilbert, & R. D. Kirkby. Dept. of Psychology, U. of Victoria, BC, Canada, V8W 3P5.*

With concurrent alternate stimulation of two forebrain sites, one of the sites (dominant) supports typical progressive seizure generalization (kindling), whereas the other site (suppressed) supports only focal or partial seizures (antagonism). Because antagonism may reflect an actual arrest of kindling from the suppressed site (Burchfiel & Applegate, Neurosci. Biobehav. Rev., 1989), we have attempted to replicate these important observations.

We implanted two bipolar electrodes into adult male Long-Evans rats, which were stimulated once per day with 1-sec trains of square-wave pulses (biphasic, 60 pps). Stimulation site alternated daily until six consecutive generalized seizures were provoked from one of the sites.

Kindling antagonism was displayed by animals stimulated in the septum (SE) and either the corpus callosum (6/8) or amygdala (AM) (18/31), with the AM being dominant in all cases. The cingulate cortex, however, was always dominant over the AM (n = 3). Whereas one rat receiving bilateral amygdaloid stimulation exhibited a clear pattern of antagonism, the five remaining rats inconsistently displayed generalized seizures from one or both sites (mutual antagonism; Burchfiel et al., Exp. Neurol., 1982). Surprisingly, antagonism was not observed in any rats stimulated in the SE and either the entorhinal cortex (n = 5), angular bundle (n = 5), dorsal hippocampal commissure (n = 4), or contralateral SE (n = 7).

We have replicated kindling antagonism, but found that it was site-specifically less robust than did Burchfiel and Applegate, who applied monophasic pulse trains (100 pps) to Sprague-Dawley rats multiple times daily. These methodological differences may account for the somewhat discrepant findings.

71.10

LONG LASTING CHANGES IN GENETIC EXPRESSION PRODUCED BY KINDLING. R.J. DeLorenzo*, J.B. Perlin, C. Gerwin, and R. Vick, E.R. Jakoi. Dept. of Neurology, Medical College of Virginia, Richmond, VA 23298.

Kindling is a model of long-term plasticity changes in brain and involves, in part, a potentiation of excitatory amino acid (EAA) synapses, especially the N-methyl-D-aspartate (NDMA) receptors. We have recently shown that EAA and NMDA receptor activation in hippocampal neurons in culture can produce long-lasting and selective regulation of the expression of a gene encoding the membrane associated protein ligatin (Brain Research, in press, 1992). To test the hypothesis that repeated subconvulsant stimulations required to induce kindling can permanently alter gene expression of hippocampal neurons, we measured steady state mRNA levels encoding several phenotypic proteins including ligatin by Northern and in situ hybridization analyses. At six weeks following kindling, hippocampal steady state ligatin mRNA levels were decreased by approximately 30% in comparison to control and sham animals. Northern blot analysis of total mRNA isolated from sham and kindled animals verified selective recognition of the 2.4 Kb ligatin transcript and showed that kindling produced a reproducible decrease in the ligatin transcript. In situ hybridization studies were performed 3 months after the last kindling stimulus showed significantly reduced ligatin mRNA levels bilaterally in specific hippocampal neurons while not effecting the level of α CaM Kinase mRNAs in adjacent sections. A significant decrease in the immunocytochemical labeling of ligatin protein in hippocampal neurons of kindled brains. These results provide the first direct evidence that the physiological alteration in neuronal excitability that occurs in kindling can alter gene expression in a long-lasting manner in identified hippocampal neurons.

71.11

CHANGES IN GLUTAMATE RECEPTOR AND PROENKEPHALIN GENE EXPRESSION AFTER KINDLED SEIZURES. S. Lee, J. Miskovsky, J. Williamson[1], R. Howells, E. Lothman*[1] and S. Christakos. Dept. of Biochemistry, UMDNJ-New Jersey Med. Sch., Newark, NJ 07103, [1]Dept. of Neurology, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908

Changes in gene expression after kindled seizures were examined using microdissection of discrete brain areas and Northern and slot blot analyses. Experimental animals were kindled with either of two protocols: 1) a paradigm in which 50 Hz/10 sec. stimulus trains were delivered every 30 minutes through hippocampal electrodes (12 stimulations every other day for 4 days) and 2) a traditional approach in which 50 Hz/10 sec. stimulus trains were given to the hippocampus three times daily for 16 days. Rats were sacrificed 24 hours or 30 days after the last kindled seizure. We first examined the possibility that kindling may affect transcription of mRNA for neurotransmitter receptors. We found significant decreases (1.7-4.7 fold) in AMPA/kainate activated glutamate receptor mRNAs (GluR-1, -2, -3 mRNAs) in hippocampus, amygdala/entorhinal cortex and in frontoparietal cortex 24 hours but not 30 days after rapidly kindled seizures. However, changes in GABA receptor α_1, α_2, α_4 or β_1 mRNAs were not observed in any brain region 30 days after traditional kindling or 24 hours after rapidly kindled seizures. In addition, we also tested whether changes in the expression of proenkephalin could be detected after kindling. We found a significant 3.5-3.6 fold induction in proenkephalin mRNA in the hippocampus and in the amygdala/entorhinal cortex 24 hours but not 30 days after rapidly kindled seizures. Our findings suggest that changes in glutamate receptor and proenkephalin gene expression are robust, acute sequelae to kindled seizures and may perhaps be involved in kindling. Supported by NIH NS-20270

71.12

CHANGES OF mRNAs ENCODING EXCITATORY AMINO ACID RECEPTORS IN AMYGDALA KINDLED RATS DETECTED BY IN SITU HYBRIDIZATION. M.Hikiji, Y.Fujiwara*, H.Tomita, I.Kohira, M.Ono and K.Akiyama. Dept.of Neuropsychiat.Okayama Univ.Medical Sch., Shikata-cho 2-5-1 Okayama 700, Japan

To investigate lasting changes of excitatory amino acid receptor subtypes at their mRNA level in kinding, we performed in situ hybridization with synthetic oligonucleotide-probes complimentary to NMDAR1 (Moriyoshi et al. 1991), GluR-2 (Keinanen et al. 1990), KA-1 (Werner et al. 1991) and mGluR1 (Masu et al. 1991). Amygdala kindling-established rats were sacrificed 28 days after the last kindled seizure. As a result, kainate receptor mRNA detected by KA-1 probe increased on both sides of hippocampal CA3 region and there were no significant modifications of NMDA (NMDAR1), AMPA (GluR-2) and metabotropic glutamate (mGluR1) receptor mRNAs. This change consists with the report of increase of [^{3}H]kainate binding in hippocampus of amygdala-kindled rat (Represaet al. 1989) and might indicate that new excitatory neural circuit mediated by kainate receptor is formed in hippocampus by kindling.

71.13

HUMAN ASTROCYTES CULTURED FROM EPILEPTIC FOCI ARE PHENOTYPICALLY DISTINCT AND EXHIBIT ENHANCED GLUTAMATE TURNOVER. S. N. Magge, M. L. Brines*, A. Cornell-Bell, M. J. During, D.D. Spencer, and N. C. de Lanerolle. Section of Neurosurgery, Yale University School of Medicine, New Haven, CT 06510.

Since astrocytes are important in regulating extracellular levels of excitatory neurotransmitters, neuronal hyperexcitability in epilepsy could arise from primary abnormalities of glial glutamate handling. Surgical specimens obtained from epilepsy patients offer a unique opportunity to test this hypothesis. Therefore, tissue was obtained from tumor-associated neocortical and medial temporal lobe (MTL) seizure foci. Using EEG criteria, neocortex was divided into tumor, adjacent hyperexcitable, and normal regions, while MTL tissue was divided into hippocampus, parahippocampus, and normal temporal neocortex. A series of enzymatic digestions were then used to generate primary astrocyte cultures.

Immunohistochemical characterization of these cultures revealed two principle glial types--flat protoplasmic cells that stain weakly for GFAP and strongly for vimentin and fibrocytic process bearing cells that stain intensely for GFAP and weakly for vimentin. In neocortical cultures, the numbers of fibrous astrocytes were 2-4 fold greater in hyperexcitable and tumor regions compared to normal. Fibrous cell density in MTL-derived cultures varied considerably among different patients.

HPLC analysis of conditioned media showed that astrocyte glutamate synthesis was related to in vivo hyperexcitability. Tumor-derived astrocytes produced glutamate concentrations greater than twice that of normal cultures, with some tumor cultures producing glutamate to concentrations over 300µM. In MTL cultures, similar 2-3 fold increases in glutamate were observed from hippocampus and parahippocampus over normal.

Overall glutamate uptake into cells was assessed using depletion of ^{3}H-glutamate from media over time. Uptake occurred rapidly with hyperexcitable and tumor cultures showing about 1.5 and 2 times the capacity of normal cultures for uptake respectively. MTL cultures also showed a gradation of glutamate uptake capacity with parahippocampus > hippocampus > normal cortex.

These differences in glutamate handling by astrocytes cultured from specific brain regions suggest that in vivo derangements of glial function may contribute to epileptogenesis.

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71.14

LASER PHOTOBLEACH-RECOVERY SHOWS INCREASED GAP JUNCTION COUPLING ON ASTROCYTES FROM HUMAN EPILEPTIC FOCI WHICH EXHIBIT HYPEREXCITABLE CA^{2+} RESPONSES. S. Lee, S.Magge, D.D.Spencer and A.H. Cornell-Bell*, Cell Biology and Neurosurgery, Yale University School of Medicine, New Haven, CT 06510

Human temporal lobes obtained from epilepsy surgeries provide hippocampus, surrounding parahippocampus (hyperexcitable EEG activity) and normal cortex (normal EEG activity) for culture. Primary astrocyte cultures reflect their seizure experience for up to 7 weeks in vitro. Sclerotic hippocampal astrocytes have a decreased response to glutamate whereas parahippocampal cells show a dramatic elevation in the frequency of intracellular Ca^{2+} oscillations and intercellular Ca^{2+} waves. Laser photobleach-recovery was used to quantify the degree of gap junction coupling in the astrocyte syncitium cultured from epileptic and normal tissues. Normal cortical astrocytes refilled (to Time x 1/e) after photobleach to a final level that was 25% of the original cell flourescence within 375.4 sec (n=17). Sclerotic hippocampal cells were somewhat slower (T x 1/e = 428 sec) and refilled to a level 26% of the original flourescence(n=7). Interestingly, Ca^{2+} hyperexcitable parahippocampal cells (n=14) refilled in less time (T x 1/e = 218 sec) and to a level that was 42% of original cell flourescence indicating a comparatively higher degree of coupling . Astrocyte cultures were loaded with 6-carboxyfluorescein diacetate AM ester (7 ug/ml from Molecular Probes). Fluorescent molecules de-esterified intracellularly are no longer diffuseable. A desired target cell is photobleached with a laser on a Bio-Rad MRC600 that is zoomed to 8 and is focused on a small area. Fluorescence is bleached with 15 scans of the laser taken 1 sec apart. Dye refilling is monitored using time-lapse confocal scanning laser microscopy and is corrected for bleach and offset errors (see Finkbeiner, 1992).

DEGENERATIVE DISEASE: ALZHEIMER'S—β-AMYLOID II

72.1

SUBSTANCE P INDUCES DEPHOSPHORYLATION OF TAU IN RAT BRAIN CEREBRAL CORTICAL SLICES. M.L. Miller and G.V.W. Johnson*. Department of Psychiatry, University of Alabama at Birmingham, Birmingham, AL 35294-0017.

Substance P belongs to the tachykinin family of neuroactive peptides and is present in neuronal systems in many parts of the CNS including the cerebral cortex, striatum and basal forebrain. Substance P is a potent depolarizing agent, has been postulated to modulate second messenger systems, and appears to protect against certain neurotoxic damage. Interestingly, the β-amyloid protein found in Alzheimer's disease brain contains a conserved domain that is homologous to a conserved region in tachykinins. In this study we examined the effects of substance P on the phosphorylation state of two heat-stable, microtubule-associated proteins, tau and MAP-2.

Cortical slices were incubated in the absence or presence of various concentrations of substance P, the reactions stopped and the heat-stable proteins extracted. After determination of protein concentration, the samples were subjected to back phosphorylation with the catalytic subunit of cAMP-dependent protein kinase in the presence of ^{32}P-ATP. There was a significant increase in the in vitro incorporation of ^{32}P into tau which had been treated with substance P compared with controls. This increase was inhibited by the addition of spantide, a specific substance P antagonist. Peptide mapping suggests that the increase in phosphorylation is confined to a single peptide. No significant differences in the phosphorylation of MAP-2 were apparent. These results indicate that substance P induces a selective dephosphorylation of tau.

Supported by NIH grants NS27538 and AG06569, and grants from the American Health Assistance Foundation and Alzheimer's Association.

72.2

THE AMNESTIC EFFECTS OF BETA-AMYLOID FRAGMENTS ON PASSIVE AVOIDANCE RESPONDING IN MICE ARE BLOCKED BY SUBSTANCE P. M. E. Hall and J. M. Stewart. Dept. of Biochem. and Neuroscience Training Program, University of Colorado Medical School, Denver, CO 80262.

Recent findings have strongly implicated the peptide beta-amyloid (bA) in the etiology of Alzheimer's Disease (AD). This peptide and some of its fragments have been shown to have neurotoxic effects in vitro and in vivo. Furthermore, Flood et al, (PNAS, 88:3363-3366, 1991) have reported that acute treatment with bA fragments can cause amnestic effects in mice. Immediate post-training intraventricular injection of the bA fragment bA(18-28) resulted in a dose-dependent impairment of PAR, which was statistically significant at a dose of 10 nanomoles (nmol). Based on reports that substance P (SP) can block the cytotoxic effects of bA, the effects of SP on bA-induced impairment of PAR were examined. Co-injection of several doses of SP resulted in a dose-dependent block of the amnestic effect of 10 nmol of bA(18-28). SP fragments were also co-injected with bA(18-28) to determine whether the N- or C-terminal portion of SP mediates its protective effect. It was observed that the N-terminal SP fragment SP(1-7) was as effective as SP in blocking the amnestic effect of bA(18-28), while the C-terminal SP fragment $pGlu^{6}$-SP(6-11) and the C-terminal SP analog DiMe-C7 were ineffective. These results suggest that the anti-amnestic effects of SP are mediated by its N-terminal region and not its C-terminal, tachykinin region.

72.3

MUTATIONAL ANALYSIS OF ß-AMYLOID PRECURSOR PROTEIN PROCESSING. Lore Lewis-Higgins* and Kevin M. Felsenstein. Dept. of Biophysics and Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492.

Alzheimer's Disease (AD) is a debilitating neurodegenerative disorder that afflicts more than 4 million in the U.S. alone. These individuals exhibit progressive cognitive impairments which are predominately associated with degenerating neurons in the basal forebrain, cerebral cortex, and hippocampus. Post-mortem examination of AD brains reveal neurofibrillary tangles, neuritic plaques, and amyloid deposition in meningeal blood vessels. Most cases of AD are sporadic, however, several families with autosomal dominant inheritance patterns have been identified. To date, four single point mutations in the ß-APP gene have been identified. Three substitutions at amino acid 717 are linked to early onset AD, while a single substitution at amino acid 693 results in hereditary cerebral hemorrhage with amyloidosis of dutch-type (HCHWA-D). The neuritc plaques and cerebrovascular amyloid are composed of an ~4.2 kDa protein derived from the proteolysis of the ß-amyloid precursor protein (ß-APP). The ß-protein region of ß-APP is normally proteolytically cleaved by the activity referred to as APP-secretase at lys^{16}. This cleavage results in the disruption of the ß-protein and would prevent any formation of ß-amyloid. Thus, the generation of intact ß-protein is the result of alternative proteolytic processing of the ß-APP molecule.

The goal of the present study was to examine the effects of the HCHWA-D and FAD mutations and mutations at or near the APP-secretase site on normal and alternative processing of ß-APP. Using a novel fusion protein expression system data will be presented that suggest certain mutations may lead to changes in the processing pattern of ß-APP.

72.4

DEVELOPMENT OF A FUSION GENE SYSTEM FOR THE ANALYSIS OF ß-AMYLOID PRECURSOR PROTEIN PROCESSING. Kevin M. Felsenstein, Susan B. Roberts, Kim M. Ingalls, Darcy A. Grisel, and E. Edward Baetge*. Dept. of Biophysics and Molecular Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492.

Alzheimer's Disease (AD) is the fourth leading cause of death and the leading cause of memory loss and dementia in the United States. Throughout the world it accounts for one-half to two-thirds of all cases of progressive dementia. The deposition of ß-protein (senile plaques and cerebrovascular deposits) and the formation of neurofibrillary tangles in cortex are the major pathological hallmarks AD. The 4.2 kDa ß-protein is derived from the 110-130 kDa ß-amyloid precursor protein (ß-APP). ß-APP is differentially processed to yield both non-amyloidogenic and potentially amyloidogenic products. Non-amyloidogenic processing is defined by a cleavage within the ß-protein sequence by a proteolytic activity referred to as APP-secretase. The cleavage releases the protease nexin II domain of ß-APP, and a non-amyloidogenic fragment is retained in the cell membrane and degraded by the cell. However, alternative proteolytic pathways exist that process the ß-APP protein such that potentially amyloidogenic fragments containing intact ß-protein are formed. Neither the cellular pathways nor the proteolytic activities involved are fully characterized.

To study ß-APP processing and the generation of ß-protein, we have developed and validated a novel fusion protein expression system that mimics the processing of ß-APP. The reporter system has provided extremely sensitive methods for detecting small quantities of products and clearly confirming the existence of multiple processing pathways. We will present data for alternative cleavages and propose a model for ß-APP processing.

72.5

EVIDENCE SUGGESTING THAT β-PROTEIN IS GENERATED IN CELLS EXPRESSING THE CARBOXYL TERMINAL 100 AMINO ACIDS OF THE AMYLOID PRECURSOR PROTEIN (APP). T.L. Martin*, D. Franco and K.M. Felsenstein. Bristol-Myers Squibb, Wallingford, CT 06492.

Alzheimer's disease is characterized pathologically by diffuse cerebral atrophy and the presence of senile plaques and neurofibrillary tangles in cortex. Data from a series of recent genetic linkage studies has provided powerful support for the notion that the APP gene plays a central role in the pathogenesis of Alzheimer's Disease. The hypothesis that C- terminal fragments of APP may themselves be capable of mediating the cell death characteristic of the AD brain has been proposed on the basis of studies conducted by several groups. Data surrounding a precise definition of the culprit species is mixed; half suggest that the intact or aggregated C- terminal 100 amino acid fragment is toxic while others implicate β-protein, the 42 amino acid molecule coded for by the amino terminus of the larger 100 amino acid fragment described above as the critical moiety. We will present data that will consolidate these apparently variable observations. Cells stably transfected with the APP-C100 fragment were subjected to immunological analysis employing antibodies to the C- terminal domain of APP. Cells expressing this construct were observed to contain two unique bands running at apparent molecular weights of 14 & 9 kD. Based on the uniqueness of these two bands to the transfected cell lines, their reactivity with multiple C- terminal APP antibodies and the relative molecular weights, we hypothesize that the 14 kD parent molecule undergoes proteolytic cleavage resulting in the generation of a 9 kD C- terminal fragment and a ~5kD amino terminal moiety potentially analogous to β-protein.

72.6

MUTANT AMYLOID PRECURSOR PROTEIN INCREASES PRODUCTION OF POTENTIALLY AMYLOIDOGENIC FRAGMENTS. I.T. Durkin, T. Loh, S.P. Trusko, M.J. Savage, M.A. Glicksman*, R.W. Scott and R. Siman. Cephalon, Inc., West Chester, PA 19380

The amyloid plaques deposited in the brains of Alzheimer's disease patients are largely composed of the β/A4 peptide. Secretion of the precursor to this peptide, the amyloid precursor protein (APP), involves cleavage within the β/A4 domain. Amyloid deposition therefore requires processing of APP by other pathways. We are modeling aberrant processing of APP by expressing APP mutants in cultured cell lines. DNAs coding for mutants of APP751 were inserted into CMV expression vectors and transfected by $CaPO_4$ co-precipitation. Western blots of conditioned media and cell lysates were probed with APP antibodies, and the relative amounts of various forms of APP quantitated by laser densitometry. A double mutation near the secretory processing site, K668E/F671D, was defective in secretion: the ratio (secreted APP in medium)/(fully-glycosylated APP in lysate) was less for the mutant than for the wild-type. Either mutation alone had no detectable effect on secretion. Antisera against the COOH-terminus of APP specifically immunoprecipitated from cell lysates a 9.5 kDa fragment, the mobility of the product of secretory processing. The antisera also precipitated smaller amounts of larger fragments, one of which co-migrated with the COOH-terminal 100 residues of APP (which contain the entire β/A4 domain). Cells transfected with the K668E/F671D construct produced more of these larger fragments, and less of the secretory processing product, than cells transfected with wild-type APP. These results demonstrate that the secretory protease is not sequence-specific, but may be sensitive to disruption of secondary structure near the substrate cleavage site. The mutant APP K668E/F671D is a poorer substrate for secretory processing, and so provides a useful model for elucidating alternate routes of APP processing.

72.7

BETA-AMYLOID PRECURSOR PROCESSING: MULTIPLE LYSOSOMAL PROTEASES GENERATE AND DEGRADE POTENTIALLY AMYLOIDOGENIC FRAGMENTS. R. Siman*, S. Mistretta, I.T. Durkin, M.J. Savage, T. Loh, S. Trusko and R. Scott. Cephalon, Inc., West Chester, PA 19380

Processing of the beta-amyloid precursor proteins (APPs) occurs in both secretory (Weidemann et al., Cell *57*, 115 (1989)) and lysosomal/endosomal (Golde et al., Science *255*, 728 (1992)) pathways. Secretory processing destroys the beta/A4 domain, but lysosomal proteolysis generates APP fragments that are potentially amyloidogenic, and could be precursors for amyloid deposition. We have examined routes of APP processing in human 293 cells stably transformed to overexpress APP751. Lysosomotropic agents and protease inhibitors of distinct specificities have been used to identify proteolytic events that may form or degrade potentially amyloidogenic fragments. Additionally, subclones of stable transformants were identified which differ markedly in efficiencies of APP secretion. APP fragments were identified by metabolic labelling and immunoprecipitation with antibodies to two different domains near the COOH-terminus, followed by high resolution Tris/Tricine SDS-PAGE. While secretory processing produced a single 9.5 kDa APP fragment, at least 6 fragments were generated within lysosomes, the largest four of which contain the entire beta/A4 domain. Lysosomotropic agents (NH_4Cl, chloroquine) prevented the appearance of all 6 fragments. However, inhibitors of lysosomal cysteine proteases (E-64, Z-Phe-Ala-CHN_2) caused accumulation of 5 of the fragments, suggesting that these fragments are generated by non-cysteine protease(s), and then are cleared by cysteine protease(s). In the presence of lysosomotropic agents, APP fragments failed to accumulate upon protease inhibitor treatment, confirming that the 5 fragments are formed within lysosomes. Low concentrations of Z-Phe-Ala-CHN_2 were used to selectively block the lysosomal cysteine protease cathepsin L, while higher concentrations were used to also inhibit the cysteine protease cathepsin B. Cathepsin L appears to be the major protease responsible for breakdown of potentially amyloidogenic fragments within lysosomes. The finding that a number of potentially amyloidogenic fragments of APP are routinely generated and then degraded by lysosomes suggests that altered lysosomal function could contribute to amyloid deposition.

72.8

TISSUE DISTRIBUTION IN THE RAT OF A MEMBRANE-ASSOCIATED METALLOENDOPEPTIDASE THAT MAY BE INVOLVED IN METABOLISM OF THE ALZHEIMER AMYLOID β/A4 PROTEIN (Aβ P). T. Yamamoto*[1], F. Kametani[2] and D. Allsop[3]. [1] Lab. of Molecular Recognition, Yokohama City Univ., Yokohama 236, Japan, [2] Dept. of Molecular Biology, Tokyo Institute of Psychiatry, Tokyo 156, Japan, and [3] Division of Biochemistry, The Queen University of Belfast, Northern Ireland, UK.

We have previously reported the presence of a membrane-associated amyloid β/A4 protein-cleaving protease(AβP-CP) in rat cerebral cortex (Soc. Neurosci. Abst. 17, 1448, 1991). This enzyme is a metalloendopeptidase that cleaves between His(14) and Gln(15) of a β/A4 8-17 synthetic peptide. We have now determined the regional tissue distribution of this enzyme in the adult rat. In the brain, cerebral cortex and hippocampus had the highest level of activity, while the striatum had only modest activity, and no significant activity could be detected in the cerebellum. Also no significant activity was observed outside the brain in peripheral tissues such as kidney, lung and pancreas. These data strengthen the idea that AβP-CP may play a physiological role in the proteolytic processing (degradation?) of AβP or its precursor since the gross regional profile of the enzyme mirrors the distribution of amyloid deposits in Alzheimer's disease.

72.9

β-Amyloid Precursor Protein is Processed by an Alternative Potentially Amyloidogenic Pathway Peter Seubert, Tilman Oltersdorf, Michael G. Lee, Robin Barbour, Cheryl Blomquist, David L. Davis, Karin Bryant, Douglas Galasko*#, Leon J. Thal#, Lawerence Fritz, Ivan Lieberburg, and Dale Schenk Athena Neurosciences, Inc. South San Francisco CA 94080. #Department of Neurology, Veteran's Administration Hospital, San Diego, CA 92162

Secretion of the β-amyloid precursor protein (β-APP) has previously been shown to be accompanied by cleavage within the β-amyloid protein (β-AP) sequence. Amyloidogenic metabolism of β-APP must then involve a different scheme. To test the idea that an alternative secretory pathway exists, which leaves β-AP intact, the conditioned-medium of human fetal mixed-brain cultures was depleted of β-APP possessing the β-AP 1-15 sequence by use of immuno-affinity methods. Material lacking the β-AP 1-15 epitope, but reactive with antibodies to other β-APP regions was detected. Immunological data suggest this material may be truncated at or near the N-terminal of the β-AP sequence.

The apparently truncated β-APP is secreted with a similar time course of maturation and cleavage as β-APP cleaved within the β-amyloid region, suggesting post-secretion events are not responsible for this form. A similarly truncated β-APP was found in CSF and, to a lesser extent, in conditioned medium from 293 kidney cells overexpressing either the 695 or 751 forms of β-APP. We suggest this alternative secretory event may be the first step in amyloid formation.

72.10

EVIDENCE THAT THE KPI DOMAIN IS NOT INVOLVED IN THE PROCESSING OF ALZHEIMER PRECURSOR PROTEIN. US Ladror[1], AM Manelli[1], TF Holzman[1] DE Frail[1*], GT Wang[1], WL Klein[2], GA Krafft[1]. [1]Abbott Labs. Abbott Park, IL 60064. [2] Dept. Neurobiol. Northwestern Univ., Evanston, IL 60208.

The A4 peptide, which accumulates in amyloid plaques in brains of Alzheimer disease (AD) patients, is the product of proteolytic cleavage of a larger protein called Amyloid precursor protein (APP). In this work we tested whether the Kunitz protease inhibitor (KPI) domain, which is present in 3 of 4 alternatively spliced forms of APP, affects protease activities in extracts from normal and AD brains against substrates that mimic the amyloidogenic cleavage site (AMYL) and the secretory cleavage site (SECR).

APP695 and APP751 were obtained from transfected HEK293 cells. Equal amounts of APP695 and APP751 were obtained from the media after 2 days of growth, suggesting an equal rate of secretion. This result indicates that the KPI domain in APP 751 did not inhibit the SECR cleavage in transfected cells. Protease activities in normal and AD brains were assayed at pH of 7.5 and at pH 6.0, using fluorogenic substrates. APP 695 and the KPI-containing APP751 had no effect on protease activities against the SECR and AMYL sites in extracts from both normal and AD brains. However, KPI-containing APP751, at similar concentrations, completely inhibited the activity of trypsin against these substrates. Activity against the SECR substrate in extract from normal brains at pH 7.5, produced a major cleavage at a site identical to that of trypsin, indicating that the cleavage is identical to the secretory cleavage. Under similar conditions the AMYL substrate was cleaved two positions down-stream from the AMYL site. These results suggest that the KPI domain is not involved in either the secretion of APP or the amyloidogenic cleavage of the APP protein.

72.11

CHARACTERIZATION OF ALZHEIMER-RELATED PROTEASE ACTIVITIES IN NORMAL AND ALZHEIMER BRAINS. RE Kohnken[1]*, US Ladror[1], GT Wang[1], TF Holzman[1], WL Klein[2], BE Miller[1], GA Krafft[1]. [1]Abbott Labs. Abbott Park, IL 60064. [2] Dept. Neurobiol. Northwestern Univ., Evanston, IL 60208.

Using fluorogenic substrates, we have examined protease activities against the secretory (SECR) site of the amyloid precursor protein (APP, positions 664-671 of APP751) and the amyloidogenic (AMYL) site of APP (positions 649-656) in normal and Alzheimer's (AD) brains. Protease activities in the high speed supernatant against the SECR site had an optimum at pH 3.0-4.0, and another broad optimum at pH 6.0-7.5. Protease activities against the AMYL site had an optimum at or below pH 3.0 and another at pH 6.0. Activities at low pH were significantly higher in AD brains relative to controls, however, at pH 6.0-7.5 normal brains had significantly higher activities than AD brains, using both substrates. Percent inhibition by TLCK, TPCK and PMSF and by sulfhydryl reagents $CuSO_4$, MMTS and PCMB, at pH 7.5 of activities against the SECR and AMYL sites is shown in the table. Pepstatin inhibited both activities at pH 3.5 94%, but did not affect activities above pH 5.5. Apparent K_M for the activities against the SECR site at pH 4.0 was 5-8 μM in extracts from normal and AD brains. The activity at pH 4.0 was reversibly stimulated about 3 fold by preincubation at the assay pH. The stimulation was time dependent and was reversed by increasing the pH to 7.0.

Inhibitor	SECR	AMYL
TLCK	16	31
TPCK	32	57
PMSF	18	25
$CuSO_4$	94	89
MMTS	79	87
PCMB	96	92

These results indicate that multiple activities are present in brain extracts that may be involved in APP processing *in vivo*. Higher acidic activity and lower alkaline activity in AD brain may result from the pathological state of AD brains.

72.12

***IN VITRO* PRODUCTION OF AMYLOIDOGENIC FRAGMENTS FROM ALZHEIMERS BETA-AMYLOID PRECURSOR PROTEIN (APP)** A. M. Brown+, A. Potempska#, A.J. Blume*+, D. Miller# and M.P. Vitek+ *Dept. of Molecular Pharmacology, Lederle Laboratories, Pearl River, NY 10965. #New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Recently several groups have isolated potentially amyliodogenic C-terminal fragments of APP from normal and AD brains [Estus et al Science **255**, 726 (1992)]. We have constructed an assay system that allows us to evaluate the ability of isolated proteases or proteolytic activities to generate amyloidogenic fragments of APP from full length APP and then compare them to endogenously produced fragments of APP. The assay employs purified APP from rat brain [Potempska et al. JBC **266**, 8464 (1991)] and rat brain extracts that are enriched in C-terminal APP fragments by immunopurification. Using this assay we have demonstated that Cathepsin-G is able to produce both amyloidogenic and non-amyloidogenic C-terminal fragments from full-length APP. Two major C-terminal products of approximately 9 and 11kd were found, which are similar in electrophoretic mobility to C-terminal fragments isolated from rat brain. The identity of these fragments was confirmed and the location of their N-termini was characterized by epitope mapping. The exact location of their N-termini is being determined by sequencing. When the proteolytic fragments were probed with antibodies to the N-terminus of APP we were able to observe conversion of the full length APP to a fragment of molecular weight similar to the extracellular form of APP with kinetics that were comparable to the conversion of the C-terminus to the short (9 & 11kd) forms. We have also begun to characterize the reaction between APP and other proteases whose localization in the brain or association with amyloid deposits make them candidates for the formation of BAP from APP.

72.13

DESIGN AND SYNTHESIS OF INTERNALLY QUENCHED FLUOROGENIC SUBSTRATES FOR NEURAL PROTEASES. GT Wang[1], US Ladror[1], TF Holzman[1], WL Klein[2], GA Krafft[1]*. [1]Abbott Labs. Abbott Park, IL 60064. [2] Dept. Neurobiol. Northwestern Univ., Evanston, IL 60208.

The formation of β/A4 amyloid from its precursor protein (APP) involves at least 2 distinct proteolytic events, and may result from altered processing or trafficking of APP. In order to characterize proteases that may be relevant to normal and amyloidogenic processing of APP, we have synthesized a series of internally quenched fluorogenic substrates that encompass putative cleavage sites for constitutive processing (secretase) and for β/A4 amyloid formation (amyloidogenic). In this paper, we describe experiments, using these fluorogenic substrates, to demonstrate that proteolytic activity in normal brains against the amyloidogenic substrate at pH 7.5 was stimulated by Ca^{2+}, and that the stimulation resulted form an effect of Ca^{2+} on the substrate and not on the protease involved in the cleavage. Activity against substrate version 1 was Ca^{2+}-stimulated 200-400%. Replacing two Glu's with Gln's (version 2) reduced Ca^{2+}-stimulation to 150-200% and inhibited the activity about 25%. Replacing all four Glu's with Gln's (version 3) reduced Ca^{2+}-stimulation to 30% but stimulated the activity to levels close to the Ca^{2+}-stimulated activity of version 1. Substrate version 1 was cleaved by brain extract at the N-side of Ala-8, two positions down stream from the amyloidogenic cleavage. These results suggest that the stimulatory effect of Ca^{2+} is facilitated by binding to the substrate.

Version	Substrate	No Ca^{2+}	1mM Ca^{2+}
J=Glutamyl-EDANS; B=Lysyl-DABCYL		*Relative rate*	
1	Ac-EJ**EVKMDAEF**BE-NH_2	1.00	2.95
2	Ac-QJ**EVKMDAEF**BQ-NH_2	0.70	1.86
3	Ac-QJ**QVKMDAQF**BQ-NH_2	2.70	3.62

72.14

BETA AMYLOID PEPTIDE (ßAP) IN VITRO TOXICITY: LOT-TO-LOT VARIABILITY. P.M. Robison*, D. Waters, B. Gitter, L. Simmons, G. Becker, J. Small and P.C. May, Depts. of CNS & Molecular Biology and Biotechnology Research, Lilly Research Labs, Eli Lilly and Co., Indianapolis, IN 46285

ßAP is associated with the neuritic plaques of Alzheimer's Disease (AD) and may contribute to the degeneration of neurons. Conflicting observations have been reported about the direct in vitro and in vivo neurotoxicity of ßAP. We have compared lots of ßAP preparations in high density rat hippocampal mixed primary cultures and found marked lot-to-lot differences. Cytotoxic effects of various ßAP 1-40 preparations were assessed in 10 DIV cultures treated in defined media for 4 days and toxicity was measured as LDH released into the media. Repeated experiments using different cell preparations and one lot of peptide obtained from a commercial supplier resulted in dose-dependent (1-10uM) direct neurotoxicity. Two other lots from the same supplier were essentially non-toxic at 10uM. Three additional ßAP preparations from unrelated sources also did not produce cytoxicity. Initial biochemical characterization of the 3 lots has not yet revealed any marked differences. Low levels of endotoxin contamination (1-2 EU/ml) were detected in several preparations but did not correlate with cytotoxicity. Our observation that some but not all preparations of ßAP are neurotoxic in vitro may account for much of the present controversy in this area.

72.15

LIPOPOLYSACCHARIDE-INDUCED CYTOTOXICITY IN PRIMARY RAT HIPPOCAMPAL CULTURES. **P.C. May*, P.M. Robison, R. Berry, D. Waters and B. Gitter, Dept. of CNS & Molecular Biology, Lilly Research Labs, CNS Division, Eli Lilly and Co., Indianapolis, IN 46285**

A key event in the pathogenesis of Alzheimer's disease (AD) may be the transition of ß/A4 amyloid deposits from diffuse to compacted neuritic plaques. This transition may involve an inflammatory process as numerous cytokines and acute phase proteins are found associated with the neuritic plaque. We have used lipopolysaccharide (LPS) treatment of primary mixed rat hippocampal cultures to elaborate a cytokine cascade and model the inflammatory process in AD brain. LPS treatment (10ng/ml-5ug/ml) resulted in cytotoxicity assessed by phase contrast microscopy and LDH release. LPS cytotoxicity follows a slow time course requiring >48 hours to be fully manifested. LPS cytotoxicity may be mediated through release of TNF as direct addition of recombinant murine TNFa produced cytotoxicity. Consistent with a role for cytokines, co-treatment with dexamethasone (10nM-1uM) and other glucocorticoids blocked LPS cytotoxicity in a dose dependent fashion; NSAIDs, EAA-receptor antagonists, and free radical scavengers were ineffective. These results indicate that inflammatory processes initiated by LPS treatment result in direct cytotoxicity in rat hippocampal cultures. Similar inflammatory processes may contribute to pathology in AD.

72.16

WITHDRAWN

73.1

CORTICAL DESTRUCTION IN HUNTINGTON's DISEASE. H.Braak* and E. Braak. Department of Anatomy. J.W.Goethe University, D-6000 Frankfurt/M. 70, Germany

Brains of individuals who had suffered from Huntington's disease (grade 3 and 4 according to Vonsattel et al. 1985) and control brains were examined. Frontal sections through the temporal lobe were stained for Nissl material and lipofuscin pigment. The diseased brains revealed a layer-specific loss of nerve cells in two allocortical areas. The most impressive changes were seen in the entorhinal cortex. The brunt of the destruction was born by the deepest entorhinal layer pri-γ which was found to be almost completely depleted of nerve cells. The upper cellular layer pre-α, the layer pre-γ and the subiculum showed less severe alterations. The entorhinal cortex and the subiculum are major relay stations of limbic circuits. The specific destruction of these cortical areas is considered to contribute to cognitive dysfunction seen in patients suffering from Huntington's disease.

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Vonsattel et al.(1985) J. Neuropath. exp. Neurol. 44, 559-577

73.2

THE QUINOLINIC ACID MODEL OF HUNTINGTON'S DISEASE: AN ELECTROPHYSIOLOGICAL APPROACH. F. Block, M. Schwarz, R. Töpper, J. Noth*

Besides a diminuition of amplitude of early somatosensory evoked potentials (SEPs) a decreased amplitude in visual evoked potentials (VEPs) has been described as neurophysiological changes in Huntington's disease (HD). The present study investigates whether quinolinic acid (QA) lesions confined to the striatum - an animal model of HD - are sufficient to induce these electrophysiological abnormalities. Two weeks after unilateral intrastriatal injection of QA (240 nmol) SEPs were recorded from the somatosensory cortex in response to electrical stimulation of the contralateral forelimb or VEPs response to a strobe light in rats under light pentobarbital anesthesia. Striatal QA lesion significantly reduced the amplitude of short latency cortical SEPs by about 40% without affecting the latency. The VEPs were not altered at all by QA treatment. The present results suggest that the QA animal model of HD also displays some of the SEP abnormalities of HD patients whereas VEPs are not affected.

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73.3

DIFFERENT SURVIVAL PATTERN OF STRIATAL INTERNEURONS AFTER SLOW VS FAST INTRASTRIATAL INJECTIONS OF QUINOLINIC ACID. G.Figueredo-Cardenas*, Q. Chen and A. Reiner, Dept. Anat and Neurobiol., College of Medicine, Univ. TN, Memphis, TN 38163.

A relative sparing of neurons containing somatostatin (SS)- neuropeptide Y (NPY) - NADPH- diaphorase compared to projection neurons has been found after intrastriatal injection of quinolinic acid (QA), an excitotoxin acting at NMDA receptors (Beal et al., J. Neurosci., '91). Such findings have been used in support of the excitotoxin hypothesis of Huntington's disease (HD) and to claim that intrastriatal QA produces an animal model of HD. However, other studies have reported that SS/NPY+ interneurons are highly vulnerable to QA (Davies et al., Nature, '87; Anderson and Reiner, Soc. Neurosci. Abstr., '91). Since these research groups used different intrastriatal injection methods, we examined the influence of the speed (3min vs. 15min) and the amount (1µl of 225mM QA vs. 1µl of 50mM QA) injected. After two weeks, alternate brain sections from these animals were processed for diaphorase and cresyl violet stained, and neurons of these types were drawn with camera lucida and counted in both lesioned and nonlesioned sides.

Our results showed a relative preservation of diaphorase neurons compared to Nissl-stained neurons after the large fast QA injections (88% of control diaphorase perikarya in areas with 40% of control Nissl-stained neurons). In contrast, the slow large QA injections resulted in poorer sparing of diaphorase neurons compared to Nissl-stained neurons (8% of control diaphorase perikarya in areas with 40% of control Nissl-stained neurons). Similarly enhanced relative survival of diaphorase neurons compared to Nissl-stained neurons was seen with the fast compared to slow small injections. Since rapid injections result in QA efflux, we interpret the results to suggest that brief compared to prolonged QA exposure may produce different patterns of diaphorase vs.projection neuron survival. NS-19620, NS-28721 (AR).

73.4

TRANSFORMING GROWTH FACTOR α IMMUNOREACTIVE STRIATAL NEURONS DEVELOP PROLIFERATIVE DENDRITIC CHANGES IN HUNTINGTON'S DISEASE. A. Evans*, R.J. Ferrante, K.M. Harrington, and N.W. Kowall, Massachusetts General Hospital, Boston, MA 02114.

Proliferative dendritic abnormalities affect spiny striatal neurons in Huntington's disease (HD) but the extent to which enkephalin and substance P subsets are involved is not known. It is also not clear if similar dendritic changes affect NADPH diaphorase aspiny neurons which are spared in HD. Transforming growth factor alpha (TGFα), which colocalizes with enkephalin in the striatum, more clearly labels discrete dendritic processes than either enkephalin or substance P. We therefore examined the distribution of TGFα and NADPH diaphorase in double stained sections of striatum from 6 HD patients and 5 normal controls. In HD, TGFα neurons were depleted in a dorsoventral gradient, similar to that seen with enkephalin and substance P antisera. The staining intensity of individual neurons, however, was somewhat increased over controls. Proliferative dendritic abnormalities and dysmorphic dendritic recurving affected TGFα but not NADPH diaphorase neurons. Our observations show that dendritic proliferation involves enkephalin neurons that contain a growth factor, TGFα, not present in substance P neurons. If TGFα plays a causal role in the development of dendritic proliferation, spiny substance P neurons should not show evidence of dendritic proliferation in HD. Furthermore, TGFα may exacerbate neuronal injury if dendritic proliferation leads to neuronal degeneration in HD.

73.5

EXAMINING RAPID RESPONSE GENES IN THE HUNTINGTON'S DISEASE ANIMAL MODEL P. D. Walker* & L. R. Carlock, Departments of Anatomy/Cell Biology & Molecular Biology/Genetics, Wayne State University School of Medicine, Detroit, MI 48201.

Intrastriatal injections of quinolinic acid (QA) produce a selective pattern of neostriatal damage analogous to Huntington's disease (HD), an autosomal dominant disorder localized to chromosome 4. To determine if the rodent model will be useful for identifying and defining HD candidate genes, we analyzed the QA-induced neostriatal expression pattern of a neuron-specific gene, D4S234, which maps adjacent to the HD genetic marker D4S10. The transcriptional response of this gene was compared to several intermediate-early genes (IEGs c-fos, jun-B, zif-268) that appear to be involved in the cellular reaction to excitotoxicity. Within the QA lesion site, D4S234 mRNA levels rapidly increased (161.7± 17.7% of saline controls; $p<0.02$) within 45 minutes. This early response was analogous to IEG induction and may occur via a Ca^{+2}/cAMP-linked regulatory site identified in the D4S234 promoter which is homologous to a FAP response element in the c-fos promoter.

At 4-12 hours post-QA, a decline in D4S234 expression correlated with the loss of neurotransmitter mRNAs indicative of neuronal death. From 1-7 days, D4S234 mRNA was reduced to 8.1 ± 3.5% ($p<0.0001$) of saline control neostriata. Interestingly, prior to the decline in D4S234, a second phase of IEG expression occurred between 1.5 - 4 hours and persisted up to 24 hours suggestive of glial response. These results support the use of the *in vivo* excitotoxic model for examining the expression of genes localized to the HD gene region and underscores the importance of focusing on the early-stage HD brain to determine changes in gene expression that may relate to neurotoxicity. (Supported by NS24236 to L.R.C.)

73.6

A SECOND PHASE OF INTERMEDIATE-EARLY GENE EXPRESSION CORRELATES WITH NEURONAL LOSS AND GLIAL/IMMUNE RESPONSE IN THE HUNTINGTON'S DISEASE MODEL. Y. Shan*, P. D. Walker & L. R. Carlock, Departments of Anatomy/Cell Biology & Molecular Biology/Genetics, Wayne State University School of Medicine, Detroit, MI 48201.

Intermediate-early gene (IEG) induction rapidly occurs within 1 hour following intrastriatal injections of quinolinic acid (QA). Although this early response may trigger neurotoxicity, the successful use of NMDA antagonists to inhibit neuronal death after the initial IEG phase suggests that later signaling events may induce neuronal death and glial response. Therefore, we detailed the acute expression patterns of IEG response after neostriatal QA to determine if additional phases of IEG expression could be correlated with neuronal loss and the activation of glial and immune cells.

Following the detection of the initial phase in IEG induction during the first hour following QA injection, a second and more prominent phase of neostriatal c-fos, jun-B and zif-268 mRNA elevation occurred between 1.5 - 4 hours and persisted up to 24 hours. Because a significant decline in neurotransmitter-specific mRNAs had been detected between 4 -12 hours, this second and larger wave of IEG response extending beyond 12 hours may relate to reactive gliosis. Interestingly, an additional wave of mRNA increases specific for the synthesis of interleukin-1β and heat-shock protein-70 peaked at 12 hours and gradually declined to control levels by 48 hours.

The identification of a second wave of IEG induction which appears to correlate with neuronal loss and subsequent glial/immune response suggests that a specific window of time (1-4 hours) following brain damage should be targeted in attempts to block neuronal loss and glial response to excitotoxicity. (Supported by NS24236 to L.R.C.)

73.7

FUNCTIONAL LOCOMOTOR HETEROGENEITY OF THE STRIATUM IN A RODENT MODEL OF HUNTINGTON'S DISEASE. E.E. Concepcion and W.C. Low. Departments of Neurosurgery and Physiology, and Program in Neuroscience, University of Minnesota Medical School, Minneapolis, MN 55455.

Recent studies utilizing a rodent model of Huntington's disease have demonstrated locomotor abnormalities following unilateral lesions of the striatum using neurotoxic analogs of excitatory amino acids. These abnormalities are manifested as an asymmetric rotation ipsilateral to the side of the lesion following apomorphine administration. In the present study we provide evidence for the dependence of rotational direction on the site of the striatal lesion. Sprague-Dawley rats were given lesions of the right striatum using stereotaxic injections of quinolinic acid (150 nmole in 1 μl saline). One group of rats received injections at AP 3.0 mm, ML 2.5 mm (with respect to bregma), and DV 4.5 mm (with respect to the dural surface). A second group received injections at AP 1.4 mm, ML 3.3 mm, and DV 4.5 mm. A third group received striatal injections at AP -0.2 mm, ML 4.25 mm, and DV 4.0 mm. The behavioral effects of apomorphine administration (1 mg/kg, s.c.) was examined one month after the lesion. Animals with anterior striatal lesions (AP 3.0 mm) displayed rotational biases contralateral to the side of the lesion while animals with more posterior lesions (AP 1.4 mm or -0.2 mm) exhibited ipsiversive rotations. Analysis of variance revealed significant group effects ($p < 0.05$). Post-hoc pair-wise comparisons revealed that animals with AP 1.4 mm and AP -0.2 mm did not differ in their rotational bias. Both of these groups, however, differed in their rotational biases in comparison to animals with AP 3.0 mm injections of quinolinic acid ($p < 0.05$). These results reflect a possible heterogeneity of locomotor function in the rodent striatum along its rostro-caudal axis. (Supported by NIH RO1-NS-24464).

73.8

A MORPHOMETRIC EXAMINATION OF EXTRASTRIATAL NUCLEI IN RATS WITH QUINOLINATE-INDUCED LESIONS OF THE STRIATUM. MF Mazurek* and JCS Furtado, McMaster University Medical Centre, Hamilton, ON, Canada

While it is well known that the brunt of the degenerative process in Huntington's Disease (HD) is sustained by the striatum, it has recently been recognized that many extrastriatal areas, including cerebral cortex, substantia nigra and thalamus, are also affected in HD. It is unclear whether this extrastriatal damage is reflective of independent pathology or secondary to degeneration of the striatal outflow neurons. We have begun to investigate this issue in rats with chronic unilateral quinolinate-induced lesions of the medial striatum. Nissl and AChE stains were used to outline the striatum, cortex and thalamus, while immunocytochemistry for tyrosine hydroxylase and substance P was used to delineate the boundaries of the substantia nigra. In lesioned animals, the striatum, substantia nigra and two thalamic nuclei (VA, VL) were significantly reduced in area (using the Bioquant IV programme) on the lesioned side. No changes in area were observed in the thalamus as a whole or in the frontal cortex. These findings indicate that damage to the striatum can produce morphological changes in "downstream" nuclei of the striatal-pallidal-thalamic-cortical circuit.

73.9

EARLY VULNERABILITY OF STRIOSOMES IN HUNTINGTON'S DISEASE STRIATUM. J.C. Hedreen* and S.E. Folstein. Neuropathology Lab. and Depts. of Pathology, Neuroscience and Psychiatry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

To define the early vulnerability of components of the neostriatum in Huntington's disease (HD), we have investigated the distribution of neuronal loss and accompanying fibrillary astrocytosis in neostriatal tissue from autopsy cases of HD. The earliest changes are scattered islands of neuronal loss and astrocytosis that occur prior to the previously described ventrally progressing wave of generalized neuronal loss. Examination of serial sections immunocytochemically stained for markers of the striatal patch-matrix system in an early case (Vonsattel grade 0) shows that these islands of early neuronal loss correspond to the striosome (patch) compartment of the neostriatal patch-matrix system, a finding supported by the study of ventral striatal regions in 13 additional grade 1-2 cases. Connections of striosomes suggest that the early degeneration of striosome neurons may be responsible for some of the early psychiatric and motor symptoms in HD.

73.10

EXCITATORY AMINO ACID RECEPTORS IN HUNTINGTON'S DISEASE NEOCORTEX. M.V. Wagster*, J.C. Hedreen, W. Paschen, D.L. Price, S.E. Folstein and C.A. Ross. Neuropathology Lab. and Depts. of Pathology, Psychiatry, Neurology, and Neuroscience, The Johns Hopkins University School of Med., Balto., MD 21205.

Several lines of evidence indicate the presence of lamina-specific neocortical pathology in individuals with Huntington's disease (HD), and excitotoxic mechanisms have been proposed. Using quantitative autoradiographic methods, we have measured NMDA, kainate, and AMPA receptor binding in HD and control middle frontal gyrus and motor cortex. In middle frontal gyrus, we detected no change in NMDA-sensitive [^{3}H] glutamate binding but found a selective decrease in [^{3}H] kainate (control=201.7; HD=156.1, corrected for shrinkage) and [^{3}H] AMPA binding (control=1108.2; HD=713.3, corrected for shrinkage) in layer VI, where we previously had found neuronal loss. [^{3}H] glutamate and [^{3}H] AMPA binding values in whole motor cortex did not reveal any deficits in HD; laminar analyses are in progress. We are preparing DNA probes for glutamate receptor subtype mRNAs to attempt to corroborate these data with *in situ* hybridization.

73.11

ALZ 50 LABELS ASPINY NEURONS IN NORMAL HUMAN STRIATUM THAT RESIST DEGENERATION IN HUNTINGTON'S DISEASE. K.M. Harrington*, F. Malchiodi Albedi, A.C. McKee, R.J. Ferrante and N.W. Kowall. Massachusetts General Hospital and Harvard Medical School, Boston MA 02114.

ALZ 50 is a monoclonal antibody raised against homogenates of Alzheimer's disease (AD) brain that labels pathological inclusions in AD and other disorders. Immunoblot studies show that ALZ 50 labels normal and hyperphosphorylated forms of the microtubule associated protein tau. Although initially thought to only recognize abnormal structures in AD brain, it is clear that normal neurons in rodent, primate and human brain are immunolabeled with ALZ 50. We noted that a prominent subset of normal appearing medium sized neurons were labelled by the ALZ 50 antibody (courtesy of H. Ghanbari) in normal adult human striatum. We studied the caudate nucleus in 6 normal controls and in 6 cases of HD to determine if ALZ 50 positive neurons were spared. In all HD cases there was dramatic preservation of ALZ 50 neurons that were morphologically identical to somatostatin-neuropeptide Y-NADPH diaphorase-NO synthase neurons. ALZ 50 neurons in normal brain may be enriched in a specific postranslational form of tau protein. Alternatively, ALZ 50 may crossreact with an epitope in an unrelated molecule. In either case, the localization of ALZ 50 immunoreactivity in a subset of striatal neurons in normal adult striatum portends a role for ALZ 50 immunoreactive proteins in normal neuronal physiology.

73.12

BRAIN KYNURENINE AMINOTRANSFERASES AND KYNURENIC ACID LEVELS IN HUNTINGTON'S DISEASE. D.A. Jauch*, E. Urbańska, P. Guidetti, F. Peretti, [1]W.O. Whetsell, Jr. and R. Schwarcz. Maryland Psych. Res. Center, Baltimore, MD 21228 and [1]Vanderbilt Univ. School Med., Nashville, TN 37232.

Two distinct kynurenine aminotransferases (KAT I and KAT II) exist in the human brain (Brain Res., 542: 307, 1991). Since their enzymatic product, the neuroprotective excitatory amino acid receptor antagonist kynurenic acid (KYNA), has been speculatively linked to the pathogenesis of Huntington's disease (HD; Life Sci., 35: 19, 1984), we have examined KYNA levels and the activity of KAT I and KAT II in several regions of brains from HD patients and control donors (N = 13 each). Using [^{3}H]kynurenine as the substrate and pyruvate as a cofactor, the enzymes were determined under optimal conditions (pH 10.0 for KAT I and pH 7.4 for KAT II) in tissue homogenates. Under these conditions, KAT I activity was several-fold higher than KAT II activity in all samples. Both enzyme activities were highest in cortical areas (e.g. control frontal cortex: KAT I: 104 ± 15 fmoles/mg tiss/h; KAT II: 20 ± 2 fmoles/mg tiss/h). Activities of both enzymes, as well as KYNA levels, expressed on a mg tissue basis, showed significant decreases (32-56%) in HD striata ($P < 0.05$). The effects were less pronounced when data were expressed per mg protein. These findings suggest diminished endogenous neuroprotection in the HD striatum. (This work was supported by USPHS grants NS 28236 and MH 31862).

73.13

EVIDENCE FOR ABNORMAL METABOLISM IN PATIENTS WITH HUNTINGTON'S DISEASE USING LOCALIZED ^{1}H NMR SPECTROSCOPY. **W.J. Koroshetz*, B.G. Jenkins, M.F. Beal, B.R. Rosen. Dept of Radiology and Neurology, Mass. Gen. Hospital, Boston Ma. 02114.**

Huntington's Disease (HD) is an autosomal dominantly inherited degeneration of striatum accompanied by generalized cerebral atrophy. Excessive excitatory stress and/or impaired mitochondrial function have been hypothesized to contribute to neurodegeneration in HD. Either mechanism might cause elevated lactate in the brain of patients with HD.

Using water suppressed STEAM-spectroscopy (Frahm et al,[1989] Magn Reson Med,,9:79) we studied 18 patients with various clinical stages of HD. Proton-spectra from voxels including occipital cortex demonstrated elevated lactate peaks at 1.33 ppm in all symptomatic patients. Average estimated lactate in individuals with clinically evident HD was 1.3 $\pm$ 0.5 mM (N=16). Normal lactate levels (0.4 $\pm$ 0.15 mM, N=12) were seen in 2 assymptomatic individuals who had a diagnosis made by DNA linkage analysis. Lactate levels in occipital cortex correlated best with duration of disease ($p<.0005$). Because of the relatively high content of heavy metal in basal ganglia, spectra from voxels containing basal ganglia have a poorer signal to noise ratio as compared to those containing occipital cortex. In 17/19 patients no significant lactate peak at 1.33 pm was seen, in 2/19 patients a broad peak was seen in the basal ganglia. Intersubject averaging improved the signal to noise ratio without revealing a significant lactate peak in basal ganglia spectra from most patients or controls.

Elevated lactate occurs reliably in the occipital cortex of patients with HD. If lactate increases as a signature of metabolic stress then suppression of lactate may retard the neuronal degeneration in HD. Ongoing attempts to pharmacologically suppress brain lactate in HD patients will be discussed.

73.14

GLUCOSE TRANSPORTER ISOFORM EXPRESSION IN HUNTINGTON'S DISEASE BRAIN

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Several reports over the past decade have suggested that decreased glucose utilization in the basal ganglia of Huntington's Disease (HD) patients may be a factor in the cellular atrophy of the caudate and putamen. We examined the expression of the two major glucose transporter isoforms of brain, GLUT 1 and GLUT 3. GLUT 1 is found in all cells of the brain, while GLUT 3 is localized primarily in neurons. Membranes were prepared from postmortem samples of HD caudate and cortex and non-HD caudate and cortex. Equivalent amounts of membrane protein were seperated on 7.5% SDS polyacrylamide gels, transferred to MSI membranes by western blotting and probed with antisera to GLUT 1 and 3. In all cases (n=6) there was a significant increase in GLUT 1 immunoreactivity in HD caudate compared with either HD cortex or non-HD caudate and cortex. When the same membrane preparations were probed with GLUT 3, no significant differences could be detected in HD versus control samples. These data suggest a specific up-regulation in the expression of GLUT 1, a transporter known to increase in reponse to stress; i.e. hypoglycemia and hypoxia. Interestingly, the consistent expression of GLUT 3 in HD caudate despite substantially reduced neuronal numbers suggests two novel reponses. 1. The few remaining neurons in HD caudate express a greatly increased amount of GLUT 3, or 2. That glia are capable under these circumstances of expressing GLUT 3 in addition to enhanced GLUT 1 expression. Up-regulation of GLUT 1 may be a response to the increased energy demands in HD basal ganglia.

73.15

BASIC FIBROBLAST GROWTH FACTOR (FGF) AND RECEPTOR (FGFR) IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder that localizes to the chromosome (#4) that contains the genes encoding a high affinity fibroblast growth factor receptor (FGFR) (*Genomics* 11:1133-1142, 1991) and basic fibroblast growth factor (FGF) (*Biochem. Biophys. Res. Commun.* 138:644-651, 1986). Because this growth factor is a potent neurotrophic growth factor that has been implicated in neurodegenerative disease (*Biochem. Biophys. Res. Commun.* 171:690-696, 1990), we examined the presence of basic FGF and its high affinity receptors in control and HD brains. Immunocytochemical localization of basic FGF with a specific polyclonal antibody (773) shows that basic FGF-like immunoreactivity is widely distributed within the astrocytic population of the normal caudate nucleus. This staining is dramatically increased, particularly within the large reactive astrocytes that are typically seen in HD. Using a monoclonal antibody to FGFR-1, which recognizes FGFR-2 and FGFR-3, a positive reaction product is seen on the cell surface of neurons, astrocytes and endothelial cells in both HD cases and controls. The staining intensity, however, appears decreased in HD, although Western blotting establishes that the expected molecular forms of basic FGF and FGFR are present in extracts of cortex and caudate nucleus. The levels of basic FGF were then measured in tissues of HD patients and controls that were also matched for age, sex and the postmortem interval. A quantitative RIA demonstrates that the concentration of basic FGF within the caudate nucleus increases up to 6-fold in HD, but remains unchanged in cerebral cortex. These observations indicate that although the normal molecular forms of basic FGF appear to be present in HD, alterations in the regulation of basic FGF and/or FGFR may be involved in the pathogenesis of this disease. Supported by RVHPGSS3, AG10682, and DK-18811.

TRAUMA: BEHAVIORAL STUDIES

74.1

SELECTIVE COGNITIVE IMPAIRMENT FOLLOWING TRAUMATIC BRAIN INJURY IN RATS.

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Impairment of cognitive abilities is a frequent and significant sequelae of traumatic brain injury (TBI). The purpose of this experiment was to examine the generality of the cognitive deficits observed after TBI. The performance of three tasks was evaluated. Two of the tasks (passive avoidance and a constant-start version of the Morris water maze) were chosen because they do not depend on hippocampal processing. The third task examined was the standard version of the Morris water maze which is known to depend on hippocampal processing. Rats were either injured at a moderate level (2.1 atm) of fluid percussion brain injury or surgically prepared but not injured (sham-injured control group). Nine days after fluid percussion injury, injured (n=9) and sham-injured rats (n=8) were trained on the one-trial passive avoidance task with retention assessed 24 hr later. On days 11-15 following injury, injured (n=9) and sham-injured (n=8) rats were trained on a constant-start version of the Morris water maze that has the animals begin the maze from a fixed start position on each trial. Additional injured (n=8) and sham-injured (n=8) animals were trained on days 11-15 after injury on the standard (i.e., using variable start positions) version of the Morris water maze. The results of this experiment revealed that performance of the passive avoidance and the constant-start version of the Morris water maze were not impaired by fluid percussion TBI. However, performance on the variable-start version of the Morris water maze was significantly impaired by TBI. Thus, a moderate level of fluid percussion TBI does not produce a generalized deficit in learning and memory. These findings suggest that the hippocampus is selectively vulnerable to traumatic injury.

Supported by NS 12857

74.2

DIFFERENTIAL SENSITIVITY OF LOCOMOTOR RECOVERY AND DESCENDING MOTOR EVOKED RESPONSES FOLLOWING SPINAL CORD CONTUSION IN RATS.

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Behavioral and electrophysiological recovery were evaluated in rats after experimental spinal cord contusion injuries in order to provide data for comparison with other contusion models, and to evaluate motor evoked responses as a measure of functional recovery. Ten 300 gm female rats under ketamine/xylazine anesthesia were injured using a controlled contusion device. A 2 mm diameter impactor head applied approximately 217 k dynes of force to compress the cord 0.8 mm. Histologically, the cord cross-sectional area was reduced by 70% at the impact site. Animals were rated using a 15-gradation open field locomotor score between 2d and 4 wks. They became capable of support by 8d and near-normal coordinated walking by 15d. Auditory startle responses (ASR) and cerebellar stimulated myoelectric evoked potentials (CMyEP) were recorded myographically from vastus lateralis and tibialis anterior hindlimb muscles. CMyEP were recorded under anesthesia. ASR and CMyEP were abolished up to 6 wks after injury. However, substantial myoelectric responses from cutaneous muscles of the lower trunk, innervated from cervical spinal roots, were present. Dorsal cord field potentials (DCFP) were recorded at the sciatic n. entry zone to cerebellar and tibial n. stimulation. Tibial responses were normal or perhaps enhanced, whereas cerebellar responses were abolished. ASR and CMyEP are thus more sensitive to SCI than locomotor function, suggesting they are mediated by distinct pathways, or that segmental excitability was altered. The injuries produced by this device were very consistent, and correspond roughly to an "intermediate" level injury produced the predecessor OSU impactor.

Supported by grants from NIH [NS10164 (JAG) & NS10165 (BTS)] and Paralyzed Veterans of America Spinal Cord Research Foundation (JAG).

74.3

THE BEHAVIORAL AND ANATOMICAL EFFECTS OF CONTUSION INJURY TO THE MEDIAL FRONTAL CORTEX OF RATS. S.W. Hoffman*, Z. Fülöp, D.G. Stein. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102.

Contusion injury to the medial frontal cortex (MFC) results in profound impairments of spatial navigation performance. Male rats (90 days of age) received bilateral contusion-injury to the MFC. Seven days after injury the rats were tested on a Morris water maze task for 10 days (2 trials/day; 20s on platform; 20s intertrial interval) and compared with sham-operated controls. Activity monitoring was performed on days 1, 5, 10, and 15 postinjury. At 18d postinjury, the brains were processed for reactive astrocytes and microglia. ANOVA revealed that the contused rats were impaired on both latency and distance ($p < 0.05$) to locate the platform as compared to shams. The swim strategy of the contused rats was also found to be impaired. These rats swam a significantly greater percentage of distance in the outer annulus; ie they swam closer to the wall of the maze. These water maze deficits were more severe following contusion than after bilateral aspirations of the same region of cortex. The mean overall time to reach the platform during the ten days of testing for aspiration-lesion rats was 30.3±4.6s and 54.8±3.4s for the contusion-lesion rats. ANOVA of open-field activity revealed that brain injured rats spent a longer period of time in the center of the apparatus compared to shams ($p < 0.05$). Descriptive histological analyses showed that after 18d survival, the lesion could be characterized by an inverted-wedge shape. This cavity was lined with GFAP-positive astrocytes and silver impregnated microglia. There was also a circular patch of gliosis in the dorsomedial caudate that extended through the entire rostral to caudal length of this structure. This region contained both astro- and microglia, which were evenly distributed over the cellular and fascicular areas. The presence of macrophage/microglia in the brain is an indication of an inflammatory immune reaction. This intense and widespread immune response could be a reason for the more severe deficits observed in this model of MFC injury.

74.4

SPATIAL LEARNING DEFICITS IN RATS WITH CORTICAL CONTUSION INJURY. R.L. Sutton*, R.J. Sutherland, G. Quintana, T. Gutierrez and D.M. Feeney. Division of Neurosurgery and Departments of Psychology and Physiology, University of New Mexico, Albuquerque, NM 87131.

Adult male rats received a traumatic brain injury (TBI) centered over the right motor cortex [Br. Res. (1981) 211:67]. Four days postsurgery sham injured and TBI rats were tested for 12 days (2 blocks of 4 trials per day) in a Morris water maze (MWM) task. The location of the escape platform was changed daily. TBI rats had longer escape latencies than shams during the 2nd trial block from Day 1-12 and showed little improvement over 8 trials in daily test sessions (deficient acquisition of spatial learning set). Groups did not differ in latencies to a visible platform on Days 13-14. Rats pretrained in the MWM exhibited only mild, transient deficits during postTBI reacquisition tests. At 18-22 days no electrophysiological abnormalities in the strength or plasticity of perforant path-dentate gyrus connections were detected in the contralateral hippocampus. This MWM deficit postTBI may not be due to secondary hippocampal injury alone [Neurosci. Abst. (1989) 15:69].

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74.5

MILD EXPERIMENTAL BRAIN INJURY: MEMORY DYSFUNCTION CORRELATES WITH HIPPOCAMPAL NEURONAL LOSS IN RATS.

R.R. Hicks*, D.H. Smith, D.H. Lowenstein, K.G. Perlman, R.L. Saint Marie, and T.K. McIntosh, Surgical Research Center, Univ. of Conn. Health Center, Farmington, CT 06030 and Dept. of Neurosurgery, Univ. of Penn., Philadelphia, PA 19104.

Memory dysfunction following human traumatic brain injury is believed to result from damage to key structures in the brain, including the hippocampus. Profound memory impairment has recently been observed following moderate (2.3 atm.) and severe (2.6 atm.) lateral fluid-percussion (FP) brain injury in rats. In the present study, we examined the effects of FP injury of mild severity (1.0 atm.) on memory dysfunction and cell loss in the hippocampus. Male Sprague-Dawley rats (n=9) were trained in the Morris water maze to find a submerged platform using external cues. 2.5 hours after training animals were subjected to mild FP brain injury (1.0 atm.; n=6) or "sham" (surgery with no injury; n=3). Memory function was evaluated 42 hours after injury/sham injury. Animals were sacrificed and their brains removed 6 hours after the memory tests (48 hours post-injury). Sections (40 um thick) were stained with a Nissl stain (thionin), and the number of surviving (stained) neurons in the hilus and injured area of CA3 were counted manually and using an image analysis system (Imaging Research, Inc.), respectively. Linear regression analysis was performed by comparing each animal's memory score to the amount of cell loss in the hilus or CA3. All animals that received FP brain injury demonstrated some hippocampal neuronal loss and a significant correlation between memory scores and hilar cell loss ($r=0.810$, $p=0.008$) and CA3 cell loss ($r=0.878$, $p=0.002$) was observed. These results suggest that there may be an important link between the magnitude of hippocampal cell loss and the severity of memory dysfunction following experimental brain injury. Furthermore, this combined assessment may prove to be a valuable measure in determining the efficacy of therapeutic interventions following traumatic brain injury. This work was supported in part by NIH NS26818 and a grant from the Brain Trauma Foundation.

74.6

EXPERIMENTAL BRAIN INJURY INDUCES LONG-TERM MEMORY DYSFUNCTION ASSOCIATED WITH BILATERAL HILAR NEURONAL LOSS.

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Although long-term memory loss following traumatic brain injury is thought to result from bilateral damage to the hippocampus, precise histologic evidence has yet to be demonstrated. In the present study, we measured the effects of lateral fluid-percussion (FP) brain injury in rats on memory function two weeks following injury, and subsequently examined the hippocampus for neuronal loss. Male Sprague-Dawley rats (350-400 g, n=22) were trained in a Morris water maze (MWM) to find a submerged invisible platform (20 trials over 2 days) using external visual cues. 2.5 h after training, animals were subjected to lateral FP brain injury of moderate severity (2.2 atm, n=11) or sham treatment (surgery with no injury, n=12). Two weeks following injury or sham injury, animals were tested in the MWM for memory retention of the pre-injury learned task. Brains from these animals were then sectioned (30 um) and stained with toluidine blue (Nissl stain). The number of surviving (stained) hilar neurons were counted manually using light microscopy. Memory scores from the injured and sham groups were evaluated using the Mann-Whitney U test. Linear regression analysis was then used to examine the relationship between memory scores and the number of hilar neurons. Injured animals demonstrated a highly significant ($p<0.001$) memory loss compared to sham animals. *Bilateral* hilar neuronal loss was observed in all injured animals; no other hippocampal regions demonstrated obvious bilateral cell loss. Moreover, there was a significant correlation between memory score and hilar cell counts ($r=0.855$, $p=0.002$). These results suggest that 1) FP brain injury induces long-term memory dysfunction lasting at least two weeks, and 2) FP brain injury also leads to permanent bilateral loss of dentate hilar neurons that correlates with the degree of memory impairment. This work was supported, in part, by a VA Merit Review grant and a grant from the Brain Trauma Foundation.

74.7

A HISTOLOGICAL AND BEHAVIORAL STUDY OF EXPERIMENTAL TETHERED SPINAL CORD. G. Mandybur, S. Yamada, R. Adey, and B. Liwnicz*, Division of Neurosurgery, Loma Linda University School of Medicine, Loma Linda, CA 92350.

Tethered cord syndrome is manifested by neurologic deficits in legs and incontinence associated with an elongated spinal cord and thick filum terminale. Oxidative metabolism is impaired in cat model and human tethered cord. In this report laminectomy and ligature of the filum terminale allowed traction of the lumbosacral cord. A ligature was passed over the pulley and connected to one of weights (3 g, 5 g, and 8 g) and dropped 1.0 cm. The animals were sacrificed 24 hours - 2 weeks after surgery. L2, L7, S3, and coccygeal cord segments were removed and examined under light and electron microscopy (LM and EM). Only high grade (8g) drop traction resulted in urinary incontinence and tail anesthesia. Edema and lymphocytic infiltration in gray matter neurons were noted by LM; and numerous neuronal membrane breaks and loss of mitochondrial cristae found by EM. This is the first report to describe histological damage to the gray matter in cat cord tethering and to correlate these findings with permanent incontinence in tethered cord patients.

75.1

IN VIVO ROLES OF THROMBIN DURING WOUND HEALING IN THE CENTRAL NERVOUS TISSUE.

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This investigation was carried out to clarify the in vivo effect of thrombin during wound healing in the central nervous system (CNS). Thrombin, buffer, plasmin and albumin were infused into rat caudate nucleus by an osmotic mini-pump. Brains were examined by immunohistochemistry using antibodies for BrdU, GFAP, vimentin and laminin. The following data were quantitatively analyzed: the area of GFAP-positive cells, the area and number of vimentin-positive cells, the number of BrdU positive cells. We found that thrombin caused proliferation of immature mesenchymal cells, infiltration and recruitment of inflammatory cells, induction of angiogenesis, and an increase in vimentin-positive reactive astrocytes. These histological changes following thrombin infusion resembled the inflammation, scar formation and reactive gliosis which are part of wound healing in the central nervous system. These results suggest that thrombin may play an important role in neuronal regeneration and in the secondary injury caused by the inflammatory response following CNS injury.

75.2

Conduction failure in traumatized earthworm giant axons is mediated partly by Na^+ influx via voltage-gated Na^+ channels, with recovery dependent on Na^+-K^+ATPase.

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The initial phase of loss of direct conduction in traumatized mammalian spinal axons occurs within the first sec of impact, with recovery within 30-60 min, implying a direct effect on nerve membrane (J.Physiol.1991,427:58P). To define the basic mechanisms, a 1gm weight was dropped on *Lumbricus* giant axons, monitoring conduction, extracellularly, in median giant (MGN) and lateral giant fibers (LGN). On impact, a high frequency discharge occurred (~200Hz for <1sec) , followed by a period of conduction loss varying with the height (4-25mm) of the weight drop. For a given weight drop, recovery was hastened by exposing the nerve cord to Tetrodotoxin (~500nM) prior to the trauma and then washing after trauma (n=4). Recovery period was delayed in nerve cords (n=4) treated with Strophanthidin (10^{-4} M) suggesting that membrane bound Na^+ - K^+ ATPase is necessary for recovery of conduction. In a preliminary cat experiment, pretreatment with Strophanthidin completely blocked recovery of primary afferents in the dorsal column, following a small weight drop.

75.3

SURVIVAL OF MAMMALIAN CNS NEURONS AFTER NEURITE TRANSECTION IN MODIFIED IONIC ENVIRONMENTS (MIE)

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Reducing external Ca^{+2} ($\leq$ 30 µM) delays but does not prevent death of spinal cord (SC) neurons after dendrotomy (J. Neurotrauma 7, 1990). Reducing Ca^{+2} and Na^+ or Cl^- prevents destruction of the smooth endoplasmic reticulum (Exp. Brain Res. 86, 1991). We are now studying lesioned neuron survival in low Ca^{+2} MIE with low Na^+ or Cl^-.

Studies involved sets of experiments; each experiment used three 21-28DIV mouse SC cultures seeded on the same date. Ten neurons/culture were chosen for laser microbeam transection of a primary dendrite 100 µm from the soma and ten were unoperated controls. Surgery was performed in a Hepes-buffered MIE. KCl replaced NaCl in low Na^+ MIE, and Na^+ isethionate replaced NaCl in low Cl^- MIE. Survival was assessed by erythrosin B (EB) and phase microscopy (to identify moribund cells).

Time	Normal Ions (NI)	Low Ca+2 (LC)	LC/low Cl-	LC/low Na+
2h (5 sets)	54% ± 13	68% ± 4		92% ± 8
2h (3 sets)	50% ± 10	57% ± 15	77% ± 6	
6h (5 sets)	42% ± 8	56% ± 11		58% ± 8
6h (3 sets)	53% + 6	63% + 6	60% + 10	

Thus, 2h EB-minus-moribund survival: (a) was higher in any MIE than in its matched NI control group, and (b) was 20-25% higher when Na^+ or Cl^- was also reduced than in the matched LC control group. However, protection by Na^+ or Cl^- reduction was lost by 6h. The results indicate that Na^+ and Cl- contribute to the very early neuronal death after injury. Delayed death was probably caused by residual Ca^{+2}, but <1 µM Ca^{+2} seems to accelerate Na^+ and Cl^- damage (ibid.). Studies: (1) in MIE with ionic or nonionic substitutes for all 3 ions, and (2) of NI restoration after 2h, are in progress. NIH NS29683 and Intl. Fndtn. for Ethical Res.

75.4

EITHER ACUTE MUSCARINIC BLOCKADE OR CHRONIC CHOLINE SUPPLEMENTATION ACCELERATES RECOVERY OF MOTOR FUNCTION FOLLOWING CORTICAL IMPACT IN RATS.

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In the rat fluid-percussion model, substantial evidence suggests that traumatic brain injury produces a brief period of excessive excitation of specific receptor subtypes, including muscarinic receptors, that may produce excitotoxic neural injury. However, in the rat cortical impact model, it is not known whether cholinergic excitotoxic mechanisms contribute to the induction of functional deficits. The first study was to determine if blockade of excitation of muscarinic receptors would reduce beam walking deficits. Ten rats were injected with scopolamine (1 mg/kg) 15 min prior to a lateral cortical impact. Ten sham control animals were surgically prepared, but not injured. Beam walking deficits were assessed daily for 4 days. Animals treated with scopolamine returned to preinjury levels significantly ($p<0.05$) earlier than sham controls. Thus, cholinergic excitotoxic mechanisms may contribute to the induction of chronic functional deficits in the rat cortical impact model.

Acute excitotoxic insult could ultimately result in prolonged reduction in cholinergic neurotransmission. To test this hypothesis, 20 rats were similarly injured then treated with CDP-choline (300 mg/kg/day), a choline supplement (n=9) or saline (n=10). Treatment started 24 hours postinjury and continued daily throughout the 5 day beam balance assessment period. Rats treated with CDP-choline demonstrated significantly ($p<0.05$) less beam balance deficits than saline treated animals. These data support the hypothesis that following the acute stage of neural injury, a chronic state of deficient cholinergic neurotransmission may persist during which agents supplementing neurotransmission may improve outcome. Supported by CDC CCR606659.

75.5

FUNCTIONAL SIZE OF THE HUMAN INTERVERTEBRAL FORAMEN.

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It is commonly believed that neural structures within the intervertebral foramen (IVF) occupy only a small fraction of that compartment. The presence of transforaminal ligaments (TFLs), ligamentous structures that traverse the IVF, has not been considered when calculating the functional size of the IVF. Four lumbar spines, including T12 and in one case T11, were obtained from embalmed human cadavers and carefully dissected to expose the contents of the IVFs. All ligamentous structures in the vicinity of the IVFs were preserved. A total of 57 TFLs were observed. Of 49 IVFs examined, at least one TFL was present in 34 (69.4%).

The greatest superior-to-inferior dimension (SI) (perpendicular to the plane of the intervertebral disc) and the greatest anterior-to-posterior dimension (AP) (parallel to the plane of the intervertebral disc) of each IVF were determined. When present, the TFLs helped define a compartment of the IVF that contained the spinal nerve root. The SI and AP of these compartments were also determined. 34 IVFs had at least one TFL that ran in an anteroposterior direction, and the SI of the compartment for the spinal nerve root was an average of 31.5% less than the SI of the IVF. 13 IVFs had at least one TFL that ran in a superoinferior direction, and the AP of the compartment for the spinal nerve root was an average of 5.7% less than the AP of the IVF. Therefore, in the presence of TFLs, there is less space in the IVF for the nerve root than originally thought. This may increase the incidence of sypmtoms after trauma or degenerative changes.

76.1

TRAUMATIC BRAIN INJURY CAUSES A DECREASE IN MUSCARINIC (M1 and M2) RECEPTOR BINDING IN THE RAT BRAIN M.M. DeAngelis, B.G. Lyeth*, L.W. Jenkins and R.L. Hayes[1]. Division of Neurosurgery, Medical College of Virginia, Richmond, VA 23298; [1]Division of Neurosurgery, University of Texas Medical School at Houston, TX 77030.

Traumatic brain injury (TBI) results in excessive release of acetylcholine (ACh). Previous studies have indicated that muscarinic receptor mediated processes are involved in TBI pathophysiology. We examined the effects of moderate fluid percussion TBI (2.2 atmosphere) on radioligand binding specific to M1 and M2 muscarinic receptor subtypes. After TBI or sham injury, animals were sacrificed by *in situ* freezing at 3h or 24h post-injury. Slide mounted tissue sections were incubated in either [^{3}H]-pirenzipine (23nM) for M1 or [^{3}H]-AFDX384 (9nM) for M2 receptor subtype labeling. Localization of M1 and M2 receptor subtypes, in the hippocampal formation and adjacent cortex was analyzed by quantitative autoradiography. There were statistically significant ($p < .05$) decreases in binding to M1 receptors in cingulate cortex, midparietal neocortex and amygdala at 3h post-TBI and to M2 receptors in the apical dendrites of sector CA 2,3 pyramidal cells and molecular layer of the dorsal blade of the dentate gyrus at 24h post-TBI. These results demonstrated temporal and spatial differences in receptor subtype binding alterations after TBI and suggest that M1 and M2 receptor subtypes may be differentially affected in the pathophysiology of TBI. Supported by NIH grant NS12587

76.2

EARLY CHANGES IN CATECHOLAMINE TURNOVER FOLLOWING TRAUMATIC BRAIN INJURY (TBI) IN RATS. S. Pan, A. Dunn-Meynell, B.E. Levin*. Neurol. Serv. (127), VA Med. Ctr., E. Orange, NJ 07018; Dept. of Neurosciences, NJ Med. Sch., Newark, NJ 07103.

Manipulation of norepinephrine (NE) and dopamine (DA) activity alters the outcome of TBI. Since little is known about how TBI affects NE and DA metabolism, brain turnover (TO) rates were assessed by HPLC assay of the decline of NE and DA levels after synthesis inhibition with α-methyltyrosine at 6h and 24h after right sensorimotor cortex contusions produced by rapid, 2mm depression of cortex with a 5mm dia. plunger. Lesion site NE TO decreased by 53% ($p<0.001$) and ipsilateral striatal DA TO was undetectable at 6h. Both recovered by 24h. NE TO increased at 6h and returned to sham control levels at 24h in contralateral anterior cortex (56%; $p<0.005$) and bilateral cerebellum (39%; $p<0.005$) and medulla (344%; $p<0.001$). Ipsilateral hypothalamic NE TO increased by 151% ($p<0.001$) at 6h and remained elevated at 24h. Bilateral hypothalamic DA TO increased at 6h (212%; $p<0.001$) but returned to control levels at 24h. Thus, TBI produced early, largely transient changes in brain NE and DA TO which might explain the effects of pharmacological manipulation of NE and DA on the outcome of TBI. Supported by the Research Service of the Department of Veterans Affairs.

76.3

IS SPINAL CORD INJURY INDUCED LOSS OF RATE-SENSITIVE DEPRESSION RELATED TO GABAb RECEPTOR DYSFUNCTION? **F.J. Thompson*[1], R. Parmer[1], J. Graham, R.G.Fessler[2]. Depts. of Neuroscience[1], Neurosurgery[2], College. of Medicine., Univ. of Florida, Gainesville, Florida 32610-0244**

We recently reported several neurophysiological alterations in lumbar reflex excitability subsequent to midthoracic contusion or hemisection injury (Thompson et al., 1991; 1992a). These changes were characterized by progressive onset and permanent duration. The most significant change was the decrease in the magnitude of rate-sensitive depression of monosynaptic reflexes. By one month post injury, this process is significantly less effective and the reflex magnitudes produced by repetitive sensory inputs are 350 to 400% increased over those in normal animals. Since proprioceptive signals include slow adapting, repetitive inputs, this loss of rate-sensitive depression is proposed to contribute significantly to the development of hyperreflexic syndromes subsequent to spinal cord injury.

To identify a more specific substrate for this regulatory process, analysis of rate-sensitive depression was tested before and following focal application of CPG35348, a specific GABAb blocker, to the pial surface of the fifth lumbar spinal cord segment, in normal ketamine anesthetized adult rats. Following application of doses as small as 10 μg, decreased rate-sensitive depression of tibial monosynaptic reflexes (MSRs) was recorded. The decrease in rate-sensitive depression resulted in 10 Hz MSRs that were 300% larger than the pretreatment controls. Preliminary findings indicate that focal application of a specific GABAb blocker produced alterations in rate-sensitive depression which mimicked part of the change produced subsequent to thoracic cord lesions. (Supported by NIH-NINCDS (NO1-NS-7-2300 and 5-PO1-NS-27511) and the Florida Impaired Drivers and Speeders Trust Fund.

76.4

IN VIVO MICRODIALYSIS MEASUREMENTS OF CEREBELLAR NOREPINEPHRINE FOLLOWING CORTICAL CONTUSION AND AMPHETAMINE. K.A. Krobert*, R.L. Sutton and D.M. Feeney. Depts. of Physiology, Psychology, and Division of Neurosurgery, University of New Mexico, Albuquerque, NM 87131.

Rats had concentric dialysis probes (3 mm X 250 um fiber; in vitro recovery = 11.8 - 13.5%) placed bilaterally in the cerebellum (Cb) immediately after contusion [Br.Res. (1981) 211:67] of the right sensorimotor cortex or sham surgery. Analyses of 20 min dialysate samples (artificial CSF; flow = 1.5 ul/min) with HPLC (20 ul sample loop) revealed that mean ± SEM basal levels in shams was 1.23 ± 0.24 pg in left and 1.08 ± 0.11 pg in right Cb. Contusion reduced basal NE levels to 0.57 ± 0.27 in left and 0.48 ± 0.08 pg in right Cb. D-amphetamine (2 mg/kg, i.p.) 24 hr after surgery bilaterally increased NE levels by 174 to 1051% in shams and 425 to 1188% in contused rats. Peak NE levels occured 20-40 min postdrug, declined to basal levels by 3-4 hrs, and remained at predrug levels at 22-24 hours postdrug. These data indicate that amphetamine releasable NE stores are present in Cb despite injury-induced depression of spontaneous release. This finding may relate to amphetamine facilitated behavioral recovery after cortical injury. Supported by Army Grant DAMD17-91-7-1006.

76.5

CONTROLLED CORTICAL IMPACT INDUCES SUSTAINED ELEVATIONS IN THE EXTRACELLULAR CONCENTRATIONS OF EXCITATORY AMINO ACIDS **P.E. Swedlow, M.L. Botscheller, D.W. Marion, R.J. Sclabassi* and A.M. Palmer.** Brain Trauma Research Center, Departments of Clinical Neurophysiology, Neurological Surgery, Psychiatry Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

Traumatic brain injury (TBI) is associated with short-term elevations in extracellular concentrations of excitatory amino acids ($[EAA]_e$) in both epidural compression and free weight drop models (*J Neurosurg* 73: 889-900; *J Cereb Blood Flow Metab* 10: 631-7). We used controlled cortical impact to define certain neurochemical and behavioral consequences of TBI in a rodent model. Maintaining a constant impact velocity (3.2 - 3.5 m/s), we varied depth of brain deformation (DBD). Rats were randomized to groups of severe (DBD=3.5 mm, n=6), mild (DBD=1.5 mm, n=7) and sham (n=9) injuries. Serial measurements of $[EAA]_e$ were collected by *in vivo* tissue microdialysis, and a beam balancing task was used to measure changes in behavior. Only severely injured animals displayed significant behavioral deficit 10 days after injury. However, both mild and severe groups demonstrated significant injury-dependent increases over mean pre-injury values (mean fold increase ± SEM) in extracellular concentrations of aspartate (mild, 32 ± 12; severe, 65 ± 13) and glutamate (mild, 71 ± 23; severe, 107 ± 20). **$[EAA]_e$ returned to baseline within 20 min after mild impact but remained significantly elevated for at least 60 min after severe impact.** A non-transmitter amino acid (serine) showed a similar pattern of injury-induced change, suggesting that trauma-induced increases in $[EAA]_e$ are attributable, at least in part, to cell lysis. A prolonged elevation of $[EAA]_e$ has not been reported in other models of TBI. (This work was supported by NINDS Grant 1P20NS30318-01).

76.6

LATERAL FLUID PERCUSSION BRAIN INJURY CAUSES CHOLINERGIC FOREBRAIN NEURON DEATH. M.S. Grady*, J. Leonard, D.Q. Maris Dep't. of Neurosurgery, Univ. of Wash., Seattle, WA 98104

Traumatic brain injury (TBI) results in widespread damage within the human CNS. Fluid percussion injury is used as a model of human TBI in experimental animals. This accepted model has been characterized extensively, but little attention has focussed on distal effects of the injury on specific neuron populations. Human TBI frequently causes memory disorders, with some data showing a causal relationship between TBI and Alzheimer's disease. The behavioral results of fluid percussion injury include spatial memory dysfunction. In the rat, cholinergic neurons located in the forebrain are known to play a role in memory function. We investigated the results of fluid percussion injury on this neuron population.

Anesthetized rats underwent a 2.5 atmosphere lateral fluid percussion injury and were sacrificed at intervals of 7, 10 ,14 and 28 days after injury. Sections were taken through the medial septal nucleus (MSN), site of injury and hippocampus and submitted for histology, including ChAT and NGFR immunocytochemistry, AChE histochemistry and Nissl stain. Cell area and number was analyzed in the MSN and AChE density in the hippocampus. Comparison was made to the opposite, uninjured side of the brain as well as to control animals.

There was a time dependent loss of ChAT in the ipsilateral MSN, beginning at 7 days and extending through 28 days. NGFR stained cells were also depleted though significant loss did not occur til 10 days post injury. There was a concomitant loss of AChE terminal density in the hippocampus. These results suggest that loss of specific neuron populations may account for some of the behavioral deficits in TBI, and may be amenable to reversal with pharmacologic intervention.

77.1

CELLULAR REACTIONS AT SPINAL CORD LESION SITES: NEUTROPHILS, MACROPHAGES, MICROGLIAL CELLS AND ASTROCYTES. I. Dusart* and M.E. Schwab. Brain Research Institute, University of Zurich, August Forel str. 1, 8029 Zurich, Switzerland.

Local spinal cord lesions are often greatly enlarged by "secondary damage", a process which is poorly understood and results in scar and cavity formation and absence of tissue repair. From 24 hours to 3 months after transection of the dorsal 2/3 of the adult spinal cord, the different types of cells present in the lesion were identified by immunohistochemistry.

As soon as 24 hours after the lesion, massive cell death was observed in a region up to 200 µm from the primary mechanical lesion. At this time and throughout the next 24 hours, the only non-CNS cellular type present in the lesion are neutrophils (polymorphonuclear leukocytes). This cell type is present throughout the areal extent of the lesion and has been observed in this site for approximately 4 days.

Within two days, a recruitment of blood-borne monocytes (ED-1+) began to be apparent, simultaneously with a proliferation of cells. Some of these proliferating cells were OX42+ and were located in the periphery of the lesion which suggests that they are microglial cells. The number of microglial/macrophagic cells was very high between 4 and 8 days at the lesion site. During this first week, very few astrocytes remained in the lesion site itself. However, in the surrounding area, numerous activated astrocytes and some activated microglial/macrophagic cells were present.

After one week, the astrocytic processes were dense around the lesion site, but only few of them entered the necrotic tissue area. The reactive microglial/macrophagic cells progressively disappeared from the lesion site leading, in most of the cases, to the formation of a cavity.

Our results suggest an early role for neutrophils in tissue damage after spinal cord transections. The described CNS inflammatory process seems to correspond to that observed in the periphery. However, in contrast to the fibroblast bed forming in peripheral lesions, astrocytes wall off the necrotic CNS tissue resulting in the formation of a cavity.

77.2

CHARACTERIZATION OF LEUKOCYTE INFILTRATION IN TRAUMATIC BRAIN INJURY IN THE RAT. W.C. Horner*, P.E. Setter, L.C. Fritz and D. Hines. Athena Neurosciences, Inc., S. San Francisco, CA, 94080.

Leukocytes have been implicated as factors contributing to the secondary damage which accompanies brain injury. We used immunhistochemical (IHC) and biochemical analysis to examine the time course of infiltration of neutrophils (N's) and monocytes (M's) after a 10gm weight was dropped from 10 cm onto the right sensorimotor cortex of the adult rat. At various times post injury (p.i.) the animals were anesthetized and perfused with periodate-lysine-paraformaldehyde fixative. The brains were removed, fixed and paraffin embedded. Serial brian sections (6um) were stained with anti-Leukosialin (anti-N antibody) and mAb ED1 (anti-M antibody). Four hrs p.i. N's were present only in the area of hemorrhage. At 16 & 24 hrs p.i. N's were seen at the center of the hemorrhagic/necrotic area as well as in nearby vessels, in the ipsilateral ventricle and in the surrounding parenchymal tissue (deep layers of parietal and occipital cortices). Distal to the focal necrosis, N's were localized in the optic tract, the external capsule and CA2 & 3 pyramidal cell fields of the hippocampus. Staining from animals sacrificed 2,3,4 and 7 days p.i. revealed that the N's were localized to injured tissue and were not seen migrating into uninjured neuropil. Furthermore, at 16 and 21 days p.i. there was no evidence of N's. By this time the injured area had resolved from a necrotic zone to a cavitation. There were no N's in or surrounding the cavitation. We also measured the change in myeloperoxidase (MPO) activity as a marker of the presence of N's in injured brain. The time course of the increase in brain MPO was the same as that of the IHC detection of N's : MPO activity (U/gm tissue) was 0.025±0.003; 0.3524±0.049; 0.597±0.091; 0.710±0.105; 0.557±0.080 and 0.063±0.021 at 4,16,24,48,72 and 168 hrs p.i.

M's showed a different pattern of localization and time course of infiltration as compared to N's. At 24 hrs p.i. there was some minor M infiltration, primarily in the CA2 & 3 region of the hippocampus. Two and 3 days p.i. M's appeared in the perimeter of the focal area of injury, but not in the focal area. Monocytic infiltration was robust 4 days p.i., at which time M's were in and around cuffed vessels similar to those seen in other brain inflammatory disorders. On day 7, large, rounded, vacuoled M's bordered the tissue surrounding the cavitation; this pattern persisted through 21 days p.i.

77.3

INCREASED KININOGEN IMMUNOREACTIVITY IN INJURED SPINAL CORD IN RAT. Z-H. Li, J. Xu, J. Chao, N. Bhat* and E.L. Hogan. Neurology, Med Univ of SC, Charleston, SC 29425.

Bradykinin is a mediator of inflammation with receptors discovered in substantia gelatinosa and dorsal root ganglion of spinal cord (Stersanka et al., 1988). We have found an increase in kininogen, the bradykinin precursor, in traumatized cord (Xu et al., 1991).

Using the ABC immunocytochemical technique to study kininogen immunoreactivity (IR) in rat compression spinal cord trauma models, it was found that kininogen IR was increased in the injured spinal segment compared to sham (laminectomy). The kininogen IR was increased in the superficial layer of dorsal horn, mainly in the margin area, and in substantia gelantinosa when compared to sham. There was strong IR staining of astrocytes in both white and gray matter and IR positive endothelial cells in small blood vessels. In dorsal root ganglion, IR staining surrounded sensory neurons and a few axons. IR was increased in the superficial layer of the dorsal root and dorsal root ganglion. The increased kininogen IR was observed 30 minutes after trauma and its density was roughly proportional to the magnitude of compression. The results provide evidence that trauma may induce an increase in kininogen levels. The increase may be a protective response to inhibit protease, etc., or lead to increase of kinin peptides that mediates inflammation. These possibilities are being studied. Supported by NS-11066.

77.4

BRAIN HYDROXYL RADICAL GENERATION AFTER EXPERIMENTAL HEAD INJURY. P.A. Yonkers, P.K. Andrus and E.D. Hall*. CNS Res., The Upjohn Co., Kalamazoo, MI 49001.

The time course and intensity of brain hydroxyl radical (•OH) generation were examined in male CF-1 mice during the first hour after concussive head injury. Hydroxyl radical production was measured using the salicylate trapping method in which the production of 2,3 and/or 2,5 dihydroxybenzoic acid (DHBA) in brain 15 min after salicylate administration was used as an index of •OH formation. In mice injured with a concussion of moderate severity, a 60% increase in 2,5 DHBA formation was observed by 1 min post-injury compared to in uninjured mice. The peak in DHBA formation occurred at 15 min post-injury (+67.5%, $p<0.02$ compared to uninjured). At 30 min the increase in DHBA lost significance, indicating that the post-traumatic rise in brain •OH formation is a transient phenomenon. In severely injured mice, the peak increase in DHBA (both 2,3 and 2,5) was observed at 30 min post-injury, but also fell off thereafter. Injury had no effect on plasma levels of DHBA. However, saline perfusion of the injured mice to remove the intravascular blood before brain removal eliminated the injury-induced increase in 2,5 DHBA. This implies that the source of the increased DHBA in the injured mice is the microvasular endothelium. The administration of the 21-aminosteroid U74006F, which possesses .OH scavenging properties, also attenuated the post-traumatic rise in DHBA. These results are a direct demonstration of the occurrence and time course of increased •OH production in injured brain.

77.5

DAMAGE TO SPINAL CORD NEURONS BY HYDROXYL RADICALS GENERATED IN SITU. D. Liu*, R. Yang, X. Yan and D. J. McAdoo. Marine Biomedical Institute and Dept. of Human Biological Chemistry and Genetics, Univ. of Texas Med. Branch, Galveston, TX 77550.

Our experimental model for studying secondary damage and drug actions on secondary injury uses microdialysis to sample substances released, to administer damaging substances and to administer drugs together with the damaging substance. We use electrophysiological recording to record decline in conduction, neurochemical analyses to obtain the identity and time course of released substances, and histological examination to assess neuron damage to characterize secondary damaging substances and study the effective mechanisms of the drugs. In this study we administer H_2O_2 and $FeCl_2$/EDTA through two parallel microdialysis fibers within the spinal cords of deeply anesthetized rats; the ·OH radical is generated when these substances mix between the fibers. The amount of ·OH generated is indicated by the amounts of tyrosine isomers formed from phenylalanine. ·OH generated by our procedure blocks electrical conduction, increases release of amino acids and destroys neurons. $FeCl_2$/EDTA and H_2O_2 administered alone have lesser or no effects on the parameters measured. Our method provides a model for studying damage by ·OH and testing the effects of drugs on damage by ·OH in vivo.

Supported by the Spinal Cord Research Foundation, the American Paralysis Association and the NIH (NS11255).

77.6

PHOSPHORYLATION OF A 61,000 DALTON PROTEIN KINASE C (PKC) SUBSTRATE INCREASES FOLLOWING TRAUMATIC BRAIN INJURY (TBI). K. Yang[1*], W.C. Taft[1], C.E. Dixon[1], R.K. Yu[2], R.L. Hayes[1]. Neurosurgery[1], UT Medical School at Houston, Houston TX 77030; Dept. Biochemistry[2], Medical College of Virginia, Richmond VA 23298.

We have reported activation of PKC following moderate TBI in rat brain (J Neurotrauma 9:72, 1992). In order to investigate the consequences of PKC activation at the substrate level, we have examined *in vitro* phosphorylation patterns following injury. Male Sprague-Dawley rats were subjected to a moderate level (2.2 Atm) of fluid percussion injury. One hour after injury the animals were sacrificed by *in situ* freezing with liquid nitrogen, and dorsal hippocampi were dissected and homogenized at 4°C. Endogenous phosphorylation of homogenate protein was examined at 30°C; reactions were initiated with the addition of gamma-[^{32}P]-ATP. Phosphorylations were stopped after 30s and the phosphorylated proteins were separated by 10% SDS-PAGE. A significant increase in the phosphorylation of a novel 61,000 dalton protein (TBI61) was observed. TBI61 phosphorylation was 120% higher in injured samples compared to sham control (Injury:3.86 ± 0.67; Sham control:1.75 ± 0.50 [arbitrary dens. units]; P⟨0.05). TBI61 phosphorylation could be stimulated by the administration of exogenous phosphatidylserine and diacylglycerol. TBI61 also could be phosphorylated by exogenous PKC. In all cases, TBI61 phosphorylation was inhibited by the PKC inhibitor, staurosporine. Thus TBI enhances the phosphorylation of a novel PKC substrate, TBI61.(Supported by NIH grant NS21458).

77.7

REGIONAL ACTIVITY OF PROTEIN KINASE C AFTER EXPERIMENTAL BRAIN INJURY IN THE RAT. M. R. Prasad, C. Tzigaret, D. Smith, M. Thomas* and T. K. McIntosh. Surgical Research Center, University of Connecticut Health Center, Farmington, CT 06030-1110.

Extracellular excitatory amino acids (EAA) and their receptors have been implicated in secondary neuronal damage after traumatic brain injury. Several *in vitro* studies suggest that excitatory EAA-stimulated neuronal damage of cultured neurons may involve protein kinase C translocation from cytosol to membrane. We examined the regional levels of lactate (a biochemical marker for cellular injury) and the distribution of protein kinase C (measured by phorbol-ester binding assay) or calcium-phospholipid-dependent protein kinase C (measured as co-factor stimulated ^{32}P-phosphorylation of histones) between cytosol and membrane fractions after lateral fluid-percussion (FP) brain injury. Adult rats were subjected to FP brain injury of moderate severity (2.2-2.4 atm). At 30 min post injury, the brains of injured (n = 4) and sham operated (n = 4) animals were frozen using *in situ* freezing techniques. The ipsilateral (injury site) left cortex (LC), contralateral right cortex (RC), and left and right hippocampi (LH and RH) were dissected out carefully at 0°C. The lactate levels of injured cortex were 3-fold higher than those of sham operated animals ($p < 0.05$). However, no significant differences were observed in lactate concentrations in other regions. Furthermore, no significant differences were observed in the distribution of protein kinase C between cytosols and membranes of LC, RC and LH of brain-injured animals, when compared to those of sham operated animals. These results suggest that at 30 min following FP brain injury, cellular damage is present only at the injury site, and translocation of cellular protein kinase C may not be involved in the pathogenesis of this neuronal damage. (Supported by funds from the Department of Surgery, a grant from UConn and, in part, by NIH NS26818 and a VA Merit Review Grant.)

77.8

EFFECTS OF PROTEIN OR RNA SYNTHESIS INHIBITION ON SURVIVAL OF MAMMALIAN SPINAL CORD (SC) NEURONS AFTER NEURITE TRANSECTION J. H. Lucas*, G. Wang and H. Azzazy Physiology Dept., Ohio State Univ., Columbus, OH 43210.

Hypothermia (2h at 17°C followed by rewarming to 37°C) and thiopental (TP, 200 μM) significantly increased survival of SC neurons after dendrotomy (Brain Res. 517, 1990; Soc. Neurosci. Abst. 17, 1991). Cooling and TP both reduce cell metabolism. We are now evaluating interventions which inhibit specific aspects of metabolism.

Experiments utilized pairs of mouse SC cultures (21-28 DIV) seeded on the same date. Ten neurons/culture had laser microbeam transection of a primary dendrite 100 μm from the soma; 10 others were unlesioned controls. The maximum nontoxic concentration (no death or stress of uninjured neurons exposed for 96h) of a protein synthesis (puromycin, PMN, or cycloheximide, CHX) or RNA synthesis (Actinomycin D, AD) inhibitor was added to one culture of the pair. Measurements of ^{35}S-Met uptake were employed to monitor inhibition.

PMN (0.05 μg/ml) or CHX (1.0 μg/ml) was added 1h before surgery. Survival at 24h was 82% ± 8 S.D. with PMN and 78% ± 8 with CHX vs 54% ± 13 and 48% ± 13 in the respective control groups ($p < 0.01$, independent T test). Percentages are means of 5-8 experiments (50-80 lesioned cells). Removal of inhibition at 24h (^{35}S-Met confirmation) caused no additional death at 48h. If CHX was added immediately or 1h after surgery, survival fell to 63% and 53% respectively vs 53% and 50% in the control groups (12 experiments). When AD (0.01 μg/ml) was added 12h before surgery (maximum effect on protein synthesis), survival at 24h was 63% vs 43% (6 experiments). Other RNA inhibitors will be studied. NIH NS29683.

77.9

CNS EXPRESSION OF IMMEDIATE EARLY GENE *c-fos* IN RESPONSE TO DIFFERENT SITES OF PERIPHERAL NERVE INJURY. S. Zhao, H. W. Thompson‡, R. W. Beuerman‡ D.H. Kim and D.G. Kline* Dept. of Neurosurgery, Louisiana State University Medical School, and ‡Lab. of Molecular Biology, LSU Eye Center, New Orleans, LA 70112.

The brachial plexus of 30 rats was cut bilaterally, either just above or 1 cm below the dorsal root ganglia. Following nerve transection, individuals from each group were sacrificed at half, one and two hours post-operatively. Expression of *c-fos* protooncogene in the spinal cord and brain was determined by two methods: Northern blot hybridization to detect *c-fos* mRNA and immunohistocytochemistry to identify the *c-fos* protein product. When both groups of animals were compared to the untransected control animals, we found an increase in *c-fos* gene expression in both the spinal cord and brain half an hour after nerve transection. These levels continued to rise at one and two hours post-nerve transection. While both groups demonstrated greater c-fos expression than controls, the group with nerve transection above the dorsal root ganglia showed a greater elevation than those with a transection below the ganglia. Additionally, immunocytochemistry showed that the increased *c-fos* protein appeared mainly in the dorsal horn of the spinal cord, somatosensory and pyriform cortex, hippocampus, and thalamus of the brain. Our results suggest that peripheral nerve transection near the spinal cord may have distinct effects on the central nervous system when compared to transection more lateral to the spinal cord.

77.10

CYTOSKELETAL PROTEIN LEVELS DECREASE FOLLOWING TRAUMATIC BRAIN INJURY (TBI). W.C. Taft*, C.E. Dixon, M. Wiesmann, K. Yang, R.L. Hayes. Department of Neurosurgery, Univ. of Texas Health Science Center at Houston, Houston TX 77030.

Disruption of neuronal cytoarchitecture has long been implicated in the pathophysiology of TBI, but little is known about the specific molecular features of TBI-induced cytoskeletal derangements. In order to investigate the effect of TBI on cytoskeletal proteins, we have quantitated protein levels of predominant microtubule protein components. Male Sprague-Dawley rats were subjected to moderate fluid percussion injury (2.1 atm), a magnitude which produces a spectrum of long-lasting neurobehavioral deficits not associated with hippocampal neuronal death. Animals were sacrificed 3 hours later by *in situ* freezing with liquid nitrogen. Hippocampi from naive, sham and injured animals were dissected and homogenized at 4°C. Hippocampal proteins were balanced for protein content, separated by SDS-PAGE and transferred to nitrocellulose for quantitative immunoreactivity (IR) measurements. We observed a 44.3 ± 9.2% decrease (compared to sham controls, $p < 0.005$) in hippocampal MAP2 levels. In addition, we found alterations in the labelling pattern of higher MW tau proteins. In contrast, we observed no changes in the labelling of β tubulin III, a neuron-specific tubulin isoform. The data are consistent with the conclusion that even sublethal levels of TBI can trigger the activation of neuron specific proteases which reduce cytoskeletal protein content in injured neurons. Supported by NIH NS 21458.

77.11

SUBCORTICAL NEURONAL DEGENERATION FOLLOWING EXPERIMENTAL RAT BRAIN INJURY. Holly D. Soares* and Tracy K. McIntosh,Ph.D. *Dept Surgery, Univ CT Health Cntr, Farmington, CT 06030 and Dept of Neurosurgery, Univ of Penn, Philadelphia, PN 19104.

A common clinical neuropathological event following human traumatic brain injury includes gross involvement of subcortical structures. Our lab uses experimental lateral fluid percussion (LFP) in the rat to examine neuropathological sequelae following brain injury. Although LFP brain injury is known to result in focal cortical and hippocampal damage, we investigated whether neuronal degeneration also occurs in subcortical structures. Adult male Sprague-Dawley rats were anesthetized i.p. with 60 mg/kg sodium pentobarbital and subjected to moderate (2.3-2.4 atms) LFP brain injury. Following trauma, animals were reanesthetized and perfused with 4% paraformaldehyde immediately (n=4), 2 hrs (n=4), 24 hrs (n=4), and 72 hrs (n=4) post-injury. 35μ vibratome coronal brain sections were subjected to cell body silver degeneration techniques (Gallyas et. al., 1980; Nauta and Gyaux, 1954). Ipsilateral neuronal degeneration was observed in ventral and posterior thalamus, lateral geniculate, medial geniculate, superior colliculus, anterior pretectal, and parafascicular nuclei. Neuronal degeneration was also observed in ipsilateral caudate putamen and bilateral substantia nigra. In conclusion, subcortical structures exhibit significant neuronal degeneration following experimental LFP rat brain injury. Supported, in part, by a VA Merit Review grant and the Brain Trauma Foundation.

78.1

IMPAIRMENT IN REGIONAL ENERGY METABOLISM FOLLOWING SPINAL CORD COMPRESSION INJURY IN THE RAT.

A.Mautes and A.C. Nacimiento*
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We demonstrated, using bioluminescence imaging of ATP, glucose and lactate in serial spinal cord tissue sections, a clear impairment of regional energy metabolism after a 3h exposure by laminectomy (Soc.f.Neurosci.Abstr.1990,16,206.5) With this baseline we studied under identical experimental conditions the corresponding posttraumatic changes following compression at L4 with the method of Nacimiento et al (J.Neurosurg.1985, 62:, 898.)Results: a) ATP content decreased significantly in both lumbar and thoracic (left intact) segments; b) glucose content showed a clear, but not significant, tendency to increase in both regions; c) lactate increased significantly, both in lumbar and thoracic segments, by about 40% over laminectomy-only levels. Conclusions: i) non-adjacent intact segments may be involved in the posttraumatic changes; ii) the combination of inhibited glucose degradation, low ATP and high lactate indicates regional recirculation; iii) reperfusion tissue damage may contribute to posttraumatic metabolic changes. Supported by DFG-Grant Na 115/5-1.

78.2

METABOLIC ALTERATIONS ACCOMPANY IONIC DISTURBANCES DURING A HYPOXIC INSULT TO THE RETINA: AN IN VITRO STUDY. I. Fineman*, C. Doberstein, B. Badie, D.A. Hovda, N. Martin, and D.P. Becker. Div. of Neurosurgery, UCLA School of Medicine, Los Angeles, CA 90024, USA.

In order to further our understanding of the ionic and metabolic derangements that occur following brain injury we utilized a retina in vitro model of hypoxia. Rats were administered a lethal dose of pentobarbital (150 mg/kg, IP), and the retinas dissected into oxygenated (95% O_2, 5% CO_2) Ames medium. Retinas were randomly assigned to either experimental (95% N_2, 5% CO_2) or control conditions. All retinas were incubated at 37°C. Changes in extracellular potassium (K^+)using flame spectrometry , intracellular calcium (Ca^{++}) using ^{45}Ca (5uCi/ml in incubating solution) autoradiography/optical densitometry, and lactate using light spectrometry were measured as well as the alteration in glucose uptake using [14C]-2-deoxy-D-glucose (2DG, 1 uCi/ul) following 10, 20, and 30 minutes of hypoxia (5 experiments per time point). All measurements were conducted pre- and post-hypoxic insult. The results indicate that compared to controls hypoxia produced an increase in: (1) the concentration of extracellular K^+ (2) the concentration of intracellular Ca^{++}, (3) the difference in pre- compared to post-insult concentrations of ^{14}C and, (4) the amount of lactate. All of these effects were statistically significant ($p < .05$) and exhibited a dose response relationship with the exception of intracellular calcium which did not become significantly different until 30 minutes post-hypoxia. These results suggest that utilizing an in vitro model of hypoxia, ionic and metabolic derangement can be demonstrated which are similar to those seen following an in vivo traumatic brain injury. With the use of this model the mechanisms behind these ionic-metabolic relationships can be addressed at the molecular level. (NS27544, NS30308, Brain Trauma Foundation)

78.3

CHANGES IN LOCAL CEREBRAL BLOOD FLOW FOLLOWING CONCUSSIVE BRAIN INJURY. C. Doberstein, F. Velade, H. Badie, D.A. Hovda, and D.P. Becker*. Division of Neurosurgery, UCLA School of Medicine, Los Angeles, CA 90024, USA.

Previous work has indicated a prolonged metabolic and ionic disturbance following concussive brain injury. The present study is a continuation of this previous work using a concussive injury in rats and describes our preliminary results regarding cerebral blood flow (CBF) using the iodo[^{14}C]antipyrine autoradiographic technique. CBF was assessed within the cerebral cortex and comparisons were made between the two hemispheres as well as between different time points. A total of 11 male Sprague-Dawley rats (male, 230-275 g) were studied beginning immediately after, and up to 10 days following, a left parietal fluid percussion injury (FPI; 4.1-5.0 atm). Immediately following FPI, a bilateral reduction in cortical CBF was noted. Furthermore, a marked asymmetry was evident in the parietal and occipital regions which revealed nearly a 50% decrease in CBF compared to the corresponding uninjured right side. Cortical CBF recovered within 24 hours with no apparent asymmetry. From 2 to 5 days following FPI the injured cortex demonstrated reduced blood flow which, although not as severe (~ 35%) as that seen immediately following injury, fell to approximately 71 ml/100gm/min in the left parietal cortex. By 10 days after injury blood flow had normalized and cortical CBF values were comparable to those seen in the control animals. Our data suggest that CBF exhibits complex, prolonged changes following concussive brain injury. Secondly, CBF and metabolism are uncoupled immediately following FPI but become recoupled within 24 hours. Finally, that like cerebral metabolism, blood flow is reduced 2 to 5 days following concussive injury recovering to normal values by 10 days after the insult. (NS30308, NS27544 & The Brain Trauma Foundation)

78.4

CEREBRAL HEMODYNAMIC CHANGES FOLLOWING PERCUSSIVE BRAIN INJURY IN ANESTHETIZED PIGLETS. M. Shibata*, S. Einhaus[a], S. L. Zuckerman[b], W. M. Armstead, and C. W. Leffler. Laboratory for Research in Neonatal Physiology, Departments of Physiology & Biophysics, and [a]Neurosurgery, University of Tennessee, Memphis and [b]Division of Critical Care and Pulmonary Medicine, St. Jude Children's Research Hospital, Memphis, Tennessee.

Effects of fluid percussion injury (FPI) to brain were studied using newborn pigs. Under α–chloralose anesthesia, FPI was applied to the left occipital cortex in animals equipped with closed cranial windows over the right occipito-temporal cortex. The magnitude of FPI ranged from 2.07 and 2.56 atmospheres. Diameter changes of pial arterioles under the window were recorded using an intravital system for 3 h following FPI. Cerebral blood flow (CBF) was determined using radiolabelled microspheres, and periarachnoid cerebrospinal fluid (CSF) prostanoids were measured by radioimmunoassay. Immediately (within 1 min) following FPI, pial arteriolar diameter began to decrease reaching a minimum 23% below the baseline levels at 140 min post-FPI. The diameter remained unchanged thereafter. CBF in the cerebral cortex, thalamus, hypothalamus, midbrain, pons, medulla oblongata, and the cerebellum decreased 48% (mean) below the baseline values. Levels of CSF prostaglandin (PG) E_2, 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, and thromboxane B_2 increased reaching their maximum within 60 min following FPI by, respectively, 218, 321, 452, and 361% above the baseline levels. Only thromboxane B_2 fell to its pre-injury level by 3 h post-FPI. These results may suggest that the vasoconstriction and decreased CBF that follow FPI could induce asphyxic/ischemic brain damage, and that enhanced prostanoids could modulate FPI-induced CBF alteration. (Supported by NIH Grants)

78.5

INTERSTITIAL CO_2 AND pH TRANSIENTS LINKED TO SPREADING DEPRESSION IN RAT HIPPOCAMPAL SLICES. T. Taira*, J. Voipio, P. Paalasmaa and K. Kaila, Dept. of Zoology, Div. of Physiology, Univ. of Helsinki, 00100 Helsinki, Finland.

Spreading depression (SD) in the brain is accompanied by large shifts in interstitial pH (pH_o). We have used a novel CO_2/H^+-sensitive microelectrode for simultaneous monitoring of CO_2 and pH in the interstitial space of rat hippocampal slices (thickness 300-400 μm), kept in an interface chamber at a temperature of 32 °C. The physiological solution contained (in mM): NaCl 124, KCl 3.0, $CaCl_2$ 1.5, $NaHCO_3$ 25, NaH_2PO_4 1.1, $MgSO_4$ 2.0, and D-glucose 10. It was equilibrated with 95% O_2 + 5% CO_2 (P-CO_2 38 mm Hg; pH 7.4). The recordings were made in stratum pyramidale of area CA1. Spreading depression was induced either by repeated trains of stimuli applied to the Schaffer collaterals or by microdrop application of 2 M KCl. The change in pH_o during SD was biphasic, with an initial alkaline transient followed by an acid shift and, finally, by a slow recovery of pH_o to its original baseline level. The initial alkaline transient was associated with a slight fall (about 5 mm Hg or less) in interstitial P-CO_2, and the subsequent acid shift was paralleled by a rise in P-CO_2 to about 60-70 mm Hg. Inhibition of extracellular carbonic anhydrase activity by bath applied benzolamide or by microdrop application of prontosil dextran 5000 (see Kaila et al., NeuroReport 3: 105, 1992) enhanced the initial alkaline shift, and there was now a large (20 mm Hg) fall in P-CO_2. These results indicate that substantial transmembrane fluxes of CO_2 take place during SD, and that extracellular carbonic anhydrase plays a key role in SD-linked changes in interstitial pH and CO_2.

78.6

THE CEREBRAL METABOLIC RATE OF OXYGEN AS AN INDEX OF EFFECTIVE HIGH-DOSE PENTOBARBITAL THERAPY IN PATIENTS WITH SEVERE HEAD INJURY. S.P.Gopinath, C.S.Robertson, J.C.Goodman*, R.G Grossman, Department of Neurosurgery and Pathology, Baylor College of Medicine,Houston,Tx,77030.

High-dose pentobarbital is one of the treatment modalities to control the refractory intracranial hypertension in patients with severe head injury.To achieve the therapeutic end result, plasma barbiturate levels and EEG monitoring for burst suppression activity are usually considered. Experimental studies have shown that cerebral metabolic rate of oxygen(CMRO2) decreases progressively by increasing doses of barbiturates until the EEG was completely suppressed.

The intent of the present investigation was to know the effect of high-dose pentobarbital on CMRO2 in patients with severe head injury and to know whether CMRO2 could be used as an index in defining the therapeutic endpoint.

29 severely head-injured patients having refractory intracranial hypertension were studied. Pentobarbital was given in a dose of 10 mg/kg over 30 minutes, followed by 5 mg/kg/hr for 3 doses, followed by 1-2 mg/kg/hr. The parameters recorded include ICP, BP, CPP, CBF, cerebral A-V O2 difference, CMRO2, prior to and after the loading dose of pentobarbital, and while the plasma concentration of pentobarbital was higher .

The CMRO2 significantly decreased after the loading dose of pentobarbital by 34% and the change in CMRO2 was closely related to the pretreatment value ($n=29$, $r=0.83, p<.001$). CMRO2 levels ≥0.7μmol/gm/min were associated with decrease in CMRO2 of approximately 50% ($n=18$, $r= 0.79$, $p=<0.001$) while CMRO2 levels < .7μmol/gm/min did not significantly change with pentobarbital ($n=11, r=0.11$) .

The present study demonstrates that only those patients with a CMRO2 ≥ 0.7μ mol/gm/min had a reduction in CMRO2 with barbiturates, and that no additional reduction in CMRO2 was achieved at the higher plasma levels of pentobarbital. The CMRO2 could thus be employed as a therapeutic end-point in patients requiring high-dose pentobarbital therapy.

78.7

THE ABILITY OF BRAINSTEM ELECTRICAL STIMULATION TO DESYNCHRONIZE THE EEG IS TRANSITORILY INTERRUPTED FOLLOWING FLUID PERCUSSIVE INJURY. M.R. Park*, J.B. Schweitzer, S.L. Einhaus, J.T. Robertson. Depts. Pathology, Anatomy & Neurobiology, and Neurosurgery, Univ. Tenn., Memphis, College of Medicine, Memphis, TN 38163.

Ascending cholinergic fibers play a principle role in controlling thalamic function and, therefore, behavioral state. Loss of consciousness, often for prolonged periods, is a hallmark of blunt head trauma. It is known that immediately following head injury, a pulse of acetylcholine (ACh) can be detected in the the cerebral spinal fluid, suggesting that cholinergic systems might be profoundly affected. One well-known test of the integrity of ascending brainstem cholinergic systems is to monitor the effectiveness of brainstem electrical stimulation in causing EEG desynchronization. In the present experiments, we followed the changes of the desynchronizing effect, in cats, during the period following administration of a fluid percussive injury. The animals were maintained on propfol anesthesia. Preparatory surgery included the stereotaxic placement of concentric or paired-wire stimulating electrodes in the tegmentum at sites that are effective in causing EEG desynchronization. A single pressure pulse, in the range of 2.6–2.9 atmospheres, was then administered. Immediately following the injury, the EEG is characterized by a slow-wave (3–4 Hz), large-amplitude pattern. Within 6–10 minutes alpha rhythms (ca. 10 Hz) come to predominate. During this time and during the slow-wave phase, brainstem stimulation is no longer able to desynchronize the EEG. However, this is an acute effect. Recovery of the responsiveness to brainstem stimulation returns 20–60 minutes post-injury, preceding behavioral recovery by several hours. It is unlikely that this is a specific effect on ACh pathways. The animals are apneic following the injury, suggesting disruption of brainstem respiratory centers. Motor artifacts from the brainstem stimulation remain, but these can be attributed to direct stimulation of the oculomotor nerve.

TRAUMA: DRUG TREATMENT

79.1

EFFECTS OF NIMODIPINE ON TWO NEUROLOGIC MEASURES SENSITIVE TO SENSORIMOTOR CORTEX DAMAGE. C. Nelson, S. Finger*, and Daniel Simons, University of Wyoming, Psych. Dept., University Station Box 3415, Laramie, WY 82070, Washington Univ., Psych. Dept., St. Louis, MO 63130, and Univ. Pitts. Med. School, Physiol. Dept., Pittsburgh, PA 15261.

Rats were given sensorimotor cortex lesions centered in the forepaw regions, or sham operations, and were tested on their ability to run along a narrow bridge without slipping, and to hang on to a small diameter dowel suspended above the ground. 24 hours after surgery, half of the animals received daily oral treatments of the calcium channel blocker, nimodipine, for 2 weeks while the remaining rats received vehicle alone. Repeated measurements over the first three postoperative weeks showed that rats that received lesions performed worse on the bridge and dowel than animals given control operations. Among the animals with lesions, rats that received nimodipine performed significantly better than those that received vehicle alone. Examination of the barrel fields did not reveal differences in the lesions between the groups. These results extend previous findings from this laboratory, which have shown that the effects of acute brain injuries may be reduced by rapid nimodipine treatment.

79.2

NALMEFENE TREATMENT AFTER SENSORIMOTOR CORTEX CONTUSION IN THE RAT. E.C. Benzel*, M.P. Weisend, R.L. Sutton and D.M. Feeney. Division of Neurosurgery and Department of Psychology, University of New Mexico, Albuquerque, NM 87131.

Ninety-six male Sprague Dawley rats were anesthetized with halothane and received contusion injury [Br. Res. (1981) 211:67] to the right sensorimotor cortex (N=60) or sham injury (N=36). Animals were given a single intraperitoneal injection of either saline or one of five doses of the long-lasting opiate receptor antagonist nalmefene (NAL; 0.05, 0.1, 0.5, 1.0, or 3.0 mg/kg) 45 min postsurgery. Neurobehavioral performance on beam-walking, inclined plane and a wire grip test was evaluated for 28 days postdrug. No dose of NAL treatment improved neurologic outcome. Higher doses of NAL increased contralateral forepaw wire grip time (pathological grasp). Injury-induced weight loss increased with increasing NAL dosage. Loss of hippocampal CA3 pyramidal cells ipsilateral to cortical contusion was attenuated by the 3.0 mg/kg dose of NAL.

Supported by Baker Cummins Pharmaceutical.

79.3

EFFECT OF LAZAROID (21-AMINOSTEROID U-74389F) PRETREATMENT IN COMPRESSION TRAUMA TO THE RAT SPINAL CORD. SS Haghighi*, XZ Geng, LE Spollen, JJ Oro. Div of Neurosurgery & Dept of Pathology, University of Missouri-Columbia.

Data on the early time course of steroid action on injured spinal cord is limited. In our study, the effect of U74389F (21-aminosteroid) treatment in rats with acute spinal cord trauma was studied. Cortical somatosensory evoked potentials (CSEPs), mean arterial blood pressure, and heart rate were recorded before and after a weight-induced compression injury of 200 grams up to 5 hrs after injury. Treatment with U74389F was a single intravenous injection (15mg/kg) 1 hr prior to injury, followed by a continuous infusion at the rate of 3mg/kg for 5 hrs after injury. The CSEPs were abolished after the injury in all animals. The majority of treated animals (86%) demonstrated a return of the CSEPs within the 2nd hr post-injury. Control group showed 14% CSEP recovery at this time period. Recovery rate remained the same for the 2 groups at the 3rd, 4th, and 5th hrs post-injury. Histologically, parenchymal disruption, hemorrhage, and edema were present in all specimens, nevertheless, these changes varied in severity from animal to animal. A histological difference was seen between the treated and non-treated groups in 2 animals.

We conclude that administrations of 21-aminosteroids ensuing 1 hr prior to spinal cord injury facilitated recovery of the spinal cord function as measured by the CSEPs in the compression model of acute spinal cord trauma.

79.4

EFFECTS OF METHYLPREDNISOLONE & YM-14673 AFTER SPINAL CORD INJURY IN RATS. D.L. Behrmann,* J.C. Bresnahan, M.S. Beattie. Neuroscience Prog., Div. of Neurosurgery, Ohio State U., Columbus, OH 43210.

A model of acute spinal cord injury (ASCI) in rats that uses preset differences in spinal cord displacement to produce a rapid (≈20 msec) contusion injury (spinal T9) was used to evaluate behavioral and histologic effects of Methylprednisolone (MP) and the TRH analogue, YM-14673.

Methods: Compounds were administered IV at 5 min, 2, 4, and 6 hrs after the injury. Functional recovery was evaluated for 29 days using open field walking (OFW), inclined plane (IP), and grid walking (GW - Ex. 2 only); percent tissue spared was determined at the lesion epicenter.

Exp. 1: MP administered at 30 mg/kg (n = 16) and 60 mg/kg (n = 16) resulted in better OFW-scores compared with controls (n = 16) over days 8-29 ($p < .01$) and at day 29 ($p < .05$); both performed better on IP over days 8-29 ($p < .01$); the MP-30 mg/kg animals scored higher at day 29 ($p < .05$). Histology showed greater sparing of white matter ($p < .05$) for MP-60 mg/kg animals (23.4%) compared to controls (17.3%).

Exp. 2: A better IP score was observed for the MP group (60 mg/kg, 30 mg/kg x 3; n = 15) over days 8-29 ($p < .05$). No improvement in OFW or GW scores of MP animals was observed at this relatively milder injury level as compared with Ex. 1. YM-14673 animals (1 mg/kg x 4 & 1 mg/kg/day; n = 15) scored better than both MP and control animals (n = 15) in OFW over days 8-29 ($p < .01$); these animals also performed better than controls on IP (days 8-29, $p < .01$; day 29, $p < .05$) and GW (day 29, $p < .05$). MP and YM-14673 produced no additive effect. Histologic analysis is pending.

Both MP and YM-14673 produced positive behavioral results. This study demonstrates the utility of this model for preclinical assessment of pharmacologic agents and indicates that YM-14673 in addition to MP may be beneficial after clinical ASCI. Supported by NS-10165 and training grant NS-07291.

79.5

METHYLPREDNISOLONE PREVENTS THE EXCESSIVE SPINAL CORD DESTRUCTION AFTER MECHANIC-ENZYMATIC MANIPULATION OF SUBACUTE SPINAL CORD CONTUSED AREAS IN RATS. I. Grijalva, G. Guizar-Sahagun, I. Madrazo, R.E. Franco-Bourland*, H. Salgado-Ceballos, A. Ibarra, and A.L. Espitia. CAMINA Research Center, IMSS, UNAM, INNSZ. Mexico, D.F., Mexico.

Methylprednisolone (MP) is an effective neuroprotector given early after spinal cord (SC) injury. Here we assessed the effect of MP on previously injured SC areas, after mechanic-enzymatic manipulation (M-EM) as a preliminary step for transplantation. Adult rats were subjected to severe SC contusion. Nine days after SC injury all rats were subjected to mielotomy at the injured site. The necrotic and scarring tissues were removed by light aspiration, and by the use of a mixture of collagenase (0.25%) and hialuronidase (0.1%) for 20 min. After a 30 sec treatment with 0.1 M EDTA the area was irrigated with a saline solution. MP (30 mg/kg) was administered first IP and then IM to ten rats immediately before this procedure and 2, 4, 8 and 24 hours after. Ten lesioned rats subjected to M-EM, that did not receive MP, were the controls. All rats were prepared for SC histology 9 days after M-EM. The SC parenchyma destruction observed in MP-treated rats was significantly smaller than that observed in the control group.

79.6

METHYLPREDNISOLONE ALTERS INTRACELLULAR ELEMENTAL SHIFTS 6HR AFTER SPINAL CORD TRAUMA.

L.G. Walsh* and W.B. Greene. Neuropath Research, Dept. of Pathology, Medical Univ. of S. Carolina, Charleston, SC 29425-2245.

Electron probe x-ray microanalysis was used to measure the subcellular composition of dorsal axons 6 hr after 20 g·cm spinal cord impact trauma followed by high dose methylprednisolone (MP) in rats. In shams, relative to untreated, the agranular reticulum (AR) had ↓ total Na, Mg, Cl, K and most vesicles no longer contained elevated Ca. Similarly, mitochondria (mito) had ↓ Mg and were less dense and myelin had ↓ Na, Mg and Fe and ↑ K and Cl.

Two distinct groups of axons were found after trauma with or without MP. One group was entropic and had lost all normal elemental gradients in axoplasm (axo), AR, mito and myelin. As in untreated trauma, the intact group of axons could be further divided by their appearance. With MP, the less dense axons had ↓ axo P and density relative to both shams and less dense untreated axons. The AR had ↓ P relative to MP shams and ↓ Na, Mg, P, Cl, K and Ca relative to untreated. With MP both mito Na and density were < than shams. In untreated both were > than shams. The MP myelin was not different among shams, less or more dense axons but had ↓ density, P and Mg relative to untreated. With MP, the more dense axons were more similar to shams.

Our study indicates that in shams, MP inhibited Ca sequestration by the AR and stimulated Na/K pumping within myelin. In traumatized axons, improved mitochondrial homeostasis and uniform alterations in myelin were indicated.

79.7

DOSE-RESPONSE STUDY OF METHYLPREDNISOLONE IN ACUTE SPINAL CORD INJURY IN THE RAT. I. Koyanagi, C.H. Tator*. Div. of Neurosurgery and Playfair Neuroscience Unit, The Toronto Hospital and Univ. of Toronto, Toronto, M5T 2S8, CANADA.

High dose (30mg/kg) methylprednisolone (MP) has recently been found to have therapeutic effects in acute spinal cord injury (SCI) in a cat model and also in humans. However, the effect of this drug has not been determined in a rat model of SCI. The purpose of the present study was to assess the effect of MP on posttraumatic spinal cord blood flow (SCBF) and spinal cord electrophysiology in a rat model of SCI. Twenty-five Wistar male rats were subjected to a 51g clip compression injury at C8-T1, and were randomly divided into five treatment groups which received one of the following intravenous bolus injections 30 minutes after injury: vehicle, or 30, 60, 120, or 240mg/kg of MP. SCBF at the injury site and the adjacent level (C7) was measured by the hydrogen clearance technique. Evoked potentials were recorded over the somatosensory cortex (SSEP) and cerebellar surface (CSEP) following sciatic nerve stimulation. Descending volleys were recorded from the T9-10 spinal cord following posterior fossa stimulation (PFEP). SCBF measurement and evoked responses were performed before and 1, 2 and 3 hours after injury. The SCBF at both levels decreased after injury in the control group (pre-SCI mean SCBF: 56.8±2.6 at C8/T1 and 54.5±4.7 at C7; post-SCI 1 hour mean SCBF: 27.3±2.7 at C8/T1 and 38.0±4.1 at C7; scale: ml/100g/min), and there was no recovery at 3 hours. No significant increase in SCBF was observed in any of the MP groups. SSEP, CSEP and PFEP were abolished following SCI in all rats, and did not recover in the control or treatment groups. This study demonstrated that a single bolus dose of MP up to 240mg/kg had no beneficial effect on posttraumatic SCBF and electrophysiological activity in this model.

79.8

GM1 TREATMENT PROTECTS MOUSE SPINAL CORD NEURONS EXPOSED TO GLUTAMATE: LDH RELEASE REDUCED and GM1 MEMBRANE SURFACE DISTRIBUTION MAINTAINED.

C. Vorwerk, H. Laev, J. Bonheur and S.E. Karpiak* Div. Neuroscience NYS-PI, & Dept. of Psychiatry, Columbia U. (Physicians and Surgeons), NY, NY.

As an *in vitro* model for spinal cord damage, spinal neurons cultured from Swiss Webster mice were challenged with glutamate (10mM), and the levels of released LDH [*lactate dehydrogenase*] were monitored at 24 and 48hrs. Analysis of neuronal plasma membrane disorganization (damage) was determined by the distribution of endogenous GM1 ganglioside using cholera toxin anti-toxin immunohistochemistry. The effects of GM1 ganglioside on LDH release due to membrane damage were studied to further elucidate the mechanisms underlying this lipid's neuroprotective effects. Such GM1 effects are of particular interest due to the clinical reports which indicate the efficacy of GM1 treatment for spinal injury. Spinal cell cultures derived from ±13 day mouse fetuses were subjected to 10mM glutamate for 30min. Parallel cultures were treated with 40 or 80uM of GM1 ganglioside. Both glutamate damage and GM1's neuroprotective effects were assessed at 24 and 48hrs. Released LDH was expressed as the percent of the total LDH for each cultured plate. Without GM1 treatment 18-20% of the total LDH was released, indicating that <20% of the cultured spinal cells posses glutamate receptors. GM1 treatment significantly reduced the LDH release. The membrane structure changes observed by the GM1 immunohistochemistry paralleled the LDH release data. GM1 treated cultures showed significant structural integrity as evidenced by continuous staining of GM1 along the perykariya and processes. These data further support our hypothesis that GM1 treatment reduces plasma membrane damage.

This work was supported by a grant from the FIDIA Research Foundation.

79.9

GM1 IMPROVES INJURY-INDUCED METABOLIC DEFICIT IN RAT OPTIC NERVE. E. Yoles, V. Lavie* and M. Schwartz . Department of Neurobiology, The Weizmann Institute of Science, 76100 Rehovot, Israel.

The present study demonstrates the earliest reported effects of GM1 treatment of crush-injured mammalian axons. In order to assess the ability of GM1 to attenuate the immediate injury-induced response, we employed a noninvasive optical technique in which changes in NADH redox state during a 2-min episode of anoxia are used to monitor real-time metabolic responses resulting from optic nerve injury and subsequent GM1 treatment. Injury of the adult rat optic nerve was followed by a decrease in nerve metabolic activity adjacent to the injured site, as indicated by a reduction in the NADH response to anoxia. The metabolic activity was examined hourly from 1 to 4 h postinjury in two groups of GM1-treated injured nerves (where GM1 was administered intraperitoneally immediately after injury, n=6, or 30 min prior to injury, n=5) and in one group of control animals (where saline was administered intraperitoneally immediately after injury, n=11). ANOVA analysis revealed a significant beneficial effect of GM1 treatment (F=10.13, P=0.001) on the metabolic response after injury, but no statistical difference was found between the two GM1-treated groups. A positive interaction was found between the treatment effect and the time period after injury (F=6.74, P=0.0001). In the GM1-treated groups, the metabolic response increased with time from 65.6±5.3% and 56.5±9.8% of the preinjury response 1 h after injury to 72.7±3.6% and 88.7±11.6% 4 h after injury in the groups injected prior to and immediately after injury, respectively. In the saline-treated control group there was no significant change in the metabolic response with time (41.9±5.1% of the preinjury response 1 h after injury vs. 37.8±3.9% 4 h after injury). The results of this study show that intraperitoneal administration of GM1 30 min before or immediately after axonal injury reduces the metabolic deficit resulting from the injury.

80.1

STRUCTURAL CHANGES OF ASTROCYTIC PROCESSES IN RESPONSE TO GRAFTED FETAL BRAIN CELLS. N. Hawrylak*, T. Schwarz and W.T. Greenough Beckman Inst., Neurosci. Prog., Dept. of Psych. and Cell & Struct. Biol., Univ. of Illinois, Urbana 61801.

Previous studies in our laboratory using the stab wound model of gliosis demonstrated a remodeling of astrocytic processes with reference to the damage site which first appeared at four days after injury (Isaacs K.R., et al., Soc. Neurosci. Abs. 1991 17:168). The present study examined the orientation of astrocytic processes in reference to a host-graft interface zone following grafting of fetal brain cell suspensions into the adult cerebral cortex.

Immunohistochemical (GFAP) and computerized drawing techniques were employed to examine the orientation of astrocytic processes. Graft sites in 25 adult host rats were examined. Astrocytic somata with well stained processes were identified and digitized in three dimensions with a computerized tracking system. Twenty astrocytes from the grafted hemisphere and five astrocytes in the opposite (control) hemisphere were drawn. Processes and process terminals on cells located 150-300 µm distant from the interface zone were analyzed at 1, 2, 4, 10, and 20 days post grafting and compared to control cells (n=5 for all post grafting intervals examined).

Processes and terminals showed no statistically significant changes at 1, 2, 4 and 10 days post grafting. An increase in processes (14%, $p<0.001$) and terminals (12%, $p<0.01$) oriented toward the interface zone was significant at 20 days post grafting.

These results, taken together with the previous report, suggest that astrocytic process remodeling with a specific orientation is a significant component of glial scarring and that grafting of fetal brain tissue may in some way arrest or delay this aspect of astrocytic scar formation. Supported by ONR N00014-89-J1556

80.2

ASTROCYTIC RESPONSE FOLLOWING CORTICAL CONTUSION IN THE RAT. S. Baldwin, T. Carbary, and S.W. Scheff. Dept. Anatomy & Neurobiology,and Center on Aging, Univ. Kentucky, Lexington, KY 40536-0230.

Astrocytes play a key role in normal brain function by regulating amino acid uptake, the concentration of various ions and the blood brain barrier. These cells also respond to injury in the CNS. The injury response of the CNS is complex and wide spread. Presently, little is known about the degree of astrocytic reactivity in damaged cortex. An electronic controlled pneumatic impact (ECPI) animal model for cortical injury has been developed which manifests many of the neuropathologies seen in human closed head injury. To further characterize the pathology of nervous system injury we examined possible changes in cortical astrocytes.

Fisher 344 rats were subjected to a cortical contusion on the parietal region using the ECPI. Coronal brain sections were stained with GFAP antibody 1,2,4 and 10 days after the cortical damage. Cortical areas within the damaged cortex and ventral to the injury site both ipsilateral and contralateral to the contusion were examined for possible hyperplagia and hypertrophy of astrocytes. Image analysis revealed a time dependent increase in GFAP positive cells not only within the damaged cortex but in areas quite remote from the impact site. These results suggest that cortical regions remote from the initial injury site may be prone to secondary injury.

80.3

TWO STEP ACTIVATION OF THE RESIDENT AND INVADING MICROGLIAL CELLS FOLLOWING BRAIN CONTUSION. Z. Fülöp*, R. Duvdevani, S.W. Hoffman, and D.G. Stein. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102.

In contused brain large volumes of chemical by-products of tissue breakdown diffuse into undamaged areas causing secondary degeneration. Gliosis may be an important marker to evaluate the efficacy of possible treatment. Therefore, we studied the interaction between neuronal death, microglia, and astroglia in adult male rat brains perfused at 2, 6, 24h, 3, 7, 18d after frontal cortex contusion. During the first 3 days after injury, dead and dying cells, swelling neuropil, broken capillaries and scattered small hematomas characterized the impact area, but no stained microglial and/or astroglial cells were present. However, among some well defined neuronal groups in the neighboring cortical areas and axon bundles in the corpus callosum, cingulum, and striatum, showing mild degeneration, activated microglial cells were seen already at 2h (1st Activation). Beginning from the 3rd day a large number of macrophages appeared primarily in and around the lesion area. From this time on, both the number and size of microglial cells increased dramatically and they have dispersed to remote areas (2nd Activation), which was followed by reactivation of astrocytes. By day eighteen the impact area was transformed into a cavity around which an astroglial scar, intermingled with microglial cells and macrophages, had formed. We believe that the early activated microglial cells were intrinsic, while those which appeared after the third day may have been transformed from the invading macrophages and capillary pericytes. These data point on the importance of third posttraumatic day as a starting time of the inflammatory response in the contused brain.

80.4

MICROGLIAL ACTIVATION OCCURS ONLY IN SPINAL CORD TRACTS UNDERGOING WALLERIAN DEGENERATION. S.R. Whittemore* & M. Koshinaga, The Miami Project and Dept. Neurological Surgery, Univ. Miami School of Medicine, Miami, FL 33136

The role of microglia in the response to CNS injury is poorly understood. We characterized the response of adult spinal cord microglia to a lesion which eliminated the dorsal columns and corticospinal tract at T8. In control spinal cord, significant numbers of OX42-positive, ramified microglia are found throughout the white and grey matter. Two days after lesion (DPL), the microglia in the severed T4-5 f. gracilis were ameboid and expressed intense OX42 and slightly increased class I major histocompatibility (MHC) antigen immunoreactivities. No activated microglia were seen in the intact f. cuneatus or the retrogradely degenerating corticospinal tract. In the f. gracilis 5DPL, OX42 immunoreactivity was slightly decreased and class I MHC expression was markedly enhanced. By 12DPL, OX42 and class I MHC immunoreactivities were near control levels. At L1-2, activated microglia and increased class I MHC expression was restricted to the corticospinal tract and was maximal 5DPL, returning to near control levels by 12DPL. In the medulla, microglia activation and enhanced class I MHC expression was seen in the n. gracilis, but not the n. cuneatus, at 2DPL. At 5DPL, OX42 immunoreactivity was markedly decreased, but class I MHC expression was still enhanced. Thus in both a descending motor and ascending sensory tract, microglial activation was seen only in those areas undergoing Wallerian, and not retrograde, degeneration. Microglial activation is a very early lesion-induced event in the CNS, and we suggest that cytokine(s) released from activated microglia may play a role in initiating part of the neuroprotective response to CNS injury (see Koshinaga et al, adjacent poster). Supported by The Miami Project, The Daniel Heumann Fund for Spinal Cord Research, and NS26887.

80.5

ALTERED ACIDIC AND BASIC FIBROBLAST GROWTH FACTOR EXPRESSION FOLLOWING SPINAL CORD INJURY, M. Koshinaga*, H.R. Sanon & S.R. Whittemore, The Miami Project and Department of Neurological Surgery, Univ. Miami School of Medicine, Miami, FL 33136

In normal spinal cord, aFGF immunoreactivity was localized in the cytoplasm of ventral motor neurons and sensory fibers in the dorsal columns. Basic FGF immunoreactivity was restricted to astrocyte nuclei and the cytoplasm of a few neurons in the intermediate gray matter. Spinal cord lesions resulted in complete destruction of the dorsal columns and corticospinal tract at T8. Two days post-lesion (DPL), aFGF immunoreactivity was increased in ventral motor neurons and was now seen in intermediate gray matter neurons. Acidic FGF was not detected in the lesioned f. gracilis at T4-5, but markedly increased in the f. cuneatus. At L1-2, aFGF-positive fibers in the f. gracilis dramatically increased. This aFGF immunostaining was maintained 5 and 12DPL. The lesion-induced loss of aFGF immunoreactivity in the n. gracilis suggests that aFGF is anterogradely transported in sensory afferent fibers. Two DPL, GFAP immunoreactivity increased at the lesion site, as well as T4-5 and L1-2, with no change in bFGF staining. Five DPL, increased bFGF-immunoreactivity appeared at the edge of the cystic cavity and the dorsal columns at T4-5 in both the nucleus and cytoplasm of reactive astrocytes, and was increased at 12DPL. Western blots detected an 18 kDa bFGF-like protein in normal spinal cord, which increased at the lesion site. Full length and aminotruncated aFGFs (18.6, 17.1, 14.9 kDa) were detected, with unchanged levels following injury. We suggest that the differential temporal and spatial expression of aFGF and bFGF following spinal cord lesion are mediated by cytokine(s) released from activated microglia (Whittemore & Koshinaga, adjacent poster). Supported by The Miami Project, The Daniel Heumann Fund for Spinal Cord Research, and NS26887.

80.6

BLOOD-BRAIN BARRIER PERMEABILITY AND EDEMA FORMATION IN EARLY AND LATE PHASES OF FRONTAL CORTICAL CONTUSION. **R. Duvdevani*, R.L. Roof, Z. Fulop, S.W. Hoffman, and D.G. Stein. Brain Research Laboratory, Institute of Animal Behavior, Rutgers University, Newark, NJ 07102.**

Blood-brain barrier (BBB) integrity may play an important role in both the injury and recovery processes following cerebral trauma. BBB integrity and edema formation *in vivo* were evaluated in sham rats and following bilateral frontal medial frontal contusion in adult male rats . Rats were sacrificed 2 hr, 24 hr, 3 days, 7 days and 18 days after injury. BBB integrity was assessed in these rats by measuring the area stained by Evans blue (EB) reaction 30 min after intravenous EB injection. Edema was assessed in fresh brains immediately following decapitation using the wet weight-dry weight tissue punch technique. EB was present in the injured area as early as 2 hrs, peaked at 3 days, and disappeared by 18 days. Edema was also observed in the injured area as early as 2 hrs, peaked at 24 hrs-3 days and disappeared by 7 days. Our findings suggest that medial frontal contusion is accompanied by changes in BBB permeability and brain water content and that these changes endure at least 7 days post-injury. We conclude that the duration of BBB permeability can either serve as a "window of opportunity" for allowing entry of therapeutic substances into the CNS, or impair recovery by introducing inflammatory agents into the CNS.

80.7

GENDER INFLUENCES OUTCOME OF BRAIN INJURY: PROGESTERONE PLAYS A PROTECTIVE ROLE. R.L. Roof*, R. Duvdevani, & D.G. Stein. Brain Research Lab, Inst. of Animal Behavior, Rutgers Univ., Newark, NJ 07102.

Edema is a common and serious complication following brain injury, producing intracranial pressure, axonal swelling, demyelination, and can be a major cause of secondary brain damage. In this experiment, male, normally cycling female (**NC**), and pseudopregnant (**PP**) female rats were examined for brain edema 24 hrs after a medial frontal cortical contusion injury. Tissue from the area immediately adjacent to the injury site as well as a distal site was ex amined using the wet weight/dry weight tissue punch technique. The edemic response differed depending on sex and hormonal condition. Tissue from the injured area in males showed a 6.4% increase in water content compared to tissue from the distal area. Tissue from the injured site in **NC** females, given contusion injuries on the day of proestrus, showed a 3.5% increase in water content. **PP** females showed almost no edema (0.3%). To determine whether the reduced edemic response observed in the **PP** females was due to the high levels of progesterone (**P**) present during **PP**, 12 additional female were ovariectomized to eliminate endogenous estrogen (**E**) and **P**. Of these, four were given **E** implants, four given the **E** implants as well as injections of 4 mg **P** for 6 days, beginning 2 days after ovx. 4 additional ovx rats received oil injections. These rats were then given cortical contusion injuries. Tissue punches taken 24 hrs after injury in the ovx, and ovx+**E** groups showed 4% and 5% increases in water content. Tissue punches from the rats in the ovx+**E**+**P** group showed a much lowered ($p<0.01$) edemic response (1.7%). The high levels of **P** present in the **PP** and ovx+**E**+**P** groups nearly eliminated the post injury edema. The lower levels of **P** present in **NC** females significantly reduced post injury edema compared to males. We conclude that **P** plays a protective role in the brain following traumatic injury.

80.8

EFFECT OF SPINAL CORD INJURY ON CSF/TRACER INFLUX. RH Tirgari, CP Barrett*, ML Rennels, N Haynes, and RP Rees. Dept. of Anatomy, Univ. Md.Sch. of Medicine, Baltimore, MD 21201

Tracer substances injected intrathecally distribute throughout the entire spinal cord via perivascular spaces and pericapillary basal laminae. In regions of spinal cord injury, however, this trace influx does not occur, and is diminished in adjacent segments that appear normal. We compared injured and adjacent intact cord segments in rats injected intrathecally with HRP (10 m prior to sacrifice) at post injury times ranging from 1 h to 7 d. Fluid/solid influx into the spinal cord was assessed by immunostaining with anti-HRP (shown by the PAP method). Performed also were antibody staining to endothelial barrier antigen and routine histological evaluation. At 1-12 h, anti-EBA staining showed a disrupted vascular pattern in the lesion epicenter, but a normal pattern in the segments rostral and caudal to the lesion. However, unlike control spinal cords or segments very distant from the lesion epicenters, HRP influx into the segments near lesions was minimal at one hour and was further reduced in the adjacent 4-5 segments over the 4-12 h period after injury. At 7 d, some recovery of HRP influx was seen 4 segments from the epicenter, but not in the 1st to 3rd segments which showed histopathological degeneration. Thus, HRP uptake in revealing a change in CSF flow may have signaled subtle changes caused by spinal cord injury that led to secondary damage.

TRAUMA: HYPOTHERMIA

81.1

BRAIN TEMPERATURE BEFORE AND AFTER TRAUMATIC BRAIN INJURY IS RELATED TO OUTCOME. M.P. Weisend* and D.M. Feeney, Depts. of Psych. and Physiol., Univ. of New Mexico, Alb., NM 87131.

We examined the effect of traumatic brain injury (TBI) on brain and body temperature (TMP) and the relationship between TBI induced TMP changes, neuronal pathology, and behavioral recovery.

Male, Sprague Dawley rats (300-360g) were anesthetized with halothane followed by: scalp incision only (n=8); scalp incision and craniectomy (n=8); or scalp incision, craniectomy and focal cortical impact to the right sensorimotor cortex (n=10). Body core TMP was measured from the rectum and brain TMP inferred from the temporalis muscle during, and for 6 hours after surgery. Beam-walking agility and "pathological grasp" were measured for 28 days after surgery. Rats were then sacrificed for thionin histology.

Surgery alone lowered brain, but not body TMP. TBI induced an additional transient (2-6 min.) drop in brain TMP, with no comparable effect on body TMP. However, during the first hour after TBI both brain and body TMP were elevated compared to controls. Additionally, TBI induced an abnormal pattern of identical brain and body TMP throughout the 6 hour measurement period whereas controls showed the normal pattern of higher brain than body TMP. Mean brain TMP before TBI was positively correlated with the severity of hippocampal and thalamic pathology. In contrast, absolute brain TMP after TBI was not correlated with hippocampal and thalamic pathology. The mean difference between pre- and post-TBI brain TMP was negatively correlated with hippocampal and thalamic pathology.

These data indicate that low brain TMP prior to TBI reduces secondary pathology while prolonged depression of brain TMP after TBI indicates a more severe injury.

Supported by U.S. Army Grant DAMD17-91-Z-1006.

81.2

HYPOTHERMIA ATTENUATES LOSS OF MICROTUBULE-ASSOCIATED PROTEIN 2 (MAP2) FOLLOWING TRAUMATIC BRAIN INJURY (TBI). R.L. Hayes*, W.C. Taft, C.E. Dixon, K. Yang, G.L. Clifton. Dept. of Neurosurgery, Univ. Texas Health Science Center at Houston, Houston TX 77030.

Moderate hypothermia improves neurobehavioral outcome of rodents subjected to moderate fluid percussion injury (J.C.B.F. Metab. 11, 114, 1991). To investigate the effect of hypothermia on the molecular events which occur following TBI, we have quantitated protein levels of MAP2. Parallel groups of sham and injured rats were maintained at normothermic (37°) or hypothermic (30°C) temperatures, as measured within temporalis muscle. Hypothermia was initiated prior to injury and continued for 60 min. Animals were sacrificed 3 hours post-injury by *in situ* freezing with liquid nitrogen. Hippocampi were dissected and homogenized at 4°C. Hippocampal proteins were balanced for protein content, separated by SDS-PAGE and transferred to nitrocellulose for quantitative immunoreactivity (IR) measurements. Normothermic injury produced a 44.3 $\pm$ 9.2% decrease in hippocampal MAP2 levels ($p < 0.005$, compared to sham controls). In animals maintained hypothermic, no change in MAP2 was detected after injury (injured value 103.3 $\pm$ 9.9% of sham controls; $p > 0.1$). A similar pattern was observed in both membrane and cytosolic protein fractions. The data suggest that moderate hypothermia is effective at reversing indices of proteolysis, as well as some persistent neurological deficits, that occur following TBI. Thus hypothermia may represent a useful therapeutic approach to treatment of head injured patients. Supported by NIH NS 21458.

81.3

CHRONIC ALTERATION AND CATASTROPHIC FAILURE OF NETWORK ACTIVITY AFTER HYPOTHERMIA. G.W. Gross* and J.H. Lucas. Dept. of Biological Sciences, Univ. of North Texas, Denton, TX 76203 and Dept. of Physiology, Ohio State Univ., Columbus, OH 43210.

Hypothermia has been used to treat a variety of CNS pathologies but has recently been reported to cause neuronal cell death if cells are maintained at 10°C for over one hour (Lucas et al., Brain Res. 517, 1990). We have used multielectrode recordings to further elucidate this phenomenon.

Monolayer networks derived from dissociated embryonic mouse spinal tissue and grown on multielectrode surfaces self-organize to generate complex spontaneous spike activity. Such networks display stable histiotypic responses to pharmacological manipulations (Gross and Kowalski, In *Neural Networks, Concepts, Applications, and Implementations*, Vol. IV, Prentice Hall, 1991) and remain electrophysiologically responsive after six months in culture. However, after 1h at temperatures below 17°C, irreversible changes in network activity, including channel loss, can be demonstrated after return to normothermic conditions (with temperature ramp times of 10-15 min). After 2h at 10°C, cell show extensive swelling and no spontaneous activity can be recovered at 37°C. This hypothermic injury is prevented by N-methyl-D-aspartate (NMDA) antagonists, indicating that irreversible network failure may be the result of NMDA complex-mediated Ca++ damage. Supported by grant NS29683-01 and the Hillcrest Foundation of Dallas, TX.

81.4

ULTRASTRUCTURAL EFFECTS OF HYPOTHERMIC INJURY ON CULTURED MOUSE SPINAL NEURONS. D.G.Emery,* J.H.Lucas and G.W.Gross, Zoology and Genetics, Iowa State Univ., Ames, IA 50011; Physiology, Ohio State Univ., Columbus OH, 43210; and CNNS, Univ. of N. Texas, Denton, TX 76203.

Cultured mouse spinal neurons swell progressively when cooled below 17° (J. Neurotrauma 7:1990). At 10° all of SC neurons swell, and 74% die when the cultures are rewarmed to 37°C. N-methyl-D-aspartate (NMDA) antagonists reduce the death rate to 10%. We have examined the ultrastructure of neurons cooled to 17° or below with transmission EM.

Neurons fixed after cooling to 17° for 2h (+/- rewarming) appeared nearly normal. Some after 2 h at 10° had slight dilation of the Golgi cisternae and electron lucent foci in the mitochondrial matrices. A few neurons were dead, and only their nuclei remained intact. Neruons cooled to 10° and returned to 37° for 4h developed 3 patterns of ultrastructural change. Some were obviously dead and degenerated. Some developed electron dense cytoplasm, a condition associated with mortal injury and indicating a loss of cell volume, possibly caused by K^+ efflux. However, no other signs of lethal stress were present. The endoplasmic reticulum, although slightly dilated, was not vesiculated, and there was no mitochondrial swelling.

The NMDA antagonist D-2-amino-5-phosphonovalerate (D-APV, 100μM) prevented most ultrastructural effects of cooling and rewarming. Some cells had increased numbers of lysosomal bodies, indicating organelle damage, but no damaged organelles were seen. The ultrastructure of neurons subjected to cooling will be compared to that of neurons exposed to toxic concentrations of NMDA and to the calcium ionophore A23187. Supported by NIH grant NS29683-01.

82.1

R. DESCARTES AND THE ORIGINS OF MODERN CHRONOBIOLOGY. E. Barrera-Calva*, B. Barrera-Mera. Esc. Nal. Preparatoria No. 2. ECQ. and Depto. de Fisiología. Fac. de Medicina. U.N.A.M A. Postal 70250, México 04510. D.F.

In his studies on natural sciences "Le Traite de L'Homme (1646) and "Les Pasions de L'ame" (1648), Descartes theoretical designs on autonomic reflex activity, engrams, and sensory perceptions, reveal an original and clever faculty of thought.Of particular interest is the Cartesian proposal regarding the pineal gland (PG) whose complex photosensitivity and endocrine properties have already been revealed. Recent advances in PG studies give the Cartesian model for PG function significant reality. Cerebral neural paths, drawn, for example in the Cartesian model, represent the cerebral cyclical activity as modified from PG time moment to moment during the day. Descartes suggested that the animal corporeal components constitute a machine automatically controlled by a structure of the PG which, like a clock, is working to tell the time. By illumination PG, by removing and reimplanting it(1), or simply by the oral ingestion effects of melatonine in birds(2), the coordinating role of that gland in the neuroendocrine processes devoted to regulate the locomotor cyclic control is indeed proved. Therefore, PG functions, recently revealed, emerge as a proof of the glorious traces of Cartesian thought which have inspired several generations of famous scholars not only in mathematics and philosophy but also in the field of experimental natural sciences.1.-Proc.Nat.Acad.Sci.59.414 1968. Ann.Rev.Physiol.40.501 1968;2.-Experientia 41.1615 1985.

82.2

THE CONTRIBUTION OF VITTORIO MARCHI TO EXPERIMENTAL NEUROANATOMY. Marina Bentivoglio* and Mario Lambiase°. Institute of Anatomy, University of Verona and °Division of Neurology, Hospital of Castellammare di Stabia, Naples, Italy.

Vittorio Marchi (1851-1908), a pupil of Golgi and of the physiologist Luciani, dedicated himself to clinical work since 1888 and left a limited number of publications on the structural organization of the CNS. These studies demonstrate that Marchi, although in favour of the reticular theory, has been a thorough, versatile and creative neuroscientist. In 1884 and 1885 he prepared in Golgi's laboratory a protocol based on the use of osmic acid and potassium bichromate for the selective staining of the myelin sheath of degenerating fibers. With this method, in 1886 Marchi provided together with Giovanni Algeri, a remarkable description of the "descending" degeneration following lesions of different areas of the cerebral cortex in dog and monkey. The anatomical data are accompanied in this study by a detailed behavioral description of the motor and sensory deficits observed after various cortical ablations. In 1887, using the Golgi impregnation in a large number of mammalian species including man, Marchi provided the first description of the "intimate structure" of the thalamus. In this study Marchi described Golgi type I and type II thalamic neurons and emphasized that the former were clearly prevalent in the thalamus. A careful observer, Marchi stands out as a pioneer of modern neuroanatomy.

82.3

THE ANATOMY OF THE EYE: ALHAZEN AND VESALIUS. I. S. Russell*. G. A. Russell, Department of Humanities in Medicine, 164 Reynolds Bldg., Texas A&M University, Texas, 77843-1114.

In his work on optics, the Kitab al-Manazir, Ibn al-Haytham (Latin: Alhazen) gave an accurate description of the biconvexity and the forward position of the lens in the eye. Historically, however, a diagram of a concentric onion eye with the lens dead in the center was attributed to him as the 'Alhazen eye'. Although neither the text nor the illustration in the Arabic original supports such an 'eye', the diagram appears in the printed Latin translation : the Risner ed. of Alhazen's Opticae Thesaurus (1572), which in turn can be traced to Vesalius' De humani corporis fabrica (1543).

Curiously, Vesalius places the diagram next to his dissection of the eye, where the lens is also in the middle of the globe. For Vesalius to make such an error, two possibilities emerge: either his illustration was not really based on dissection; or under the influence of the Graeco-Arabic medical tradition, he depicted what he expected to see. (The crystalline lens as the principal organ of vision was logically placed in the middle of the globe.)

Risner seems to have inserted the erroneous diagram in order to 'update' his edition of Alhazen on the authority of Vesalius' contemporary reputation. It is an historical irony that the misrepresentation of Ibn al-Haytham's eye is due to an error by the father of scientific anatomy.

82.4

THE HERITABILITY OF DEGENERACY AS REPRESENTED BY THE ABSINTHISM IN NINETEENTH CENTURY FRANCE; INFLUENCES UPON CONTEMPORARY SOCIETAL PERCEPTIONS OF DRUGS OF ABUSE. Randall B. Murphy and Linda H. Schneider, Dept. Chemistry and Center for Neural Science, New York University, and E.W. Bourne Laboratory, New York Hospital-Cornell Medical College, Dept. of Psychiatry, White Plains, New York 10605

The sources of a perceived increase in the worldwide incidence of mental disorder in the mid 19th century were widely discussed, both in the medical and popular media. The heritability of insanity was a mainstream current, particularly in the psychiatric literature of France. A unique component of these views was the fundamental heritability of acquired traits, which today would be viewed as neo-Lamarckist but was, in fact, fully compatible with conventional Darwinian concepts of that period. A paradigm of this concept is the case of absinthism in France, which, although not fully controlled by legislation until this century, was the topic of controversy as early as the 1860ties. This controversy, which is best known today by its representation in art, had as its central thesis the concept that a form of epileptic insanity was promoted by excessive consumption of absinthe; this degenerate trait was thought to be fully heritable through damage of the " germ plasm ". The pharmacologically active component of absinthe, the terpene thujone, is indeed a potent convulsant, and excessive consumption of the drink may have produced acute or possibly chronic neurological changes. However, the alleged validation of the thesis of the heritability of degeneracy was so strongly reinforced by this episode that its consequences continue to persist, and may be traced to present-day views as to the definition of drugs of abuse and the intervention to prevent such abuse.

82.5

THE 1906 NOBEL: PRIZE AND PARADOX. J.L. Culberson*, D.O. Overman and D.E. Haines. Departments of Anatomy, West Virginia University, Morgantown, WV 26506 and University of Mississippi, Jackson, MI 39216.

That Camillo Golgi and Santiago Ramon y Cajal shared honors as co-recipients of the 1906 Nobel prize is widely known. It is also generally understood that the prize was based mainly on Cajal's extensive studies using Golgi's renowned technique of "black staining." Much less well known is the dramatic contrast between the Nobel lectures presented by Golgi and Cajal on the occasion of the awards. Golgi, speaking first, on *The Neurone Doctrine-Theory and Facts*, recited a litany of his objections to the neuron theory, which he had always opposed. He chose this unlikely title, he said, because "(at this time) this doctrine is generally recognized to be going out of favor." Cajal spoke a day later on *"The Structure and Connexions of Neurons."* His lucid summary of modern (1906) understanding of neuroscience would have fit comfortably into the program of our 1992 meeting; few revisions of the basic principles he presented that day have been necessary. The differing personalities of Golgi and Cajal and their divergent views of the nervous system set the stage for this particularly fascinating occasion, a moment in the history of neuroscience when two noted investigators shared the Nobel stage without sharing any ideas about the fundamental organization of the nervous system. This presentation will review the circumstances of the 1906 prize and highlight the differences in the views of these distinguished neuroscientists, using illustrations and text from their Nobel lectures.

82.6

ENDBULB OF HELD. A.M. Berglund, Eaton-Peabody Lab., Mass. Eye and Ear Infirmary (Boston,MA 02114) and Res. Lab. of Electronics, MIT (Cambridge, MA 02139).

In 1891, using Golgi's new technique, Hans Held depicted large endings of the auditory nerve in the cochlear nucleus. A reticularist, Held used other techniques to argue that these "basketlike" axonal terminals joined with the apposing cell body. Cajal (1909), however, recognized these "endbulbs of Held" as unique terminals of individual neruons. He and later Lorente de No (1933; 1981) found that the bushy cells in the cochlear nucleus receive 1-3 endbulbs, thus indicating that a very few primary auditory neurons dominate the input of the target cell. Pfeiffer et al. (1966) found a physiological correlate: a positive potential, which represents the invasion of the endbulb by the parent fiber's action potential, occurs 0.5 ms prior to the target cell's response. Electron microscopy confirms the presence of numerous small synapses of excitatory morphology between the endbulb and the bushy cell (Lenn and Reese, 1966); immunocytochemical data suggest that the endbulb transmitter is glutamate (Altschuler et al., 1984). Thus, the endbulb of Held provides a secure excitatory input from primary to secondary neuron.

Initial Golgi observations had revealed a spoon-shaped terminal projecting a number of thin filopodia (e.g. , Morest et al., 1972). Ryugo and Fekete (1982), comparing Golgi preparations from young animals and endbulbs labeled with horseradish peroxidase from adult animals, showed that the mature endbulb is a "delicate reticulum which appears to enclose the postsynaptic cell body". The holes in the endbulb lattice are presumably filled with inhibitory terminals of CNS origin (Ibata and Pappas, 1976; Schwartz and Gulley, 1978; Cant and Morest, 1979; Adams and Mugnaini, 1987). Sento and Ryugo (1989) showed that endbulb complexity reflects whether the parent fiber has high or low spontaneous activity. Detailed knowledge of the endbulb of Held, derived from over a century of observation, provides an important model for demonstrating structural-functional relationships.

(Support: NIH grant §T32DC00006.)

82.7

SUBSTRATES OF CONSCIOUSNESS: 1850 to 1950. L.H.Marshall* Neuroscience History Program, UCLA, Los Angeles, CA 90024-1761.

Initial steps that considered seriously the neural substrates of consciousness began with Laycock's *Mind and Brain* (1860) and Carpenter's *Principles of Human Physiology* (1842). Carpenter suggested the thalami, which received the sensations and had a reciprocal relation with cortex. Head and Holmes (1911) added the emotions to the concept, and Ranson and Cannon shifted consciousness to the hypothalamus as the site of a "drive" that maintains a conscious state. AMA posters in 1948 concluded from clinical pathology that coma results from damage where mesencephalon, subthalamus, and hypothalamus meet. To those who placed consciousness in the cortex, Stanley Cobb (1952) said: "A healthy cortex cannot by itself maintain the conscious state." And about Penfield's centrencephalon he wrote: "There can be no center.... It is the streaming of impulses in a complex series of circuits...." This concept echoed the "stream of thought" of the psychologists. In spite of many attempts, it is apparent that the resolution of the hoary mind-brain problem is yet to come.

82.8

SIGMUND FREUD AND THE HISTORY OF NEUROTECHNIQUE: THE "LOST YEAR". B. Quinn, Division of Neuropathology & Laboratory for Neuroimaging, UCLA Medical School, Los Angeles, CA 90024.

From the 1870's through the early 1900's, fiber architectonics was a vigorously researched field which developed in step with studies of Nissl- and Golgi-stained material. While the cortical areas of Brodmann are still referenced frequently, myeloarchitectonic works from the same era are forgotten, perhaps because optimal myelin stains have always been more complex and capricious than the straightforward Nissl stain. Freud spent many months in 1883/84 laboriously altering the gold chloride myelin stain of Gerlach (1870) and Flechsig (1876) and his three papers on this stain, with his letters of the period, show he was convinced it would bring him great fame. Ironically, the stain, described by Freud as a "method which will never fail",[1,2] was never reproduced and was soon described as "so unreliable as to be useless"[3]. I hypothesize that Freud's method, similar to that of Schmued[4], may also have been catalyzed by trace peroxide or a similar trace reactant[5]. Close examination of Freud's letters to his fiance show he may have experienced failures of the method, even as he reviewed his galleys declaring it "would never fail". The abysmal failure of his method may have disgraced Freud before those he most wanted to impress, such as Meynert, and the importance attached to novel stains in this era (Golgi, Nissl, etc.) may have been underappreciated by Freud's biographers. Ironically, within months he published his paper extolling the virtues of cocaine, which within two years was called "the third scourge of humanity" after alcohol and morphine. The failure of Freud's painstakingly described stain might reverberate in the absence of explicit technical instruction in his analytic work.[6]

1) Freud, 1884, Brain 7. 2) Freud, 1884, Arch Anat Physiol 5. 3) Upson, 1888 J Nerv Ment Dis 13. 4) Schmued, 1990 J Histochem Cytochem 38. 5) Quinn & Graybiel, 1991 J Neuropath Exp Neur 50:239. 6) Sulloway, 1991 Isis 82.

82.9

THE AMAZING FATE OF NIKOLAI BERNSTEIN AND HIS BOOKS. M.L. Latash, Jr.* and L.P. Latash, Sr. Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612, University of Illinois, Chicago, IL 60612

N. A. Bernstein, the founder of theoretical neuroscience as an independent field of studies, wrote a number of books several of which were not published during his lifetime. One of the books was devoted to polemics with Pavlov, and Bernstein himself aborted its production after Pavlov had died. Another book, "On Dexterity and Its Development" was banned during the anti-Western and anti-semitic campaign of Stalin in the end of the forties. The latter book was saved by the family of Bernstein and found by one of his closest friends and students, I. M. Feigenberg. This book has recently been published in Moscow. The tragedy of scientific fate of Bernstein was determined by two major factors. First, his intellectual power and creativity allowed him to penetrate so deeply into the analysis of the principles of organization of brain functions, especially those related to control of voluntary movements, that he found himself ahead of his time by about 50 years. As a result, he could not be understood by most of his colleagues. Second, he was unfortunate to live and work in conditions of one of the most anti-humaine, totalitarian regimes which decided, in particular, what was right and what was wrong in science. The miraculously saved book, "On Dexterity..." represents a unique example of reader-friendly handling of the most complicated issues of motor control, motor development, motor skill acquisition, and more general aspects of brain activity. These issues have only recently moved into the center of attention of the scientific community. The scientific and historical value of the book is beyond any competition.

82.10

NEUROSCIENTISTS PORTRAYED ON COMMEMORATIVE MEDALS. R.R. Sonnenschein and C.H. Sawyer, Depts. of Physiology and Anatomy & Cell Biology, UCLA Sch. of Med., Los Angeles, CA 90024-1751.

Since the late 17th Century, it has been customary in most of Europe, and to a lesser extent in the U.S.A., to honor eminent individuals, including scientists and physicians, by the casting or striking of medals carrying their portraits. These may be privately commissioned by friends and colleagues; given as awards by scientific organizations; issued as mementos of scientific congresses; or produced for sale by private or governmental mints. Such medallic portraits of leading figures can give an historical overview of a scientific field. This exhibit will consist of photographs of medals in the private collection of R.R. Sonnenschein, of some important contributors to neuroanatomy, neurophysiology, neuroendocrinology, sensory and behavioral physiology, psychiatry and clinical neurology.

82.11

SPITZKA AND SPITZKA ON THE BRAINS OF THE ASSASSINS OF PRESIDENTS. D. E.Haines. Dept. of Anatomy, Univ. of Mississippi Med. Ctr., Jackson, MS 39216

Edward Charles Spitzka (1852-1914) and his son Edward Anthony Spitzka (1876-1922) were prominent neurologists/neuroanatomists in the late 1800s and early 1900s respectively. At the early age of 29 E. C. Spitzka was called upon to testify for the defense at the 1881 trial of Charles Julius Guiteau the assassin of President James Garfield. He was a quick and competent witness who held his own against an aggressive and experienced prosecutor. He was the only expert that argued forcefully that Guiteau was insane. This was an unpopular position as the general populace did not want Guiteau to escape execution via an "insanity dodge". Spitzka's view did not prevail; Guiteau was convicted and executed. Subsequent examination of the evidence supports Spitzka; Guiteau was almost certainly a paranoid schizophrenic. Almost exactly 20 years later (1901) the son, E. A. Spitzka, performed the autopsy on Leon F. Czolgosz the assassin of President William McKinley. Spitzka (E. A.) wrote a detailed account of the assassin's brain and concluded there was no anatomical indications of a diseased state. He concluded that Czolgosz was "socially diseased and perverted, but not mentally diseased". Spitzka (E. A.) studied the brains of criminals executed by electrocution; this earned him the enmity of underworld figures and repeated death threats. This poster highlights the lives and accomplishments of E. C. and E. A. Spitzka with particular emphasis on their involvement in the "neurology" of the assassins of Garfield and McKinley.

82.12

CLARENCE LUTHER HERRICK: THE FATHER OF NEUROSCIENCE IN AMERICA. D. E. Haines. Dept. of Anatomy, Univ. of Mississippi Med. Ctr., Jackson, MS 39216

In the late 1800s there were a number of U.S. scientists, such as H. H. Donaldson, B. G. Wilder, E. C. Spitzka, C. L. Herrick, and others, whose primary or sole interest was in the nervous system. Of these Clarence Luther Herrick, with his tireless approach to investigation, his integrated structural-functional view of the nervous system, and his founding of influential and enduring scientific journals, clearly had the greatest long-term influence on the subsequent development of the field of neuroanatomy/neuroscience in the U.S. Herrick approached the nervous system from the standpoint that seemed, at that time, most appropriate. That was the broad based use of comparative studies and attempts to correlate structural features of the nervous system with functional or behavioral abilities. Although Herrick did not use the emerging techniques of that time (his hands-on neuro work was largely compressed into the period 1888-1893) it is likely that he would have done so had he not become seriously ill in 1893. He founded 3 scientific journals, the most notable being The Journal of Comparative Neurology; the impact and influence of JCN was immediate. Herrick's weekly meetings with his students to discuss research foreshadowed our journal clubs. Neuroscience today, that being attempts to understand structure and function of the nervous system using a multi-technique approach on a wide variety of forms is clearly Herrick's vision. Taken collectively Herrick's concepts, accomplishments, and initiatives formed the basis for the subsequent development of neuroanatomy/neuroscience in this country.

82.13

THE DISCOVERY OF ELECTRORECEPTION, OR MAN'S PROBLEMS WITH UNDERSTANDING A SENSE ABSENT IN HUMANS. B. Fritzsch* and P. Moller#, *Div. Anat., Creighton Univ., Omaha, NE 68178 and #Am. Mus. Nat History, New York, NY 10024.

Ideally, science should be a continuous accumulation of insight towards a more refined understanding of the world. In reality, however, science sometimes loses information because no agreement upon its value and meaning is achieved. Belated rediscoveries are best exemplified by the early work of Gregor Mendel published in 1865 and forgotten until 1900.

An equally remarkable example is the history of a sense present in many aquatic vertebrates that enables them to detect weak electric fields: the electroreceptive sense. Discovered by Lissmann in 1958 it is only since 1981 that we have been aware of its widespread existence in vertebrates. This presumably ancient vertebrate sense was lost in all terrestrial vertebrates, including man, presumably owing to the different physical properties of air as compared to water. While struggling to understand the stunning effects some fish have on man, suggestions in favour of a unique sense were raised repeatedly and rejected. Rejections were largely made by scientists who were otherwise extremely influential; particularly in furthering our understanding of phenomena surrounding the fish's stunning effects now known to be due to their electric organs. We will present quotations from 17th-18th centuries papers which explicitly argued in favour of such a unique sense. We will also present how the *Zeitgeist* led eminent experts to reject explicitely the existence of an electric sense.

82.14

SAVING THE SEALS: LOGOS OF NEUROSCIENCE SOCIETIES AND ORGANIZATIONS. R.A. Johnson[1,2] and L.H. Marshall[2]. Graduate School of Library and Information Science[1] and Neuroscience History Program[2], Brain Research Institute, UCLA, Los Angeles, CA 90024-1761.

Logos, emblems, and insignia are unique, readily-identifiable, primarily non-verbal markings which represent and distinguish products, organizations, publications, and services. Undoubtedly, one instantly recognizes the most effective of these: the medical caduceus, the snake-embraced rod or staff of Aesculapius. We will present both familiar and unusual examples of logos of *neuroscience*-related organizations and publications. This will be coupled with explanations of the significance and overall appearance and design of these graphic representations, in which the human brain motif predominates. A collection of entries from the first logo contest for the Society's *Neuroscience Newsletter* over twenty years ago will be complemented by descriptions and interpretations by the designers. The origins of several other attempts to capture the essence or define the scope of the neurosciences for specific organizations or publications will be traced. Attendees will be invited to discuss the characteristic elements that could be incorporated into a logo which reflects members' activities and interests and the Society's role in advocating neuroscience research.

82.15

HISTORY OF NEUROSCIENCE IN MEXICO IS REPRESENTED BY ONE OF ITS LEADERS, PROF. J.J. IZQUIERDO. X. García* and E. Gijón Department of Physiology, Sch. of Med., U.N.A.M., A.P. No. 70-250, 04510 México, D.F. MEXICO

Professor J.J. Izquierdo (1893-1974) studied medicine graduating in 1917 in the city of Puebla, where he was born. He was initiated in works of physiology at the School of Medicine, and he looked for further training abroad during 1927-1930, in the United States of America, England and Germany. He promoted educative reformation with his book "Curso de Fisiología de Laboratorio", 1929, and with works as "Balance Cuatricentenario de la Fisiología en México", 1934, "Harvey iniciador del método experimental", 1936, "Análisis experimental de los fénomenos fisiológicos fundamentales", 1939, "Bernard, creador de la medicina científica", 1942. Other writings as "Montaña y los orígenes del movimiento social y científico de México" 1955, "El Brownismo en México", 1956, "El Hipocratismo en México", 1957, y "La primera casa de las ciencias en México", 1958, allowed him to give knowledge to the historical scientific and phylosophical basis of his line of thinking and to reveal unknown aspects, not analyzed or interpreted by the ancient mexican sciences. He was founder of several societies like Mexican Physiological Sciences Society in 1957. He was one of the most prominent physiologist in Mexico, without any doubt. He published more than 400 works He gained the most prominent prize as Emeritus Professor for the National Autonomous University of Mexico.

82.16

ADRENAL GRAFTS: A 20TH CENTURY ADVENTURE IN NEUROSCIENCE. A. Márquez-Orozco, Ma.C. Márquez-Orozco, G. Reyes and B. Barrera-Mera. Deptos. de Embriología y Fisiología, Fac. de Medicina, UNAM, A.P. 70-250, 04510 México, D.F. MEXICO

The adrenal glands originally described as "Glandula Renibus Incrumbentes" by Bartholomeus Eustachius (1520-1574) were soon recognized as a valuable biological structure. These glands in which not only the molecular structure of a neurohormone was reported, but also its crude extract was first obtained, purified, cristallized, and "in vitro" synthesized (see Garmichel, 1989) have been studied. This gave the basis to use the medullar portion as a suitable neuronal model in nervous functional restitution. First, we must recognize that before the neonatal involution (Scheel 1908), and the functional initiation of adrenals were known (Lutz and Case, 1925), the survival conditions of the implanted adrenal glands (Wyman and tum Suden, 1932), and their maintainance depend on the integrity of the hypophysis host (Pomeratz et al, 1942). The fact that the adrenals are also endowed with a selfsustained activity (Andrews and Folk, 1964; Kendall, 1964), is a promissory fact. The neuronal autonomic activity could free-run in a coordinated manner with cerebral rhythmic activity during the day. So that the interest currently oriented to use the fetal adrenal cromaffin cells as a substitute neuron in the human brain (Backlund, 1985) is a suitable possibility. The use of fetal adrenal tissues was first considered at an early time of the presente century.

82.17

THE IMPACT OF 19TH CENTURY EXPERIMENTAL NEUROPHYSIOLOGY ON SLEEP RESEARCH OF THE 1960s. **C. V. Alma. Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020.**

In the 18th and 19th centuries, the phenomenon of sleep was often explained by a combination of physiological concepts and metaphysical constructs. The successful introduction of the ablation procedure by the 19th centrury neurophysiologist Jean-Pierre Flourens lent support to the physiological explanations of sleep function. The ablation method involved the lesioning of certain brain loci and the formation of inferences concerning the function of the specific area by noting changes in behavior. This structure-function-behavior link was used in studies where a part of the cerebral lobe was ablated and the "deprivation of dreams" was observed. In the 1960s, localization studies were performed with kainic acid lesions in order to support the role of certain cell groupings of the pontine reticular formation in REM sleep generation. Jean-Pierre Flourens believed in both the principles of limited localization of function and cortical equipotentiality with respect to sleep function. The ablation studies of Jean-Pierre Flourens supported the localization of function of such states as the comatose and soporific states. His experimental work and localization of function ideas paved the way for modern sleep studies, which view an anatomically distributed system with some localization of function.

82.18

EUGENIO TANZI AND THE FIRST SYNAPTIC HYPOTHESIS OF LEARNING AND MEMORY. G. Berlucchi* and M. Bentivoglio°, Institutes of Physiology and °Anatomy, University of Verona, Italy.

In 1893, in his review article '***The facts and inductions on the today's histology of the nervous system***' the neurohistologist and psychiatrist Eugenio Tanzi (1856-1933), contrary to the opinion of the Italian neuroscientists community led by Golgi, provided a lucid overview, based mainly on Cajal's work, in support of the neuron theory. With a passionate belief in *'the microscopic interval that divides each neuron from the other'* (the term synapse had not yet been coined), Tanzi clearly enunciated for the first time the synaptic (defined by him 'physio-psychological') hypothesis of learning and memory. He proposed that each external stimulus acts on the nervous system by producing, in addition to a temporary modification, a longer lasting impression that may become permanent *('a permanent trace, a sort of residual, sometimes even indelible')*. According to Tanzi's theory, neurons activated by the stimulus tend to grow in length, and the reduction of interneuronal distance brings about an increase in the capacity of transmitting the *'nervous wave'*. These phenomena are only temporary when a single activation occurs, but they produce a relatively permanent facilitative associative link between neurons when there is a repetitious activation, thus providing a basis for memories and learned motor skills. Tanzi's hypothesis, later referred to by Cajal (1911), arose little interest. Only after more than half a century Hebb (1949) revived interest in the possible importance of the modifiability of synaptic transmission in learning mechanisms.

83.1

NEUROPHYSIOLOGY LECTURE DEMONSTRATIONS. J. Hore, Dept. of Physiology, Univ. Western Ontario, London, ON N6A 5Cl, Canada

One way to successfully interest students in neurophysiology and thereby to improve student ratings of your lectures is to perform demonstrations in lectures. Advantages of demonstrations include the following: 1) they can illustrate important principles, 2) they grab the attention of the students, 3) they can make students actively think (especially if used together with "buzz" groups), 4) they improve your rapport with the class. Even such simple demonstrations as getting students in class to observe each others eye movements, vibrating a students arm to produce a false illusion of arm position and acting as if you have Parkinsonian akinesia can have a dramatic effect on student interest.

A list of possible demonstrations together with the principle they illustrate will be presented and given as hand-outs. A few will be presented live on request. The audience will be invited to add to the list demonstrations they currently use or have witnessed.

83.2

THE ACTION POTENTIAL THEATER -- ELECTROPHYSIOLOGICAL PERFORMANCE ART. M.L. Collaer. Behavioral Neuroscience Program, Psychology Department, UCLA, Los Angeles, CA 90024-1563.

An obstacle that many students encounter in trying to understand complex biological processes, such as electrophysiological events, is the inherent difficulty of visualizing seemingly arcane subcellular elements and the subtleties of their interactions.

In an effort to make their understanding of postsynaptic potentials and action potentials more concrete and comprehensible, students participating in weekly discussion sections of an upper division Psychobiology course at UCLA act out these processes.

Although the actual performance (with students acting out the flow of ions, opening of ion specific channels, neurotransmitter release and receptor binding) requires only about 15 minutes, background instruction and group discussion occur on the day of, and session preceding, the play. Students are given an overview of intra- and extracellular communication, membrane properties (emphasizing different types of ion channels) and the ionic basis of the resting, action and postsynaptic potentials.

The "Action Potential Theater" can be adapted for groups of students ranging in size from 12 to 30. A large outline of a cell is taped onto the floor or chalked onto a sidewalk. Students start in positions appropriate to their role, either inside or outside of the cell or in the membrane, and act out their role. They explain who they are, what they are doing and why. Although the exercise is approached in a lighthearted and nonthreatening way, the fear of looking foolish is a strong motivator for encouraging advance preparation.

Student evaluations have rated this exercise a 9 (out of 9) in its effectiveness as a learning tool and an 8 (out of 9) in its "enjoyableness."

83.3

INNERVATION OF A CRAYFISH POSTURAL MUSCLE: A STUDENT LABORATORY EXERCISE TO EXAMINE SYNAPTIC TRANSMISSION AND NERVE-TARGET MATCHING. B.R. Johnson* and R.R. Hoy. Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

We are organizing a series of undergraduate laboratory modules for the neurosciences using the crayfish (Hoy et al. 1991. Neurosci Abst. 17: 516, 1991). These modules emphasize quantitative methodology, principles of nervous system function and physiological and morphological techniques of experimental neurobiology. The superficial flexor muscle in the crayfish abdomen is a postural muscle innervated by a purely motor nerve containing only 6 motor neurons. In this module, students simultaneously record spontaneous extracellular action potentials from the motor nerve and intracellular junctional potentials from muscle fibers. Action potentials of specific amplitude classes are matched with stereotyped muscle junctional potentials that are evoked as students sample recordings from different muscle areas. Students observe the cause (nerve action potential) and effect (synaptic potential) of synaptic transmission, temporal and spatial summation of excitatory and inhibitory synaptic activity leading to muscle contraction, and non-random distribution of multi-neuronal innervation. Tactile excitatory and inhibitory inputs modulate the level of spontaneous activity and these can be mapped out. These results lead to discussions of synaptic transmission and integration, sensory modulation of motor activity, invertebrate and vertebrate motor organization, receptive field organization, and principles of neural development. Supported by NSF grant BNS8809445, the Howard Hughes Medical Institute, the Grass Foundation and Apple Computer, Inc.

83.4

SOME ADVANTAGES AND DISADVANTAGES OF USING THE IN VITRO HIPPOCAMPAL SLICE AS A MODEL TO TEACH METHODS IN NEUROPHYSIOLOGY. Sally Veregge*, Department of Biological Sciences, San Jose State University, San Jose, CA 95192.

Graduate or upper division courses in neurophysiological methods often use multiple animal models, each appropriate for the specific technique to be learned. The advantage to this approach is that the student is exposed to the different model systems used in neurophysiology. However, a student may spend as much time becoming proficient with each dissection or preparation technique as with the recording methodology. For our techniques course, we exclusively used the hippocampal slice preparation. With this in vitro model, it is possible to instruct the students in the principles and techniques of field potential, single-unit, intracellular, and patch-clamp recording. In addition, each student can become proficient with the dissection technique and learn the intricacies of maintaining tissue in vitro. The major disadvantage of using the slice preparation is that laboratory periods must be 4 to 6 hours long due to the 1 hour incubation period required for slices to recuperate from the trauma of dissection.

83.5

DATA ACQUISITION, ELECTROPHYSIOLOGY, AND CELL CULTURING USING A GIANT AXON PREPARATION. S. A. George*, K. E. Long, and J. L. Carroll, Jr. Neuroscience Program, Amherst College, Amherst MA 01002.

We use the ventral nerve cord of the common earthworm in a series of laboratories in the undergraduate Neurobiology course. The nerve cord has a median giant axon and fused lateral axons. Students begin with extracellular recording of the nerve cord *in situ*, using suction electrodes, to investigate the fundamentals of impulse initiation and propagation in the two giant axons. Because of the importance of data acquisition in modern neurobiology, students also learn simple programming using a PC-based data acquisition system including a MetraByte data acquisition board. During the following week, excised nerve cord preparations placed in a recording chamber are used to study the extracellular action potential waveform. Students then record intracellularly from the median giant axon, in order to learn the ionic mechanisms of impulses and impulse afterpotentials, which are prominent in this axon. Finally, students learn to prepare dissociated cell cultures of large neurons from the nerve cord. We have found this simple, inexpensive preparation to be very useful in teaching fundamentals of excitability in the Neurobiology course.

83.6

YET ANOTHER LABORATORY EXERCISE IN SOMATOSENSORY PSYCHOPHYSICS THAT IS EXPEDITIOUS, INEXPENSIVE, AND SUITABLE FOR LARGE CLASSES J.D. Greenspan, Depts. of Neurosurgery and Physiology, SUNY Health Science Center, Syracuse, NY 13210

A procedure is described which allows a large class (>100) to quickly and easily demonstrate the punctate nature of cutaneous sensitivity. This laboratory was conducted by a first year medical school class (N=128), but could be used for undergraduate or high school students. Each of the three students in a group took turns as the subject, experimenter, and record keeper. They were given a handout that included a drawing of a 4x5 grid on the dorsal surface of the middle phalanx of a digit. They were instructed to envision this grid on the third digit of the right hand. While the "subject" kept his/her eyes closed, the "experimenter" would use his pen (or pencil) to lightly touch the subject's skin at each of the 20 blocks formed by the grid. The subject, then, would respond after each contact whether he/she felt the touch as cool or not. The record keeper kept track of where on this grid the subject experienced a cool sensation.

All the students appreciated that some spots on the skin were more sensitive to cool than others. The average number of cool sensations reported out of 20 test spots was 8.3 (std. dev. = 3.0). This lab, along with a demonstration of two-point discrimination (Greenspan, Soc. Neurosci. Abst., 14:516, 1991), provides easily observable properties of the somatosensory system.

83.7

USE OF EASY-SHAPING MATERIAL IN THE TEACHING OF EYE DEVELOPMENT IN GRADUATE COURSES. Ma.C. Márquez-Orozco, M.A. Lástiri* and A. Márquez-Orozco. Embryol. Dept. Sch of Med. and CISE Natl. Univ. of Mexico. Mexico 04510 D.F.

Easy-shaping material is a didactic resource used to support theaching in eye development at the ophtalmology specialty level, graduate studies of the School of Medicine (UNAM), imparted at the General Hospital (Ministry of Health).

This resouce eases the teaching-learning process, as it allows tridimensional modelling of the embryonary structures, and explanation of their evolution during development. A previous study with freshman students of the same School of Medicine had demonstrated its effectiveness as a didactic resource. Our aim was to evaluated its usefulness for the teaching of eye development, which contains a great deal of neuroembryology. Evaluation was performed through a structural interview of students taught with the use of this material. Results indicate that this teaching method fosters and eases comprehension of the contents of the eye embryology curriculum at a graduate level.

83.8

USE OF CASE STUDIES TO ENHANCE AN UNDERSTANDING AND COMPARISON OF HUMAN AMNESIA AND LIMITLESS MEMORY. M.R. Foy*. Dept. of Psychology, Loyola Marymount University, Los Angeles, CA 90045.

The instruction of memory in neuroscientifically-based courses typically involves the discussion of memory deficits (amnesia) produced by some type of brain damage. The importance of the hippocampus and temporal lobes in human memory is clearly demonstrated in the handful of reports describing patients with bilateral hippocampal and/or temporal lobe damage. A discussion of these reports which detail the different types of amnesia from an individual such as H.M. aid the student in explaining some specific instances of amnesia, and how the study of the neurobiology of memory is concerned with the identification of brain structures involved in the formation and storage of memories. However, a more complete approach to the understanding of memory entails not only a discussion of individuals who have an extremely poor memory (such as H.M., who could not form new memories), but individuals who have demonstrated an extremely exceptional memory (such as S., who simply could not forget). In this presentation, a way to instruct students as to the extremes of human memory will be explored.

83.9

ELECTROPHYSIOLOGICAL MARKERS OF HYPERPARATHYROIDISM. K.M. Perryman, L.J. Fitten*, A. Brinkman, S. Ganzell and D. Ganzell. Sepulveda VAMC Cognitive Neurophysiology Laboratory (116A-8A) and UCLA School of Medicine, Sepulveda, CA 91343.

Quantitative EEG (QEEG) and P300 evoked potentials were recorded from patients with primary hyperparathyroidism (PHP) and aged-matched controls (AC) during a resting, eyes closed (EC) state and while they were performing a auditory detection task (oddball paradigm). PHP and AC subjects were required to detect pseudo-random occurrences of 1kHz tones delivered 20% of the time among more frequent (80%), 600 Hz tones. Subjects motoric reaction times (RT) were also a performance measure of detection. P300 latencies were collected from midline scalp electrodes Fz, Cz and Pz and averaged across 30 presentations of the odd tone. QEEG activity was collected from frontal (F3, Fz, F4) and parietal (P3, Pz, P4) scalp regions and averaged across 4 second sampling epochs during the EC condition. PHP patients were divided into young (18-40yrs), middle (41-60yrs) and aged (61-80yrs) groups of 10 each for comparison against AC subjects.

Only the aged PHP group demonstrated significant electrophysiological differences compared to their aged-matched control group. There was significant ($p<.05$) slowing of their dominant EEG rhythms in the frontal regions and a increased latency of the P300 potentials recorded from Fz when compared to the AC group. Repeated electrophysiological measures are presently being collected following parathyroidectomy on some of these patients to evaluate possible effects of brain calcium levels.

83.10

DEVELOPING AN ANALOGUE MODEL OF THE NERVOUS SYSTEM BY MAPPING THE ORGANIZATIONAL STRUCTURE OF A UNIVERSITY. S.A. Frutiger, Denison University, Granville, OH 43023.

Students in introductory neuroscience courses are often overwhelmed by the complexity of nervous system structure. This exercise encourages students to develop a conceptual model of nervous system organization which emphasizes the functional role of the nervous system as a communication network.

Students interviewed a representative of several university offices/departments (i.e. executive office, student support services, academic support services, physical plant, academic departments in the arts, humanities, sciences and physical education) with a standard set of interview questions designed to provide information about role definition, communication network, and functional output. Information from interviews was used to develop an organizational map for the institution and to develop a model of potential analogue structures in the nervous system which had similar functional roles. Potential discussion topics during model development include: hierarchical versus parallel organization, spatial and temporal summation of input, involvement of a single structure in several functional circuits, procedural versus declarative memory/learning systems, evolutionary pressures and adaptive modifications, etc.

83.11

UNDERGRADUATE NEURAL SCIENCE AT NEW YORK UNIVERSITY.

L. Kiorpes, C. Aoki, E.E. Coons, C.S. Leonard, and J. A. Movshon*. Center for Neural Science and *Howard Hughes Medical Institute, New York University, 4 Washington Place, NY, NY 10003.

We have developed a multidisciplinary undergraduate curriculum to provide broad training in the biological and behavioral sciences with a focus on neuroscience. Our aim is to attract students to research careers in science and medicine, and to prepare them for graduate and professional programs or other technical careers. The program is research-oriented — students learn current techniques and methods in neuroscience through laboratory courses. Also, students spend at least one semester involved in ongoing research in the active laboratories of the Center. Students take 3 core courses: *Introduction to Neural Science, Cellular and Molecular Neuroscience,* and *Behavioral and Integrative Neuroscience,* the latter two of which have an integrated laboratory component. Students can emphasize either behavioral or biological aspects of neuroscience by choosing elective courses and seminars in either Psychology or Biology. Additional requirements include foundation sequences in Biology, Chemistry, Physics, and Mathematics.

Program development supported by the Howard Hughes Medical Institute.

83.12

A FLEXIBLE LABORATORY SEQUENCE FOR TEACHING NEUROSCIENCE AT THE UNDERGRADUATE LEVEL. C.A. Paul*, M. Sharma, J. Jenkins and J.E. Sweeney. Dept. of Biology, Wellesley College, Wellesley MA 02181.

It is challenging to provide students with a broad foundation in neuroscience at a small undergraduate institution due to constraints of staffing and equipment. In order to maximize resources, we have designed a **flexible** laboratory sequence which could accompany different neuroscience courses; this year it accompanies an upper level course on the neural mechanisms of learning and memory. This lab integrates different techniques to examine the consequences of lesioning the medial septal area (MSA) in rodents. The four major components of the laboratory include: 1) stereotaxic surgery to lesion the MSA and implant recording electrodes in the hippocampus, 2) electrophysiological recordings of hippocampal theta waves in the behaving animal, 3) behavioral assessments using a passive avoidance and a spatial navigation test, and 4) lesion verification and efficacy using Nissl and acetylcholinesterase (AChE) histology and/or quantitative measurements of AChE and acetylcholine levels in the hippocampus.

This laboratory fulfills several teaching goals. First, students address real research questions and generate novel data. Second, students are exposed, first-hand, to the interdisciplinary nature of current neuroscience research. Third, this sequence is flexible and could accompany different types of neuroscience courses. Next year, the same basic sequence will be used in conjunction with a neuropharmacology course; a relevant pharmacological agent will be administered such as an AChE inhibitor. Students will then examine whether this drug restores brain electrophysiology, behavior and neurochemistry following the MSA lesion. We envision, in upcoming years, that this sequence could also be taught with a course on the neurobiology of gender (e.g. comparing females and males in the laboratory). This type of flexible laboratory sequence maximizes resources at a small institution and most importantly, expands opportunities in the undergraduate curriculum.

83.13

DEVELOPMENT OF AN INTERDISCIPLINARY MAJOR IN NEUROSCIENCE AT A LIBERAL ARTS COLLEGE. P. Naour, L. Normansell and D. Quinn. Muskingum College, New Concord, OH 43762.

Neuroscience is an interdisciplinary field in which scientists from a diversity of disciplines investigate the full spectrum of structure and function in the brain. The small undergraduate liberal arts institution provides an excellent opportunity to properly integrate an interdisciplinary neuroscience major. The major we developed was designed to provide the undergraduate student a basic core of courses which serve as the foundation for common seminar experiences and advanced research opportunities with the faculty. The student becomes a part of a research team and engages in scientific inquiry rarely experienced by undergraduate students.

The neuroscience major at Muskingum College consists of three components. In the Basic Science Core, students are provided a foundation in physical, life, behavioral, and computational sciences by completings courses in chemistry, biology, psychology, and computer science. In the Neuroscience Core, students gain an in-depth understanding of brain organization and function. They do so in a combination of formal lecture and laboratory courses, informal seminar experiences, participation in an international forum on the brain (the annual meeting of the Society For Neuroscience), and the conduct of a comprehensive individual research project which serves as the capstone for their undergraduate experience. In the Neuroscience Distribution component, students may individualize their program by selecting from a group of advanced courses which provide either a behavioral, cognitive, computational, or molecular orientation to the basic major.

83.14

TEACHING OF COGNITIVE NEUROSCIENCE A.C.N. Chen. NeuroCognition Institute, Los Angeles, CA 91403, USA

Background: Since 1982, the author has been teaching a graduate seminar on "Cognitive Neuroscience," originally at the University of Washington in Seattle. This 4-credit course covers 20 topics during an academic quarter. The themes of this course are found to parallel those in the Journal of Cognitive Neuroscience inaugurated in 1989.

Objectives: This course integrates theoretical developments and research findings in cognitive psychology as well as human neuroscience in recent decades. Major objectives of this course are to direct students in graduate school and medical schools towards understanding of brain function and dysfunction in human cognition.

Themes: Principles and practices of neurometry, brain imaging, and neural modeling are presented. Neurometry is focused on quantitative EEG analyses (cortical power spectrum, CPS; event-related potentials, ERP; magnetoencephalography, MEG). Brain imaging relates mappings of EEG/MEG activities (coherence mapping, CPS mapping, ERP mapping), neurochemical activities (PET, SPECT, rCBF) and structural anatomy (CAT, MRI). Modeling of neural signal processing in the brain is examined under the information processing paradigm. Information processing is employed as a unifying thread for understanding the hierarchy of human cognition: sensory information, attention and arousal, perceptual and conceptual knowledge representation, memory and language, thinking and reasoning, intelligence and creativity, as well as emotion/motivation/belief.

Significance: In contrast to the micro-analysis fathomed in molecular neurobiology, the thrust of cognitive neuroscience emphasizes the molar integration of neural events that occur in the model systems. Modeling of brain function subserves modeling of human cognition. Major efforts of this course thus stress the science of normal brain function. Understanding of brain dysfunction can then be quantitatively evaluated via neurometry, brain mapping, and neural activity source localization. Implication of these assessments to human pain, headache, stress, affective/cognitive disorders, head injury, brain trauma, chemical dependency, and environment neurotoxicity are discussed, with case presentations.

83.15

AN "ANIMAL RIGHTS" ATTITUDE SURVEY OF UNDERGRADUATE PSYCHOLOGY STUDENTS. M. Vigorito*, D.T. Juliano, and D.M. Murph. Dept. of Psychology, Seton Hall University, South Orange, NJ 07079.

The "Animal Rights" issue is a topic of great concern to researchers and teachers of neuroscience. Yet little empirical work on students' attitudes toward "Animal Rights" issues have been conducted. Thus, an attitude survey on the use of animals in research and other related issues were given to students who had completed their first college introductory psychology course (n=112) and to psychology majors in their junior or senior year (n=63). Few significant differences in attitude were seen between the two groups. A notable exception was that more majors (80%) than introductory psychology students (40%) disagreed with the statement "Psychological experiments with animals are wasteful since they cannot be generalized to explain human behavior". Also, fewer majors (10%) than introductory psychology students (36%) agreed with a statement that biomedical research with animals is immoral and should be stopped. Seventy-seven percent of all students said they supported the animal rights movement and only 8% indicated that they did not support it. These and other findings of the survey suggest the need to discuss animal rights and animal welfare issues in the classroom.

83.16

STUDY OF A CHANGE ON STRATEGIES FOR TEACHING HUMAN PHYSIOLOGY TO MEDICAL STUDENTS. E. Gijón*, X. García, and M.A. Lastiri. Department of Physiology, Sch. of Med. and Research and Educational Services Center. UNAM. Ap. P. 70-250 México, D.F. 04510 MEXICO.

The professor, students, objectives, contents and strategies are elements of the teaching-learning process of human physiology, that interact to obtain learning in two axis; one horizontal axis or learning axis, and one vertical axis or teaching axis. The learning axis is formed by the relation student-content-objective and the teaching axis is represented by the professor-content-strategy relationship. The strategies are selected by the professor but are prefigured by the educative institution. During 1992 some institutional changes have been incorporated on strategies for teaching human physiology. The active participation of students directed by one professor was changed to traditional lecture by four professors. The Human Physiology course was divided in four sections, nervous, endocrine hematology and immunologic systems, cardiovascular-respiratory and renal-digestive systems. One professor for each section during 2 hours lecture twice a week; 10 weeks for the nervous system section and 8 weeks for each of the other sections. Emphasis was given for using audiovisual support to every lesson with slides selected by a group of professors giving each section, according to the contents and objectives of the program.

83.17

STAINED BRAIN SECTIONS PLASTINATED FOR TEACHING HUMAN NEUROANATOMY AND NEUROEMBRYOLOGY. N. Ulfig (SPON: European Neuroscience Association) Dept. Anatomy, J.W.Goethe-University, D-6000 Frankfurt/M., F.R.G.

Teaching human neuroanatomy and neuroembryology is accompanied by two problems: Wet brain tissue is quite inconvenient to handle and not resistant enough when handled several times. Subcortical nuclei and their subdivisions are difficult to recognize in unstained sections. These difficulties can be overcome by combining staining of brain tissue sections with subsequent plastination.

Adult human brain: Staining of 1-4 mm thick frozen sections (or thicker sections made with a macrotome) with astra-blue or aldehydefuchsin provides a sharp contrast between white and grey matter. Aldehydefuchsin stains varying amounts of lipofuscin pigment (pigmento-architectonics).

Fetal human brain: Tissue is embedded in celloidin (because of the high vulnerability of the tissue). 0,5 - 2 mm thick sections are stained with Darrow-red for the demonstration of Nissl-substance.

The sections of adult and fetal brains are dehydrated and transferred to acetone. Then they are impregnated according to the standard S10/S3 plastination procedure (von Hagens, G: Heidelberger Plastinationshefter, 1985, Anatomisches Institut, Universität Heidelberg, Im Neuenheimer Feld 307, D-6900 Heidelberg).

83.18

A SIMPLE PLASTIC MODELS SHOW A COMPLICATE NERVOUS PATHWAYS IN THE NEUROANATOMY TEACHING. Z.Q.Liu, X.G.Luo*, Z.H.Liu. Dept. of Anatomy & Neurobiology, Hunan Medical University, Changsha, Hunan 410078, P.R.C.

The medical students often feel difficult, when facing the study of the complicate internal structures and pathways of the central nervous system (CNS). It will be necessary for the teachers to make their teaching picturesque. Here comes the question. How can we express the complex connections of CNS in a simple form? A set of plastic models of nervous pathways made by ourselves have been successfully applied in teaching, and the effect seems very good. Based on the teaching specimens of CNS and description of plane diagram. We made ten typical plane samples (coronal and horizontal) of CNS with plates of organic glass. The internal structures were drawn with different colour on the plates. The bodies of neurons are represented by plastic beads, and nerve fibers by plastic thread which pass through the corresponding region in the different planes. We have designed and produced each set of 8 models of motor and sensory pathways. They not only show the location of neurons in space, but also the connection between the centre and periphery. Indeed, it is very convenient for the students to understand and study the 3-dimensional structures and benefit them to achieve a better comprehension. In the meantime, the observation of models are combined with cases analysis. It evokes the students great enthusiasm and interest in study neuroantomy. It is also helpful to improve their ability for analysizing and solving the problems. Supported by Teaching Grant from HMU.

83.19

NEUROANATOMY FLASH CARDS M. E. McNeill, East Carolina University School of Medicine; Greenville, NC 27858-4354

The visual format of flash cards capitalizes on the holistic or gestalt perception that is attributed to the right cerebral hemisphere. Nobel laureate Roger W. Sperry, in contrasting left/right hemispheres, said: "The main theme to emerge ... is that there appear to be two modes of thinking, verbal and nonverbal represented rather separately in left and right hemispheres, respectively, and that our educational system, as well as science in general, tends to neglect the nonverbal form of intellect" (in McGuigan & Schoonover, eds., The Psychophysiology of Thinking, NY Academic Press, 209-29, 1973).

The artistic, non-intimidating simplicity of a 62 page spiral bound collection of 8.5 x 11 inch flash cards has been an effective aid in teaching neuroanatomy. These cards: (1) Have visual clarity (2) Offer self-testing (3) Are convenient to use (4) Convey spatial organization (5) Facilitate repetition (6) Employ proprioceptive learning through the use of student work sheets. The use of flash cards has minimized the difficulties of students beginning to unravel the mysteries of the most complex system in the body. Student response has been enthusiastic. "A little picture is worth a thousand words", Chinese proverb.

TEACHING OF NEUROSCIENCE: COMPUTER-ASSISTED INSTRUCTION

84.1

THE GRAPHIC BRAIN: A COMPUTER GENERATED NEUROANATOMY TEXT. T.J. Voneida, *Neurobiology Dept., NEOUCOM, Rootstown, OH 44272.*

It has been our experience in teaching neuroscience to medical and graduate students that one of the most difficult areas for them to master is the integration between the three dimensional anatomy, the normally functioning central nervous system, and the diseased or injured brain and spinal cord.

Part One of this MS-DOS text is an interactive, self-testing **ATLAS** which allows one to select from a 3-D array of Weil stained sections of human spinal cord, hind- mid- and forebrain (in horizontal, sagittal and coronal planes). Cursor selection of any section causes it to "slide out" of the array (like a slice of bread), and enlarge. Each structure is numbered, and its name appears by cursor activation, along with "Input/output?" for nuclei and "Origin/termination?" for tracts. Activation of either of these provides the major connections for each labeled structure.

Part Two, **FUNCTIONAL SYSTEMS**, contains 3-D displays of major sensory/motor systems and cranial nerves. Each sensory system begins with a digitized neurological examination, showing the stimuli required to activate its receptors. Activation results in a 3-D view of the peripheral path entering and coursing through the CNS. Synapses are highlighted by colored flashes. Cross referenced Weil stained sections can be activated from the Atlas at any point for a more detailed view of a specific pathway.

Part Three, **CLINICAL CORRELATIONS**, includes case study descriptions of various neurological problems. In addition, digitized images of televised patient examinations are used to demonstrate the pathophysiology of several major neurologic disorders. These will include interactive questions, with specific cross-references to material covered in Parts I and II.

Special thanks is given to Alive Centers of America and to Ms. Sharon Johnston for their invaluable assistance in this project.

84.2

A COMPUTER AIDED INSTRUCTION PACKAGE DEMONSTRATING ANIMATIONS THROUGH A HIGH RESOLUTION THREE DIMENSIONAL DIGITAL MODEL OF THE WHOLE HUMAN HEAD.

S.Narayan*, D.Sensharma, A.Lee, B.A.Payne, E.M.Santori and A.W.Toga,

Laboratory of Neuro Imaging, Dept. of Neurology, UCLA School of Medicine, Los Angeles CA 90024.

We present here an interactive Computer Aided Instruction (CAI) package in neuroanatomy providing visualization of animated images through a three dimensional digital model of the unstained human head.

Full color digital images were taken of the blockface of a cryomicrotomed frozen human head every 200 microns. From this series a three dimensional digital volume with a resultant pixel resolution of 200μm^3 was created on a UNIX workstation. Resampled images were then computed along orthogonal axes and written sequentially to a write-once-read-many (WORM) times videodisc unit. Playback of this customized videodisc dataset provides an animated view of these slices. Other three dimensional surface models of brain structures, reconstructed from MRI data, were also included. A mouse driven CAI package was written for the IBM PC, and interfaces to this database. This tutorial program illustrates topics in basic and applied neuroanatomy by accessing videodisc frames to display animations and labeled still images. Animation and interaction as applied to these three dimensional data provides an appreciation of three dimensional relationships, and have great potential as teaching tools in the neurosciences.

84.3

COMPUTER ASSISTED INSTRUCTION IN BASIC NEUROLOGICAL ANATOMY: INTERACTIVE PROGRAMS ON THE MOTOR SYSTEM. P.A. Young*, P.H. Young, S.L. McClain, J.C. Young, Department of Anatomy and Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Over the past year a series of interactive programs have been developed to enhance the understanding of the anatomical basis of motor system disorders at the level required in neuroanatomy courses offered to medical and allied health professions students. Each program allows the user to access three different areas: anatomy, circuitry, and disorders. The anatomy sections feature gross specimens, myelin-stained sections, magnetic resonance images, and anatomical illustrations. In the sections on circuitry, input, output, and where applicable, the interconnections are presented using graphics and animation. The disorders sections include descriptions, illustrations, and video demonstrations of various motor system abnormalities. Animated renditions of the abnormal circuitries enable the user to visualize and understand the CNS mechanisms responsible for the disorders. Included in the programs are review quizzes consisting of multiple choice questions and the identification of structures in gross specimens, myelin-stained sections, and magnetic resonance images. (Supported in part by Practical Anatomy and Surgical Techniques Workshops of St. Louis.)

84.4

VETERINARY NEUROANATOMY: AN INTERACTIVE ATLAS OF THE BRAIN AND NEURAL PATHWAYS. S.L. Cummings*, M.J. Guinan, D.J. Magliano, and R.L. Kitchell. Veterinary Anatomy and Cell Biology, University of California, Davis, CA 95616.

An interactive Macintosh computer program has been designed as an instructional adjunct for veterinary neuroanatomy and neurology students. The program may be used during laboratory periods as a reference guide and outside of formal class time as a study and review aid. Veterinary Neuroanatomy presents full color digital images of whole brain and brainstem of the sheep, gross transverse sections of sheep brain, and 36 stained histological transverse sections of the canine brain. Individual structures present in each image may be identified by using a mouse to indicate either the region of interest or a term from an accompanying list on the screen. Informational descriptions have been written for each labeled structure. Neural pathways are visualized in animated tutorial programs which are interactive with the Atlas. Relevant gross or histological sections may be viewed from any point in all pathways. The Ascending Pathways tutorial currently includes dorsal column-medial lemniscal, spino-cervico-thalamic, and ventral trigeminothalamic pathways. The Descending Pathways tutorial currently includes corticospinal, corticobulbar, and corticorubral pathways. The Visual Pathways program includes tutorials on visual perception, visual motor reflexes, and autonomic regulation of pupillary diameter. Requires Macintosh II, IIx, IIsi, IIci, or IIfx; 5MB RAM; 8 bit 13" color display; hard disk with 15MB of free space; high density floppy disk drive.

84.5

A FANTASTIC VOYAGE THROUGH THE HUMAN BRAINSTEM AND BASAL FOREBRAIN. C.F. Da Silva*[1], P. Dikkes[2], R.A. Pearlstein[3] and R.L. Sidman[4]. [1]University of Sao Paulo, Sao Paulo, Brazil; [2]Children's Hospital, Boston, USA; [3]Eidetics Inc., Watertown, USA; [4]Harvard Medical School, Boston, USA.

Serial section cinematography was used as a method for visualizing the internal structure of the human brain. Three normal brains obtained from autopsies were fixed by immersion in formalin and, after dissection, were embedded in block in Paraplast Plus according to Bergman et al (Science Tools, 27:46, 1980). A LKB Multirange Microtome was used to cut 25 µm serial sections which, after being mounted on glass plates, were stained with gallocyanin/methyl-green (Augulis & Spinwall, Stain Technol.,46:137, 1971). The slides (more than 12,000) were aligned and registered onto a 35 mm cine film with a "blink comparator" similar in kind to those used by astronomers for star plate analysis. Briefly, the slides were mounted in pairs in the instrument. A beam chopper and splitter arrangement (Levinthal & Ware, Nature, 236:207, 1972) allowed rapid sequential viewing of the image pair. One slide of the pair was then translated and rotated relative to the other until the apparent "blinking" that results from out-of-register areas being seen in rapid succession was minimized. Section 1 of the pair being registered was then photographed on a single frame of the cine film, after which was replaced by section 3 and the process iterated, overlapping pair by pair, throughout the series. The final product was a cine film in which each frame equals one serial section in tissue register. The film (now in a video disc format) can be viewed forwards and backwards repeatedly as a movie, allowing one to follow fiber tracts and nuclei into the brain at different speeds; it is also being used to create 3-D computer reconstructions.

84.6

PROTOTYPE FOR INTERACTIVITY: A MULTIMEDIA PLATFORM AND A TIERED CASE STUDY DESIGN FOR MEDICAL NEUROBIOLOGY. W.F. Hughes, T. Tom, J. Kerns, H.A. Paul, and T.J. Hoeppner* Rush-Presbyterian-St. Lukes Medical Center, Chicago, IL 60612

The objective of this project has been to provide a platform for small group discussion and to extend this style of interaction to a large group setting. Neuroanatomy graphics tutorials and case studies incorporating animations, sound, and video sequences were developed on a ***Commodore-Amiga*** computer featuring simple yet powerful multimedia authoring tools. Displayed with an RGB projector, these tutorials provide a focus for full-class (100 students) workshops and lectures, as well as independent small group study sessions facilitated by student group leaders. Three levels of case study target clinical applications of neuroanatomy and neurophysiology as a stimulus to developing solving strategies: **1)** ten **anatomically-oriented *cases,*** presented in small groups coordinated with neurology residents, are available in computerized versions for group discussion; **2)** two large-group ***case-oriented workshops*** emphasize basic neurophysiology, evoked potentials, and EEG; and **3)** two ***multidisciplinary case studies*** (neuro-immuno-microbiology) employ small group techniques developed in the problem-based "Alternative Curriculum" at Rush Medical College

Emphasis on open discussion linked to these computer-based tutorials (also available for independent student review) has transformed the character of one of our most popular basic science courses and re-energized student responsiveness in a large group.

84.7

THE GRAPHIC BRAIN - NEUROPHYSIOLOGY. T.J.Teyler, *Neurobiology Dept., NE Ohio Coll Med, Rootstown, OH 44272*

The **Graphic Brain - neurophysiology** is a computer-assisted instructional aid for teaching neurophysiology to medical and graduate students. It was first used with students in 1991. The MS-DOS based courseware covers twelve topic areas, ranging from such elementary topics as diffusion and charge separation and resting and action potentials, to the dynamic actions of second messenger systems, auditory and visual sensory systems, and cerebellar circuitry. Animated graphics are used to convey difficult concepts, particularly of a spatial or time-varying nature (ion flow, network activity, etc). The courseware has been used in two ways: as an adjunct to classroom lecture, and interactively by individual or small groups of students. For interactive use, the courseware contains a didactic segment followed by an interactive self-test with remediation loops. For classroom use, the the didactic courseware is used alone.

We have found the classroom use to be most effective when first used to introduce a topic, followed by a lecture which expands upon the information in the courseware. For complex topics, we often close the lecture by presenting the unit again. Even with this redundant presentation, we cover the same material in 75% of the time required in years past using traditional teaching methods. More importantly, the students rate the courseware highly and appear to have a better mastery of the material based on their exam scores increasing to 80.1% correct, from a previous average of 73.5% correct. Students are free to preview or review all of the courseware in library-based interactive computers established for this purpose.

The courseware will be demonstrated in the poster session.

84.8

NEUROSYS: A Simulation of Neuronal Electrical Activity. H. Levitan* and Z. Eldadah. Zoology Department, University of Maryland, College Park, MD 20742.

The ability of nerve cells to generate impulses for communication is based on the complex relationship between membrane permeability and membrane potential. Gaining an understanding of the characteristics and consequences of this relationship is an essential part of a neuroscience course. As in most areas of science, this understanding is greatly enhanced when students perform laboratory exercises that are designed to illustrate the basic concepts and that provide an opportunity to test the validity and implications of the concepts. The program, NEUROSYS, allows students to initially control all the variable parameters that contribute to the interdependence of membrane potential and permeability. The initial characteristics of the cell membrane, the membrane's environmental conditions, and the characteristics of two current or voltage stimuli applied to the neuron at specified times, can all be specified and modified. Using a modification of the Hodgkin-Huxley mathematical model, the complex patterns of impulse activity that arise spontaneously can also be studied. The spontaneous simulation mode allows students to gain an appreciation of how apparently random or chaotic patterns can emerge from a completely deterministic system, and of the transitions that occur between regular, pacemaker, and bursting patterns of impulse activity and very irregular, unpredictable patterns. The results of the simulation are displayed as variations in the membrane potential, and membrane conductances or intracellular calcium concentration, as a function of time. This innovative program encourages students to design and carry out their own experiments, and demonstrates a creative application of computers for original research.

84.9

A SPREADSHEET MODEL OF SIMPLE MEMBRANE POTENTIAL RELATIONSHIPS. J. S. Thomas, ADSS, Meharry Medical College, Nashville, TN 37208.

An EXCEL spreadsheet "workgroup" has been developed which can be used to illustrate the effects of changes in specific "causal" factors (Cm, [out]/[in], Gion, Istim, I_int) on a variety of defined/derived electrical parameters (DPion, Gtotal, chordVm) and the resulting changes in Vm in a classroom or tutorial setting. The availability of multiple graphical "windows" allows display of the time evolution of all response parameters in response to a change in one or more of the triggering parameters, and the triggering parameter changes themselves can be conveniently manipulated with a mouse by directly altering ("dragging") the values of a bar graph display of the "causal" values. The "outlining" and "cell annotation" facilities of the EXCEL program also facilitate the use of these spreadsheets in self-paced simulated laboratory exercises. Since all required manipulations can be made directly with a mouse (or from mouse selected macros) completion of prescribed exercises and exploration of novel combinations of parameters can be mastered with a minimum of computer specific instruction.

84.10

Axon Engineer™: A versatile simulator of cellular ion fluxes and ionic signaling. K. Miller[1] and P. Pennefather*. Nerve Cell on Synapse Group, U of Toronto, ON, M5S 2S2, [1]Aeon Software, 1553 Bailey Hill, Eugene, Or. 97402.

Considerable effort has been expended in the last decade to quantify the dynamic properties of elements such as ion channels, ion transporters and ion buffers that interact to shape ionic signals in cells. In addition, advances in molecular biology have given form to those elements that traditionally have been dealt with as mathematical abstractions. There is a real hope that structural features can be linked to kinetic components and that explicit structure-function hypotheses can be formulated. Despite these many advances, it remains difficult to package and present the available information in a manner that effectively communicates its significance. Interactive simulations are useful in helping to develop an intuitive appreciation of the factors that shape ionic signals. At last years meeting, we described an open ended general simulator of cellular ionic signals. Using this program and a 486 computer, a complex simulation of a vertebrate neuron that considered many multi-state channels and transporters as well as intracellular buffering and diffusion of calcium could be executed in 10-100 × real time. As many important ionic signals occur on the ms. to s. time scale the rate of feedback is sufficient to keep the students attention. The parameters defining the simulation configuration are formulated in a standardized fashion from an intuitive user interface that facilitates the definition of the number of states of the channels or transporters and the influence of independent variable such as ions, chemicals, voltage and temperature on the transition rates between states. All parameters and assumptions are readily accessible to inspection and modification by the student. The program has now been extensively debugged and documented. We are in the process of developing learning modules that take advantage of the program to illustrate important advances in cellular neurobiology. For example, one module will compare the traditional Hodgkin-Huxley view of the action potential in the squid giant axon with a more modern view that is based on extensive voltage clamp, single channel and gating current measurements.

84.11

UNDER-GRADUATE NEUROBIOLOGY LABORATORY USING MACINTOSH/MACLAB TO FACILITATE INDEPENDENT EXPLORATIONS IN INTRACELLULAR RECORDING. **Diana Card Linden**. Dept. Biology, Occidental College, L.A., CA 90041.

An undergraduate neurobiology laboratory was designed to provide hands-on experience for students to record membrane and synaptic physiology. The emphasis was on close integration of lecture and laboratory materials, and to provide a series of experiments that inexperienced, but intelligent students could master. Lectures stressed molecular mechanisms of membrane physiology, biophysics and membrane biochemistry. The exercises were reliable and easy to execute. In each three hour laboratory the student needed to master dissection of tissues, manipulation and use of the electronics, computer, and collection of data (and problems associated with intracellular recording).

All projects used the frog sartorius; as the students dissections improved during the 10 week course so did their experimental results. Experiments involved recording of V_{rest} varying extracellular K^+, Rb^+, and Li^+; measurement of spontaneous and evoked EPPs varying extracellular Ca^{++}, Mg^{++}, and eserine; visualization of neuromuscular junction morphology and biochemical properties using histochemical stains, and fluorescent ligands. More sophisticated techniques, such as voltage clamp, were studied using highly interactive software simulating Hodgkin-Huxley modeling of squid axon membrane.

What makes this laboratory experience unique is that the students did all of the work themselves in one or sometimes two 3-hour sessions. The equipment for this laboratory was purchased from funding by a NSF/ILI grant to DCL and matching funds provided by Occidental College.

84.12

THE MYOTOTIC REFLEX. C.D. Barnes*, R.D. Kirkpatric, K.B. Campbell and J.G. Meador. Departments of VCAPP and Electrical Engineering and Computer Science, Washington State University, Pullman, WA 99164-6520.

Traditionally students have difficulty grasping the functional consequences of feedback as it is exhibited with its various nuances in the myotatic reflex. They have particular difficulty understanding the modulatory effects of the gamma motor system on the reflex. As a teaching aid, an easy-to-use computer simulation of this reflex system was developed under Microsoft Windows. This graphical interface allowed real-time animated simulation. In the simulation, a variable static force can be applied to the muscle or the force can be applied as a "falling weight." "Supra spinal" inputs to the alpha motor neuron can be dynamically varied, as can the γ- static and γ-dynamic motor neurons to the muscle spindles. The reflex can be opened by interrupting the Ia from the muscle spindle. Output of each of the variables has its own real-time digital read-out and there are four "strip chart" channels of output for Ia discharge, alpha motor neuron discharge, muscle length and force. These can be run continuously or triggered by the weight drop with accumulated traces in different colors. Classroom use of this simulation has proven to be more effective than didactic lectures in teaching these difficult concepts.

84.13

HYPERCARD PROGRAM TO TEACH NEUROSCIENCE. R.J. Ilmoniemi and M. Perko. Low Temperature Laboratory, Helsinki University of Technology, 02150 Espoo, Finland.

We have developed a package for computer-aided learning to supplement lectures on neuroscience. The program runs as a HyperCard application in all Apple Macintosh computers. Special emphasis has been given to pedagogic issues. At each stage, there is an effort to motivate the student as well as to orient her to the topic before exposure to all the details. The student is an active participant in the teaching/learning process and is given a possibility to test her progress.

The package consists of sections (Hypercard stacks), each of which covers one topic such as gross anatomy or the synapse. The system can be expanded by adding stacks or adding material to existing sections.

The student is guided through the sections, but it is possible to pick any stack or subsection at any time. The program keeps track of where the student is and how much time has been spent in different parts of the program. Since text is best read from books, we put emphasis on graphics and animation in explaining how the brain is organized and how it works. If the student is confused, help is available. Background material, e.g., on chemistry or physics, can be accessed as needed. At any time, the student can test his progress by invoking various kinds of tests. Success is rewarded by special displays, demonstrations, or games.

Besides learning material, stacks have been developed for student registration, for literature, background material, glossary, user's notes, etc. The teacher has the right to read registration information as well as to modify the material. There is an auxiliary stack containing high-level programming tools for the teacher who wishes to do his own programming.

84.14

"NEURAL COMMUNICATION" : A NEW, HIGHLY VISUAL COMPUTER-ASSISTED PROGRAM BASED ON HYPERMEDIA PRINCIPLES. P.I. Magistretti[1]* and B. Zaerpour[2], Institut de Physiologie[1], Université de Lausanne and OPAL [2], 5 rue d'Evian, Lausanne, Switzerland.

A series of computer programs has been developed with the specific aim of promoting associative learning. "Neural communication" is the first such program. Using hypermedia principles, the information content of the program is distributed throughout an interactive network. The program is organized in over 40 windows, each containing images, text and animations connected to each other and to information in other windows through "active zones". This results in the connection of bits of information to dozen of others within the program. Various tools allow the user to wander throughout the program following his/her own associative thinking, rather than a sequential, predetermined and rigid pattern. Each user can create his/her own knowledge trail. A self-assessment option allows the student to test his/her knowledge through multiple-choice questions. In case of an incorrect answer, a "tutorial" option directly brings him/her back to the part of the program where the subject of the question is treated. New questions can also be prepared with the "question-maker" option. A "slide-show" option, through which a sequence of windows can be created, is also available for lectures, since, with a simple computer-projector interface, windows can be projected on a large screen. The program is available for PC (optimal configuration 386, 16 MHz, 2 MB RAM and VGA color screen) as well as for Macintosh. "Neural communication" is part of a series of OPAL modules (Open Programs for Associative Learning) that can be automatically interconnected when loaded on a computer. Presently other modules include "Calcium as a messenger" and "Immune cells". New modules are currently being developed.

84.15

SLICE OF BRAIN, A VIDEODISC FOR NEUROSCIENCE EDUCATION. S. S. Stensaas, Cornell Univ. Med. Coll., Dept Path., P. Burrows, Univ. Utah, Instructional Media Serv., J. R. Bolles, Univ. of Wash. Health Sci. Cent. Educ. Res., J. W. Sundsten, Univ. Wash. Dept. Biol. Struc. and E. C. ALVORD, Jr., Univ. Wash., Dept of Path.

A cooperative videodisc, *Slice of Brain*, has been produced using material previously published on three other videodiscs: *Slice of Life; The Digital Anatomist: Human Brain Animations;* and *Disorders of the Nervous System: Motor*. In addition, unpublished images in neuropathology (6000), gross anatomy of the head and neck (500), and serial sections of a monkey brain have been added. A generic videodisc allows users to select equipment and develop interactive instructional materials to meet their unique needs. A generic disc is one that has no specific content or design, but can be used in different ways for independent study, or in multiple courses using a variety of hardware. When controlled by a computer (DOS, Macintosh, Amiga, Next) it becomes a powerful vehicle for independent and cross discipline teaching. The possibility now exists to introduce a series of courses of increasing complexity that appeal to beginners and also permits the more advanced students to review content at all levels as desired.

Slice of Brain uses a mixture of stills and motion sequences and is directed at the professional, graduate and undergraduate neuroscience community. The disc contains motion sequences in addition to the extensive collection of stills. Approximately 7 minutes of reconstructed digital brain animations demonstrate the interrelationships of major brain structures as they rotate. Patient sequences demonstrate dynamic pathologic terms such as ataxia, hyperreflexia and hemiplegia. Still images emphasize gross and microscopic anatomy, radiology and pathology.

The videodisc will be demonstrated together with preliminary software that the authors are developing. It is hoped that these demonstrations will stimulate members to contribute material to future editions of the disc or to develop educational projects using the disc that can be distributed to enhance neuroscience education.

84.16

SYNAPSE – THE MOVIE: GRAPHICAL ANIMATIONS OF SYNAPTIC FUNCTION FOR GRADUATE AND MEDICAL NEUROSCIENCE EDUCATION. R. P. Hammer, Jr., Lab. Cell. & Molec. Neuropharm., Dept. Anatomy & Reprod. Biol., Univ. Hawaii Sch. Med., Honolulu, HI 96822.

Computer-assisted self-learning has become a valuable tool by which to provide graphical, multimedia resources. Extensive use of such electronic resources in our problem-based learning medical curriculum has shown that optimal usage and student performance is attained from highly visual applications which utilize the unique capacities of the computer interface. In particular, graphical animations of physiological processes which are difficult to visualize in three dimensions are most useful. Current models of synaptic function at the cellular and molecular levels provide an ideal basis for such animated modules. *Synapse – The Movie©*, created using Aldus SuperCard®, is a Macintosh® application which illustrates the physiological and molecular events underlying the process of neurotransmitter function at the synapse using a series of colored graphical animations created with MacroMind Director®. The user navigates around the synapse either by viewing basic synaptic functions or by examining details of pre- and postsynaptic function, such as voltage-gated calcium channels, exocytosis, ligand-gated ion channel or G-protein-mediated receptor function (e.g., muscarinic and nicotinic acetylcholine, dopamine, or serotonin receptors), and presynaptic terminal, autoreceptor or transporter function. The sequence of events occurring upon receptor activation (e.g., D_1-mediated $G_{s\alpha}$ activation, adenylate cyclase stimulation, and cAMP-dependent protein kinase-induced phosphorylation of DARPP-32) is graphically illustrated in simple 3D-rendered "cartoons." Textual and audio explanations are presented at each step, and users can control forward progress or replay a segment as needed. *Synapse – The Movie©* is a useful instructional tool in both graduate and medical neuroscience courses, and might be a valuable adjunct to continuing medical education and advanced undergraduate neuroscience curricula.

84.17

ENHANCEMENTS TO NEURODATABASE FOR TEACHING.
S.L. Wertheim* and R.L. Sidman. Division of Neurogenetics, N.E. Regional Primate Research Center, Harvard Medical School, Southborough, MA 01772-9102.

NeuroDatabase is a software system for managing images, related graphics and text for research and teaching. Its teaching functionality has been substantially enhanced since it was shown at the 1991 Soc. for Neurosci. meeting. NeuroDatabase allows teachers to incorporate their own image sets and to store a caption, three types of overlay graphics, and labels for each image. Icons of all images in a series are viewable in a Series Browser. Choosing an icon displays the original image. Any series can have an associated orientation image for indicating the plane of section and moving through the series. Two self-testing modes have been added. "Structure names" presents a list of all structures defined for the current image and shows their outlines in random order, asking the student to choose the correct name from the list. "Structure borders" highlights a random structure name in the list and asks the student to indicate that structure in the image. The vocabulary system has been substantially enhanced. Classification information is kept for each term, allowing users to distinguish anatomy terms from neurochemistry or pathology terms while searching. It is possible to satisfy reasonably abstract requests such as "Show me all the fiber bundles in this image". Storing certain relationships between terms, allows users to see structures in a particular region or those related to a given functional system. Guided Tours provides a narrative for a particular topic, associating each text block with a set of images and graphics selected by the instructor. Creating Guided Tours has been made easier with a new user interface. A student tracking facility records the student name, date, session duration, time spent using the self-tests, and Guided Tour topics chosen. Supported by NIH Grant NS02820.

84.18

THE SECOND MESSENGER SYSTEM. Sheldon S. Ball* & Vei H. Mah. Dept. of Pathology, University of Mississippi, Jackson MS 39216 & Dept. of Neuropathology, Thomas Jefferson University, Philadelpia, PA 19107.

The Second Messenger System is an interactive computer program for students, educators, and research investigators in the molecular neurosciences. The application allows an individual to ask a sequence of questions in a single interactive session, thereby facilitating the development and testing of several hypotheses in a short period of time. Graphical representations of molecules and molecular events enable individuals to grasp spatial and functional relationships (and in the future, temporal relationships) among molecules, cellular compartments, and cell regions. Fairly simple but well-defined reasoning capabilities allow an individual to ask sophisticated questions and to predict novel molecular events or pathways. The SECOND MESSENGER SYSTEM (SMS) contains information about: 1) the molecules of signal transduction processes and the motifs that impart function to these molecules; 2) the molecular events of signal transduction processes; 3) cell-specific expression of genes critical to signal transduction processes. Signal transduction is a particularly appealing area to develop a molecular neurosciences information system for two reasons: 1) the complexity of regulatory cascades that result from numerous but relatively simple interactions provides a system relatively straightforward to represent, and at the same time, exploits something computers do well, keep track of details; 2) the central role that abberrations in signal transduction play in disorders of development, aging, and mental health. SMS is being developed through object-oriented programming in a portable programming environment supported by COMMON LISP and the COMMON LISP INTERFACE MANAGER.

TEACHING OF NEUROSCIENCE: ELEMENTARY AND SECONDARY GRADES

85.1

TEACHING NEUROSCIENCES TO THIRD GRADERS IN THE INNER CITY.
V. Lemmon*, S. Burden, A. Garner, D. Orentas, S. Piszczkiewicz, E. Wong, Dept. of Neurosciences, Case Western Reserve University, Cleveland, OH, 44106

We have developed a program to bring a hands-on scientific experience to elementary students with little or no previous science training. Our goals include teaching basic concepts about nervous system organization and function, showing that science is fun and providing positive role models for the children. The presentation begins with a 12-15' chalk talk where 4 concepts are introduced. These are 1) the brain has different parts that do different jobs, 2) it takes time for brain signals to move from one brain region to another, 3) the brain uses electrical and chemical signals, and 4) neurons come in many shapes and sizes. Students are involved in the presentation to capture their attention. In order to reinforce these concepts, the students are then divided into small groups that spend about 5-7' at each of 5 different hands-on activities. The activities include 1) a human brain and spinal cord, 2) a series of brain models from different vertebrates, 3) measurement of student reaction times, 4) a spirited Hypercard program that illustrates the effects of different drugs on chemical transmission and 5) a microscope for observation of different neuronal cell types. Each station is managed by a graduate or medical student. We are particularly fortunate to receive the assistance of members of the local chapter of the Student National Medical Association. At the end of the session the student with the fastest reaction time receives a prize. Detailed information, including the Hypercard program, can be obtained from V. Lemmon

85.2

KIDS JUDGE! NEUROSCIENCE EXHIBIT. UIC Graduate Students,[1] D.A. Twombly,[3] and D.L. Colbern*[1,2]. Committee on Neuroscience[1] and Dept. of Physiology and Biophysics[2], Univ. of Illinois at Chicago (UIC), IL 60680; Dept. of Pharmacology[3], Northwestern Univ., Chicago, IL 60611.

Science literacy is a critical problem facing today's society. At least part of this problem is due to a lack of communication between biomedical scientists and the public at large, particularly children being educated in the school systems. As a means of addressing this issue, we organized a "Kids Judge! Neuroscience Exhibit", geared toward children at the elementary school level. The program was designed to stimulate an interest in science, and to allow children to interact directly with university scientists.

Nineteen graduate students in the UIC Neuroscience program participated in the Kids Judge! exhibit. Each student created a display to illustrate concepts about the brain, in a fashion that would be both exciting and understandable for schoolchildren. In preparation for the exhibit, we obtained display-making guidance from professional staff of the Chicago Museum of Science and Industry. The exhibit itself was attended by 180 fourth grade children from three of the Chicago Public Schools. Many of these students were visually impaired; the displays were designed to be interesting and accessible to these children as well. In addition to the displays, the program included a "workshop" in which kids constructed brain parts with clay, a presentation on careers in science, and an introduction to the BEEM-Net (Brain-Exchange Electronic Mail) computer system (see Colbern et al. 1992). A novel aspect of the exhibit was to allow all of the kids to serve as judges. They evaluated displays on a number of indices, and voted for their favorite. The most popular exhibitors received awards in a ceremony at the end of the program. Based on the enthusiastic reactions from the schoolchildren, their teachers, and their principals, the exhibition was an overwhelming success. This forum proved to be an effective means of promoting interchange between scientists and representative members of the lay public.

85.3

BEEM-NET: BRAIN-EXCHANGE ELECTRONIC MAIL NETWORK FOR KIDS AND SCIENTISTS. D.L. Colbern[1], C. Collins[2], J. Emerton[2], and D.A. Twombly[3]*. Dept. of Physiology and Biophysics, Univ. of Illinois at Chicago[1], 60680; BioTechNet - Eaton Publishing[2], Natick, MA 01760; Dept. of Pharmacology, Northwestern University[3], Chicago, IL 60611.

Electronic mail is rapidly becoming a common mode of communication in the scientific and business communities. We are utilizing this technology to develop an electronic mail network between research neuroscientists and students in the school systems. This network is intended to provide opportunities for information exchange, and an environment where students can have direct access to university scientists. With the assistance of BioTechNet - Eaton Publishing, we have established the "Brain-Exchange Electronic Mail Network" (BEEM-Net), which is being linked to schools in the Chicago Public School System. BEEM-Net allows students using computers in their schools or at home to access a consortium of neuroscientists and a number of on-line educational resources. Students may address questions or commentary to the network at large or to specific scientists. Participants may engage in interactive sessions and leave mail for one another. This dialogue could include questions concerning science fair projects, information learned in the classroom or at home, or even practical issues such as careers in science. The BEEM-Net system was first introduced at the Kids Judge! Neuroscience Exhibit (see UIC Graduate Students et al., Soc. Neurosci. Abstr., 1992). In preparation for this exhibit, 6 eighth grade students were trained to operate the BEEM-Net system. These students in turn demonstrated the system to 180 fourth graders who attended the Kids Judge! exhibit. At this event, students used BEEM-Net to send messages to neuroscientists at the University of Scranton, to BEEM-Net representatives in the Far East, and to parents with Internet access. We expect BEEM-Net to be a valuable supplement to science education, as well as a means of cultivating an interest in science and an appreciation for its value to society.

85.4

BRAINLINK: NEUROSCIENCE TEACHING MATERIALS FOR ELEMENTARY GRADES. L.M. Miller, K.H. Taber*, J.H. Dresden, W.A. Thomson. Departments of Community Medicine and Radiology, Baylor College of Medicine, Houston, TX 77030.

BrainLink is a three year ADAMHA Science Education Partnership Award-funded science education project designed to present the latest factual information about the brain, while at the same time conveying the excitement of "doing science" to students in grades 1-6. The materials are being designed to teach normal brain function and the effects of injury and disease with an emphasis on how neuroscience research is helping to develop new insights and treatments. A total of six units will be developed. Instructional materials are designed to increase the participation and interest of students in the classroom setting through a creative combination of "hands on" activities and student-focused stories. An associated activities book for use in the home setting will provide opportunities for one-to-one parent/child explorations focusing on aspects of the nervous system and neuroscience related careers. An outline and summary of the entire project will be presented, as well as prototype materials.

85.5

HANDS-ON NEUROSCIENCE DEMONSTRATIONS FOR ELEMENTARY AND SECONDARY SCHOOL CLASSROOMS. J A Fiez*, A Berkowitz, S T Carmichael, H P Goodkin, J G Keating, A Lewis, J S Solomon, L E White. Div. of Biol. and Biomed. Sciences, Washington Univ., St. Louis, MO 63110.

We have used a participatory, hands-on approach to introduce neuroscience to more than 150 students (5th-8th graders). Our goal was to create activities (see below) which are both educational and fun.

Human Psychophysics: A series of short computer programs show how performance measures, such as reaction time and accuracy, reveal information about higher brain functions, such as attention and language.

Visual and Motor Illusions: Visual illusions demonstrate how the brain can be "fooled" and how it adapts to stimuli. Vibration of leg and arm tendons produce motor illusions which demonstrate the role of proprioception in determining body position and producing postural adjustments.

Sensory-motor adaptation: Prism goggles are used to distort visual input, and students attempt to hit a target throwing beanbags. Students deduce the importance of visual guidance and motor activity for the learning process.

Neuroanatomy: Brain anatomy is demonstrated using a human and various animal brains. An MRI demonstrates how living brains can be imaged and the location of damage determined. Slides of neurons show what neurons look like and how the connections between neurons can be traced.

Neuropharmacology: A dissected turtle heart is used to show how heart rate and force can be affected by neurotransmitter agonists and antagonists.

Neurophysiology: An EKG shows that the electrical activity of muscles can be measured, while EMGs show the effects of force, movement, and reflexes on muscle electrical activity. The discharge of electric fish is recorded to show that electrical activity can also be used by an animal to sense its surrounding.

85.6

ANIMAL MODELS IN BASIC RESEARCH: A WORKSHOP FOR TEACHERS. E.M. Granger, P.C. Hayward, K.D. Videgar, J.C. Smith*. Depts. of Biological Sciences and Psychology, Florida State University, Tallahassee, FL 32306.

We have developed a series of workshops for middle and high school science teachers addressing, first, what basic research is and why it is done and, second, why responsible animal use is needed in basic as well as applied behavioral and biomedical research. Resource materials for dissemination to teachers include written materials, video materials, and hands-on exercises for classroom use. These materials are being distributed to classroom teachers through workshops held in local school districts. All middle- and high-school science teachers in Florida will have the opportunity to attend a workshop in their local school district. The long-term goals of the project are to increase scientific literacy by promoting public understanding of what basic research is, why it is necessary for progress toward improving health, and why the responsible use of animals is necessary in basic as well as in applied behavioral and biomedical research. These goals are essential for the Neuroscience community as well. Progress toward these goals will be assessed through the analysis of pre- and post-tests that have been developed for administration to all of the teachers before and after their participation in the workshop and to their students before and after classroom implementation. Workshop administration manuals and resource materials will be available to all interested Society for Neuroscience members by 1993.

85.7

A FUN BOOK FOR TEACHING NEUROSCIENCE TO 8-12 YEAR OLD STUDENTS.

Philip R. Kennedy, Neuroscience Lab., Bioengineering Center, Georgia Institute of Technology, 400 10th. St. N.W., CRB Rm 325 - Atlanta, GA 30332.

Previous approaches taken by authors of non-fiction books on the central nervous system have attempted to describe an aspect of its anatomy or physiology as a simplified text. Titles might include "How we Hear", "How we See", and so on. This approach is rejected as being too didactic and impersonal for children of the 8 to 12 years age group. The present approach is based on the belief that the factual content is less important than the "imagination content". To stimulate the imagination, the reader is taken on an explanatory "walk" through his or her brain, spinal cord and muscles as he or she performs aspects of an everyday experience.

The present effort is a 9,000 word, non-fiction manuscript consisting of 9 chapters with 28 figures, titled "Get a Move On, Neuron!". The reader becomes involved in self-testing with such maneuvres as the finger-nose test, the patellar reflex test and so on. After each chapter, parental involvement is encouraged.

The author is an MD, PhD, and a graduate of The Institute of Children's Literature of Redding Ridge, CT. The book was inspired by the author's desire to write a simple explanation of brain function for his children. It is expected that this approach will benefit the Society's efforts to teach neuroscience to middle and high school students.

85.8

A VISION OF TOMORROW IN TODAY'S STAR STUDENTS: SCIENCE AS A MULTICULTURAL DISCIPLINE.

R.D.Brinton*°, D.C Moot+ and M. Mayo+. °Dept. of Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California and + Francisco Bravo Medical Magnet High School , Los Angeles, CA 90033.

The declining numbers of persons entering fields of science is coupled with an equally drastic change in the ethnic and sexual composition of the pool of persons eligible to enter fields of science. Our approach in the Science Technology And Research (STAR) Program has been to address the decline of interest in science in tandem with the challenge of the new demographics of potential scientists. The STAR Program is a cooperative science education venture with Francisco Bravo Medical Magnet High School which is to the USC Health Sciences campus. The STAR Program provides junior and senior high school students interested in learning about scientific exploration the opportunity to participate in research projects. The ethnic composition of the STAR Program is 9% African-American, 45% Asian-American, 9% East Asian-American, 4% Asian Pacific-American and 32% Hispanic-American. Gender composition of the STAR Program is 59% female and 41% male. The full ethnic and gender diversity of the STAR Program was reflected in the STAR students winning first place in the Bravo and STAR Science and LA County Science Fair competitions.

A fundamental goal of the STAR Program is to provide STAR students with a mentor that will be a consistently available presence in the life of the STAR student as she or he proceeds through college, graduate and postgraduate education onto their ultimate career choice. Therefore, an important criterion for selecting investigators to participate in the STAR Program is their capability and commitment to act as a mentor to the STAR student in their laboratory.

The multicultural composition of the STAR Program fosters an awareness and respect for other cultures as well as providing a forum for intellectual debate of varied perspectives. Evidence of multicultural harmony and mutual respect is evident in the pride that STAR students have in the accomplishments of their colleagues regardless of gender or race. Video and photographic presentation of STAR students and science competitions will be presented along with a description for implementing a similar program .

Supported by NIH grant SO3 RRO3011 to R.D.B.

85.9

THE SCIENCE EDUCATION PARTNERSHIP: TEACHING NEUROSCIENCE IN THE SAN FRANCISCO PUBLIC SCHOOLS. D.C. Bowen* and W. P. Milestone°. Program in Neurobiology, University of California, San Francisco and °Science Department, Abraham Lincoln High School, San Francisco.

The Science Education Partnership seeks to promote teaching partnerships between scientists at UCSF and teachers in the San Francisco Unified School District (SFUSD). In this abstract, we describe strategies which have proven effective in providing practical demonstrations of concepts in neuroscience and general biology. Given the linguistic heterogeneity of the SFUSD, it has proven critical to present information in a manner that is as independent of language as possible. We have found this goal to be best accomplished by designing projects conducted by small groups of students working together in close contact with both members of the teaching partnership. Two examples of this approach are described briefly below.

(1) ***Drosophila* genetics**. Student lab groups worked together to determine the phenotypes of parental and offspring flies in a test cross using a sex-linked marker for eye color. This experiment emphasized collaboration among groups and testing hypotheses by comparing predicted results to actual data. (2) **Neuroanatomy**. Students were first familiarized with the cytoarchitecture of the brain then examined a *post mortem* human brain and discussed its functional organization. Since these experiments utilized materials that are available to most neurobiologists, we hope they provide helpful models for others interested in similar teaching partnerships. Supported by the UCSF Chancellor's Office, the Crocker Foundation and the American Honda Foundation (to SEP), the NSF and NIH (to DCB).

86

SYMPOSIUM. PROTEIN PHOSPHATASES AND THE REGULATION OF NEURONAL EXCITABILITY. D.L. Armstrong, NIH (Chairperson); A. Nairn, Rockefeller Univ.; J.H. Byrne, Univ. Texas; I.B. Levitan, Brandeis Univ.; M.P. Charlton, Univ. Toronto.

Phosphoprotein phosphatases are proving to be important regulators of neuronal excitability and synaptic transmission. At the same time knowledge of protein phosphatase structure, function and regulation is beginning to explode with wide ramifications for cell signalling, membrane transport, cell cycle control, oncogenesis and environmental health (Shenolikar & Nairn '91 *Adv. Second Messenger & Phosphoprotein Res.* 23: 3-121). Two important classes of biological toxins polluting both salt and fresh water are potent inhibitors of phosphoserine/phosphothreonine protein phosphatases.

The structure and regulation of these enzymes will be reviewed (**Nairn**). Novel mechanisms of phosphatase activation by neuropeptides will be presented (**Armstrong**). Ion channel regulation by protein phosphatases will be described at the cellular level (**Byrne**) and analyzed at the molecular level using purified enzymes on single channel proteins in a reconstituted system (**Levitan**). Finally, the functional consequences of these changes in excitability on synaptic transmission will be demonstrated at peripheral synapses in both mammalian and crustacean nervous systems (**Charlton**).

87

SYMPOSIUM. MOTILITIES OF THE AUDITORY PERIPHERY. R. Hallworth, Northwestern University (Chairperson); J. Schacht, University of Michigan; J. Assad, University of Rochester; M. Ulfendahl, Karolinska Institutet.

Hair cells of the inner ear are not simply passive mechanoreceptors. They exhibit several kinds of motilities that are expressed as shape changes or as force generation. Hair cells may use motilities to modify their responses to mechanical input. This symposium will explore the various forms of motility in hair cells and their possible roles in transduction. Dr. Hallworth will describe membrane-potential driven length changes in cochlear outer hair cells in vitro. The possible location and mechanism of the motility will be discussed. Cochlear outer hair cells also undergo tonic length changes. Dr. Schacht will highlight the role of intracellular calcium and efferent neurotransmission in the maintenance of outer hair cell length. In contrast to somatic length change, some hair cells show motility of their cilia. Dr. Assad will discuss a possible motor mechanism that controls adaptation in saccular hair cell cilia. Finally, a number of stimuli modify motility in isolated cells. Dr. Ulfendahl will present the results of experiments that use these agents to indicate what role motility plays in cochlear transduction.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION

90.1

POLYSIALIC ACID NEURAL CELL ADHESION MOLECULE (PSA-NCAM or EMBRYONIC NCAM) IS EXPRESSED IN THE REGION OF THE GnRH PULSE GENERATOR OF THE PUBERTAL RHESUS MONKEY. A.D. Perera[1], C.F. Lagenaur[2] and T.M. Plant[1*]. Depts. of [1]Physiology and [2]Neuroanatomy and Cell Science, University of Pittsburgh, Pittsburgh, PA 15261.

Puberty in primates is triggered by the reinitiation of pulsatile GnRH release: a neuroendocrine event that we hypothesize reflects a reorganization of the neural substrate underlying the GnRH pulse generator (PG). A precedent for such postnatal plasticity in neuroendocrine systems has been established for the magnocellular oxytocinergic neurons, where the capacity for structural change in adulthood has been linked to the expression of PSA-NCAM (PNAS 88:5494,1991). Further evidence that PSA-NCAM is indicative of postnatal plasticity is provided by the finding that in mature rodents this molecule is found in hippocampus and olfactory bulb, two areas where neuronal plasticity continues into adulthood. Here, we tested the hypothesis that the pubertal reinitiation of the PG in primates is also a plastic event, and therefore might be associated with the expression of PSA-NCAM. Using a monoclonal antibody raised against PSA-NCAM, intense immunoreactivity for this molecule was observed in the arcuate-median eminence region (ARC-ME) of the pubertal male monkey (n=3). Double-staining revealed an overlap of PSA-NCAM immunoreactivity with GnRH perikarya and nerve terminals in the ARC-ME: a region where the PG is thought to reside. Reduction in the intensity of PSA-NCAM staining after desialylation with neuraminidase, and Western blot analysis confirmed the presence of PSA-NCAM in the ARC-ME. The role, if any, of this molecule in the initiation of primate puberty is probably of a permissive nature, since PSA-NCAM was also observed in ARC-ME of the juvenile (n=1). These findings are consistent with the notion that neuronal plasticity in the ARC-ME may initiate primate puberty.

90.2

PATTERN OF GNRH SECRETION IN THE PITUITARY PORTAL CIRCULATION OF THE GROWTH-RETARDED FEMALE SHEEP DURING HYPOGONADOTROPISM. J.M. Manning*, C.G. Herbosa, C.R. Friedman and D.L. Foster. Reproductive Sciences Program, and Departments of Obstetrics and Gynecology, and Biology, University of Michigan, Ann Arbor, MI 48109-0404.

Dietary-induced growth retardation produces hypogonadotropism and delayed puberty. In the sheep, even in the absence of ovarian steroid feedback, growth-retarded females exhibit slow luteinizing hormone (LH) pulses compared to normally-growing, ovariectomized females (*Endo.* 116:375, 1985). The infrequent LH pulses could reflect either: 1) correspondingly infrequent GnRH pulses, or 2) normal frequency GnRH pulses of a depressed amplitude, such that only an occasional GnRH pulse attains an amplitude sufficient for LH release. To determine by which mechanism (frequency vs. amplitude) growth retardation suppresses GnRH secretion, we simultaneously collected pituitary portal blood for GnRH and jugular blood for LH over a 4-hr period in growth-retarded (28kg, n=8; "28kg") and normally-growing (60kg, n=7; "60kg") ovariectomized lambs at 52 wks of age, 20 wks beyond the normal age of puberty. As expected, the 28kg females were hypogonadotropic and exhibited a low LH pulse frequency (0.5±1.0 pulses/4h, mean ± SE), compared to the 60kg females (6.4±0.4 pulses/4h). The pattern of GnRH secretion corresponded with that of LH secretion, in that 28kg females exhibited slow GnRH pulses (0.5±1.0 pulses/4h), compared to 60kg females (7.0±0.4 pulses/4h). The failure to find high frequency, low amplitude GnRH pulses which are subthreshold for LH release supports the hypothesis that the *frequency* of GnRH secretion is suppressed by growth retardation. (Supported by NIH HD-07517, -18394, and -18258).

90.3

ESTRADIOL REDUCES THE AMPLITUDE AND INCREASES THE FREQUENCY OF PULSATILE GONADOTROPHIN RELEASING HORMONE (GnRH) SECRETION PRIOR TO THE PREOVULATORY GnRH SURGE IN THE EWE. N. P. Evans,* G. E. Dahl and F. J. Karsch. Reproductive Sciences Program, University of Michigan, Ann Arbor, 48109.

During the follicular phase of the ovine estrous cycle, GnRH is secreted into pituitary portal blood as pulses that increase in frequency and decrease in amplitude, followed by a sustained surge that triggers the preovulatory luteinizing hormone discharge. This study tested the hypothesis that the follicular phase rise in circulating estradiol (E) stimulates these changes in the pattern of GnRH secretion. Mid-luteal phase ewes were ovariectomized, treated with sc Silastic implants to mimic luteal phase concentrations of progesterone (P) and E, and implanted with an apparatus for collection of pituitary portal blood. One week later P was removed to simulate luteolysis, and the ewes received one of three E treatments: NO E (n=5) luteal phase E implant removed; BASAL E (n=5) luteal phase concentration of E maintained; INC E (n=5) E increased from basal to peak follicular phase concentrations by sequential addition of E implants. GnRH was monitored in pituitary portal blood sampled at 10 min intervals over a 20 hr period beginning 16 hr after P removal. E treatment significantly reduced ($P < 0.05$) the total amount of GnRH collected during the 20 hr period: NO E: 4.1 ± 0.9 ng; BASAL E: 2.1 ± 0.6 ng; INC E: 1.4 ± 0.2 ng. Moreover, E significantly ($P < 0.01$) reduced the size and increased the frequency of GnRH pulses. Analysis of GnRH secretion over time in the INC E group indicated the suppressive effect of E on both the amount of GnRH collected and GnRH pulse size were dose dependent. These results support the conclusion that E contributes to the increase in GnRH pulse frequency between luteolysis and the GnRH surge in the ewe, and that the follicular phase rise in circulating E causes diminution of the amount of GnRH secreted per unit time, due to a progressive reduction in GnRH pulse amplitude. (USDA-90-37240-5507, NIH-HD18337 and HD18258)

90.4

EFFECTS OF ESTRADIOL AND ENDOGENOUS OPIOIDS ON PULSATILE GNRH SECRETION DURING THE BREEDING SEASON OF THE EWE. R.L. Goodman*, N.P. Evans, G.E. Dahl, and F.J. Karsch Department of Physiology, West Virginia Univ., and Reproductive Sciences Program, Univ. of Michigan, Ann Arbor, MI 48109

In breeding season ewes, estradiol (E) inhibits the amplitude of luteinizing hormone (LH) pulses. This action of E may involve endogenous opioid (EOP) neurons: EOP antagonists increase LH pulse amplitude in E-treated ovariectomized (OVX), but not in OVX, ewes. This study determined if these inhibitory actions of E and EOP neurons reflect changes in the secretion of gonadotropin-releasing hormone (GnRH) by administering naloxone to OVX and OVX+E ewes (6/group). Luteal phase ewes were OVX, an apparatus for hypophysial portal blood collection implanted, and steroids replaced with Silastic implants when needed. Two weeks later, portal blood samples were collected every 10 min for 6 hr before, 6 hr during, and 4 hr after iv naloxone infusion (1 mg/kg/hr) into OVX and OVX+E ewes. E treatment decreased the total amount of GnRH collected during the first 6 hr from 515 ± 126 to 248 ± 83 pg by inhibiting GnRH pulse size (OVX: 94.2 ± 27.7; OVX+E: 24.8 ± 9.3 pg/pulse). E increased GnRH pulse frequency from 5.8 ± 0.4 to 8.2 ± 0.6 pulses/6 hr. Naloxone did not alter pulse frequency, but increased GnRH pulse size, and did so in both OVX (250 ± 53 pg/pulse) and OVX+E (72.7 ± 20.5 pg/pulse) ewes. In summary, during the breeding season, E stimulates GnRH pulse frequency, while inhibiting the amount of GnRH released in each pulse. In contrast, EOP neurons appear to selectively inhibit GnRH pulse size and do so independently of E. Thus, EOP neurons may not mediate the feedback actions of E on pulsatile GnRH secretion. (NIH HD17864, HD18337, HD18258, USDA-90-37240-5507).

90.5

GnRH PULSE FREQUENCY AND THE DIFFERENTIAL REGULATION OF LH AND FSH IN THE MALE DJUNGARIAN HAMSTER. J.M. Meredith*, F.W. Turek, J.E. Levine, Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.

In the Djungarian hamster photostimulation has been shown to evoke differential release of the gonadotropins, with FSH levels rising rapidly following the onset of photostimulation and LH levels rising significantly later. We hypothesize that GnRH pulse frequency gradually increases following photostimulation and that the differential rise times in the gonadotropins may correspond to time points when GnRH pulse frequencies are preferential for either LH or FSH release. To test this hypothesis, photoinhibited hamsters were given various GnRH treatments consisting of chronic 1 minute GnRH pulses administered s.c. at frequencies of 1 pulse every 45 (fast), 90 (medium), or 180 min (slow). Animals were then sacrificed at 0, 3, 5, 10, 20, and 30 days following treatment. LH levels following 10 d of fast frequency GnRH pulses (3.26±0.30 ng/ml) were significantly greater than LH levels in animals exposed to 10 d of medium, and slow GnRH pulse frequencies (1.57±0.19 and 1.17±0.21). On the other hand, 10 d of medium GnRH pulse frequency produced FSH levels which were significantly greater than 10 d of fast frequency GnRH pulses (15.21±2.02 vs. 9.81±1.68). Animals which had been exposed to GnRH for 30 d at the fast and medium pulse frequencies had testes weights (871.02±102.03 and 965.10±19.63 mg) which were significantly greater than animals which had been exposed to 30 d of the slow GnRH pulse frequency (619.85±55.22). Testosterone levels at 30 d also differed according to the GnRH pulse frequency, with supraphysiological levels (32.93±6.59 ng/ml) in the fast frequency group, significantly lower levels in the medium frequency group (8.63±2.72), and nondetectable levels in the slow frequency group. These results are consistent with studies suggesting that slower GnRH pulse frequencies facilitate the release of FSH while faster GnRH pulse frequencies facilitate the release of LH. In addition, the present results suggest that GnRH pulse frequency can secondarily regulate testicular functions through regulation of relative proportions of LH and FSH release. (NIH HD00879, HD20677 & HD21921)

90.6

PULSATILE LHRH RELEASE IS INDEPENDENTLY CONTROLLED BY THE ALPHA-ADRENERGIC AND NEUROPEPTIDE Y (NPY) NEURONAL SYSTEMS IN THE RHESUS MONKEY. A. K. Yamane and E. Terasawa*, Reg. Primate Research Ctr., Univ. of Wisconsin, Madison, WI 53715

Previously we have shown that both the α-adrenergic and NPY neuronal systems play a role in the control of pulsatile LHRH release: The α_1-adrenergic antagonist prazosin suppressed, while the α_1-adrenergic agonist methoxamine (MTX) stimulated LHRH release (*Endocrinology* **123**: 1808, 1988); a specific antiserum to NPY (aNPY) suppressed, while NPY stimulated LHRH pulses (*Ibid.* **130**: 2333, 1992). However, it is unclear whether these neuronal inputs are independently involved in the regulation of LHRH release or whether one system is more proximal to the other. In the present study, using a push-pull perfusion method, we have conducted 2 experiments to examine the possible interactions between these two neuronal systems on the control of LHRH pulsatility in ovariectomized monkeys. Experiment I: To test whether the α-adrenergic neuronal system mediates NPY input, NPY at 10^{-6}M was directly infused into the stalk-median eminence (S-ME) before and 2 h after injection of prazosin. The results indicate that NPY infusion clearly stimulated LHRH release during the prazosin-induced LHRH suppression. The response of the NPY-induced LHRH release after prazosin did not differ from that after vehicle injection as a control for prazosin. Experiment II: To test whether the NPY neuronal system mediates α-adrenergic input, MTX at 10^{-5}M was directly infused into the S-ME 10 min after infusion of aNPY. MTX stimulated LHRH release during the aNPY-induced LHRH suppression and the response was similar to that after NRS infusion as a control for aNPY. These results suggest that the α-adrenergic and NPY inputs maintain a parallel relationship and are not dependent on each other in the control of pulsatile LHRH release. (Support: NIH grants RR00167 and HD15433.)

90.7

FACILITATION OF LHRH-INDUCED LH SURGES BY NEUROPEPTIDE Y (NPY) DEPENDS UPON PREOVULATORY PROGESTERONE SECRETION. A.C. Bauer-Dantoin*, B. Tabesh, and J.E. Levine. Dept of Neurobio & Physiol, Northwestern Univ, Evanston, IL 60208.

We have demonstrated that NPY potentiates LHRH-induced LH secretion only under the endocrine conditions in which preovulatory LH surges are generated. The present study was designed to test the hypothesis that the actions of progesterone (P) in particular are requisite for NPY's facilitory actions on LH secretion. Female rats were fitted with atrial catheters on diestrus. On proestrus, hourly blood samples were collected from 1100-2100h. At 1230h, rats received a s.c. injection of the P antagonist RU486 (6 mg/kg BW), or oil. At 1330h, rats received pentobarbital (PB; 40 mg/kg BW) to block hypothalamic LHRH release, or saline (SAL). Every 30 min from 1400-1800h, PB-treated rats received i.v. pulses of LHRH (15 ng/pulse) or SAL, along with concurrent pulses of NPY (5 μg/pulse), or SAL. Administration of RU486 to rats treated with SAL at 1330h completely blocked the endogenous LH surge. In oil-treated, PB-blocked rats, concurrent administration of NPY with LHRH significantly ($p<.01$) potentiated the ability of LHRH to restore LH surges. However, the potentiation by NPY of LHRH-induced LH surges in PB-treated rats was completely blocked by the administration of RU486 at 1230h. RU486 slightly attenuated the ability of LHRH alone to restore LH surges in PB-blocked rats. These results demonstrate that one function of preovulatory P secretion is to up-regulate pituitary sensitivity to the facilitory actions of NPY. We hypothesize that these actions of P, together with an increase in NPY neurosecretion, mediate the acute increase in pituitary sensitivity to LHRH that occurs just prior to the LH surge (NIH HD20677, HD21921, HD00879, and MH10177).

90.8

NEUROPEPTIDE Y STIMULATES LHRH RELEASE FROM SUPERFUSED HYPOTHALAMIC GT1-7 CELLS. L.M. Besecke*, A.M. Wolfe, M.E. Pierce, J.S. Takahashi and J.E. Levine. Dept. of Neurobio. & Physiol. Northwestern Univ. Evanston, IL 60208.

Neuropeptide Y (NPY) has been shown to stimulate LH release by actions at both the hypothalamic and pituitary levels. At the hypothalamic level, this effect is exerted via stimulation of LHRH release. To assess the possibility that these hypothalamic effects can be exerted by direct action on LHRH neurons, we used an *in vitro* superfusion system to examine the effects of pulses of NPY on LHRH release from immortalized GT1-7 cells (Mellon *et al.*, 1990). We have found that addition of NPY (10^{-12}-10^{-6}M) produced a dose-dependent increase in LHRH release from these cells in three-day culture. A 10^{-8}M dose produced a 300% increase in LHRH release, a 10^{-8}M dose produced a 115% increase, and a 10^{-12}M dose elicited a modest 25% increase in LHRH release over baseline. Estimated EC_{50} for this NPY effect on LHRH release in these cells is 10^{-8}M NPY. Robust LHRH responses were also seen when pulses of the LHRH secretagogues veratridine (50 μM), prostaglandin E_2 (25,100 μM), and norepinephrine (10^{-5},10^{-7},10^{-8}M) were administered in the superfusate media. However, substance P (10^{-6}M) and arg-vasopressin (10^{-6}M) did not significantly alter LHRH release profiles. These data demonstrate that NPY directly stimulates LHRH release from superfused GT1-7 cells, and suggest that the mechanism by which NPY stimulates LHRH *in vivo* may be through direct action on the LHRH cell. In current studies we are attempting to characterize NPY receptor subtype(s) and signal transduction mechanism(s) involved in the NPY-induced LHRH release from GT1-7 cells. (NIH HD20677, HD00879 & HD21921)

90.9

A POSSIBLE ROLE OF GABA IN TONIC INHIBITION OF LHRH RELEASE BEFORE THE ONSET OF PUBERTY IN THE FEMALE RHESUS MONKEY. D. Mitsushima, S. Chongthammakun* and E. Terasawa, Reg. Primate Research Ctr., Univ. of Wisconsin, Madison, WI 53715.

It has been hypothesized that during the prepubertal period in the rhesus monkey a dominant inhibitory neuronal system controls LHRH neurons, suppressing pulsatile LHRH release. In the present study we have examined the role of γ-aminobutyric acid (GABA), known inhibitory neurotransmitter, in the control of LHRH release during puberty using a push-pull perfusion method. GABA (10^{-5}M, 10^{-7}M) or the GABA-A antagonist bicuculline methiodide (10^{-5}M, 10^{-7}M) was infused into the stalk-median eminence for 10 min in 4 prepubertal (14.5±0.6 mo) and 7 midpubertal (40.2±1.7 mo) monkeys. Results: While in prepubertal monkeys neither 10^{-5}M nor 10^{-7}M GABA infusion altered LHRH release, in midpubertal monkeys GABA at both doses significantly suppressed LHRH pulses (10^{-5}M; $p<0.01$; 10^{-7}M: $p<0.05$). LHRH suppression occurred within 10 min after GABA infusion and lasted for at least 60 min with 10^{-5}M and 30 min with 10^{-7}M. In contrast, bicuculline infusion at both doses in prepubertal monkeys consistently induced a prompt LHRH increase (10^{-5}M: $p<0.025$; 10^{-7}M: $p<0.02$). The LHRH increase peaked during the infusion and returned to preinfusion levels within 20 min after the infusion. In midpubertal monkeys, bicuculline at 10^{-5}M also stimulated LHRH release ($p<0.01$), but bicuculline at 10^{-7}M was without effect. The results are interpreted to mean that in the prepubertal period there is a powerful GABA inhibition of the LHRH neurosecretory system: bicuculline removes endogenous GABA inhibition thereby stimulating LHRH release, while exogenous GABA is not effective because LHRH release is already inhibited by endogenous GABA. It is concluded that the LHRH neuronal system may be chronically inhibited by GABA neurons before the onset of puberty in primates. (Support: NIH grants HD11533 and RR00167.)

90.10

GLUTAMATE-IMMUNOREACTIVE TERMINALS SYNAPSE WITH GNRH NEURONS IN THE MONKEY HYPOTHALAMUS.

P.C. Goldsmith*, K.K. Thind, and A.D. Perera[1]. Reproductive Endocrinology Center, Univ. Calif., San Francisco, CA 94143 and [1]Dept. Physiol. Univ. Pitts. Sch. Med., Pittsburgh, PA 15261.

Glutamate (GLU) is the most prevalent excitatory transmitter in the brain. GLU agonists rapidly stimulate reversible GnRH neurosecretion in sexually mature and even pre-pubertal animals. To determine whether GLU acts directly on GnRH neurons, we investigated GLU-GnRH interactions in hypothalami from adult female and juvenile male macaques. Monoclonal antibody specific for glutaraldehyde-fixed GLU (Glu-2) was used to label vibratome sections with an ABC peroxidase technique. GLU-immunoreactive (-ir) neurons were numerous throughout the diencephalon, most noticeably in the diagonal band, suprachiasmatic, supraoptic, paraventricular, and arcuate (ARC) nuclear regions. GLU-ir staining occurred chiefly in neuronal cytoplasm, and to a variable degree in many GLU-ir cell nuclei. Fiber density was greatest in the median eminence, where GLU receptor concentrations are reportedly high (Soc. Neurosci. Abstr. 16(1):284, 1990). Prior absorption of Glu-2 with a GLU-glutaraldehyde-BSA conjugate completely abolished immunostaining. To examine neurointeractions, GnRH-ir structures were localized with the polyclonal antibody LR2, PAP reagents and DAB. GLU-ir elements were identified with Glu-2 and immunogold (15 nm). Electron microscopy revealed numerous GLU-GnRH contacts and synapses, almost exclusively axo-dendritic in the lamina terminalis, but occasionally also axo-somatic in the ARC region. Postsynaptic membranes seemed rarely specialized, but GnRH-ir granules sometimes congregated nearby. Since few unlabeled inputs were noted, we conclude that GLU-ir afferents exert a major excitatory influence on GnRH neurons in the monkey hypothalamus. Their role during the normal menstrual cycle or reactivation of pulsatile GnRH secretion at puberty remains to be determined. Work supported by NIH grant HD10907 (P.C.G.)

90.11

NORADRENERGIC INNERVATION OF GNRH NEURON-CONTAINING AREAS: A COMBINED IMMUNOCYTOCHEMICAL AND RETROGRADE-TRACING STUDY, D.E. Wright* and L. Jennes. Dept. Anatomy and Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0084

Norepinephrine (NE) influences the activity of gonadotropin-releasing hormone (GnRH) neurons during the female rat estrous cycle at both the axon terminal and the GnRH perikarya. Recent evidence suggests that NE-neurons in the A_2 region may be important in conveying the feedback signals of estrogen to GnRH neurons. We therefor studied the relationship between the A_2 region and areas containing GnRH neurons using two neuroanatomical tracers, fluorogold (FG) and fluororuby (FR) combined with immunohistochemistry (IHC) for tyrosine hydroxylase (TH), dopamine beta hydroxylase (DBH) and GnRH. Female rats received pressure injections of either FG or FR in the septum-diagonal band and rostral preoptic area. The proximity of injection sites to GnRH neurons was confirmed using GnRH-IHC. Both FG and FR injections resulted in retrogradely-labeled TH or DBH-immunoreactive neurons in the A_2 region. The majority of these double-labeled cells were located in caudal regions of A_2 ipsilateral to the injection site. A few double-labeled neurons were also observed in the A_1 region. The results suggest that NE neurons in the A_2 region possess significant projections to areas containing GnRH neurons, supporting the hypothesis that noradrenergic A_2 neurons play an important role in modulating GnRH neuronal activity in the female rat brain. Supported by NIH HD24697.

90.12

DO SCN EFFERENTS CONTACT GnRH CELLS IN THE HAMSTER? A COMBINED PHA-L TRACT TRACING AND IMMUNOCYTOCHEMICAL STUDY. E.L. Meyer, M.N. Lehman, and E.L. Bittman*, Dept. of Zoology, Univ. of Massachusetts, Amherst 01003, and Dept. of Anatomy and Cell Biology, Univ. of Cincinnati Medical School, Cincinnati OH 45267.

A circadian pacemaker in the suprachiasmatic nucleus (SCN) regulates the timing of the preovulatory luteinizing hormone (LH) surge in the golden hamster. We used a combination of tract tracing and immunocytochemistry to determine whether SCN efferents control the timing of the surge by directly contacting gonadotropin-releasing hormone (GnRH) neurons. The lectin anterograde tracer, *Phaseolus vulgaris* leucoagglutinin (PHA-L) was iontophoretically applied to the SCN of 6 ovariectomized hamsters. Hamsters were perfused 10-14 days later with paraformaldehyde and sections (50um) were processed for simultaneous immunocytochemical detection of PHA-L and GnRH or vasopressin using a double-label immunoperoxidase technique. In several animals, PHA-L iontophoresis sites were limited to the SCN and in these cases PHA-L labeled fibers could be followed to a number of previously described efferent targets of the SCN, including the preoptic area (POA) and the subparaventricular zone of the hypothalamus. Intra-SCN connections, including efferents to the contralateral SCN, were also seen. Thus far, we have seen only rare close associations between SCN efferents and GnRH cells, although PHA-L-labelled SCN efferents form clusters of terminal boutons in adjacent areas of the POA. We speculate that SCN efferents may contact other preoptic or hypothalamic cells, such as those which express estradiol receptors, which then regulate the timing of the GnRH release. We are presently attempting to identify appositions of PHA-L labelled SCN fibers onto estrogen-receptor immunoreactive neurons in the POA. Supported by NS 24292 and 28175 to M.N.L. and by NIMH 44132 to E.L.B.

PEPTIDES: BIOSYNTHESIS, METABOLISM, BIOCHEMISTRY I

91.1

IDENTIFICATION AND CHARACTERIZATION OF NEUTRAL ENDOPEPTIDASE-LIKE ACTIVITY IN *APLYSIA CALIFORNICA*. W. Bawab, P. Crine, and L. DesGroseillers*. Department of biochemistry, University of Montreal, Montreal, Canada, H3C 3J7.

We are investigating the role of ectoenzymes in the inactivation of neuropeptides in *Aplysia*. Last year, we reported the characterization of a first neuropeptide-degrading enzyme in the kidney, ovotestis, heart and central nervous system of *Aplysia*. This enzyme has the same enzymatic characteristics as the mammalian aminopeptidase N (Biochem. J. 1992. In press). Using [^{3}H]leu-enkephalin (YGGFL) as substrate, we now describe a second neuropeptide-degrading enzyme in the kidney and CNS of *Aplysia*. This enzyme cleaves leu-enkephalin at the Gly^4-Phe^4 bond, as determined by HPLC analysis of metabolites. The degradation of the substrate was abolished by the neutral endopeptidase (NEP) inhibitors HACBO-Gly, thiorphan and phosphoramidon, and by the divalent cation chelating agent 1-10 phenanthroline, suggesting that this enzyme is similar to mammalian NEP. Phase separation experiments, using Triton X-114, showed that more than 60 % of the membrane-bound NEP activity in the *Aplysia* kidney comes from an integral membrane protein. The presence and size of the neutral endopeptidase can be revealed by a very potent inhibitor of NEP, [^{125}I]RB104, after migration on SDS-PAGE and transfer unto nitrocellulose membranes. Using this technique, we showed that the NEP-like enzyme in *Aplysia* kidney membranes has an apparent molecular mass of about 130 Kda. Finally, a partial cDNA has been isolated from a CNS cDNA library and the DNA sequence revealed about 50 % identity between the *Aplysia* and mammalian enzymes. The presence of a NEP-like enzyme may contribute to the extracellular degradation of *Aplysia* neuropeptides.

91.2

PROTEOLYTIC ENZYME INHIBITORS SUPPRESS *IN VITRO* GROWTH OF SMALL CELL LUNG CANCER WHICH CONTAINS PROHORMONE CONVERTASE 1 AND 2 mRNA. D. A. Clark*, R. Day, N. G. Seidah, and T.P.Davis. Dept. of Pharmacology, Univ. of Arizona Coll. of Med., Tucson, AZ 85724 USA and Clin. Research Instit. of Montreal, Montreal, Quebec H2W 1R7 Canada.

Gastrin releasing peptide (GRP) functions as an autocrine growth factor in small cell lung cancer (SCLC) cells which contain GRP receptors. GRP is synthesized as a preprohormone and enzymatically processed to the active peptide in SCLC cells where we have measured the mRNA for prohormone convertase (PC) 1 and 2. In this study the effect of the specific enzyme inhibitors bestatin, Bowman-Birk inhibitor (BBI) and aprotinin on the *in vitro* clonal growth of two SCLC cell lines which contain GRP receptors was investigated. The cell lines NCI-H345 and NCI-N592 were mixed with a single dose of bestatin, BBI or aprotinin and incubated for 9-14 days in 35mmX10mm plastic petri dishes in a humidified atmosphere of 5% CO_2/95% air. The SCLC cell colonies were counted with an automated FASII image analysis system. A dose-dependent and significant ($p<0.05$) inhibition of growth was observed in both cell lines with the aminopeptidase inhibitor bestatin inhibiting up to 75% of SCLC clonal growth at the 100μM dose. The trypsin-chymotrypsin inhibitor BBI inhibited up to 86% of SCLC clonal growth at 100μg/ml. Aprotinin, a trypsin inhibitor, decreased clonal growth of NCI-N592 at a dose of 100μg/ml and NCI-H345 at 500 μg/ml. A dose of 75-100μg/ml BBI also decreased GRP and proGRP radioimmunoassay levels and decreased the level of mRNA for PC1 and PC2 by 50%.

These data suggest that processing and metabolic enzymes serve an important role in regulating growth of SCLC and that enzyme inhibitors may be potent suppressors of SCLC. (Supported by NIH DK36289, HD26014 and DA06284)

91.3

INDUCTION OF PEPTIDYLGLYCINE ALPHA-AMIDATING MONOOXYGENASE (PAM) BY NERVE GROWTH FACTOR (NGF) IN PC12 CELLS. T.A. Ford and G.P. Mueller*. Dept. Physiol., Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Pheochromocytoma PC12 cells undergo dramatic neuronal differentiation in response to NGF. In this process gene expression is altered to establish specific neural properties. Here we sought to determine whether NGF induces the activity of PAM (EC1.14.17.3), the enzyme that α-amidates neuropeptide Y, a peptide expressed during differentiation. PC12 cells were grown with or without NGF for 5-7 days to allow for maximum differentiation. Medium, whole cell extracts and soluble and membrane bound cell fractions were assayed under optimal conditions for PAM; activity was found to be primarily membrane bound (five-fold greater than soluble) and very little activity was released into the medium. As compared to control cells, PAM activity was increased significantly by NGF (0.71 ± 0.03 vs. 0.49 ± 0.03 pmol/ug/hr) ($p<0.05$). Through kinetic analysis, it was determined that the NGF-induction of PAM was due to an increase in V_{max} with no change in K_m. It has been previously observed that glucocorticoids inhibit some, but not all, of the changes that occur during NGF-induced differentiation. We found that dexamethasone (DEX) decreased basal PAM activity and completely prevented its induction by NGF. Interestingly, DEX treatment did not alter the changes in cell morphology induced by NGF. These findings suggest that the process of differentiation in PC12 cells is a useful model for studying the expression of PAM.

91.4

'PROHORMONE THIOL PROTEASE' AND NEUROPEPTIDE PRECURSOR PROCESSING: PEPTIDE CLEAVAGE AT DIBASIC and MONOBASIC SITES, and EXPRESSION OF RECOMBINANT PROENKEPHALIN AND ß-PROTACHYKININ. V.Y.H. Hook, L. Mende-Mueller+, Rama Kannan*, and T.J. Krieger. Dept. of Biochem., Uniformed Services University of the Health Sciences, Bethesda, MD. 20814, and +Dept. of Biochem., Medical College of Wisconsin, Milwaukee, WI.

Processing of the precursors of peptide neurotransmitters requires cleavage at dibasic (Lys-Arg, Arg-Arg, Arg-Lys, and Lys-Lys)and monobasic (Arg) sites. A novel 'prohormone thiol protease' (PTP) has been demonstrated to be involved in enkephalin precursor processing (T.J.Krieger and V.Y.H. Hook *J. Biol. Chem. 266*, 8376-8383, 1991). Examination of PTP's cleavage site specificity with the enkephalin-containing peptides peptide F, peptide E, and BAM-22P as substrates was conducted by HPLC separation of products followed by peptide microsequencing. PTP cleaved at dibasic Lys-Arg, Lys-Lys, and Arg-Arg sites, as well as at a single Arg; the preferred P1' position was Arg. These findings demonstrate that PTP possesses almost all of the necessary specificity for dibasic and monobasic sites required in neuropeptide precursor processing. To allow future characterization of PTP with authentic precursor substrates at estimated in vivo levels of precursor (requiring milligram amounts of precursor), high level expression of proenkephalin and ß-protachykinin in *E. Coli* has been achieved. It will be important to determine PTP's cleavage sites with authentic precursor substrates.

91.5

THE ONTOGENY OF ENZYMES INVOLVED IN THE POST-TRANSLATIONAL PROCESSING AND METABOLISM OF PEPTIDES IN THE BRAIN. M.G. Oakes*, P.N.M. Konings and T.P. Davis. Dept. of Pharmacology, University of Arizona, College of Medicine, Tucson, AZ 85724.

We quantitated the level of activity of specific peptidases to determine if the enzymes involved in the post-translational processing and metabolism of peptides are involved in the age-related regulation of bioactive peptide levels in specific regions of the developing rat brain.

Carboxypeptidase H (E.C.3.4.17.10, CPH), a processing enzyme, showed a significant increase in activity from P0 (birth) to P7 in the hypothalamus (20.1 to 26.7 pmoles/mg protein/min) and cortex (11.5 to 18.9) but a decrease of activity in the cerebellum (19.1 to 16.2). The activity decreased in the cortex from P7 to P30 (18.9 to 15.0). CPH is expressed at high levels at birth and generally levels off after P7 to adult levels which may signify the involvement of this processing enzyme throughout the life of the rat in the synthesis of neuropeptides.

The metabolic enzymes, Metallo Endopeptidase (E.C.3.4.24.15, MEP) and Neutral Endopeptidase (E.C.3.4.24.11, NEP) do not present the same activity pattern as the processing enzyme CPH. MEP activity increased only in the cortex after birth (946.3 to 1268.8 pmoles/mg protein/min), leveled off, and then decreased in all three regions from P30 to P90. NEP activity increased in the hypothalamus after birth (1657.8 to 3080.4 pmoles/mg protein/min) but decreased in the other two regions. At P7 to P30 a significant decrease in NEP activity occurs in the cerebellum (779.0 to 319.9), no change in the hypothalamus was noted. These two metabolic enzymes are detectable at birth and may function in the degradation of peptides released. The presence of these processing and metabolic enzymes at birth and the pattern of their activity during development suggests a potential regulatory role in the production and degradation of bioactive peptides in the developing rat brain. (Supp. by NIH grant HD28310 and DA06284).

91.6

AGE-RELATED CHANGES IN THE CONTENTS OF NEUROPEPTIDES IN THE PITUITARY AND THE BRAIN OF THE RAT. F. Tang* and Z.P. Wang, Department of Physiology, Faculty of Medicine, University of Hong Kong, Hong Kong.

To study the effect of age on neuorpeptide contents, male Sprague-Dawley rats aged 3 months, 12 months, and 22 months were kept on a 12 hr light: 12 hr dark lighting cycle (light on at 6:00 a.m.) and were sacrificed at 10:00 a.m. and 10:00 p.m. ß-endorphin (ß-E), leu-enkephalin (LE), met-enkephalin (ME), substance P (SP), somatostatin (SRIF) and cholecystokinin (CCK) were measured in various brain regions and the anterior (AL) and neurointermediate (NIL) lobes of the pituitary (except SP and CCK) by RIA (Tang and Man, Neuropetides 19, 287-291, 1991). As expected, ß-E, ME and LE in the NIL, and ME and LE in AL increased with age, but whereas the increases were both in the day and at night for ß-E and ME, the increase for LE was at night only. Hypothalamic ß-E and ME decreased with age in the day only. In addition, brainstem ß-E and LE, and cortex LE decreased with age at night, and striatal LE decreased with age in the day, whereas hippocampal ME decreased with age both at night and in the day. While there was an age-related decrease in SRIF and SP in the corpus striatum and the hypothalamus only in the day, the decrease in CCK with age in the hippocampus and the hypothalamus was only confined to night levels. This study demonstrates that there are age differences in the neuropeptide levels (generally, a decrease, except for opioid peptides in the pituitary) and that these changes may follow a diurnal rhythm. As these neuropeptides may play a stimulatory or inhibitory role in neurotransmission, their changes with age and with diurnal rhythm may be related to alteration in the activity of the animal.

91.7

POSTTRANSLATIONAL PROCESSING OF PROENKEPHALIN IN AtT-20 CELLS: EVIDENCE FOR CLEAVAGE AT A LYS-LYS SITE. John Paul Mathis* and Iris Lindberg. Dept. Biochem. and Mol. Biol., Louisiana St. Univ. Med. Ctr., New Orleans, LA 70112.

Proteolytic processing of proenkephalin was examined in several subclones of AtT-20 cells stably transfected with rat proenkephalin cDNA (AT/PE cells). Proenkephalin is synthesized in both glycosylated and unglycosylated forms as demonstrated by treatment with tunicamycin. Radioimmunoassays and Western blot studies showed that AT/PE clones process proenkephalin at some but not all Lys-Arg sequences in a limited processing profile reminiscent of the chromaffin cells of the bovine adrenal gland. Pulse-chase studies using Met^5-enk-Arg-Gly-Leu antiserum showed that 50% of the precursor is processed within 1 h and processing is complete after 2.5 h with the production of the 5.3 kDa peptide. Radiosequencing results verified the efficient cleavage of a Lys-Lys site within proenkephalin which resulted in the production of the 5.3 kDa peptide. Proenkephalin cleavage products stored within cells, which included the 5.3 kDa peptide, could be released upon stimulation of cells with $BaCl_2$ (2-fold above basal levels), 8-Br-cAMP or cortical releasing factor (8-fold above basal levels) and a mixture of $BaCl_2$ and 8-Br-cAMP (15-fold above basal levels). An important difference between the processing of proenkephalin and POMC in AtT-20 cells is efficient cleavage of a Lys-Lys site in proenkephalin and not in POMC. The ability of AT/PE to process proenkephalin in a natural manner makes it a suitable model system to determine whether structural elements are involved in the processing of proenkephalin at the Lys-Lys site. This work was supported by *NIDDK35199*.

91.8

PROCESSING OF PRODYNORPHIN BY THE PROHORMONE CONVERTASES PC1, PC2 AND FURIN. R. Day*, A.C. Dupuy, H. Akil, M. Chrétien, N.G. Seidah. Clinical Research Institute of Montreal, Montreal, Quebec, Canada, H2W 1R7 and Mental Health Research Institute, University of Michigan, Ann Arbor, MI, USA., 48109.

Limited proteolysis of inactive precursors at pairs of basic residues is a general intracellular mechanism utilized to produce active proteins and peptides. The convertases involved have recently been described as a family of serine proteinases of the Kex2/subtilisin type. Two of these convertases, PC1 and PC2, are primarily expressed in neural and endocrine tissues while another, Furin, is thought to be ubiquitously expressed. PC1 and PC2 have been shown to cleave POMC into β-endorphin, β-LPH, α-MSH and ACTH. Our study examines the processing of another opioid peptide precursor, prodynorphin (proDyn), by PC1, PC2 and Furin. Four pairs of basic residues are found in proDyn flanking the opioid end products, α-neo-endorphin (αNE), dynorphin A 1-17 (Dyn A 1-17) and dynorphin B 1-13 (Dyn B 1-13). A recombinant vaccinia virus (VV) vector was used to co-express PC1, PC2 and Furin together with proDyn in the constitutively secreting cell line BSC-40 and in the endocrine tissue-derived cell lines PC12 and AtT-20. ProDyn processing was monitored in cells and media, before and after separation by HPLC with RIAs to αNE, Dyn A 1-17, and Dyn B 1-13. Our results demonstrate partial proDyn processing by one or more enzymes present endogenously in each cell line tested and that Dyn AB 1-32 was the major product. However, the proDyn processing pattern was modified when the cells were also infected with each VV:convertase. A differential processing profile was obtained with each enzyme. Our data demonstrate the distinct cleavage specificities of PC1, PC2 and Furin in proDyn processing. The proDyn cleavage specificity of each convertase will be discussed and the results will be correlated to the known *in vivo* processing of proDyn.

91.9

EXPRESSION OF *FOS* AND CRF mRNA IN THE BRAIN OF FEMALE RATS SUBJECTED TO SINGLE OR REPEATED RUNNING SESSIONS. S. Rivest*, S. Lee, C. Rivier and D. Richard. Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037 and Dépt de Physiologie, Univ. Laval, Québec, Canada G1K 7P4.

The regulation of early gene *FOS* and CRF biosynthesis in the rat brain by exercise and exercise-training was examined by immunocytochemistry and *in situ* hybridization. Exercise consisted in sessions of running on a rodent motor-driven treadmill during 2 h/day for a maximum of 21 days. Thirty min, 2, 12 and 24 h after the beginning of the first (acute exercise), the 8th (1 week of training) and the 21st exercise session (3 weeks of training), rats were sacrificed. Thirty min after the beginning of the acute running session, *FOS* expression was increased in several brain regions, including the arcuate nucleus, the bed nucleus of the stria terminalis, the central nucleus of the amygdala, the dorsomedial hypothalamic nucleus, the lateral hypothalamic area, the paraventricular nucleus of the hypothalamus (PVN), the suprachiasmatic nucleus and the ventral tegmental area. In the PVN, *FOS* was mainly located within the nuclei of CRF-immunoreactive (IR) perikarya. At the end of the acute 2 h-running session, *FOS* expression was still strongly activated in the above listed brains regions, and in the PVN c-*fos* proteins were located in both the parvo- and magnocellular subdivisions. Ten h following the acute running period, *FOS* had largely vanished, while an increase in CRF mRNA levels was noted in the PVN. Thirty min into the 8th running session, *FOS*-IR cells were observed in the PVN; the number of cells expressing this gene was lower than that of rats sacrificed 30 min following the acute session. In animals subjected to exercise sessions over a 3 week period, no notable change in *FOS* expression was induced by the last 2 h-running period, while CRF mRNA levels in the PVN were increased at the end of this exercise session. Thus, acute running induced both *FOS* and CRF mRNA expression in the brain. The same exercise regimen did not notably influence *FOS* expression in long term trained female rats, but it induced a marked and rapid accumulation of CRF mRNA in the PVN. Although c-*fos* gene could be involved in the regulation of CRF biosynthesis in the PVN during exercise, the present results suggest that CRF gene expression may occur independently of the stimulation of *FOS* in exercise-trained rats. *(Supp. by NIH, NSERG and MRC of Canada)*

91.10

PROCESSING OF CHOLECYSTOKININ IN THE SK-N-MCIXC CELL LINE P.N.M. Konings*, M.C. Beinfeld, M.J.C. Hendrix, R. Day, N.G. Seidah, B. J. Merrill and T.P. Davis. Dept. of Pharmacol and Anatomy, Univ. of Arizona, Tucson, 85724, Univ. Med. Center, St. Louis, Dept. of Biochem., CRI of Montreal, Quebec.

The human cholinergic neuroepithelioma cell line SK-N-MCIXC, expresses high levels of cholecystokinin (CCK) mRNA, with a robust band of approximately 0.8 Kb as shown with Northern Blot analysis. CCK mRNA is translated by the SK-N-MCIXC cells into proCCK fragments e.g. glycine extended CCK and bioactive amidated CCK, as detected by HPLC coupled to RIA using region specific antibodies of proCCK and CCK. Immunohistofluorescence demonstrated the presence of glycine extended CCK in distinct storage granules of SK-N-MCIXC cells which were grown on coverslips. The immunostaining was blocked by preincubation of the antibody with the glycine extended CCK peptide sequence showing the specificity of the staining.

To identify candidate processing enzymes in the SK-N-MCIXC cell line, the presence of mRNA for the intracellular processing enzymes, prohormone convertase 1 and 2 (PC1, PC2), and Furin were measured using Northern Blot analysis. As for PC1, we observed one band migrating at 4.0-4.2 Kb. This pattern was also found in human tissue. We observed two bands for PC2 of approximately 2.8 and 4.8 Kb in the SK-N-MCIXC cells, as was previously found in brain and pituitary. Furin appears as a band of 4.4 Kb. PC1, PC2 and Furin are subtilisin-like enzymes which preferentially cleave prohormones at the carboxyl side of pairs of basic amino acid residues. The abundance of PC1-, PC2-, and Furin mRNA suggests that these enzymes cleave at the dibasic amino acid pair Arg-Arg of the C terminal part of proCCK, as is predicted from the most frequent proprotein processing motifs. (Supported by grants DK 36289, MH 42600, HD 26013 to T.P.D., NS 18667 to M.C.B., MRC of Canada grant MT 11268 to R.D.)

91.11

HALOPERIDOL AND CHLORPROMAZINE TREATMENT ALTERS NEUTRAL ENDOPEPTIDASE 24.11 ACTIVITY IN NUCLEUS ACCUMBENS AND CAUDATE PUTAMEN. Thomas P. Davis,*Mary Oakes and P.N.M. Konings. Department of Pharmacology, University of Arizona College of Medicine, Tucson, AZ. 85724

Dopamine receptor blockade by chronic haloperidol or chlorpromazine treatment can affect both CCK- and neurotensin levels in specific regions of the rat brain. Haloperidol and chlorpromazine treatment has also been shown to alter central peptide metabolism/degradation (Konings et al. Eur. J. Pharmacol. 1990:191:115-128). In the present study, the effects of two doses of haloperidol (1 or 3 mg/Kg per day) and chlorpromazine (4 or 20 mg/Kg per day) were studied on the activity of the mammalian ectoenzyme neutral endopeptidase 24.11 (NEP) in nucleus accumbens, caudate putamen and hypothalamus. The contribution of NEP to neurotensin metabolism is markedly elevated in caudate putamen as compared to nucleus accumbens (Davis et al. J. Neurochem. 1992:58:608-617). Chronic haloperidol treatment (1mg/Kg per day x 12d) significantly increased NEP activity in nucleus accumbens. Chronic chlorpromazine treatment (4 mg/Kg per day x 12d) significantly increased NEP activity in caudate putamen. No effect was noted in hypothalamus. The increase in membrane associated NEP activity after haloperidol and chlorpromazine treatment may lead to increased peptide metabolism/degradation of synaptically released neuropeptides including the putative neuroleptic peptides CCK and neurotensin. (Supported by N.I.H. grants MH42600 and DA06289).

91.12

CHARACTERIZATION OF A NOVEL DBI PROCESSING PRODUCT PRESENT IN RAT BRAIN EXTRACT.
E. Slobodyansky*, V.Sarisky, M.Whalin and E.Costa. Fidia-Georgetown Institute for the Neurosciences, Georgetown University Medical School, 3900 Reservoir Rd., N.W., Washington, D.C. 20007.

Some of the biological actions of DBI might be mediated by specific DBI processing products. Extracts from rapidly fixed rat brain contain a number of DBI processing products; some of them interact with an antibody raised against DBI 33-50 (ODN) which is termed anti-ODN. One of them, TTN (DBI 17-50)(J.Neurochem. 53:1276, 1989) displaces both ^{3}H-4'-chlorodiazepam and ^{3}H-isoquinoline carboxamide (^{3}H-PK 11195) from a mitochondrial DBI receptor (MDR) that regulates mitochondrial steroidogenesis in glial and endocrine cells. Using HPLC and anti-ODN immunoaffinity chromatography, we purified from rat brain extract another ODN-LI peptide and established its sequence as DBI 39-75. The estimated amount of DBI 39-75 in rat brain is 5-10 pmol/mg prot. Direct binding of ^{125}I- DBI 39-75 to crude synaptic membranes reveals the presence of high affinity binding site(s). This view is supported by bifunctional crosslinking of ^{125}I-DBI 39-75 in pmole amount that shows two major crosslinking species(around 22 and 50 kDa), which appear to be displaced by cold DBI 39-75. The ^{125}I-DBI 39-75 binding profile to mitochondrial preparations from brain, adrenals and kidney will be presented.

91.13

PROTEIN CHARACTERIZATION BY AMINO ACID HYDROLYSIS: NEW SOLUTIONS. L.R.Murthy*. Dept. of Psych.,Mt. Sinai/VA Medical Center, Bronx, New York 10468.

My studies on the nature of phosphoamino acids in phos phoproteins from Alzheimer's disease brains poses some methodological problems, when phosphoproteins are hydrolysed in 6N HCl at 100° C, such as incomplete hydrolysis of proteins, degradation of labile amino acids and conversion of some amino acids through dephosphorylation and deamination. Therefore, I developed an enzymatic hydrolysis procedure for phosphoproteins to overcome these problems. β Casein,a phosphoprotein, is subjected to enzymatic hydrolysis by incubation at ambient temperature, with a series of proteases, endo and exopeptidases. These enzymes are coated on a solid support such as nitrocellulose(NC) or polyvinylidene difluoride membrane (PVDF). PVDF exhibits higher enzyme retention than NC. Proteases and peptidases bound on a solid support prevent the digestion of one protease or peptidase by another. The reaction between the protease and peptidase coated membranes and β casein in solution are terminated by removing the enzyme coated membranes from the protein hydrolyzate. The latter along with the corresponding blank are derivatized with phenylisothiocyanate and subjected to HPLC analysis. This hydrolyzed β casein showed enhanced phosphoamino acid level as compared to that hydrolysed with 6N HCl at 100° C for 4 hours.

BIOLOGICAL RHYTHMS AND SLEEP II

92.1

BRIGHT LIGHT SUPPRESSES MELATONIN AND IMPROVES COGNITIVE PERFORMANCE DURING NIGHTTIME HOURS IN HUMANS P Hannon*, G Brainard, R Childs, W Gibson, J French, J Hanifin and M Rollag. Northern Arizona University, Coll. of Health Prof., Flagstaff, AZ.,86011; Armstrong Laboratory/CFTO-Brooks AFB, Jefferson Medical College, and Uniformed Services University-Health Sciences.

This study assessed the effects of bright light on biological and behavioral measures to determine if bright light can reduce fatigue and enhance human work performance. Female subjects (N=37) were exposed to one of 3 lighting conditions in a between groups research design. Subjects in the bright light groups were exposed to 5000 lux white light from 1800 hrs to 2400 hrs (Early Bright) or from 2400-0600 hrs (Late Bright). The third group of subjects received 50 lux of red light from 1800-0600 hrs (Dim Red). Blood samples were taken every 90 minutes. Repeated measures ANOVA indicated a significant interaction effect (light x time) for tympanic temperature, (F=3.339, p=.001). The bright light conditions maintained higher tympanic temperatures from 2300 hrs through 0400 hrs. Plasma melatonin measures indicated a main effects difference of F=4.009, p=.029. Most importantly, the results showed that the "light" x "time of night" interaction was significant at F=59.436, p=.000. The suppression of plasma melatonin was greatest from 2230 hrs through 0500 hrs in the Early Bright and Late Bright groups. Cortisol was not affected by the ambient lighting conditions. Dim red light resulted in higher scores on the Stanford Sleep Scale from 2400 hrs through 0500 hrs (light x time, F= 2.595, p=.023). Subjects under the bright light conditions performed better on the cognitive measures of Code Substitution accuracy (F= 3.918, p=.030) and Column Addition accuracy (F=4.660,p=.017). These data show some improvements in cognitive performance and alertness associated with bright light exposure and occur with changes in tympanic temperature and plasma melatonin at critical time periods.

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92.2

EFFECTS OF VARYING DOSES OF EXOGENOUS MELATONIN ON HUMAN DIURNAL MOOD AND PERFORMANCE. A.B. Dollins, H.J. Lynch, M.H. Deng, K.U. Kischka, R.E. Gleason, H.R. Lieberman*, and R.J. Wurtman. Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

We examined the effects of 10, 20, 40, and 80 mg of melatonin or placebo, administered at 1145 h, on mood, performance, and oral temperature in 20 healthy male volunteers. Subjects were studied between 0930 and 1700 h on each of five separate occasions. They completed a battery of interactive computer tasks designed to assess performance and mood, the sequence and timing of which were held constant across test days. Temperature was assessed and blood samples were taken at regular intervals. Melatonin concentration was measured by radioimmunoassay. Melatonin levels (area under the time-melatonin concentration curve; AUC) differed significantly in response to the various doses. Mean AUC for the 0, 10, 20, 40, and 80 mg doses were 60, 12228, 27186, 52557, and 106223 pg/ml/7 h, respectively. All melatonin doses, relative to placebo, significantly decreased: oral temperature; number of correct responses on Wilkinson Auditory vigilance; response latency on Four Choice reaction time; and self-reported Vigor (Profile of Mood States [POMS] questionnaire). Melatonin also increased self-reported Fatigue, Confusion, and sleepiness (POMS and Stanford Sleepiness Scale). Differences were not detected among the melatonin doses. Since no temperature, mood, or performance differences resulted from the melatonin doses tested, the dose of oral melatonin necessary to cause significant short term sedative-like effects may be lower than previously believed.

92.3

MORNINGNESS/EVENINGNESS IS HERITABLE M.D. Drennan, J. Selby, D.F. Kripke, J. Kelsoe*, and J.C. Gillin. UCSD Dept. of Psychiatry, San Diego, CA 92126.

The study investigated heritability (H) of the Horne-Ostberg morningness-eveningness scale score (H-O) in humans. Questionnaires were sent to 600 twin pairs, ages 18-40 years from the Kaiser-Permanente twin registry. Questionnaires from both twins were received from 238 twin pairs. A two-question matrix classification system (Hrubec, 1976), identified 146 twin pairs as monozygotic, 74 dizygotic, and 18 unclassified. A nonsignificant ratio of the sum of mean squares ($F=1.06$, $p=.69$) showed monozygotic and dizygotic variances to be equal. The classical heritability method ($H= 2 \times (Rmz-Rdz)$) yielded a $H=.56$ ($p<.05$), with $H=.48$, $p=.07$ in females and $H=.54$, $p=.08$ in males. The within-pair estimate of genetic variance method yielded $H=.48$, $p<.05$, with $H=.41$, $p=.09$ in females and $H=.50$, $p=.11$ in males. Deleting pairs with current use of psychotropic medications, illegal drugs more than 3x/wk, or persons using alcohol more than 14 drinks/wk made no difference in H. Results support the concept that a large proportion of morningness/eveningness in humans is determined by heritable factors. Expressed human circadian phase may be influenced by genes. Supported by a NARSAD Award, and VA Research Fellowship to MDD.

92.4

EFFECTS OF CORTICOTROPIN-RELEASING HORMONE ADMINISTRATION IN PATIENTS SUFFERING FROM SLEEP APNEA SYNDROME. K. Mann, J. Röschke, M. Nink, J. Aldenhoff, J. Beyer, O. Benkert, H. Lehnert*. Dept. of Psychiatry and Dept. of Endocrinology, University of Mainz, D-6500 Mainz, Germany.

Corticotropin-releasing hormone (CRH) is known to be a centrally acting respiratory stimulant after systemic application. In a previous study this respiratory analeptic effect has been shown in normal subjects during sleep. We now extended these investigations to patients suffering from sleep apnea syndrome. Up to now 5 male patients aged 41 to 56 years participated. After an adaptation night polysomnography (EEG,EOG, EMG, ECG) was performed in two successive nights between 23.00 h and 7.00 h. While in one night placebo was applied, in the other night ovine CRH was administered intravenously as a bolus of 50 ug every hour from 0.00 h to 6.00 h. For assessment of respiration blood oxygen saturation and thoracic wall movements were measured as well as nasal and oral air flow by the thermistor method.

In all 5 patients the number of apnea periods per night was reduced under CRH. Blood oxygen saturation was clearly increased following CRH injection. The effect on sleep EEG parameters was not uniform. In most cases total sleep time was reduced with decrease of stage II and REM. In 3 patients a clear increase of slow wave sleep could be observed. Our data suggest a possible therapeutic value of CRH in sleep apnea syndrome.

92.5

DYNAMIC ANALYSIS OF RESPIRATORY PATTERNING DURING SLEEP AND WAKING STATES IN INFANTS. S.L. Raetz,* J.P. Wisor, V.L. Schechtman & R.M. Harper. Brain Research Institute and Dept of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024-1763.

Respiration undergoes marked patterning changes during the first few months of life. These changes include maturation of overall, as well as instantaneous, rate and variability during each sleep-waking state. Assessment of momentary respiratory changes provides important information about the nature of respiratory control. Thus, we characterized respiratory dynamics in normal infants using Poincaré procedures, a nonlinear method for assessing moment-to-moment interval changes. Recordings, consisting of electrocardiogram (ECG) and respiratory measures from 13 normal infants (2 - 63 days of age), were obtained over a 12 hr period. Each one-minute epoch was defined as waking (AW), quiet sleep (QS), or rapid-eye-movement (REM) sleep by a previously-developed state-classification system based upon ECG criteria. For each infant, 20 min of data from each sleep-waking state were randomly selected for analysis, and breath-to-breath intervals (BBI) were calculated from the respiratory signal. Each breath-to-breath interval (BBI_{n+1}) within a particular sleep-waking state was plotted against the previous interval (BBI_n) resulting in a Poincaré plot. Increased one breath - next breath dispersion and enhanced overall variation occurred during REM sleep when compared to QS and AW in all infants. The most marked characteristic of dynamic change occurred during AW; in younger infants (< 1mo), higher breathing rates were associated with diminished next-breath variation relative to lower rates. However, older infants showed similar next-breath dispersion at both high and low breathing rates. These data suggest that the influence of mechanisms underlying respiratory variation is diminished at higher breathing rates in younger infants. (Supported by HD22695)

92.6

DIMENSIONALITY OF SLEEP EEG DATA IN SCHIZOPHRENIA AND DEPRESSION

J. Röschke and J. B. Aldenhoff* Dept. of Psychiatry, University of Mainz, 6500 Mainz, Germany.

Neuropsychiatric sleep research has to deal with the problem that the brain's electrical activity seems to contradict the causality principle. One reason might be that the time history of the sleep EEG during a certain sleep stage is not predictable over a longer time period. Recently, it has become clear that under selected conditions unpredictability is a basic phenomenon of nonlinear dynamical systems, which are able to generate "deterministic chaos".

One of the commonly used attempts to investigate the behavior of dynamical systems is to measure the dimensionality of their attractors in phase space. Following a proposal of Grassberger and Procaccia we estimated the correlation dimension D_2 of different EEG epochs ($n=16384$ data points, $f_s=100$ Hz), each an unambigeous representative collection of one of the sleep stages II, III, IV and REM.

In schizophrenia ($n=11$, Cz) we found a slightly higher dimensional attractor during slow wave sleep and a significant decrease of the dimensionality of sleep stages II and REM. On the contrary, we found in depression ($n=9$, Cz) a significant increase of the dimensionality of every sleep stage.

These results point out that the complexity and the degrees of freedom of the sleep EEGs of schizophrenic and depressive patients depict alterations, which cannot be obtained from conventional sleep EEG analysis.

92.7

DIMENSIONALITY OF THE ELECTROENCEPHALOGRAM IN ALPHA COMA DIFFERS FROM THAT OF AWAKE ALPHA.

Y.W.Kim+, K.K.Kreible+, A.D.Rae-Grant#, and W.T.Blume*, + Lehigh University, Bethlehem, PA 18015, # Lehigh Valley Hospital, Allentown, PA 18103, * University Hospital, London, Ontario, Canada

The electroencephalogram (EEG), as a probe of scalp-recorded electrical activity arising from the cortex of the human brain, provides useful clinical information because of its temporal and spatial organization. As such, a measure of the organization embedded in the EEG pattern may characterize the state of consciousness. Recent developments in nonlinear dynamics suggest that an object can be constructed in an n-dimensional space out of a temporal sequence of data such as an EEG signal and that its organization is characterized by the dimensionality of the object. We have carried out such an analysis for a set of alpha coma EEG patterns in comparison to the EEG patterns of normal individuals. Alpha coma recorded from a single channel is visually indistinguishable from normal resting alpha rhythm. The results show that the alpha coma dimensionality reaches an asymptotic value of 5.5 ± 0.2 when the dimensionality of the space is increased to as large as 16. The normal EEG dimensionality, on the other hand, reaches 7.3 ± 0.2 at the same maximum n value of 16, and is still growing without any sign of saturation. This is entirely consistent with the fact that the EEG patterns show increasingly rhythmic organization as the level of consciousness diminishes. The change in dimensionality commensurate with the clinical condition suggests it is possible not only to quantify but also to model the EEG by nonlinear methods.

92.8

EYE MOVEMENTS DURING PARADOXICAL SLEEP IN GUINEA PIG. M. Escudero and P.P. Vidal*. Lab de Neurociencia, Univ. de Sevilla, Sevilla, Spain; and Lab. de Physiologie Neurosensorielle, C.N.R.S., Paris, France.

As the eye motor system is not inhibited during paradoxical sleep (PS), it is interesting to characterize and compare eye movements in both PS and wakefulness. Animals were chronically implanted for EMG and EEG recordings. Eye movements were measured by the scleral search technique. During PS two different types of eye movements were detected: one was similar to spontaneous saccades during wakefulness, although with higher amplitudes and velocities; the other consisted of an oscillatory eye movement of high velocity and amplitude, different from any eye movement observed during wakefulness. During the oscillation, the eyes described an ellipsoidal movement with a mean frequency of 10.8 Hz. Periods of oscillatory and saccadic-like eye movements during each episode of PS sleep showed a similar temporal distribution with a mean duration of 1.2 s. These results suggest that during PS the oculomotor system is characterized by an oscillatory behavior with a temporal pattern similar to that shown by the EEG recording during slow wave sleep.

Supported by "Acc. Integ. Hispano-Francesa"/225B grant.

92.9

Alterations of Circadian Period by Acute Infusion of Amyloid Peptide into the Suprachiasmatic Nuclei (SCN) B.A.Tate*, M.K.Lee and C.A.Marotta, Harvard College, Massachusetts General Hosp. and Harvard Med. Sch., Boston, MA 02114

The manifestations of Alzheimer Disease (AD) include dramatic disruption of circadian behaviors. The exact role of amyloid in the behavioral pathology of AD is not yet defined, but amyloid accumulation in brain is a hallmark of the disorder. Here we examined the effects on circadian rhythmicity of acute injections of amyloid into the rat SCN. Adult, female Sprague Dawley rats were implanted with stereotaxically placed injection cannulae and intraperitoneal Minimitters transmitters, by which activity was constantly monitored. Subjects housed in dim, constant light expressed normal free running rhythms. Amyloid peptide (amino acids 1-28, Peninsula Labs) or vehicle (8% DMSO) was injected bilaterally into the SCN at circadian time 13 on two consecutive days. Period, determined by periodogram analysis, was altered by amyloid.Amyloid may alter period by toxicity or disruption of normal cellular interactions. A similar phenomenon may occur in AD. Supported by PO1AG02126.

92.10

NMDA RECEPTOR ANTAGONISTS PREVENT CARBACHOL-INDUCED PHASE SHIFTS IN THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY IN THE HAMSTER. C.S. Colwell*, C. Kaufman, and M. Menaker. NSF Center for Biological Timing, Biology, Univ. of Virginia, Charlottesville, VA 22901.

A variety of evidence now indicates that excitatory amino acid receptors mediate the effects of light on the circadian system of mammals. However, in mammals, the acetylcholine agonist carbachol is the only agent which has been reported to "mimic" the phase shifting effects of light in vivo. In the present study, we found that the administration of NMDA receptor antagonists could prevent carbachol-induced phase shifts of the circadian rhythm of wheel-running activity recorded from the golden hamster. In addition, we found that carbachol, unlike light, does not cause an induction of Fos-like immunoreactivity in the suprachiasmatic nucleus (SCN) of these animals. A simple explanation for the data presented in this paper is that the intraventricular administration of carbachol causes phase shifts through a pathway distinct from that of light. Alternatively, if carbachol is acting via the light-input pathway, then it does so by a mechanism independent of Fos-induction in the SCN. In either case, elucidating the mechanisms by which carbachol acts in the circadian system may provide novel insights into the cellular mechanisms by which phase shifts are generated.

92.11

Forty Hz membrane potential oscillations and theta-like activity in basal forebrain neurones. M. Mühlethaler, A. Khateb, P. Fort, B.E. Jones*°, and A. Alonso°, *Dept. of Physiology, CMU, 1211 Geneva 4, Switzerland and °Montreal Neurological Institute, McGill University, Canada H3A 2B4*

We have recently demonstrated the distinct electrophysiological properties of identified cholinergic neurones in the substantia innominata of the basal forebrain using guinea-pig brain slices (Khateb et al., this meeting). We now report the presence in the same area of another class of cells, as yet chemically unidentified, which displays a different set of membrane properties and a very different firing pattern. These neurones, which represented approximately 1/3 of cells encountered, were characterized by the presence of a more or less prominent A-like current and a short, deep after-hyperpolarization. When they were depolarized from rest, they displayed subthreshold membrane oscillations interspersed with clusters of (3-10) action potentials. The frequency of either the subthreshold oscillations or the spikes within a cluster was within a range of 20-60 Hz and was often close to 40 Hz. The frequency of occurrence of the clusters themselves was voltage dependant and ranged from 2-10 Hz. In some cells, a further depolarization was associated with a tonic high frequency firing pattern. Although the chemical identity of these neurones remains to be established, preliminary immunohistochemical evidence suggests that biocytin injected cells having these characteristics are non-cholinergic. Given their spectrum of activity, this class of basal forebrain neurones could drive different rhythms in target structures, such as the cerebral cortex, that would include slow rhythms within the theta range (cluster pattern of firing) and fast rhythms within the gamma range (tonic mode of firing). (Swiss NSF, Fondation Fyssen, Lyonnaise des banques and Canadian MRC).

DEGENERATIVE DISEASE: ALZHEIMER'S—OTHER

93.1

EARLY DETECTION OF ALZHEIMER'S DISEASE (AD) USING REGIONAL CEREBRAL GLUCOSE METABOLIC (rCMRglc) DATA OBTAINED BY POSITRON EMISSION TOMOGRAPHY (PET). N.P. Azari*[1], K.D. Pettigrew[2], M.B. Schapiro[1], P. Pietrini[1], J.A. Salerno[1], C.L. Grady[1], J.V. Haxby[1], S.I. Rapoport[1] and B. Horwitz[1] [1]Lab. of Neurosciences, NIA/NIH, [2]Division of Applied and Services Research, NIMH/NIH, Bethesda, MD 20892

Correlational analysis of resting (eyes/ears covered) rCMRglc PET data has shown reduced rCMRglc interactions in mildly/moderately demented patients with probable AD. Individual differences in patterns of rCMRglc may be important for the early detection of AD, particularly in subjects at-risk (family history of AD). Using multiple regression and discriminant analysis to assess individual differences in patterns of rCMRglc, we compared resting rCMRglc PET data from probable AD patients and age-matched controls. The aim was to find a discriminant function that would successfully separate the AD patients from controls, and detect an AD pattern of rCMRglc in the first scan of an at-risk subject diagnosed as possible AD (who later developed probable AD). A discriminant function correctly classified patients and controls, and classified the first scan of the at-risk subject as AD. The results suggest that this statistical procedure may be useful for the early detection of AD.

93.2

LONGITUDINAL CT STUDY OF CEREBRAL CSF VOLUME CHANGES IN ALZHEIMER'S DISEASE. P.K. Shear, E.V. Sullivan, D.H. Mathalon, K.O. Lim, L.F. Davis, J.A. Yesavage, J.R. Tinklenberg, and A. Pfefferbaum*. Dept. of Psychiatry and Behavioral Sciences, Stanford University School of Medicine and DVA Medical Center (116A3), Palo Alto, CA 94304.

Neuroimaging studies have documented marked cerebral atrophy in patients with Alzheimer's Disease (AD). Few studies, however, have quantified *in vivo* longitudinal progression of cerebral atrophy. The present study examined change in volumetric brain CT indices for 41 AD subjects (26 men; 15 women; mean age 70.6±7.6 years) and 39 normal controls (NC) (24 men; 15 women; mean age 65.9±8.4 years). All participants completed two CT assessments (mean interscan interval: AD=2.1±1.0; NC=2.6±0.9 years). The AD patients met NINDS-ADRDA criteria for probable or definite Alzheimer's Disease and had a mean MMSE score of 17.5±5.6 (max=30 points) at baseline assessment. A semi-automated image analysis technique was used to quantify the CSF volume in each of four regions of interest (ROIs): frontal sulci, Sylvian fissures, parieto-occipital sulci, and the ventricular system. In comparison with the NC group, the AD group had significantly higher baseline CSF volumes in all ROIs ($p<.001$ for all comparisons). Mean CSF volume change per year over the interscan interval was computed as a slope for individual subjects on each ROI. A MANOVA performed on these slopes, with diagnosis and sex as grouping factors, revealed significantly more rapid progression of CSF volume abnormalities in the AD group, relative to the NC group ($p<.001$). No significant sex effects or interactions were identified. A final series of multiple regressions identified group differences in the relationship between age at first scan and rate of CSF volume change in the frontal cortex ($p<.03$) and the ventricular system ($p<.01$). Specifically, younger AD patients exhibited more rapid progression than did the older patients; this negative correlation between age and slope was not present in the NC subjects. *[Supported by Department of Veterans Affairs, MH40041, MH30854, MH18905, AA05965, Meyer Foundation, NARSAD]*

93.3

UBIQUITIN IMMUNOREACTIVITY IN CEREBROSPINAL FLUID (CSF) AND BRAIN TISSUE IN ALZHEIMER DISEASE. T. Kudo[1], K. Iqbal[1*], R. Ravid[2], D.F. Swaab[2], and I. Grundke-Iqbal[1]. [1]New York State Institute for Basic Research in Developmental Disabilities, New York, N.Y. 10314. [2]Netherlands Brain Bank, Netherlands Institute for Brain Research, Amsterdam, The Netherlands.

Alzheimer disease (AD) is a neurodegenerative disorder with progressive dementia. At present neither the etiology nor the pathogenesis of this disease are understood. Previous studies have shown an association of ubiquitin with the cytosketetal protein pathology in AD. In the present study we report (i) the quantitation of ubiquitin immunoreactivity in CSF from histopathologically confirmed AD and control cases, using a rapid competitive immunoassay, the enzyme-linked immunofiltration assay and (ii) the determination of the ubiquitin levels in brain tissue taken from the same case, using a competitive ELISA. These studies were carried out using monoclonal antibody 5-25 as the primary antibody. This antibody, which was raised against isolated paired helical filaments, reacts with the carboxy terminal residues 64-76 of ubiquitin. Because ubiquitin is conjugated to other proteins through the carboxy terminal, the epitope that 5-25 recognizes is likely to be less prone to proteolysis. Preliminary results show that ubiquitin immunoreactivity is significantly higher both in AD CSF and brain than the corresponding values from normal control cases. There is some correlation between CSF and brain tissue ubiquitin levels. These studies suggest that ubiquitin is elevated in the central nervous system of AD patients and that the ubiquitin level in CSF most likely reflects that in brain tissue. (Supported in part by NIH grants AG05892, AG08076, AG04220, and NS18105).

93.4

DISTINGUISHING FEATURES OF POTASSIUM CHANNELS IN FIBROBLASTS FROM ALZHEIMER, AGED AND YOUNG DONORS. R. Etcheberrigaray*, G.E. Gibson, E. Ito, K. Oka, B. Tofel-Grehl & D.L. Alkon. *Neural Systems Section*, NINDS, National Institutes of Health, Bethesda, MD 20892 and The Burke Medical Research Institute, Cornell Medical College, White Plains, NY 10605.

Since memory impairment is characteristic of Alzheimer's disease (AD), we examine here previously implicated biophysical substrates of memory storage, K^+ channels (Alkon et al., Science 1982), in fibroblasts from AD and control patients. Different cell lines from AD, aged matched controls (AC) and young donors (YC) were plated in 35 mm. petri dishes. Patch-clamp recordings were obtained only in the pre-confluent stage, 2-4 days after plating. Electrodes were filled with a high K^+ solution (140 mM K^+). Cell-attached patches obtained from AD cell lines were silent in 7 out of 11 cells, while no silent patches were found in lines from YC (n=6) or AC (n=2). In the cell-attached comfiguration, the channel seen in YC has a slope conductance (determined by linear regression) of 111 pS and at 0 mV pipette potential remains mostly in the open state with relatively brief and rapid closures. Its % open time is 74.6±3.49 (mean±S.E., n=4, ≥ 3,200 events each). The K^+ channel observed in the AD lines exhibited larger unitary currents and larger conductance (172 pS) than the channel from YC, and has a % open time of 7.28±1.64 (n=3), significantly lower than YC, $p< 0.0001$. The channel observed in cells from AC has a conductance of 116 pS, which is very similar to the conductance found in cells from YC donors. Its pattern of activity and % open time (61.96±2.67, n=2) are also similar to those of the YC channel. A small channel (≈ 1.8 pA at 0 mV. pipette potential) has been also seen in all three cell lines, with no apparent differences. Initial observations in inside-out patches have also suggested differences in Ca^{2+} sensitivity between YC and AD. These preliminary observations may lead to new insights regarding memory impairment and pathogenic mechanisms in AD.

93.5

INTRACELLULAR INJECTIONS REVEAL THE RELATIONSHIP OF GRANULE CELL DENDRITES TO NEURITIC PLAQUES IN ALZHEIMER'S DISEASE. G. Einstein* and B. Crain. Depts. of Neurobiology and Pathology. Duke University. Durham, NC 27710.

The hallmark of Alzheimer's Disease (AD) is the formation of neuritic plaques. Within the fascia dentata these are distributed in a characteristic laminar pattern in the molecular layer. Since granule cell (GC) dendrites innervate this region heavily, we wondered if GC dendrites underwent morphological changes in the vicinity of the plaques and if, in fact, they might participate in plaque formation. We therefore looked at the relationship of GC dendrites to plaques in 5 patients with AD and 4 age-matched controls. Tissue was obtained within 1 hour post-mortem from the Rapid Autopsy Program of the Joseph and Kathleen Bryan Alzheimer's Disease Research Center, immersion fixed, and sectioned at 300 μm on a vibratome. In these slabs, GC were filled intracellularly with Lucifer Yellow. To visualize plaques, slabs with filled cells were resectioned on a freezing microtome and stained with Thioflavin S. Whether they were located under plaques or in plaque-free regions, GC dendrites in AD cases were shrunken and showed a severe loss of spines compared to controls. Dendrites were swollen and dystrophic and, in some cases, bore few or no spines. However, these changes did not seem to be caused directly by the plaques since filled dendrites often stopped short of or were pushed to the sides of plaques, and when they entered plaques there was no sprouting or hypertrophy. In sum, GC dendrites appear relatively unaffected by senile plaques and probably do not participate in their formation. Instead, GC changes seen in AD most probably result from deafferentation. Supported by NIH grants AG 09216 and AG 05128.

93.6

IDENTIFICATION OF THE TRANSITIONAL AND END-STAGE FORMS OF THE NEUROFIBRILLARY TANGLE IN NORMAL AGING AND ALZHEIMER'S DISEASE. J.C. Vickers, A. Delacourte* † and J.H. Morrison Fishberg Research Center for Neurobiology, Box 1065 Mount Sinai Medical Center, New York. N.Y. 10029, U.S.A. †INSERM U156, Place de Verdun, 59045 Lille, France.

Many of the clinical features of Alzheimer's disease are likely to reflect the progressive and selective degeneration of the neuronal subclasses that provide the association pathways of the neocortex and interconnect the hippocampal formation with the rest of the brain. The neurons in the entorhinal cortex and neocortex that are particularly vulnerable to neurofibrillary tangle (NFT) formation are characterized by their selective labelling for the neurofilament (NF) protein triplet class of intermediate filament proteins. Thioflavine S staining was combined with double labelling immunofluorescence for tau and the NF proteins to demonstrate the progressive alteration of the normal neuronal cytoskeleton into NFTs within these vulnerable subclasses of neurons. Transitional NFTs were identified by their thioflavine staining and the presence of tau and both phosphorylated and non-phosphorylated NF protein epitopes, whereas end-stage NFTs were stained with thioflavine but lacked NF and tau epitopes. Transitional pathology was present in the entorhinal cortex of neurologically normal controls with no NFTs in the neocortex (47 years of age +), whereas Alzheimer's disease was characterized by end-stage pathology in the entorhinal cortex and a mixture of end-stage and transitional pathology in the prefrontal neocortex. These data suggest that the cellular correlate of dementia may be the point at which cytoskeletal changes progress from transitional to end-stage in the entorhinal cortex, and begin to involve a large enough contingent of neocortical pyramidal cells to affect corticocortical integration. Supported by AG05138

93.7

MIDKINE PROTEIN (MK) AND HEPARIN-BINDING NEUROTROPHIC FACTOR (HBNF): HOMOLOGOUS PROTEINS WITH DIFFERENT DISTRIBUTIONS IN ALZHEIMER'S DISEASE. E.G. Stopa*, I. Kovesdi, V. Kuo-LeBlanc, A. Baird and P. Böhlen. SUNY HSC, Syracuse, NY 13210, The Whittier Institute, LaJolla, CA 92037 and American Cyanamid Corp., Pearl River, NY 10965.

Midkine protein (MK) and Heparin-Binding Neurotrophic Factor (HBNF) are two recently identified heparin-binding proteins which share approximately 60% sequence homology. Their biological roles have not yet been determined, but they are thought to be involved in embryogenesis and brain function. Since Basic Fibroblast Growth Factor (bFGF), another heparin binding protein, is abnormally increased in Alzheimer's disease (AD), we examined the distribution of MK and HBNF to determine if they may also be altered in this disease. The immunocytochemical procedures were performed on 50 μm sections of prefrontal cortex, basal ganglia, thalamus and hippocampus fixed in either 4% paraformaldehyde or 10% neutral buffered formalin. Free-floating sections, obtained from 5 control and 4 AD cases, were incubated in specific polyclonal antibodies raised against MK and HBNF, and used at a dilution of 1/1000. Staining was accomplished using a modified ABC technique. The most intense staining for both proteins was seen within the ependyma. HBNF immunoreactivity was also readily observed in endothelial cells. Faint staining for both proteins was seen in cortical neuropil and selected neurons and astrocytes. MK-like immunoreactivity was extremely robust within the senile plaques and neurofibrillary tangle bearing neurons of the AD cases examined,whereas HBNF did not stain these structures. Our observations suggest that despite their structural homology, these proteins may have different biological roles, and that MK is abnormally distributed in Alzheimer's disease. AG10682

93.8

ABNORMALITIES OF THE CALPAIN-CALPASTATIN SYSTEM IN ALZHEIMER BRAIN. R. A. Nixon, K.-I. Saito, A. M. Cataldo, J. Hamos, D. Hamilton, T. Honda and A. Pope*. McLean Hospital and Harvard Medical School, Belmont, MA 02178

Calcium-activated neutral proteinases (calpain or CANP) are key enzymes in intracellular signaling cascades and potential mediators of calcium-induced cell injury. The enzyme form requiring micromolar calcium levels (μCANP), which is enriched in neurons, has been most frequently implicated. As an index of changes in the *in vivo* activity of μCANP, the ratios of the activated isoforms of μCANP to the precursor isoform were measured immunochemically in regions of postmortem human brain. This ratio was elevated 3-fold in the prefrontal cortex from patients with Alzheimer disease but not from those with Huntington disease. Other brain regions (putamen and cerebellum) where neuronal degeneration is considered minimal in Alzheimer disease also displayed significantly increased μCANP activation (Saito et al., these proceedings). Total levels of μCANP were unaltered. Calpastatin, the endogenous inhibitor of μCANP, was purified from human brain and shown by immunocytochemistry to co-localize with μCANP in neurons and dendrites. Affinity purified antibodies to human brain calpastatin revealed marked depletion of calpastatin immunoreactivity in the neocortex of individuals with Alzheimer disease. Depletion was prominent in dendrites in cortical laminae I-III, and was evident to a lesser extent in laminae IV-V. These results indicate that the CANP system may be abnormally activated in AD brain. Increased vulnerability of the neurons or their processes to degeneration under these conditions may involve a depletion or redistribution of calpastatin. Given the suspected biological roles of CANPs, persistent activation of μCANP could contribute to altered protein processing and abnormal phosphorylation in AD. Abnormal CANP-mediated proteolysis could provide a possible basis for synaptic loss and other degenerative events that underlie cognitive impairment in AD.

93.9

HEAT SHOCKED NEURONAL PC12 CELLS AS A MODEL OF THE ALZHEIMER'S DISEASE STRESS RESPONSE. G.Johnson*, L.Refolo, C.Merril, W.Wallace. Lab. of Biochemical Genetics, NIMH, Washington, D.C. and Dept. Psychiatry and Neurobiology Center, Mount Sinai School of Medicine, New York

Heat shock refers to the cellular response to a variety of insults which minimizes damage and allows for the restoration of normal cellular activities after the period of stress. In order to investigate the response of neurons to stress, we used nerve growth factor-differentiated PC12 cells incubated at either 37°C (control cells) or 45°C (heat shocked cells). The heat shocked PC12 cells exhibited several features characteristic of the stress response including a 45% reduction in total protein synthesis, the induction of heat shock protein 72, an overall decrease in protein phosphorylation, and an increased phosphorylation of the initiation factor eIF-2alpha. The Alzheimer's disease (AD) brain undergoes many changes characteristic of the heat shock response which we term the AD stress response. However, elongation factor 2, which is hyperphosphorylated in AD brain, exhibited no such increase in heat shocked PC12 cells. We have also examined the phosphorylation of amyloid precursor protein (APP). Two dimensional gel separation of immunoprecipitates of [^{32}P]-labeled PC12 cells with two different antibodies revealed four phosphorylated isoforms of APP (120-140kD, 5.3pI). APP was dramatically dephosphorylated in the heat shocked PC12 cells.

93.10

POTENTIAL ROLE OF HSP 72/73 IN THE REVERSIBLE PHOSPHORYLATION OF TAU TO FORM A68 IN HEAT SHOCKED NEURONAL PC12 CELLS. W. Wallace*, G. Johnson, C. Merril, J. Sugar, L. Refolo. Dept Psychiatry and Neurobiology Center, Mount Sinai School Of Medicine, New York and Laboratory of Biochemical Genetics, NIMH, Washington, D.C.

A68, the primary protein constituent of neurofibrillary tangles, has been proposed to be an abnormally phosphorylated form of tau, a microtubule-associated protein. We have found that A68 is produced in neuronal PC12 cells that have been subjected to heat shock (45^0 C, 30 minutes). A68, which was identified by immunoprecipitation with both tau-2 and ALZ50, exhibited the characteristic reduced electrophoretic mobility compared to normal tau as has been found in AD postmortem tissues. The A68 incorporated radiolabeled phosphate indicating that it is a phosphoprotein. Antibodies to hsp 72/73 co-precipitated the normal form of tau which indicated a stable complex formation between tau and hsp. Cell lysates that had been depleted of such hsp complexes contained A68. Thus, no A68:hsp complexes were formed. A68 was dephosphorylated and reverted to normal tau two to four hours after recovery from heat shock. In the presence of cycloheximide, A68 still formed with heat shock; however, its dephosphorylation was much more rapid during recovery.

93.11

A THEORY OF THE PROGRESSION OF ALZHEIMER'S DISEASE.

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The neurofibrillary tangles of Alzheimer's disease appear with highest density in entorhinal cortex layer II and hippocampal region CA1 (Hyman et al., *Science* 225:1168-1170). Computational modeling suggests that this selective distribution could result from a breakdown of the mechanisms of cortical associative memory, due to imbalances induced by any of a range of ætiological factors. In cortical models, previously strengthened connections can interfere with the learning of new associations, resulting in runaway synaptic modification (Hasselmo et al., *J. Neurophysiol.* 67:1230-1246). The metabolic demands or excitotoxic effects of runaway synaptic modification could be severe enough to cause degeneration. The initiation and progression of runaway synaptic modification can be described by the solution to an equation combining synaptic transmission and modification:

$$\frac{dW_{ij}}{dt} = (A_i + (1 - c)\sum_{k=1}^{n} W_{ik}A_k - H - \Omega)A_j$$

where W_{ij} = excitatory associational synapses, A_i = post-synaptic afferent input, A_j,A_k = pre-synaptic afferent input, c = suppression of synaptic transmission, H = inhibitory feedback, and Ω = threshold of synaptic modification. This yields the solution:

$$W_{ij} = Z_{ij}e^{\lambda_j t} - Z_{ij} + W_{ij}(0)$$

where $Z_{ij} = [A_i + (1 - c)\sum_{k=1}^{n} W_{ik}(0)A_k - H - \Omega]/(1 - c)\sum_{k=1}^{n} A_k$ and $\lambda_j = (1 - c)\sum_{k=1}^{n} A_kA_j$

If $Z > 0$ when $A_i = 0$, runaway synaptic modification will progress with a time course determined by Z and the time constant λ. The results of this analysis match the qualitative features of non-linear simulations of cortical associative memory function. The increased overlap in post-synaptic activity resulting in one region could then underlie the spread of runaway synaptic modification into adjacent cortical regions, with similar dynamics. *Supported by The French Foundation for Alzheimer Research.*

PAIN: PHARMACOLOGY

94.1

NALOXONE POTENTIATES VISCERAL NOCICEPTION IN NEWBORN RATS. C.M. Conway, J. Martinez, D.M. Kaiser, S.J. Evans and L.D. Lytle*. Laboratory of Psychopharmacology, Department of Psychology, University of California, Santa Barbara, CA 93106.

The importance of endogenous opiates for adult pain is well established, but nociceptive mechanisms in immature organisms are poorly understood. Development of an animal model to study nociception might therefore prove useful for understanding ontogenetic changes occurring in mechanisms mediating pain sensitivity and/or reactivity in immature organisms. The current investigation studied age-related changes in opiate mechanisms mediating visceral nociception by administering naloxone, an opiate receptor blocking drug, to developing rats.

Neonatal albino rats (5, 15, or 25 days postnatal) were pretreated with naloxone HCl (1 mg/kg; free base; i.p.) or an equal volume of its 0.9% NaCl vehicle (1 ml/kg). Fifteen min later half of each group received 12% hypertonic NaCl (1 ml/kg; i.p.) as a noxious visceral stimulus, or an equal volume of a non-noxious control solution of 0.9% NaCl. The number, duration and timing of abdominal contractions (defined as waves of muscular contractions passing caudally from the thoracic abdominal wall towards the hindlimbs and tail, as well as an arching/stretching of the back and/or a dorsolateral splaying of the hindlimbs) were assessed using 2 single blind observers for up to 10 min after the second treatment. The hypertonic saline challenge failed to elicit pain responses in 5-day-old animals, and then produced progressive, age-dependent pain responses in the two older age groups. Interestingly, the naloxone pretreatment potentiated the hypertonic saline induced pain responses of the youngest group of animals to a greater extent than the older ones. Taken together, these data replicate earlier results [Bronstein *et al., Dev. Psychobiol. 19*:473 (1986)] and suggest that endogenous opiates and/or opiate dependent mechanisms may mature early in development so as to mitigate neonatal pain.

94.2

THE DIFFERENTIAL EFFECT OF MORPHINE AND α_2 AGONISTS ON THE PREVENTION AND TREATMENT OF EXPERIMENTAL NEUROPATHIC PAIN. **M.J.C. Puke[1] and Z. Wiesenfeld-Hallin*,[2]. Karolinska Institute, [1]Dept. of Anesthesiol., Karolinska Hosp. and [2]Dept. of Clin. Neurophysiol., Huddinge Hosp., Sweden.**

We have examined the effect of intrathecal (i.t.) morphine (MO), clonidine (CL), dexmedetomidine (DEX) and saline (SAL) on self-mutilation (autotomy) behavior, a sign of neuropathic pain after sciatic nerve section, in three groups of Sprague-Dawley rats. A single dose of MO (50 μg), CL (50 μg) or SAL was injected (1) 30-60 min prior to or (2) 15 min after nerve section. Alternatively, in group (3) MO (10 μg), CL (10 μg), DEX (1 μg) or SAL was injected twice daily for 21 d, starting 24 h after axotomy.

MO reduced and delayed autotomy compared with CL and SAL in groups (1) and (2) and there was no difference between CL and SAL treatment. The beneficial effect of MO in group (1) was greater than in group (2). In contrast, CL and DEX reduced autotomy compared to MO and SAL in group (3) with DEX having a better effect than CL.

We conclude that opioid, but not α_2, agonists are beneficial in preventing neuropathic pain, whereas α_2, but not opioid, agonists are useful in treating such conditions. These differences can be related to the observations that MO is more effective in blocking "wind-up" and central sensitization of the spinal cord than CL. Furthermore, the sensitivity of the spinal cord to MO is reduced (Xu and Wiesenfeld-Hallin, Pain, 1991) and to CL increased (Xu et al., Pain, in press) after axotomy.

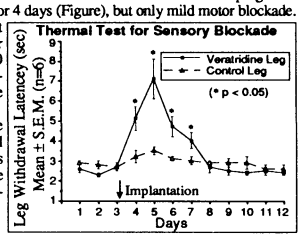
94.3

PROLONGED SCIATIC NERVE BLOCKADE USING SUSTAINED RELEASE OF VERATRIDINE FROM A BIODEGRADABLE POLYMER MATRIX. D.B. Masters*, D. Hu, S. Dutta, C.T. Griggs, G. Strichartz & C.B. Berde, Anesthesia Depts., Children's Hospital and Brigham & Women's Hospital, Harvard Med. Sch., Boston, MA 02115.

Biodegradable polymer/ local anesthetic matrices sustain drug release *in vitro* (Berde et al., Anes. 73: A776, 1990) and produce neural blockade for 3-5 days *in vivo* (Masters et al., Anes. 75: A765, 1991). Veratridine (VTD), a sodium channel activator, produces use-dependent neural block with greater potency in C-fibers than A-fibers *in vitro* (Schneider et al., Anes. 74: 270, 1991). Preliminary work also suggests analgesia in rats (Strichartz et al., Anes. 75: A771, 1991). The aim of the present study was to measure sensory and motor block of the rat sciatic nerve *in vivo*, using VTD-impregnated polymer implants.

Pellets were produced by casting molten (115 °C) 1,3 bis (p-carboxyphenoxy) propane: sebasic acid (CPP:SA; 20:80 or 50:50) polymer/VTD mixtures in Teflon molds. Anesthetized male rats (Sprague-Dawley) received implants adjacent to the sciatic nerve on one side with either 5%, 10%, or 20% VTD pellets (300 mg total); an equal mass of control pellets were implanted adjacent to the contralateral sciatic nerve. Motor blockade was assessed using a scale just prior to sensory testing. Paw withdrawal latency to a 56 °C surface was used to assess analgesia. The experimenter was blind as to the treatment.

Control pellets produced no analgesia or motor blockade. The 5% VTD-impregnated 20:80 CPP-SA pellets produced analgesia for 4 days (Figure), but only mild motor blockade. The 5% VTD/50:50 CPP:SA pellets did not produce significant analgesia or motor blockade. The 10% and 20% VTD/20:80 CPP:SA did not incorporate drug homogeneously into the polymer, and all three animals receiving 20% implants died.



VTD can produce analgesia with little compromise of motor capacity in a rat model of prolonged nerve block. Further studies are required to assess toxicity, alternative methods of VTD incorporation, and modality characteristics of the block.

(Supported by NIH grant #GM-15904)

94.4

PGE$_2$-INDUCED ENHANCEMENT OF BRADYKININ RESPONSES OF POLYMODAL RECEPTORS IS MEDIATED BY EP$_3$ RECEPTORS. T.Kumazawa*, K.Mizumura, M.Minagawa, S.Narumiya & T.Namba. Dept. of Neural Regulation, Res. Inst. of Environ. Med., Nagoya Univ., Nagoya 464-01 JAPAN

PGE$_2$ enhances bradykinin (BK) responses of polymodal receptors which signal pain. We studied what subtype of PG receptor is implicated in the PG effect, using an EP$_1$ antagonist, AH 6809; an EP$_2$ agonist, butaprost; an EP$_3$ agonist, M&B 28767. Discharges of polymodal receptors were recorded in in vitro canine testis-spermatic nerve preparations. The testis was immersed in Krebs-solution with aspirin (550 μM) to prevent release of endogenous PGs. BK (1 μM) mixed with aspirin and captopril (10 μM), an inhibitor of BK-degradation, was applied by replacing the Krebs-solution for 10-15 min. PG agonists at concentrations from 10^{-9} to 10^{-5} M were applied cumulatively when the BK-induced discharge rate reached a quasi steady state or slowly declining phase. PGE$_2$ enhanced BK-responses at concentrations above 10^{-8} M; a more potent effect was induced by the EP$_3$ agonist. The EP$_2$ agonist up to 10^{-5} M did not modify BK-responses. The EP$_1$ antagonist (10^{-6} and 10^{-5} M), which was applied during BK application, did not affect the enhancing effects of PGE$_2$ and the EP$_3$ agonist as well. It is most likely that EP$_3$ receptors mediate the enhancing effect of PGE$_2$ on the BK-response of polymodal receptors.

94.5

THE INDUCTION OF *C-FOS* EXPRESSION IN VARIOUS SUBCORTICAL BRAIN AREAS BY THE ADMINISTRATION OF COPPER SULFATE IN RATS. D.Y. Chen*, M.F. Gonzalez, J.A. Deutsch and Y. Gu, Department of Psychology, UCSD, La Jolla, CA 92093

It has been suggested that two mechanisms mediate the transduction of toxic signals to the brain. One group of emetic agents, represented by $CuSO_4$ exert their action on receptors on the gastric wall and then this signal is relayed to the nucleus of the solitary tract via the vagus nerve. Another group of nausea-inducing agents, like LiCl, may be transported via the circulatory system and act directly on the CNS. In the present study the mechanism of action of $CuSO_4$ was studied using *c-fos* immunoactivity.

Male Sprague-Dawley rats were tested after a 15 hour-fast. $CuSO_4$ isotonic to physiological saline was administered orally(300mg/kg), while corresponding volume of normal saline was given to control animals. Two hours after the injections the rats were perfused and their brains extracted and sectioned. The sections were reacted for *fos* immunocytochemistry using commercially available *fos* antibody (Microbiological Associates) and ABC reagents (Vector Lab). The most striking *fos* expression was found in the nucleus of the solitary tract, both in the frontal and far caudal parts. In addition, *fos*-positive neurons were found in the parvocellular region of the paraventricular nucleus, and amygdala. In the supraoptic area, very weak *fos* immunoactivities were also observed. It was noted that there was no apparent induction of *fos* in neurons of the area postrema.

These findings suggest an action of $CuSO_4$ on the gastric wall and the mediation of the signal to nucleus of the solitary tract via the vagus nerve, which has reciprocal connection with paraventricular nucleus. More investigation is necessary to further clarify the relay pathway of the signal, which, by now, apparently includes amygdala as one of the stations

94.6

RECURRENT NEOCORTICAL SPREADING DEPRESSION PROVOKES C-FOS-LI WITHIN V NUCLEUS CAUDALIS VIA TRIGEMINOVASCULAR MECHANISMS. M.A. Moskowitz*, K. Nozaki, R.P. Kraig. Neurology Dept., MGH, Boston, MA 02114 and Neurology, Univ. of Chicago, Chicago, Ill 60637.

KCl was microinjected into left parietal cortex every 9 min over 1 hr, and SD detected by a shift in DC potential. Cells were stained using polyclonal antisera and counted in serial sections. C-fos-LI was visualized in ventrolateral TNC, chiefly in lamina I, II$_o$ and mostly below the C-M junction, ipsil. The ratio of cells (l/r) was 1.32 after 1M KCl versus 1.06 in control animals (1M NaCl) ($p<0.01$). The ratio was also lower ($p<0.01$) after i.v. sumatriptan, a 5-HT$_1$-like agonist which blocks meningeal C-fibers and attenuates c-fos-LI after noxious meningeal stimulation. Sumatriptan did not reduce SD after KCl injection. However, combined hyperoxia and hypercapnia reduced the number of SD's, and also c-fos-LI in TNC ($p<0.01$).

Hence, multiple SD's activate cells within TNC probably by stimulation of meningeal afferents. If true, this is the first report demonstrating that events within cerebral cortex can activate brain stem regions involved in the processing of nociceptive information.

94.7

PERIPHERAL BOMBESIN INDUCES C-FOS-LIKE IMMUNOREACTIVITY (C-FOS-LI) IN THE RAT BRAIN. B. Bonaz, Y. Taché*. CURE, VA Wadsworth Medical Center, Dept. of Medicine and Brain Res. Inst., UCLA, Wadsworth, Los Angeles, CA 90073.

CCK-8 induces c-fos-LI in the hypothalamus (1) and the nucleus of the solitary tract (NTS) (2) via vagal capsaicin sensory fibers (2). Purpose: to investigate whether peripheral bombesin, known to influence food intake, induces c-fos-LI in the brain. Methods: male SD rats were injected ip either with vehicle (2 ml 0.1% BSA) or with CCK-8 or bombesin (100 μg/kg) alone or combined with systemic capsaicin pretreatment. Rats were perfused 60 min later with 4% paraformaldehyde. Brains were removed, post-fixed 3 h at +4° C and cryoprotected in sucrose. Frozen sections (30 μm) were then incubated with the primary antibody (c-fos AB-2 rabbit antibody, Oncogene Science) at a dilution of 1:2000 for 24h at +4° C. The biotinylated secondary antibody (goat anti-rabbit, 1:100) was applied for 2 h. Sections were then processed for avidin-biotin-peroxidase using diaminobenzidine as the chromogen. Results: after CCK injection (n=7), c-fos-LI was densily distributed in the area postrema, NTS (medial and dorsal subnuclei), paraventricular nucleus of the hypothalamus (PVN) and amygdala. In bombesin-treated rats (n=8), c-fos-LI was mainly localized in NTS (medial subnucleus) and the PVN (parvocellular part). Overall, fewer cells and lighter immunostaining was observed compared to CCK. c-fos-LI-induced by bombesin was not attenuated by capsaicin pretreatment (n=5), whereas that of CCK was attenuated (n=3). Conclusions: peripheral bombesin activates selected brain nuclei involved in the regulation of autonomic function.

We thank the Neuroendocrine Anatomy Core of NIH Center Grant DK 41301.

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94.8

ENHANCED LEVELS OF ENKEPHALIN & DYNORPHIN mRNA IN RAT SPINAL CORD DURING PREGNANCY. K.E. Miller*, M. Brown and B. Srinivasan. Dept. Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

During pregnancy, activation of the spinal opioid system is thought to cause attenuated responses to aversive stimuli (A. Gintzler, Science 210:193,'80). In the present study, preproenkephalin (ENK) and preprodynorphin (DYN) mRNA levels were compared between the spinal cords of pregnant and normal female rats. Spinal cords from 10 & 20 day pregnant and normal rats were removed and total RNA was extracted from lumbar and cervical enlargements. ^{32}P-labelled cDNA's for ENK (S.Sabol) and DYN (J.Douglass) were used to probe RNA blots. For normalization, blots were stripped and reprobed with ß-actin cDNA. Following densitometry and normalization, ENK & DYN mRNA levels did not change in the cervical spinal cord. In lumbar cord, ENK levels were increased by 200-300% at 10 and 20 days. DYN levels were increased similarly at 20 days. These data indicate that changes in afferent activity from the uterus during pregnancy results in enhanced production of ENK and DYN mRNA in spinal opioid neurons of the lumbar cord. The large increase in ENK mRNA is different from that observed after neuropathy or peripheral inflammation (Draisci et al., Br. Res. 560:186,'91), and may be related to visceral afferent activity.

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94.9

DORSAL COLUMN INHIBITION OF FELINE NOCICEPTIVE THALAMIC CELLS VIA GABAERGIC MECHANISMS.

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Cells in posterior parts of the feline thalamus were investigated to influences from afferents in the spinothalamic tract (STT), the dorsal column lemniscal pathway and the canine tooth pulp (TP). The cells had a spontaneous resting activity which could be increased by extracellular iontophoretic application of DL-homocysteic acid (DLH) and decreased by gamma-amino butyric acid (GABA). No effect on the spontaneous firing rate was observed following iontophoresis of the selective GABA-antagonists, picrotoxin (GABA-A antagonist) or saclofen (GABA-B antagonist). The evoked activity following stimulation of the dorsal column nuclei (DCN) was increased by simultaneous administration of picrotoxin. Such an increase in activity was not observed following STT or TP stimulation when picrotoxin was given simultaneously. The extracellular activity evoked by electrical stimulation of STT and TP was significantly depressed by preceding electrical stimulation in the DCN. This decrease in activity could be disinhibited by simultaneous administration of picrotoxin, indicating that the DCN induced inhibition is mediated via GABA-A receptors. A disinhibition of the DCN induced depression on the late responses following STT stimulation occurred after application of saclofen suggesting that the late inhibition is partly mediated via GABA-B receptors. Results in the present study indicate a thalamic interaction between low-threshold (DCN) and high-threshold afferents (STT and TP) similar to that described in the spinal cord. A thalamic "gate-mechanism" is proposed.

94.10

SUPRASPINAL MECHANISMS OF CANNABINOID-INDUCED ANTINOCICEPTION IN RATS.

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The naturally occurring as well as the synthetic cannabinoids have been shown to be potent antinociceptive agents. However, little is known of their underlying mechanism. Therefore, the purpose of this ongoing investigation was to elucidate their mechanism of action. The finding that CP-55,940 (15 μg), a synthetic bicyclic analog, produced potent antinociception (84 ± 16 %MPE) when administered directly into the ventral lateral region of the posterior periaqueductal gray (PAG) was the first demonstration implicating a brain region in the antinociceptive actions of the cannabinoids. In contrast, CP-56,667 (15 μg), the inactive stereoisomer of CP-55,940, failed to produce any appreciable antinociception, thus indicating stereoselectivity. Furthermore, this antinociceptive effect of CP-55,940 exhibits regional specificity as microinjection into either the dorsolateral region of the posterior PAG, the anterior ventrolateral region, or outside of the PAG failed to produce any antinociception. Finally, a 4 day pretreatment with pertussis toxin (0.2 μg) into the PAG completely blocked the antinociceptive action of CP-55,940, indicating that these effects are mediated through an inhibitory action on cyclic AMP at a G-protein coupled receptor. Taken together, these findings suggest that the cannabinoids produce antinociception through a G-protein coupled receptor mechanism in the PAG. This research was supported by NIDA grants DA-03672 and DA-05421.

94.11

Serum (S) ionized magnesium (IMg^{2+}) and calcium (ICa^{2+}) in headache (H) classification. Comparison of patients (P) with continuous headaches (CH) and migraines (MH).

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Due to lack of objective parameters, the classification of headaches relies on descriptions. It has been suggested that Mg may play a role in MH. Studies of intracellular and S total Mg (TMg) levels have produced inconsistent results. Using a new ion-selective electrode for Mg^{2+} we have assessed IMg^{2+} in S of P with several types of H. Measurements of ICa^{2+}, TMg and calculation of ICa^{2+}/IMg^{2+} ratios were also done. We have selected for comparison 14 P with CH, 32 P with an acute attack of MH and age matched controls. P in both H groups had a severe H at the time of blood sampling. 46% of P with MH had a low IMg^{2+} and a high ICa^{2+}/IMg^{2+} ratio as compared to 21% of P with CH (p 0.01). The S levels of TMg were normal, but were lower in P with MH as compared to P with CH or controls (p 0.05). This study suggests that IMg^{2+} may be useful in the diagnosis and treatment of headaches.

94.12

FACTORS DIFFERENTIATING BETWEEN ANALGESIA AND SEDATION IN SPONTANEOUS AND EVOKED EEG.

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The paper analyses the factorial structure of spontaneous and evoked EEG activity under the opioid meperidine (100 mg p.o.) and the tranquilizer diazepam (10 mg) in a placebo-controled double blind repeated measures study with 21 volunteers. Peri-stimulus EEG segments (Cz vs linked ear lobes) with 500 ms before and 500 ms after pain-inducing intracutaneous current pulses were spectrally analysed by means of the parametric maximum entropy method. The extracted principal component scores were correlated to pain relief and changes in vigilance. Pain relief was measured by the subjects ratings, vigilance was tested by a motor reaction task.

Results: 90 % of variance in all EEG activity could be explained by 4 varimax rotated principal components (PCs): The analgesic effect was predominantly reflected by the score of PC3 (delta power of poststimulus EEG). Decrease in vigilance could best be described by the score of PC2 (alpha power of both spontaneous and evoked EEG activity). The two other components, PC1 (beta power of spontaneous and evoked EEG) and PC4 (delta and theta power of spontaneous EEG), were not correlated with sedation or with pain relief.

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PSYCHOTHERAPEUTIC DRUGS

95.1

ELECTROPHYSIOLOGICAL EVIDENCE FOR THE DESENSITIZATION OF α_2-ADRENERGIC HETERORECEPTORS ON SEROTONIN (5-HT) TERMINALS IN THE RAT HIPPOCAMPUS FOLLOWING CHRONIC ANTIDEPRESSANT TREATMENTS.

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Previous results from our laboratory have indicated that small doses of the α_2-adrenergic agonist clonidine increase 5-HT transmission by attenuating the release of endogenous norepinephrine (NE), as a result of the activation of α_2-adrenergic autoreceptors on NE neurons, and that high doses decrease 5-HT transmission by activating directly α_2-adrenergic heteroreceptors on 5-HT terminals. The aim of the present study was to assess whether antidepressant treatments that increase the synaptic availability of NE or 5-HT change the ability of either of these two α_2-adrenoceptors to modulate 5-HT transmission. The effect of the activation of these receptors was studied by comparing the effectiveness of the electrical stimulation (200 pulses: 0.5 msec, 300 μA, 1 Hz) of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons prior to, and following, the i.v. administration of 10 and 400 μg/kg of clonidine. Rats were treated for 3 weeks with s.c. osmotic minipumps (removed 48 h before the experiment) delivering 10 mg/kg/day of paroxetine (a selective 5-HT reuptake inhibitor), 0.75 mg/kg/day of befloxatone (a reversible inhibitor of monoamine oxidase A), 10 mg/kg/day of nisoxetine (a selective NE reuptake inhibitor) or saline. None of these treatments altered the effect of the small dose of clonidine. However, the reduction of 5-HT transmission by the large dose of clonidine was abolished in rats treated with nisoxetine and befloxatone, but not in those treated with paroxetine. These results suggest that antidepressant drugs that increase NE synaptic concentration induce a desensitization of α_2-heteroreceptors on 5-HT terminals.

95.2

ATTENUATED EFFECT OF PAROXETINE FOLLOWING ITS LONG-TERM ADMINISTRATION: AN ELECTROPHYSIOLOGICAL STUDY IN THE RAT HIPPOCAMPUS.

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Long-term administration of antidepressant selective 5-HT reuptake inhibitors, has been shown to enhance 5-HT neurotransmission. The present experiments were undertaken to assess the effect of long-term paroxetine treatment on the 5-HT reuptake process. The recovery time of the firing activity of dorsal hippoccampus pyramidal neurons following microiontophoretic application of 5-HT was used as an index of reuptake activity.

In a first series of experiments, the effect of acute i.v. administration of paroxetine was assessed in control rats and in rats treated with paroxetine (10 mg/kg/day, s.c.) for 21 days, 48 h after the removal of the osmotic minipump. Before the injection of paroxetine, the effect of 5-HT was similar in treated and control rats. At the dose of 1 mg/kg, paroxetine prolonged the effect of 5-HT to the same extent in the two groups; however, at the dose of 4 mg/kg, its effect was much smaller in paroxetine-treated rats. In rats pretreated with 5,7-dihydroxytryptamine, there was a prolongation of the effect of 5-HT, and the acute administration of paroxetine did not induce any further prolongation.

In a second series of experiments, the effect of 5-HT was measured in control rats and rats treated for 2 or 21 days with paroxetine (10 mg/kg/day, s.c.), the experiment being carried out with the minipump in place. In both treated groups, the suppressant effect of 5-HT was longer than in controls, whereas the effect of the 5-HT1A agonist gepirone was unchanged. However, the prolongation of the effect of 5-HT was significantly smaller in rats treated for 21 days than those treated for 2 days.

In conclusion, these data suggest that the long-term administration of paroxetine results in a down-regulation of paroxetine binding sites.

95.3

AN EVALUATION OF SC 48,274, A NOVEL ANXIOLYTIC, IN GENERALIZED ANXIETY DISORDER (GAD). N.R. Cutler,* J.J. Sramek, A. Macpherson, R.D. Seifert, C.O. Benes, S.F. Howard. California Clinical Trials, Beverly Hills, CA 90211; Searle Res. & Dev., Skokie, IL 60077.

The data presented here as a single site study is part of a multicenter, double-blind, placebo-controlled trial to evaluate the efficacy of two fixed dosages (1.0 mg and 25 mg bid) of the unique anxiolytic SC 48,274 in GAD. Sixty-five patients at this site (52 M; $\bar{x}$ age, 33 years; range 19-64) were randomly assigned to a 4-week trial of one of the SC 48,274 dosages (1 mg, n=28; 25 mg, n=9) or placebo (n=28), followed by a 7-day single-blind placebo follow-up. Baseline HAM-A scores for the 1 mg, 25 mg, and placebo groups were 25.1, 25.9, and 24.5, respectively; after 4 weeks, HAM-A changes for the three groups were -5.1, -4.2, and -1.9. The 1 mg group showed a significant improvement compared to placebo ($F=8.93$; $p=0.004$), whereas the 25 mg group did not ($F=2.26$; $p>0.05$). Clinical Global Impression (CGI) severity of illness scores were significant for the 1 mg group vs. placebo ($X^2=3.84$; $p=0.05$), but not significant for the 25 mg group ($X^2=0.90$; $p>0.05$). Adverse events were typically mild, occurring in 67.6% of study drug subjects and in 78.6% of placebo subjects. The most frequent adverse events for drug subjects included headaches, myalgia, rhinitis, abdominal pain and nausea; however, there was no apparent pattern of adverse events distinguishing drug and placebo groups. The 1 mg dosage appeared to be slightly better tolerated than the 25 mg dosage (adverse event incidence, 64.3% vs. 77.8%). This single site study demonstrated efficacy of SC 48,274 at the 1 mg bid dosage in GAD, while the multicenter study as a whole did not demonstrate significance ($p>0.05$).

95.4

LONG-TERM MAZINDOL AUGMENTATION OF NEUROLEPTIC IN NEGATIVE SCHIZOPHRENIA. J. Seibyl*, J. Erdos, L. Karper, R. Johnson, L. Brenner, G. Heninger, D. Charney, J. Krystal. Psychiatry Service, Yale University School of Medicine and West Haven VA Medical Center, West Haven, CT, 06516

Decreased dopaminergic function has been implicated in negative schizophrenic symptoms including anhedonia, amotivation, and affective impairment. Standard antipsychotics are ineffective or even deleterious in management of negative symptoms. Preliminary work in schizophrenic patients has shown mazindol, a long-acting dopamine transporter agent, reduces negative symptoms compared to placebo. The present study evaluates the long-term efficacy of mazindol augmentation of standard antipsychotics in stable negative symptom outpatient schizophrenics. METHODS: Seventeen schizophrenics received open-label mazindol 2-4 mg/day for 4-6 months. Biweekly ratings included the Brief Psychiatric Rating Scale (BPRS), Positive and Negative Symptom Scale (PANSS), Webster Scale of extrapyramidal symptoms and the Abnormal Involuntary Movement Scale (AIMS). Fasting prolactin and HVA were obtained biweekly. RESULTS: Mazindol caused a 30-40% reduction in total and negative symptom scores while having no effect on positive symptoms. There was a trend toward the reduction of antipsychotic-induced extrapyramidal side effects, but no exacerbation of tardive dyskinesia scores. Mazindol caused an initial increase in prolactin followed by reduction to sub-baseline levels. IMPLICATIONS: This study suggests that mazindol is a useful agent for the long-term treatment of negative schizophrenic symptoms.

95.5

ELECTROCONVULSIVE SHOCK INCREASES α_{1B}-ADRENERGIC RECEPTORS BUT NOT α_{1A} OR NE-STIMULATED INOSITOL PHOSPHATE FORMATION IN RAT BRAIN. G.N. Pandey*, S.C. Pandey, L. Isaac, J.M. Davis. Illinois State Psychiatric Institute and University of Illinois, Chicago, IL, 60612

Repeated administration of electroconvulsive shock (ECS) increases α_1-adrenergic receptors, as measured by [^{3}H]-prazosin binding. α_1-adrenergic receptors have been subdivided into α_{1A} and α_{1B} subtypes. The purpose of this study is to investigate whether ECS increases one or both α_1-adrenergic receptor subtypes in rat cerebral cortex. We also studied the effect of ECS on NE-stimulated [^{3}H]inositol-1-phosphate (IP_1) formation in rat cortex. ECS (75 mA for 0.2 seconds) was administered to rats once daily for 14 days by means of earclip electrodes. The rats were decapitated 24 hrs after the last shock, and brain was removed for separation of cortices. α_1-adrenergic receptors were measured by ±ß(^{125}I-iodo-4-hydroxy-phenyl)-ethyl-aminomethyltetralone binding using 5-methylurapidil as displacer. We observed that repeated ECS administration caused a significant increase in the number of α_1-adrenergic receptors and the α_{1B}-adrenergic receptor subtype in cortex. However, it had no effect on the α_{1A}-adrenergic receptor subtype or NE-stimulated [^{3}H]-IP_1 formation. These results thus suggest that upregulation of α_1-adrenergic receptors is not associated with changes in NE-stimulated PI turnover. The lack of effects on NE-stimulated [^{3}H]-IP_1 formation in ECS-treated rats may be due to its lack of effect on the α_{1A}-adrenergic receptor subtype.

95.6

BIOCHEMICAL MEASURES RELATED TO THERAPEUTIC RESPONSE TO A SPECIFIC SEROTONIN REUPTAKE INHIBITOR IN EARLY ONSET PRIMARY DYSTHYMIA. A.V. Ravindran, R.J. Bialik*, G.M. Brown[1], Y.D. Lapierre. Institute of Mental Health Research and Dept. of Psychiatry, University of Ottawa, Ottawa, Ontario K1Z 7K4 and Clarke Institute of Psychiatry[1], 250 College Street, Toronto, Ontario M5T 1R8.

This prospective study examined biochemical correlates of the therapeutic response to a specific serotonin reuptake blocker (SSRI). The study included twenty-six patients that satisfied DSM-III(R) criteria for dysthymic disorder with no concurrent illness. They also satisfied Akiskal's criteria for primary early onset dysthymia of the subaffective type. Psychiatric rating instruments and biochemical measures were taken following a one-week washout period and again following six weeks of pharmacotherapy with an SSRI. As a group, patients had significantly lower platelet monoamine oxidase compared to normal controls and 85% showed a therapeutic response to pharmacotherapy. Responders had marked reduction in both depression and anxiety symptoms as measured by the Hamilton Rating Scale for Depression and the Hamilton Anxiety Scale. Prior to treatment, responders had significantly higher: plasma oxidase activity; serum cortisol levels following dexamethasone; urinary melatonin (as indicated by its major metabolite, 6-sulphatoxymelatonin; and urinary metanephrine levels. Responders also had lower baseline urinary levels of the major metabolite of serotonin, 5-HIAA, and showed an increase in levels following treatment. In response to treatment, urinary excretion of the major metabolite of norepinephrine, MHPG, was reduced. Non-responders showed an increase in urinary metanephrine levels following treatment, which is indicative of increased adrenal medullary output. Surprisingly, five patients showed clinical non-non-suppression of cortisol by dexamethasone following treatment. Four of these patients were responders and continued to be symptom free during one year of follow up. In summary, indices of serotonin activity were lower prior to treatment and increased in patients that responded to treatment. Other biochemical measures were also associated with either a positive or negative therapeutic response. The findings support the belief that the use of SSRI's are an important component of the treatment regime for patients suffering from the subaffective subtype of chronic low grade depression.

95.7

EFFECTS OF CHRONIC ANTIDEPRESSANT TREATMENT ON CORTICOTROPIN-RELEASING FACTOR NEURONS IN THE RAT. C.B. Nemeroff,* M.J. Owens and D.L. Knight. Lab. of Neuropsychopharmacology, Dept. of Psychiatry, Emory Univ. Sch. Med., Atlanta GA 30322.

The unique distribution of CRF and CRF receptors within the CNS, its preeminent role in coordinating the endocrine, behavioral, autonomic and immunological effects of stress and its behavioral and physiological effects after direct CNS administration all support the hypothesis that alterations in CRF neuronal systems contribute to the pathophysiology of depression and perhaps certain anxiety disorders.

We hypothesized that the efficacy of antidepressant drugs may be due, at least in part, to their actions on CRF-containing neurons in the CNS. Although acute imipramine administration was without effect on regional brain CRF concentrations in the rat, the present study sought to investigate the effects of chronic imipramine infusion. Imipramine was infused via osmotic minipumps for 28 days with plasma imipramine and desipramine concentrations maintained at levels comparable to those observed in humans (i.e., combined imipramine and desipramine concentrations of >150 ng/ml plasma). A similarly treated group was exposed to acute restraint stress on day 28 to determine whether chronic imipramine administration attenuates the effects of stress on regional brain CRF concentrations. Chronic imipramine administration, as expected, decreased (35%) the density of cortical β-adrenergic receptors, as assessed by [^{125}I]-iodopindolol binding. Cortical 5-HT_2 receptor binding, as assessed by [^{3}H]-ketanserin binding was decreased by 40% and anterior pituitary CRF receptor binding was increased 20% after chronic administration. Acute stress did not alter either β, 5-HT_2 or CRF receptor binding. *In situ* hybridization and radioimmunoassay studies in these animals are currently underway. Supported by NIMH MH-42088.

95.8

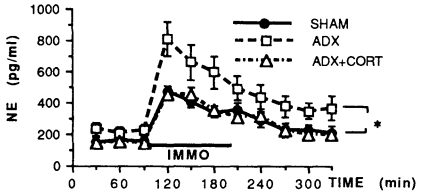
PRECLINICAL PHARMACOLOGY AND CLINICAL EFFECTS IN HUMANS OF DOPAMINE D-1 RECEPTOR ANTAGONISM. E.B. Nielsen*, P.H. Andersen, L. Farde, P. Karlsson, F.C. Grønvald, B.K. Skrumsager Pharmaceuticals Research, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark and Karolinska Hospital, S-10401 Stockholm, Sweden.

Biochemical receptor profiling of the benzazepine D-1 antagonists NNC-112, NNC-687 and NNC-756 indicated potent and selective D-1 antagonistic effect in the low nM range. The compounds were nonselective for D-5 vs D1A receptors. The selectivity and potency was confirmed in in vivo binding receptor assays in mice and rats. Animal pharmacological evaluation indicated that approximately 50% D-1 receptor occupancy exerted similar pharmacological antagonism as approximately 80% D-2 receptor occupancy in vivo. Thus, antipsychotic effects of D-1 antagonism may be expected at 50% receptor occupancy in vivo. Increasing doses (1-25 mg total) of NNC-687 were administered orally to human male volunteers. The highest doses produced approximately 50% receptor occupancy as determined by PET. Only "questionable" or mild akathisia was recorded in the subjects. Thus, 50% D-1 receptor blockade may convey antipsychotic effect in the absence of the D-2-receptor-related extrapyramidal syndromes of Parkinsonism and dystonia.

96.1

ADRENALECTOMY AUGMENTS *IN VIVO* RELEASE OF NOREPINEPHRINE IN THE PARAVENTRICULAR NUCLEUS DURING IMMOBILIZATION STRESS. K. Pacak*, R. Kvetnansky, M. Palkovits, I. J. Kopin, D. S. Goldstein. NINDS and NIMH, NIH, Bethesda, MD 20892.

Removal of endogenous glucocorticoids by adrenalectomy (ADX) activates corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) and increases hypothalamic norepinephrine (NE) turnover. Immobilization (IMMO) stress markedly increases release of NE into extracellular fluid in the PVN. This study assessed whether ADX affects release of NE in the PVN under basal conditions and during IMMO and whether cortisol (CORT) replacement attenuates the effects of ADX. *In vivo* microdialysis was used to measure microdialysate concentrations of NE, neuronal NE metabolite dihydroxyphenylglycol (DHPG), and the dopamine metabolite dihydroxyphenylacetic acid (DOPAC). Seven-10 days following ADX or ADX plus CORT (20 mg/kg/day), animals underwent 2 h of IMMO. Microdialysate collection began 24 h after probe implantation. ADX rats tended to have increased baseline NE, DHPG, and DOPAC levels and had significantly larger stress-induced responses of NE and DOPAC ($p<0.05$) than sham-operated animals. CORT replacement completely normalized both the elevated baseline NE and the exaggerated NE and DOPAC responses (Figure). These results indicate that ADX augments IMMO-induced release, metabolism, turnover, and synthesis of NE and that these effects are due to removal of endogenous glucocorticoids. Stress-induced release of NE in the PVN therefore appears to be regulated by feedback inhibition by glucocorticoids; conversely, enhanced PVN NE release after ADX is likely involved in the stimulatory effect of ADX on CRH release.



96.2

GLUCOCORTICOID ADMINISTRATION REVERSES AUGMENTED SYMPATHONEURAL RESPONSIVENESS TO IMMOBILIZATION STRESS IN ADRENALECTOMIZED RATS. **R. Kvetnansky*, K. Fukuhara, U.K. Weise, G. Cizza, K. Pacak, D.S. Goldstein and I.J. Kopin. Clin. Neurosci. Branch, NINDS, NIH, Bethesda, MD. 20892.**

The sympathoadrenal and hypothalamic-pituitary-adrenocortical systems interact at several levels. Adrenalectomy elevates basal and stress levels of plasma norepinephrine (NE), and this effect was reversed by glucocorticoid administration. The present study investigated plasma levels of catecholamine precursor DOPA, NE metabolites dihydroxyphenylglycol (DHPG), methoxyhydroxyphenylglycol (MHPG), and dopamine (DA) metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in adrenalectomized or sham-operated animals with or without glucocorticoid treatment (cortisol 25 mg/kg/24h for 7 days by osmotic minipumps) in rats at 5, 20, 60, and 120 min. of immobilization stress. Adrenalectomy eliminated the plasma levels of epinephrine and corticosterone but increased basal and immobilization-related levels of NE, DHPG, MHPG, DOPA, DOPAC, and HVA. Cortisol administration to adrenalectomized rats (plasma levels about 600 pmol/ml) reversed the increases in these parameters. The results indicate that adrenal glucocorticoids restrain stress-induced increases in catecholamine release, reuptake, metabolism, and synthesis.

96.3

ACUTE STRESS OR ETHANOL (ETOH) CAN HAVE A VERY LONG LASTING INFLUENCE ON IN VITRO DOPAMINE (DA) RELEASE FROM STRIATAL SLICES

S.M. Antelman, A.R. Caggiula, D.J. Edwards*, S. Kiss, D. Kocan, H. Barry, III *Depts. of Psychiatry, Psychology and Pharmacology/Physiology, University of Pittsburgh, Pittsburgh, PA. 15213*

We have shown that one exposure to stress or ETOH has long term effects on drug responsiveness of male rats as reflected by blunted behavioral (catalepsy) and neurochemical (increased medial frontal cortical DOPAC/DA ratio) responses to haloperidol. The present 2 studies found that when an i.p. injection of ETOH (0.5 or 2 g/kg) or a stressor (2 ml/kg saline) was given either 30-120 min or 2 wks before sacrifice, it produced a 60-80% decrease in DA release in response to amphetamine (AM; 2.5-10 μM) from striatal slices. However, when ETOH was given **BOTH** 2 wks **AND** 30 min before sacrifice, the earlier treatment significantly prevented the ability of the latter to decrease AM-induced DA release. These findings suggest that the effect on DOPAC/DA ratio earlier observed probably does reflect a change in DA release. Furthermore, by showing that prestress can act to both decrease and prevent the decrease in AM-induced DA release, they extend our previous findings on the long term, biphasic actions of stress on drug responsiveness. Supported by MH24114 & P50AA08746.

96.4

FOS-IMMUNOREACTIVITY (F-IR) IN BRAINS OF RATS EXPOSED TO INESCAPABLE SHOCK OR ADMINISTERED CORTICOTROPIN-RELEASING FACTOR (CRF). R.J. Valentino*, S. de Boer, P. Bicanich, Bowen Kang and G. Aston-Jones. Div. of Behavioral Neurobiology, Dept. of Mental Health Sci., Hahnemann Univ., Philadelphia, PA 19102, U.S.A.

Immunoreactivity of Fos, the protein product of the gene, c-fos, was compared in brains of rats exposed to 30 min of mild footshock (1.5 mA, 0.5 ms, 0.033 Hz) and rats administered CRF (3.0 µg, i.c.v.). Control rats for the 2 groups were placed in identical chambers as shocked rats but were not shocked, or were administered saline (3 µl, i.c.v.), respectively. Rats were perfused 2 h after the procedures and 30 µm coronal sections were cut and processed for F-IR. The distribution of F-IR in shocked rats included the nucleus tractus solitarius, A1 area, cuneate nucleus, nucleus paragigantocellularis,dorsal cochlear nucleus, locus coeruleus, central gray, Barrington's nucleus, periaqueductal gray, rostral Kolliker-Fuse,. habenula, lateral hypothalamus, supramammillary nucleus, paraventricular nucleus of the hypothalamus, and the cortex. Double labeling studies demonstrated tyrosine hydroxylase-IR neurons in the A1 area, nucleus paragigantocelluaris, and locus coeruleus that were also F-IR. Likewise, all F-IR neurons in Barrington's nucleus of sampled sections were CRF-IR. The distribution of F-IR in brains of rats administered CRF was similar to that observed in brains from shocked rats. However, greater numbers of F-IR neurons were observed in the spinal trigeminal nucleus, vestibular nuclei, olivary nuclei, nucleus prepositus hypoglossi, lateral parabrachial nucleus, medial central gray, pontine nuclei, central nucleus of the amygdala, and cortex of rats administered CRF. Moreover, F-IR was much less or absent in the locus coeruleus and cochlear nucleus of rats administered CRF. F-IR was virtually absent in brains from control rats. The similarities in F-IR distribution in the two conditions suggest that endogenous CRF is involved in, but is not the sole mediator of neuronal activation by inescapable shock. Supported by MH 40008, MH 00840 and NS 24698.

96.5

STRESS PREFERENTIALLY ENHANCES EXTRANEURONAL LEVELS OF EXCITATORY AMINO ACIDS IN THE PREFRONTAL CORTEX: COMPARISON TO HIPPOCAMPUS AND BASAL GANGLIA, Bita Moghaddam,* Dept. of Psychiatry, Yale Univ. Sch. Med., VA Med. Center, 116A/2 West Haven, CT, 06516.

The technique of intracerebral microdialysis was used to assess the effect of stress on the extracellular (EC) concentration of excitatory amino acids (EAAs), glutamate (GLU) and aspartate (ASP), in the rat prefrontal cortex (PFC), hippocampus, striatum, and nucleus accumbens. A 20-minute restraint procedure led to enhancement of EC GLU levels in all regions tested. The increase in GLU levels was significantly higher in the PFC than that observed in other regions. With the exception of the striatum, EC levels of ASP also were increased in all regions. Furthermore, ASP levels were higher in PFC compared to hippocampus and nucleus accumbens. In order to ensure that the above results were not an artifact of restraint that is not associated with stress (e.g. decreased mobility), we also examined the effect of swimming stress on the EC levels of EAAs in selected regions, i.e., striatum and medial PFC. Both regions displayed a significant increase in EC ASP and GLU following 20 minutes of swimming in room temperature water. This study provides direct evidence that stress enhances the EC levels of EAAs and that EAAs may be involved in adaptive stress response. These findings also may have implications for stress-induced catecholamine release and/or hippocampal degeneration.

96.6

LATERAL SEPTAL STIMULATION ABOLISHES COLD-IMMOBILIZATION STRESS-INDUCED ULCERS. E. Yadin* and E. Thomas. Department of Psychiatry, The Medical College of Pennsylvania, Philadelphia, PA 19129, and Department of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010.

The involvement of structures within the limbic system in exacerbation or mitigation of stomach ulcers induced by immobilization stress procedures in rats has been widely documented. Much of the work has focused on the central nucleus of the amygdaloid complex (Henke, Neurosci Biobehav Rev 12:143-150, 1988), stimulation of which resulted in increased number and size lesions. Food-deprived rats were restrained in a Plexiglass restrainer for 3 hours at a temperature of 4°C. Throughout the session, experimental animals received brain stimulation, 50 microamp square wave trains, 0.1 ms pulse duration, at a rate of 100 pulses/sec, delivered every 90 sec, via bipolar electrodes implanted earlier in their anterior dorsolateral septum. Implanted control animals were restrained but received no brain stimulation. At the end of three hours animals were anesthetized, blood drawn cardially for corticosteroid analysis, stomachs removed and cut along the greater curvature for ulcer formation analysis, and brains removed and fixed for electrode tip location determination. Lateral septal stimulation during the stress procedure dramatically reduced the formation of stress-induced gastric lesions. The experimental animals' stomachs had virtually no hemorrhages and ulcers, while the non-stimulated controls' stomachs showed severe ulceration. The results suggest a stress-inhibitory role for the lateral septal nucleus.

Supported in part by Ciba-Geigy Corporation.

96.7

EFFECTS OF 2DG-INDUCED GLUCOPRIVIC STRESS IN HUMANS AND THE RAT. A. Breier*, A.M. Crane, O.R. Davis, R.W. Buchanan, C. Kennedy, and L. Sokoloff. MD Psychiatric Research Cntr, Univ of MD Sch Med, Baltimore, MD. 21228.

Interruption of glucose metabolism is a potent CNS stressor. Administration of pharmacologic doses of 2-deoxyglucose (2DG) results in inhibition of glycolysis and has been used as a paradigm to examine the effects of glucoprivation in clinical and preclinical studies. We report now the results of two studies examining the effects of 2DG-induced glucoprivation on classical stress systems in man and local cerebral blood flow (lCBF) in the rat. 2DG (50mg/kg), placebo, and the high potency benzodiazepine alprazolam (1.5 mg) were administered to 10 healthy humans. 2DG resulted in 50-fold increases in plasma epinephrine, 15-fold increases in plasma ACTH, and modest increases in plasma norepinephrine. Alprazolam pretreatment significantly attenuated 2DG-induced plasma epinephrine and ACTH elevations but did not effect plasma norepinephrine. In the second study, 2DG (500mg/kg) (N=6) or saline (N=6) were administered IV to normal adult male Sprague-Dawley rats and lCBF was subsequently determined with 14-C-iodoantyprine while the animals were conscious and unrestrained. 2DG produced increases in lCBF in most regions with the largest effects in cortical areas, caudate-putamen, nucleus accumbens, and thalamus. Smaller changes occurred in amygdala, lateral lemniscus, dorsal raphe, and hypothalamus. The implication of these data will be discussed.

96.8

Prolonged stress does not reduce increased blood-spinal cord barrier (BSB) permeability in experimental autoimmune encephalomyelitis (EAE). W.D. Lo*, A.C. Wolny, A. Griffin, C.C. Whitacre. Ohio State University, Columbus, OH, 43210. Prolonged stress in female Lewis rats suppresses EAE, but the mechanism is unknown. Since stress increases plasma corticosterone, we hypothesized that stress exerts its effect by reducing the BSB permeability that occurs in this model. EAE was induced by injecting guinea pig myelin basic protein (MBP) and Freund's adjuvant. Stressed animals were restrained for 9 hrs beginning 5 days before MBP injection; controls were food and water deprived for the same period. Controls demonstrate peak signs of disease 14 days after induction while stressed animals show significantly decreased signs of EAE. We measured the PS product to 125I-albumin and 3H-AIB in separate groups with a tissue sampling method. There was no difference between the two groups for the 125I-albumin and AIB PS products. Indeed, the AIB PS product was slightly increased in stressed compared with control animals. These results suggest that stress does not inhibit the development of increased BSB permeability in EAE, and imply that the effect of stress is mediated through some other mechanism.

96.9

OPTICAL REFLECTANCE IN THE CAT DORSAL HIPPOCAMPUS AFTER EXPOSURE TO AROUSING STIMULI. M.P.Kristensen*, D.M.Rector, G.R.Poe and R.M.Harper. Interdepartmental Program in Neuroscience and Department of Anatomy and Cell Biology, UCLA Sch. of Med., Los Angeles, CA, 90024.

Electrical activity of the hippocampus is greatly modified during arousal. We measured the functional topography of cellular activity in the dorsal hippocampus following exposure to arousing stimuli by assessing patterns of 700 nm reflected light. A 1.6 mm coherent optical probe and charge coupled device camera were placed on the dorsal hippocampus. Electrodes were also placed for monitoring EOG, ECG, diaphragmatic EMG, and cortical and hippocampal EEG to determine state and EEG characteristics.

After surgical recovery, images were collected every 2-3 s from freely behaving cats at times synchronous with the ECG R-wave before, during, and after exposure to arousing stimuli of different modalities (acoustic noise, intravenous injection of saline, and complex auditory/visual stimuli). Images were digitized, averaged, subtracted between conditions, and subjected to ANOVA. All stimuli accentuated respiratory and cardiac rates as well as EMG activity. Concomitant with these physiological changes, significant overall increases in optical reflectance from the dorsal hippocampus occurred in response to complex stimuli and saline injection, indicating decreased cellular activity. The reflectance changes appeared immediately after exposure to the stimulus during waking. No such change in reflectance could be detected after exposure to simple acoustic stimuli.

These findings suggest that arousing stimuli of a complex nature and/or particular modalities reduce overall activity of the dorsal hippocampus, whereas simple stimuli of other modalities have little overall effect on this area. (Supported by HL22418)

96.10

NEOPHOBIA AND NEOPHILIA IN SOCIALLY ISOLATED RATS. FS Hall*, TW Robbins, T Humby and LS Wilkinson. Dept. of Experimental Psychology, Cambridge University, Cambridge, England.

Male Lister Hooded rats were divided into socially housed and isolation housed groups at day 21 postnatal. Testing began after 8 weeks of housing. In the first experiment half of each group of subjects were habituated to an open field, half were not. Subjects were pre-exposed to either chocolate chip cookies or cheddar cheese in the home cage. Neophobia testing was done in a 1m^2 open field for 15 minutes with food bowls containing each of the foods placed in the center of the open field (one novel and one familiar for each rat). For each food the following measures were observed: latency to contact and eat the food, the duration of contact and eating, the number of bouts of contact and eating and the amount consumed. In addition locomotion, rearing and grooming were also scored. The basic method was replicated: all rats ate more of the familiar food, for a longer time and at a shorter latency. Habituation to the environment reduced the latency to eat the familiar food, locomotor acitivity and the time between contacting each food and eating each food. Isolates showed an increased latency to contact and to eat the familiar food. There was no difference for the novel food. In a second experiment social and isolated rats were confined to one side of a two-sided apparatus for 1 hour. They were then placed in a choice chamber and allowed free movement between both sides. Isolates spent more of their time on the novel side than socially housed rats. The neophilia observed in the experiment 2 may have acted in experiment 1 to counteract neophobia for the novel food.

96.11

INVOLVEMENT OF CORTICOSTEROIDS IN THE PROCESSING OF STRESSFUL LIFE-EVENTS: A POSSIBLE IMPLICATION FOR THE DEVELOPMENT OF DEPRESSION. B.W.M.M. Peeters, C.L.E. Broekkamp and J.A.D.M. Tonnaer*. Dept. of Neuropharmacology, Organon Int. B.V. P.O.Box 20, 5340 BH Oss, The Netherlands.

In a subpopulation of endogenously depressed patients disturbances of the hypothalamus-pituitary-adrenal (HPA) axis occur. Cortisol is increased and dexamethasone suppression is blunted. However, the relationship between these disturbances and the development of depression is unknown.

Corticosteroids act mainly via two receptor types, the mineralocorticoid (MR) and the glucocorticoid (GR) receptor. These two receptors differ in a number of aspects among which, their affinity for cortisol (MR: high affinity; GR: low affinity).

By means of the behavioural despair paradigm, we investigated the role of both receptors in the processing of stressful life-events. In this paradigm, mice have to adapt to a stressful event (water stress) and retain the adaptation response until retest. It was shown that the MR receptor is critically involved in the acquisition of the adaptation response. The MR receptor agonist aldosterone increased the acquisition speed in adrenalectomized animals while the MR antagonist spironolactone had an opposite effect in controls. The GR receptor appeared to play a role in the consolidation of the response. Adrenalectomy disturbed the consolidation process and this could be restored by means of the glucocorticoid agonist dexamethasone.

Corticosteroid levels gradually increased during the test (duration 15min), resulting in peak levels at the end of the session. This also is in agreement with an involvement of the MR receptor in the acquisition process and of the GR receptor in the consolidation.

Our results indicate that both the MR and the GR receptor are involved in the processing of stressful life-events. The MR receptors seem to be involved in the adaptation process itself while the GR receptors appear to play a role in remembering the event and the displayed response. In depressed patients, cortisol levels are chronically elevated which means that GR receptors will be activated after mild stressors which induce only a small extra steroid release. Such aberrant response on mild stressors might play a role in the pathology of the depressed patient.

97.1

IN VIVO IMAGING OF BRAIN ACETYLCHOLINESTERASE WITH [^{11}C]PHYSOSTIGMINE. B. Tavitian*, S. Pappata, A. Jobert, F. Hinnen, C. Crouzel and L. DiGiamberardino. INSERM U334, CEA, SFHJ, Orsay, France.

Acetylcholinesterase (AChE) activity is dramatically reduced in specific areas of the brain in patients suffering from some neurodegenerative disorders. For example, AChE is diminished in the caudate-putamen of Huntington's disease patients and in the cortex of Alzheimer's disease patients, while there appears to be no change in its activity in Parkinson's disease. *In vivo* measurement of this enzyme's activity would thus provide a means to define and quantify regional cholinergic deficits in the brains of these patients for diagnostic and therapeutic purposes.

Our group is engaged in the search for ligands suitable for the imaging of AChE activity in the living brain using Positron Emission Tomography (PET). Previous studies using an [^{11}C]-labeled derivative of THA (tacrine) have led to the conclusion that the distribution of THA binding sites does not reflect AChE activity in the brain (Tavitian et al., submitted). In the present study, we labeled physostigmine (PHY), a competitive inhibitor of AChE, and performed PET imaging in baboons with an ECAT 953B camera.

Results from both *ex vivo* experiments in rodents and *in vivo* imaging in non-human primates demonstrate a strong match between the localization of [^{11}C]PHY and AChE activity. After injection of [^{11}C]PHY, the radioactivity is rapidly cleared from the blood and taken up into tissues (brain peak at 1.5 min). Early PET images reflect essentially the blood-flow dependant distribution, while images obtained later than 12 minutes after the injection show a specific uptake in structures known to have the highest AChE activity such as the caudate-putamen. The ratio of cerebellar to striatal uptake is 3 at 30 min. Wash out of the radioactivity is more rapid in the cerebellum than in the caudate putamen (half-lives 8 and 30 min, respectively).

In contrast to THA or its derivatives, [^{11}C]PHY thus appears to represent a good marker of brain AChE activity, which makes it of considerable clinical interest. Work is now in progress to investigate the use of [^{11}C]PHY for *in vivo* imaging of human neurodegenerative disorders.

Supported in part by the Mutuelle Générale de l'Education Nationale.

97.2

PRO-IL-1β IS PROCESSED TO ITS MATURE FORM BY THE CYSTEINE PROTEINASE CATHEPSIN B: A POTENTIAL THERAPEUTIC TARGET IN ALZHEIMER DISEASE. R. Ryan, L. Greco, Z. Stengelin and P.L. Wood*. Mayo Clinic, Jacksonville, FL 32224 & Hoechst Pharma, NJ.

Interleukin 1β (IL-1β) is a cytokine generally associated with macrophages that mediates a broad spectrum of biological actions on immune and inflammatory responses, as well as being a modulator of CNS activity. Several recent studies have indicated that IL-1β may play a detrimental role in Alzheimer's disease. Cathepsin B (CB) is a cysteine proteinase observed to be strongly induced in activated macrophages as well as being associated with neuritic plaques in Alzheimer brains. Catalytically active CB has been observed in the synovial fluid of arthritis patients. Since CB and IL-1β appear to be temporally associated in different pathological conditions we examined the ability of CB to process 36 kDa pro-IL-1β into its 17 kDa mature species.

A time dependent conversion of 36 kDa pro-IL-1β into the mature 17 kDa species by CB was observed and this conversion was inhibited by the thiol proteinase inhibitors E-64, cystatin and leupeptin. Bestatin an aminopeptidase inhibitor and the serine proteinase inhibitors secretory leukocyte protease inhibitor and elastin did not effect the processing. The addition of CB and Pro-IL-1β to boiled Alzheimer CSF also resulted in the processing of the precursor, indicating that CB may be active in the Alzheimer physicochemical environment. These data suggest that if pro-IL-1β gains access to environments rich in CB it may be processed to the 17 kDa biologically active species. [Support: Mayo Foundation]

97.3

PRETREATMENT WITH THE 5-HT3 RECEPTOR ANTAGONIST, ZACOPRIDE, BLOCKS THE EMESIS INDUCED BY THE MUSCARINIC AGONIST, CI-979, IN THE FERRET. H.J. Altman*[1], H.J. Normile[1], M. Callahan[2] and R. Davis[2]. [1]Dept. of Psychiatry, Wayne State Univ. Sch. Med., Detroit, MI 48207; [2]Dept. of Pharmacol., Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

The purpose of the study was to determine whether pretreatment with the 5-HT3 receptor antagonist, zacopride, could effectively block the emesis induced by the muscarinic cholinergic receptor agonist, CI-979, in the ferret. CI-979 was injected sub-Q at a dose of 0.1 mg/kg. At this dose all animals exhibited substantial retching. Thirty minute (sub-Q) pretreatment with zacopride significantly attenuated retching at all doses examined.

This study represents, therefore, the first successful demonstration of an antagonism of cholinomimetic-induced emesis by a drug that does not act directly on cholinergic neuro-transmission. These results are particularly important in light of the potential usefulness of cholinomimetics in the treatment of memory loss associated with Alzheimer's disease.

97.4

INCREASED LEVELS OF TRUNCATED NGF RECEPTOR IN URINE OF MILDLY DEMENTED PATIENTS WITH ALZHEIMER'S DISEASE. M.D. Lindner*, D.D. Gordon, J.M. Miller, P.N. Tariot, K.D. McDaniel, R.W. Hamill, P.S. DiStefano, and R. Loy. Univ. Rochester Sch. of Med., Monroe Community Hospital, 435 E. Henrietta Rd., Rochester, NY 14620.

Research on peripheral Schwann cells has shown that the extracellular portion of the NGF receptor is truncated and excreted in urine. Following peripheral axotomy, the number of Schwann cell NGF receptors and urine levels of the truncated NGF receptor (NGF-Rt) increase. NGF receptors are also located on cholinergic basal forebrain neurons that degenerate in Alzheimer's disease, and the present study was conducted to determine if urine levels of NGF-Rt might be altered in patients with Alzheimer's disease.

A new protocol for an enzyme-linked immunosorbent assay was developed to measure urine levels of NGF-Rt in non-demented aged controls (n=19) and patients with dementia of the Alzheimer type (n=62). Dementia severity was quantified with the expanded Clinical Dementia Rating.

Urine levels of NGF-Rt were substantially elevated in mildly demented patients and decreased to low levels in severely demented patients. The initial increase in urine levels may represent an upregulation in the production of NGF receptors, and/or an increase in the number of receptors being shed as a consequence of the degeneration of NGF receptor-containing neurons, but their precise source remains to be determined. (AG09231,AG08231,K07MH00733,AG03644

97.5

CEREBRAL GLUCOSE TRANSPORTER DEFICITS IN ALZHEIMER DISEASE: REDUCTION IN GLUT1 AND GLUT3 ISOFORMS. I. A. Simpson*, K. Chundu, T. Davies-Hill and P. Davies[1]. NIDDK /NIH, Bethesda MD. and [1]Albert Einstein College of Medicine,Bronx, NY.

PET scanning studies in patients with Alzheimers disease (AD) have revealed a reduction in glucose metabolism in parietal and temporal cortex. In this study we have determined the level of expression of the GLUT1 and GLUT3 glucose transporter isoforms in six regions (frontal, parietal, occipital and temporal cortex, hippocampus and caudate nucleus) of post mortem brains from 12 AD (68-85 y) and 12 control subjects (21-96 y). GLUT1 and GLUT3 expression was measured by Western Blot analysis using specific antibodies raised against the human erythrocyte glucose transporter (GLUT1) and the C-terminal amino acid sequence of human GLUT3. Averaging the GLUT1 data for all regions revealed a significant 45-50% ($p < 0.05$) deficit in both the microvessel (M_r 55 Kda) and the neuronal/ glial (M_r 45 Kda) forms of GLUT1. An overall reduction in GLUT3, the neuron-specific glucose transporter was also observed with significant reductions being observed in the parietal (67%), temporal cortex (65%) and the hippocampus (56%) of the AD subjects. These decreases correlate well with the deficits in cerebral glucose metabolism and neuronal function .

97.6

SIGNIFICANT DECREASE IN ADENYLATE CYCLASE ACTIVITY PRECEDES THE FULL DEVELOPMENT OF ALZHEIMER-RELATED NEUROFIBRILLARY CHANGES, T.G. Ohm[1], M. Schmitt[2], B. Lemmer[2] and J. Bohl[3] (1) Z.Morph. & (2) Z.Pharm J.W.Goethe-Univers., 6000 Frankfurt/M, (3) Neuropath, J.Gutenberg-Univ., 6500 Mainz, FRG

A water-soluble forskolin analogue (F) was used to activate adenylate cyclase (AC) in postmortem hippocampi of 41 demented and non-demented individuals. None of the former had neurological or histological signs other than Alzheimer-related changes, the latter even not these. The 41 brains were staged (Braak and Braak 1991, Acta Neuropath) and classified into controls (Contr), Stages I-IV and Alzheimer-patients (Alz). Basal as well as stimulated AC activity [pmol cAMP/mg protein/min] was measured by formation of cAMP from 0.5 mM ATP in the presence of an ATP-regenerating system, suspensions were incubated for 6 min at 37°C without and with 100μM F; cAMP was determined by radioassay and protein content measured after Lowry.

Stage	n	AC basal Mean ± SD [pmol/mg/min]	AC 100μM F Mean ± SD [pmol/mg/min]
Contr	5	32.38 ± 10.76	229.66 ± 63.48
I	9	29.36 ± 12.96	200.07 ± 66.99
II	6	22.88 ± 6.16	130.82 ± 32.06
III	9	21.80 ± 6.33	123.86 ± 43.35 *
IV	1	24.20 ± 0.00	147.30 ± 0.00
Alz	11	12.00 ± 4.68 *	98.81 ± 41.40 *

* indicates significant ($p<0.01$) vs controls
ANOVA-Scheffé-test (tested without stage IV)

The data show that the reduction in cAMP-formation is significantly correlated with the degree of Alzheimer-related neurofibrillary changes. Moreover, a considerable reduction of hippocampal cAMP-formation occurs already in stages, in which neurofibrillary alterations are almost confined to the entorhinal cortex.

Support: DFG (Oh 48/1-2)

97.7

LOSS OF DOPAMINE D2 RECEPTORS IN THE TEMPORAL LOBE AND HIPPOCAMPUS OF DEMENTING DISEASES

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We have previously described the autoradiographic mapping of the D2 receptor with [^{125}I]epidepride in human brain (Joyce et al., JPET, 253:1253 - 1263,1991). The temporal cortex, except for primary auditory cortex, shows 3-fold higher numbers of binding sites than prefrontal cortex. In the middle and inferior temporal cortex the predominant pattering is of D2 receptors in the deep laminae. At the border of the collateral sulcus this pattern changed such that D2 receptors are located in the superficial and deep laminae. The perirhinal cortex (PR) showed dense binding in the external laminae only, whereas the entorhinal cortex (EC) showed very low binding. The projection zone of the PR within the dentate gyrus (DG) exhibited D2 binding sites as did the the CA3 and subicular fields of the hippocampus and the basolateral nuclei of the amygdala. These data suggest that the D2 receptor is an important mediator of the actions of dopamine (DA) in limbic cortex. We explored this further by examining the binding in 6 cases of dementia (means of 64.8 yrs age, 9.5 hrs PMI) and 7 control cases (means of 71.5 yrs age, 9.1 hrs PMI). 4 of the 6 cases of dementia were confirmed as Alzheimer's disease (AD)and 2 cases diagnosed at autopsy as cortical atrophy. In 5 of 6 cases there was a marked reduction in the density of D2 receptors in the DG, CA3, subiculum and PR. In the AD but not the cortical atrophy cases there was , in addition, a marked reduction of D2 receptors in the basolateral nucleus of the amygdala. An additional 6 schizophrenic cases and 5 AD cases are currently being processed for D2 receptor autoradiography. Given the important roles that the PR, EC and hippocampus play in memory, it is likely that the actions of DA at D2 receptors can differentially modify memory functions in humans. This is the first direct evidence that the expression of DA receptors is modified in the limbic lobe of the dementias and could contribute to the symptoms. **Funded by MH43852, MH 43880, AG 09215.**

97.8

THE LOCUS COERULEUS AND DEPRESSION IN ALZHEIMER'S DISEASE.

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We previously reported a relationship between depression and severity of neuronal loss in the locus coeruleus (LC) in Alzheimer's disease (AD) [Ann Neurol 1988;24:233-242]. Using similar methods, we counted neuronal profiles at up to three levels of LC in 23 new AD cases. Eight depressed cases (mean age 65.9) had fewer profiles than 15 nondepressed cases (mean age 78.7) at all LC levels: 9.9(±8.8) vs 17.1(±9.6) rostral; 23.8(±18.1) vs 63.1(±21.3), p=.006 mid; and 49.0(±7.9) vs 74.0(±27.3), p=.035 caudal (1 tailed t-tests). Severity of AD-related pathology in sections of inferior temporal gyrus were comparable in the two groups. When 7 depressed and 12 nondepressed cases with definite severe dementia (last MMSE<6) were compared (post hoc), the significant mid level difference persisted. When age-matched subsets of depressed (n=4) and nondepressed (n=9) cases were compared (post hoc), significant differences at mid and caudal levels persisted. These results, similar to those of our original study, suggest that LC pathology may have a role in depression occurring in AD.

97.9

BASALOCORTICAL GABAERGIC AND CHOLINERGIC NEURONS CHARACTERIZED ELECTROPHYSIOLOGICALLY AND IMMUNOCYTOCHEMICALLY IN CULTURE.

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The existence of cholinergic magnocellular neurons projecting from the basal forebrain to the neocortex and hippocampus is well established. We have obtained evidence that in addition there are gabaergic basalocortical neurons. Basalocortical cells were labelled retrogradely in vivo with injections of DiI on postnatal day 5 or 6, and on day 8 the magnocellular basal forebrain area was dissociated enzymatically and placed in monolayer culture on small collagen islands (Baughman, et al. '91). After 14-90 days in culture, whole-cell patch recordings of membrane potentials and currents were made from 129 magnocellular basal forebrain cells. Action potential widths of the cells fell into two populations with means of 0.9±0.08 ms (n=25, 19 % of total) and 1.9±0.5 ms (n=104, 78% of total) at half height. Previously we found that APs of identified gagaergic and glutamatergic cortical neurons fell into two similar populations. Synaptic responses were studied in cocultures of basal forebrain and cortex. Of 11 narrow-AP cells studied in this way, 3 produced ipsps that were blocked with bicuculline and that reversed at about -60 mV. When cultures were stained immunocytochemically for gaba, 25% of the DiI-labelled cells were positive for gaba; this complements our previous finding that 74% of the basalocortical cells are ChAT positive. In summary, we find that a significant proportion, about 20%, of the cells in the basalocortical pathway appear to be inhibitory gabaergic neurons. Identification of the role of this system is important in view of the involvement of the basalocortical pathway in Alzheimer's disease and memory.

97.10

EXCITOTOXIN INDUCED LESIONS OF THE RAT VENTRAL GLOBUS PALLIDUS AND MEDIAL SEPTUM. AN IMMUNOCYTOCHEMICAL ANALYSIS OF LONG TERM COMPENSATORY EVENTS.

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Microinjection of quisqualic acid (QA)(60mM/ul) and ibotenic acid (IA) (25mM/ul) were made into the medial septal nucleus (.6ul) and the ventral globus pallidus (GP)(1ul) of adult male Sprague-Dawley rats. Decreased neuronal levels of brain-derived neurotrophic factor (BDNF) and glial basic fibroblast growth factor (bFGF) immunoreactivities (IR) were detected in the hippocampal formation at 1 year but not 13 days postlesion of the septum and ventral GP. In contrast to the 1 year septal lesions, which also produced a reduction in the number of BDNF IR cells in the septal area the QA and IA induced GP lesions produced an increase in the number of BDNF IR cells in this area. The 1 year QA and IA induced septal lesions were also associated with increases in c-fos IR within the lateral septal nucleus. All the induced lesions were associated with a partial disappearance of cholinergic nerve cell bodies and their nerve terminal networks within the hippocampal formation and the cerebral cortex as studied by choline acetylase and acetylcholine esterase immunocytochemistry. Within the ventral GP but not within the medial septum nucleus, the nerve cell death was associated with the appearance of calcium deposits (alizarin staining, Vankossa staining) within the large pallidal neurons. This phenomenon began already at the early time interval. At the 1 year time interval large calcium deposits were found extracellularly as rounded profiles throughout the entire ventral GP and the adjacent dorsal part.

The present findings give evidence that subcortical excitotoxic lesions of nerve cells involving *inter alia* cholinergic neurons leads to altered and reduced presence of BDNF IR and of glial bFGF IR in cortical areas 1 year later, indicating reduced trophic support of and within these brain areas. Finally, calcium toxicity may be a major factor in the mediation of excitotoxin induced nerve cell death within the GP. Thus, calcium deposits in aging brain and in patients with dementia may be a consequence of pathological enhancements of glutamate transmission. (Supported by Swed. Med. Res. Council 04X/715 and Spanish FISss 90E0593-2E; G. Bendahan and LlBoatell are fellows of the PFPI, Spain).

97.11

RAPID DEPHOSPHORYLATION AND UPREGULATION OF τ IMMUNOREACTIVITY IN HEAT-SHOCKED CEREBRAL EXPLANTS. MODIFYING EFFECTS OF HYDROCORTISONE, ESTROGEN AND BIOCHEMICAL MATURATION.

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We showed that phosphorylation of the Tau-1 epitope is similarly altered in heat-shocked rats and patients with Alzheimer's disease (PNAS 88:4543, 1991). To produce a comparable in vitro model, we heat shocked 21-day-old fetal rat cerebral explants at 45°C for 15 min, 42°C for 15 min and 42°C for 90 min. Explants were analyzed at 0, 3, and 6 h after heat shocking by immunocytochemistry and qualitative and quantitative (^{125}I-labeled protein A) immunoblotting using the Tau-1 and Tau-5 monoclonal anti-τ antibodies. At all 3 time intervals (0, 3 and 6 h) in heat-shocked explants, the amount of τ recognized by Tau-5 doubled ($P<0.001$), the nonphosphorylated Tau-1 epitope was markedly increased ($P<0.001$) due to dephosphorylation indicated by a significant ($P<0.001$) reduction of the ratio of total τ to nonphosphorylated τ and loss or attenuation of the 60-kDa and accentuation of the 50-kDa τ polypeptides. Also, heat-shocked explants were more intensely stained than controls. These in vitro changes of the Tau-1 epitope were antithetic to those observed in situ. To investigate the in situ vs in vitro differences, 21-day-old explants were treated with 1 μM hydrocortisone or β-estradiol or both for 20 h and then heat-shocked or 35-day-old explants were heat-shocked. While all 3 pretreatments prevented the Tau-5 upregulation, only hydrocortisone reduced to non-significant levels the degree of Tau-1 dephosphorylation. While in 35-day-old explants biochemical maturation of τ to its adult isoform complement (50-, 52-, 60- and 66-kDa) did not prevent the above rapid (0 h) changes, heat-shocked explants were similar to controls by 6 h. Thus, both the external milieu and the state of differentiation modify the response of τ to stressful stimuli.

98.1

PRE- AND POSTSYNAPTIC PARAMETERS DURING DEVELOPMENT OF GABAERGIC SYNAPTIC TRANSMISSION IN TECTAL CULTURES.

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The aim of this study was to establish a culture assay for GABAergic synapses with increasing efficacy of transmission, and to find out which of the most relevant parameters (quantal size g_Q, number or release sites n, probability of release p) undergoes changes in the course of synapse development. Over a period of 3 weeks in vitro (DIV5-26) the fraction of neurons generating evoked IPSCs raised from 17% to 76%. Average amplitudes of evoked unitary IPSCs (range 3-640 pA) increased during this period by a factor of 4 (about 150 neuron pairs tested), thus indicating a significant rise in the efficacy of GABAergic transmission. In contrast, no developmental change has been observed in the average amplitudes of miniature somatic IPSCs and the quantal size of evoked IPSCs (g_Q about 110 pS). No increase was found also in whole cell current densities, as determined by evaluating current responses to saturating concentrations of exogenous GABA and whole cell capacitances. Changes in single channel activity in outside-out patches were also subtle and concerned only the concentration-dependence of desensitization. In contrast, bouton density significantly increased (from about 1 bouton per neuron at DIV3 to more than 40 boutons per neuron at DIV24) and the average probability of release nearly doubled.

Our results suggest that in this culture system synapse maturation was primarily the result of presynaptic sprouting and increased p in part of the terminals. Receptor accumulation, if present, has remained very low reaching less than 3-4 times the average density of $GABA_A$ receptors.

98.2

ANTIBODIES TO SYNAPTOPHYSIN INTERFERE WITH TRANSMITTER SECRETION AT DEVELOPING SYNAPSES.

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The functional importance of synaptophysin, a synaptic vesicle-specific protein, in transmitter release at neuromuscular synapses in *Xenopus* cell culture was studied by loading of synaptophysin antibodies into presynaptic neurons. Polyclonal antibodies or their Fab fragments were loaded into spinal neurons by injection into one of the early blastomeres of *Xenopus* embryos one day prior to culturing. Immunocytochemical staining confirmed the presence of the antibody in the cultured neruons. At synapses made by synaptophysin antibody-loaded neurons, the spontaneous synaptic currents (SSCs) showed marked reduction in frequency compared to control neurons loaded with pre-immune serum or non-specific antibody or in the absence of loading. The average amplitude of the SSCs of antibody-loaded neurons was similar to that of control neurons, suggesting that the antibody reduces the frequency of spontaneous ACh secretion without affecting quantal size. Antibody-loaded neurons showed reduced amplitude and increased failure rate, or even complete failure, in the impulse-evoked synaptic response. In an alternative method, the effect of acute application of antibody was examined by direct loading of antibody through a whole-cell pipette at the soma of a presynaptic neuron. This treatment also diminished the frequency of spontaneous synaptic currents as well as the amplitude of evoked responses, indicating an immediate presynaptic effect of antibodies. Taken together, these results suggest that functional synaptophysin is required for transmitter secretion.

98.3

ANDROGENIC NOT ESTROGENIC STEROIDS ALTER NEUROMUSCULAR SYNAPSE ELIMINATION.

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In the levator ani (LA) muscle of rats, synapse elimination (SE) is regulated by androgen: treatment of castrated male rats with testosterone propionate (TP) during SE prevents much of the normal loss of multiple innervation in this muscle. The present study sought to identify the active form of TP, since TP might be converted to either an estrogenic or androgenic metabolite before affecting SE in the LA. At P7, anesthetized male rats were castrated and then treated for the next 3 weeks with 1 of 5 treatments: 1) TP, 2) dihydrotestosterone propionate (DHTP), 3) estradiol benzoate (EB), 4) EB and DHTP, or 5) the oil vehicle. At P28, rats were killed and their LA muscle dissected and stained with tetranitroblue tetrazolium salts. The pattern of innervation in the LA was assessed by counting the number of different motor axons that contact individual muscle fibers. We found that the level of multiple innervation in the LA at P28 is unaffected by EB whereas DHTP appears to mimic the effect of TP. This finding suggests that the effect of T on SE is mediated through an androgen, such as T itself or DHT, acting upon androgen receptors. Since biochemical evidence suggests that T rather than DHT is active in the LA muscle, our present findings also suggest that androgen acts at a site(s) other than the LA muscle to influence SE. Supported by NS08686 and NS28421.

98.4

A POSSIBLE ROLE FOR ATP RECEPTORS IN THE EARLY DEVELOPMENT OF CHICK NEUROMUSCULAR SYNAPSES.

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ATP receptors (ATPR) are expressed on the surface of embryonic chick skeletal muscle cells during the time that neuromuscular synapses are forming. Functional ATPR disappear by birth, but reappear if the muscles are denervated. The observation that the expression of ATPR is correlated with synaptogenesis raises the possibility that ATPR may be involved in the initial stages of neuromuscular synapse formation. We have tested this hypothesis by co-culturing neurons and muscle in the presence of an ATPR antagonist.

Our experimental approach was to pre-incubate established cultures of myotubes for 30 minutes in media containing 50 μM DIDS. At this concentration, muscle ATPR are completely blocked (Thomas et al. Brit. J. Pharmacol 103:1963). Dissociated ciliary ganglion neurons were then added, and synaptic function was tested at various times after the addition of neurons. To assess synaptic function we evoked action potentials in neurons sitting directly atop muscle fibers with extracellular stimulation, and visually assayed muscle contraction as an indicator of synaptic function. Thus our assay detected only suprathreshold synaptic contacts.

In the absence of DIDS, some neurons made suprathreshold connections within an hour, and more than half of the neurons made suprathreshold connections by 12 hours. In contrast, in the presence of DIDS, no suprathreshold connections were detected within the first 12 hours of co-culture. The effect of DIDS seems likely to be associated with its ability to block ATPR, since DIDS had no effect on the ability of acetylcholine to elicit contraction in muscle cells. Suprathreshold synaptic connections eventually formed in the continuous presence of DIDS; the earliest such responses were noted at 14 hours, and responses were common by 24 hours. Thus DIDS delays, but does not prevent the formation of suprathreshold synaptic connections.

These results demonstrate that a blocker of ATPR can delay the maturation of synaptic function, and are consistent with the idea that ATPR may play a role in the early development of neuromuscular transmission.

98.5

ELEVATION IN PRESYNAPTIC CALCIUM LEVEL ACCOMPANYING INITIAL NERVE-MUSCLE CONTACT.

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Synaptogenesis at the neuromuscular junction (NMJ) is induced by nerve-muscle (N-M) contact as shown by the immediate onset of transmitter release upon contact in vitro. Since presynaptic calcium level plays a pivotal role in transmitter release, we used N-M cultures to examine the relationship between neuronal calcium level and the earliest N-M interaction. *Xenopus* spinal cord neurons were loaded with the membrane permeant calcium indicator fluo-3/AM and monitored with confocal microscopy as an unlabeled myoball was manipulated into contact with it. In 50% of N-M contacts, an increase in neuritic fluo-3 fluorescence was observed in the vicinity of muscle contact. To obtain more quantitative information, we used fura-2 ratio imaging to correlate the presynaptic calcium level with transmitter release monitored by whole-cell patch clamping of the contacting myoball. This analysis showed a 1.5 to 6-fold increase in calcium level in the neurite upon contact with a myoball in 80% of the N-M pairs. 50% of the pairs showing calcium elevation were also positive in neurotransmission. Both the calcium rise and transmitter release were suppressed by lifting away the myoball or by calcium-free solution. Suramin, a polyanionic compound that interferes with cell surface receptors, also suppressed the calcium rise and transmitter release, suggesting a role of cell surface molecules in mediating this phenomenon. In addition to manipulated contacts, naturally formed NMJs in culture also showed an elevation in presynaptic calcium level. Thus, this elevation appears to be a physiological step in the early stage of synaptogenesis and is likely mediated by interaction of molecules at the N-M cell surfaces. (Supported by NIH grant NS23583)

98.6

DISTRIBUTION OF AGRIN mRNAs IN THE DEVELOPING CHICK EMBRYO.

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Agrin is a protein associated with synaptic basal lamina that is thought to organize the postsynaptic apparatus at the neuromuscular junction, causing aggregation of ACh receptors and other postsynaptic components. Motor neurons appear to be the major source of synaptic agrin with this activity, but most other basement membranes also contain agrin-like molecules. In the chicken, agrin-like proteins from muscle, heart and kidney induce fewer and smaller ACh receptor clusters than neural agrin (Godfrey, Exp. Cell Res. 195:99, 1991). This difference in activity may reflect differential expression of proteins encoded by 3 alternatively spliced forms of agrin mRNA, the most active of which has only been found in the nervous system (Ruegg et al., Neuron 8:691, 1992). Here we report the distribution of mRNAs coding for agrin and closely related proteins in the developing chick embryo.

In situ hybridization was performed using digoxigenin-labeled cRNA probes specific for chicken agrin mRNAs, which should hybridize to all 3 isoforms (gift of Drs. K. Tsim and U.J. McMahan). Antisense but not sense cRNA hybridized specifically to many embryonic tissues. The most heavily labeled tissues were ventral spinal cord (presumably motor neurons), kidney and heart. Somitic myotome and early skeletal muscle were less intensely labeled. At embryonic day 3 (E3, st 18) spinal cord was uniformly stained, but by E4 (st 23) ventrolateral cells were much more heavily labeled. Intensity of signal in these presumptive motor neurons appeared to decrease steadily with development, as did signal in kidney, heart and muscle. In the brain, agrin mRNAs appeared as early as E7.5 and were localized to cranial motor nuclei, laminae in cerebellum and optic tectum, and ventricular zones. We are using polymerase chain reaction to define the distribution of agrin mRNA isoforms in regions of embryonic brain and other tissues. The agrin proteins may participate in synaptogenesis and other neuron-neuron interactions in the developing brain. Supported by NIH (NS27218, HD20743).

98.7

EXTERNALIZATION OF AGRIN-LIKE MOLECULES BY EMBRYONIC NEURONS. M.W. Cohen*, F. Moody-Corbett and E.W. Godfrey. Dept. of Physiol., McGill Univ., Montreal, Que., Div. of Basic Med. Sci., Memorial Univ., St. John's, Nfld. and Dept. of Cell. Biol. & Anat., Med. Coll. of Wisconsin, Milwaukee, WI.

In order to examine neuronal externalization of agrin-like molecules, spinal cord neurons from Xenopus embryos were cultured on a substrate which binds these molecules tightly. Immunofluorescent staining revealed agrin-like molecules along the path of many neurites, even in regions of new neuritic growth less than 1-hr-old. The molecules remained associated with the surface of the culture dish following removal of the neurites by aggressive rinsing. When embryonic Xenopus muscle cells were added to cultures after complete removal of the neurons and neurites, their acetylcholine receptors (AChRs) accumulated along the paths of substrate-bound agrin-like molecules. The lengths of AChR aggregation along these paths were similar to those which occur along neurite-muscle contacts in nerve-muscle co-cultures. Besides demonstrating that embryonic spinal cord neurons externalize agrin-like molecules along most of their neuritic growth, the findings are consistent with the notion that sufficient quantities are externalized to account fully for nerve-induced aggregation of AChRs at embryonic nerve-muscle synapses.

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98.8

DIFFERENTIAL DISTRIBUTION OF MEMBERS OF THE AGRIN PROTEIN FAMILY IN THE NERVOUS SYSTEM. S.E. Horton, M.A. Ruegg, G. Escher, S. Kröger and U.J. McMahan*, Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

Several lines of evidence indicate that the agrin is synthesized and released by motor neurons to induce the aggregation of AChRs and other components of the postsynaptic apparatus at the neuromuscular junction. The agrin gene also codes for agrin-related proteins (ARPs) which are inactive in protein aggregation on muscle fibers. In chick, agrin is distinguished from ARPs by an 11 amino acid insertion which is required for agrin's activity. As a step toward learning whether agrin is involved in the induction of postsynaptic apparatus at neuron-neuron synapses and defining the role of ARPs in the nervous system, we used PCR to screen for transcripts encoding proteins having or lacking the 11 amino acid insert in regions of the nervous system having or lacking neuronal mRNA. We found that optic nerve and sciatic nerve, which contain mRNA of endothelial and glial or Schwann cells but not neurons, had transcripts encoding ARPs but not agrin. On the other hand, the retina and dorsal root ganglion, which contain mRNA not only of endothelial cells and glial or Schwann cells but also neurons, had transcripts that encoded not only ARPs but also proteins with the 11 amino acid stretch typical of agrin. The results indicate that non-neuronal cells in the nervous system synthesize ARPs. They also lead to the conclusion that neurons other than motor neurons synthesize members of the agrin family and that these members have the 11 amino acid stretch required for agrin's protein aggregating activity.

98.9

CHARACTERIZATION OF A BASAL LAMINA BINDING DOMAIN IN AGRIN. M.J. Werle*, S. Kröger, K.W.K. Tsim, M. A. Ruegg, and U.J. McMahan, Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

The C-terminal half of agrin contains the domain that induces myotubes to form aggregates of AChRs and other postsynaptic proteins including basal lamina components. The C-terminal half has also been found to contain a domain that binds to isolated sheets of chick retinal basal lamina (Kröger et al., Soc. Neurosci. Abs., 1991, 17:179). To learn whether this basal lamina binding domain binds to postsynaptic components on the surface of myotubes, we transfected myotubes with cDNA constructs coding for the C-terminal half of active agrin either having or lacking a series of 100 amino acids that are required for binding to retinal basal lamina. We found that in both cases the myotubes secreted agrin which, in turn, induced the myotubes to form aggregates of AChRs and, accordingly, the other cell surface proteins. However, myotube protein aggregates induced to form by agrin having the basal lamina binding domain had associated with them aggregates of secreted agrin, whereas the protein aggregates induced to form by agrin lacking the basal lamina binding domain did not. These findings would be expected if the basal lamina binding domain in the C-terminal half of agrin recognizes basal lamina components of the neuromuscular junction. They are also consistent with the hypothesis (Nitkin et al., 1987, J. Cell Biol. 105:2471-2478) that at developing neuromuscular junctions agrin induces the aggregation of the basal lamina components to which it binds.

98.10

FLUORESCENCE PHOTOBLEACHING RECOVERY IN LIVING MICE DEMONSTRATES ACETYLCHOLINE RECEPTOR MOVEMENT IN POSTSYNAPTIC MUSCLE MEMBRANE A.P. Brown, P. van Mier*, J. W. Lichtman, Depts. Anatomy and Neurobiology, and Neurosurgery, Washington Univ. Sch. of Med., St. Louis, MO 63110

Acetylcholine receptor (AChR) mobility in muscle membrane may be important in the formation of synapses in the embryo, and perhaps also during synaptic reorganization in later life. Studies following AChRs for up to one day *in vitro* have suggested that, once inserted in the postsynaptic membrane, AChRs are essentially immobile. By monitoring AChRs at the living adult mouse neuromuscular junction using fluorescence photobleaching recovery, we assayed for receptor mobility over much longer times and thus could detect slower diffusion rates than previously possible. In young adult mice (n=50) the sternomastoid muscle was exposed, and AChRs stained with a saturating dose of rhodaminated α-bungarotoxin (Rh-αBTx). An 8 μm diameter spot of labelled AChRs was irreversibly bleached in a superficial junction using a He-Ne laser (0.5mW, 543.5nm). In subsequent views for up to 11 days (without adding new Rh-αBTx), progressive recovery of fluorescence occurred until the bleached area was nearly indistinguishable from the rest of the junction. Despite the decrease in fluorescence of the entire junction due to receptor turnover, the absolute brightness of the bleached spots increased for several days. When the entire junction was bleached, however, no significant recovery occurred over 5 days. Control experiments ruled out that fluorescence recovery was due to the unbinding and rebinding of αBTx. Our results show that AChR mobility in the adult postsynaptic membrane *in vivo* is slow when compared to that reported during synapse formation *in vitro*. Nonetheless this movement is rapid enough to explain the disappearance of postsynaptic sites during postnatal synapse elimination. We are currently studying AChR movement at developing junctions and after denervation.

VISUAL CORTEX: FUNCTIONAL ORGANIZATION OF STRIATE CORTEX

99.1

DEPOLARIZATION-ACTIVATED POTASSIUM CURRENTS IN IDENTIFIED VISUAL CORTICAL NEURONS. J.L. Albert and J.M. Nerbonne*, Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

In vivo and *in vitro* recordings have revealed cortical neurons with distinct electrophysiological phenotypes: "regular spiking", "fast spiking", or "bursting". In order to assess directly the role of intrinsic membrane properties in determining these distinct firing patterns, we are characterizing voltage-gated outward K^+ currents in isolated identified superior colliculus-projecting (SCP) visual cortical neurons, selected to correspond to the bursting phenotype.

SCP cells were identified *in vitro* following *in vivo* retrograde labelling with rhodamine beads. Outward K^+ currents were evoked by voltage jumps positive to -30 mV from holding potentials between -90 - -30 mV. In 18 of 42 cells, currents elicited from a holding potential of -90 mV activated quickly, peaked within 10 ms, and decayed slowly during the 100 ms pulse. In the remaining 14 of the neurons, currents peaked more slowly (10-30 ms). When currents were evoked from -40 or -30 mV, both the peak and the current remaining at the end of the pulse were reduced by ~50%. Prolonged depolarizations revealed that inactivation of the K+ currents to steady state levels proceeded with a biexponential time course with time constants separated by an order of magnitude. Pharmacological experiments suggest at least 2 distinct current components: 1) a fast activating and inactivating current sensitive to 5 mM 4-AP, and 2) a more slowly activating, slowly inactivating current sensitive to 30 mM TEA. Experiments are underway to assess K^+ channel toxin sensitivity and to characterize depolarization-activated K^+ currents from cells with regular and fast spiking phenotypes.

99.2

ACETYLCHOLINE INHIBITS POSTSYNAPTIC POTENTIALS THROUGH MUSCARINIC RECEPTORS IN CULTURED VISUAL CORTICAL NEURONS F. Kimura* & R. W. Baughman Dept of Neurobiology, Harvard Medical School, Boston, MA 02115

Mammalian visual cortex recieves a dense cholinergic innervation from nucleus basalis magnocellularis in the basal forebrain. Recent *in vivo* studies demonstrated that iontophoretic application of ACh produces facilitation on visually driven responses in a majority (61-74%) of visual cortical cells. To better understand the mechanism of this modulatory effect of ACh, we looked at the effect on EPSPs and IPSPs elicited by single neuron and found that ACh inhibits both PSPs quite efficiently.

Visual cortical cultures were prepared from Long Evans rat pups 1-5 days of age. Cells were dissociated enzymatically and plated on monolayer glial islands grown on collagen dots. Electrophysiological experiments were performed with whole-cell patch pipettes in current clamp mode from 2 neighboring cells after at least 1 week in culture.

Application of ACh at 5-10 μ M reversibly depressed all EPSPs (n=14) and 6 of 7 IPSPs tested. The concentration that gave 50% reduction (EC_{50}) was in the range of 1 - 3 μ M. Since 1 μM atropine blocked this depressive effect of ACh, we carried out further experiments with pirenzepine and 4-DAMP to determine what type of muscarinic receptor is involved in this effect. With EPSPs the EC_{50} for pirenzepine was about 100 times higher than that for 4-DAMP (20 μM and 200 nM, respectively) with 5 μ M ACh, suggesting that the depressive effect of ACh on EPSPs is mediated by M_3 receptors.

99.3

THE FIRST STAGE OF SYNAPTIC INTEGRATION IN STRIATE CORTEX. J.A. Hirsch. * *The Rockefeller University, Laboratory of Neurobiology, NY, NY 10021.*

Neurons in the striate cortex respond differently to light than do the thalamic cells that convey ascending visual information. Novel cortical response properties can be explained only in part by geniculocortical patterns of convergence; circuits intrinsic to the cortex must contribute additional influence. To study the integration of thalamic and intracortical inputs in layer 4 of area 17, I have made patch recordings with dye-filled pipettes in brain slices prepared from young adult cats and ferrets. Synaptic responses were evoked by applying shocks to two sites, one in the white matter and the other in layer 6.

Recordings were made from both spiny stellate neurons and nearby pyramids; all cells fired only large, regular spikes. Weak stimulation of either site often evoked a solitary EPSP that was amplified when the membrane was depolarized above rest, an enhancement that sometimes produced a train of impulses. Stronger stimuli evoked synaptic responses comprising various combinations of polysynaptic EPSPs mixed with fast and slow IPSPs. Reminiscent of the absence or presence of end inhibition seen in simple cells *in vivo*, some neurons grew more strongly excited as stimulus strength was raised while others became more deeply inhibited. Typically, the general patterns evoked from both the white matter and layer 6 were similar for a given cell. This resemblance was not due to spatial spread of the stimulus or to excitation of a common stream of passing fibers since responses from both sites summed, and fatiguing one pathway by rapid activation failed to eliminate the response provided by the other. There were no clear-cut differences in synaptic physiology that correlated with spiny stellate as opposed to pyramidal morphology. Possible distinctions between the synaptic responses of smooth and spiny cells are being evaluated currently.

Support was from NSF grant BNS8918951 to C.D. Gilbert and NIH grant 5 R37 EY05253 to T.N. Wiesel.

99.4

Spatial and Temporal Integration of Synaptic Inputs by Visual Cortical Neurons. S.B. Nelson*, L. J.Toth and M. Sur, Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139

We have used whole-cell recording techniques to study interactions between electrically evoked synaptic responses in an *in vitro* slice preparation of rat primary visual cortex. Responses recorded under voltage clamp using pipettes that contained K^+-gluconate, ATP and Mg^{2+} (n=17) consisted primarily of a short-latency EPSC that reversed near 0 mV followed by an IPSC that reversed near the chloride reversal potential. With pipettes filled with cesium fluoride and lacking ATP and Mg^{2+} (n=21), it was frequently possible to record EPSCs in isolation. In many cases, the brief latency, smooth waveform and ablity to follow high frequency stimulation suggested they were monosynaptic. Pairs of stimuli delivered to the same electrode yielded facilitation of the second response at short intervals (<100 ms) in most cells (34/38). In about half the cells tested, suppression was seen at longer intervals (>200 ms). When sequences of stimuli were delivered to two or more electrodes displaced laterally by 200-1000 microns, most cells showed no facilitation or suppression (19/24). Hence for these cells facilitation and suppression are input-specific and may be presynaptic. In the remaining cells (5/24), however, dramatic sequence-specific interactions were observed. In these cells the net response depended strongly on the order in which spatially separated stimuli were delivered. Both sequence-specific suppression and facilitation were observed.

These results indicate that sequential synaptic inputs to cortical neurons are not always summed linearly. Paired-pulse suppression observed *in vitro* may underlie a similar suppression observed *in vivo* using pairs of visual stimuli. Sequence-selective responses could underlie direction selectivity. We are currently investigating these possibilities by using whole-cell techniques to record *in vivo* responses of cat cortical neurons to pairs of flashed visual stimuli.

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99.5

Orientation selectivity of synaptic currents during intracellular blockade of inhibition in visual cortex in vivo. L. J. Toth*, S. Nelson, B. Sheth, M. Sur. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139

A discrepancy exists as to the role of inhibition in shaping the orientation selectivity of cortical neurons. On the one hand, application of bicuculline, a $GABA_A$ antagonist, reduces orientation selectivity. On the other hand, EPSPs and IPSPs in cortical simple cells appear to be already tuned for orientation. Thus it is interesting to observe what happens to orientation selectivity if inhibitory currents within a single cell are blocked.

We have used the whole-cell technique to record intracellularly from visual cortical neurons in anesthetized, paralyzed cats while stimulating with oriented bars of light. We found that a pipette solution containing cesium fluoride and picrotoxin, and lacking ATP and Mg^{++}, dramatically reduces inhibitory ($GABA_A$ and $GABA_B$) currents, while leaving excitatory currents intact. Thus, we were able to block inhibitory inputs to a single cell while leaving inhibitory connections to neighboring cells intact. Neurons were first characterized extracellularly, while the electrode was patched to the membrane, and then intracellularly by rupturing the membrane. The absence of outward currents was confirmed by voltage clamping cells at depolarized potentials. In agreement with previous studies, we found few observable synaptic currents at orientations orthogonal to the preferred. Importantly, we found that blocking inhibitory currents in single cells does not in general lead to any dramatic change in orientation specificity.

Our results suggest a major role for oriented excitatory inputs in generating orientation selectivity, although inhibitory inputs may sharpen selectivity. It is possible that global blockade of inhibition leads to positive feedback and a loss of selectivity that is much greater than is seen when inhibitory inputs to a single cell are blocked. Supported by EY07023 and EY06363-02.

99.6

CELLS SELECTIVE FOR ORIENTATION, DIRECTION, AND COLOR IN LAYER 4Cβ OF MONKEY STRIATE CORTEX. Dan Liu, Kirk G. Thompson, Tiande Shou, Steven J. Ault, and Audie G. Leventhal* Anat. Dept., Univ. of Utah Sch. of Med., Salt Lake City, UT 84132

We recorded from single cells in the parvocellular layers of the LGNd and striate cortex of nine old-world monkeys. Both *Macaca fascicularis* and *Macaca mulatta* were studied. Broad band and colored sinusoidal gratings, bars, and spots were used as stimuli. All responses were studied quantitatively and analyzed statistically by computer.

In the parvocellular layers of the LGNd of both species many cells encountered were selective for color, orientation, and direction. Color sensitive cells were observed that responded many times better to the preferred than to the non-preferred orientation and direction.

Cells in layer 4Cβ of striate cortex were also selective for color, orientation, and direction. Their responses were qualitatively and quantitatively similar to those of the cells in the parvocellular layers providing their inputs. Penetrations that were roughly parallel to layer 4Cβ provided evidence that cells with similar preferred orientations are grouped together in that layer.

Contrary to previous reports, we conclude that cells in layer 4Cβ are sensitive to stimulis orientation. The organized arrangement of the orientation preferences of these cells strongly suggests that subcortical orientation and direction sensitivity contributes to the generation of these properties of cortical cells.

99.7

CORTICAL MECHANISMS OF RECEPTIVE FIELD EXPANSION IN THE CAT STRIATE CORTEX. *E. Volchan* and C. D. Gilbert.* Lab. of Neurobiology, The Rockefeller University, New York, NY 10021

It has been shown that the response properties of cells in the superficial layers of cat and monkey striate cortex can be modulated by stimuli lying outside the receptive field. In the adult cat, receptive field (RF) size can be altered by stimulating a large area of the visual field with an array of moving bars while occluding the cell's RF with a mask several times its diameter. Conditioning with this artificial scotoma for a few minutes, the RF area is enlarged severalfold; an effect that can be reversed by stimulating the RF's central region (*Pettet and Gilbert, Soc. Neurosci. Abstr., Vol. 17, p. 1090, 1991*). This transient increase could reflect dynamic changes at either subcortical or cortical levels. The present study favours the latter. We recorded from binocular cells in the superficial layers of the cat striate cortex. After mapping the RFs for each eye, one eye was conditioned with the artificial scotoma, while the other eye was occluded. In addition to the RF expansion in the conditioned eye we also observed an increase in field size in the occluded eye, showing that the RF expansion can be transferred from one eye to the other. The interocular transfer suggests that the substrate for the expansion is intrinsic to the cortex rather than to antecedent levels of the visual pathway.

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99.8

Ocular dominance columns in monkey striate cortex revealed by activity-dependent expression of Zif268

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The use of transcription factors for visualizing neuronal activity has been demonstrated in several non-primate species. The principal advantages of this approach are that:1) relatively short stimulation periods are required, 2) the technique can be combined with procedures for other cytochemical markers, and 3) single-cell resolution can be obtained with immunodetection.

We have used standard immunohistochemical techniques to detect the presence of one specific transcription factor, Zif268, in the visual cortex of vervet monkeys (*Cercopithecus aethiops*). Immunoreactive neurons were present in large numbers throughout the visual cortex of the normal animal, being concentrated mainly within the supragranular layers and layer VI. Simultaneous immunodetection for Zif268 and the calcium-binding proteins calbindin and parvalbumin showed a negative correlation, suggesting that the Zif268 protein may be expressed selectively within excitatory neurons.

In order to determine if Zif268 expression is affected by afferent stimulation in the monkey, we restricted visual input to one eye with the aim of revealing ocular dominance columns in striate cortex. We found that short-term monocular deprivation by either enucleation, intravitreal TTX injection, or eyelid-suturing showed dramatic changes in Zif268 levels, revealing vertically oriented columns of reduced Zif268 staining interdigitated with columns of normal expression. Furthermore, these columns were discernible after just 5 hours of monocular deprivation by all three methods of selective exposure. A comparison of the ocular dominance pattern obtained with Zif268 and cytochrome oxidase histochemistry showed a coincident reduction of both markers along columns that were precisely aligned in adjacent sections.

99.9

A NEW "BLOB" SYSTEM IN THE VISUAL CORTEX OF THE SQUIRREL MONKEY REVEALED BY NITRIC OXIDE SYNTHASE. C.W. Picanço-Diniz,† K.A.C. Martin,§ J.G. Franca,† J.A.S. Quaresma,† J.L.M. do Nascimento,† and M.J. Friedlander,*¥ †Department of Physiology, University of Para, Belem, Brazil, §MRC Anatomical Neuropharmacology Unit, Oxford University, Oxford, U.K., ¥Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294 USA.

Cytochrome oxidase (cytox) is a useful marker for different functional pathways in the primate visual system. In V1 the cytox-rich zones appear as rows of "blobs" when viewed from the cortical surface; in V2 they appear as a system of parallel stripes. Other proteins colocalize with cytox (Martin, TINS 11, 380, 1988). We examined the relationship between cytox and the synthetic enzyme for nitric oxide (NOS) by combining the two methods in single sections. The NADPH-diaphorase method (Vincent & Kimura, Neuroscience 46, 755, 1992) reveals NOS positive blobs in V1 and stripes in V2. However, in V1 of squirrel monkey the NOS positive blobs do not always overlap with the cytox blobs. The NOS blobs are slightly larger on average than cytox blobs (29,578 vs. 26,712 μm^2) and form rows that are more widely spaced than the rows of cytox blobs (583 vs. 513 μm). Most of the NOS positive neurons are in the white matter (the subplate) and have rich axonal projections to the grey matter. The NOS positive subplate neurons are larger than those in the grey matter, but both sets are sparsely spiny and probably colocalize GABA and NPY. NOS blobs contain more NOS positive neurons than the interblob zones (10.5 neurons per mm^2 vs 7.4). We are exploring the connections of the NOS system with a view to its potential importance in development and plasticity (Montague, et al., Cerebral Cortex 1, 199, 1992).

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99.10

MULTIPLE CLASSES OF NITRIC OXIDE SYNTHASE IMMUNOREACTIVE NEURONS IN MONKEY VISUAL CORTEX. S.B. Fenstemaker*[1,2], C.J. Aoki[1], T.M. Dawson[3] & S.H. Snyder[3]. [1]Center for Neural Science & [2]Howard Hughes Med. Inst, New York Univ., NY, NY 10003 & [3]Depts. of Neurosci. and Neurol., The Johns Hopkins Sch. of Med., Baltimore, MD. 21205

Nitric Oxide has been proposed as a modulator of both cerebral blood flow and of synaptic strength (Bredt & Snyder, '92, Neuron 8:3). We describe here nitric oxide synthase immunoreactivity (NOS-ir) in macaque visual cortical areas V1 and V2, and compare it with cytochrome oxidase (CO) and NADPH-diaphorase histochemistry, and ir for Neuropeptide Y (NPY). Multiple forms of NOS-ir were found in V1 and V2. Most prominent were large, densely labeled irregularly shaped neurons scattered through layers 2, 3, and 5, and concentrated in deep layer 6 and the white matter. These neurons and their long, thick processes often abutted large blood vessels. They appear indistinguishable from NPY-ir cells, except that occasional NPY neurons are found in layer 4. Smaller cells with round perikarya and shorter, finer caliber dendrites that tended to be radially symmetric were also NOS-ir. These neurons spanned the cortical layers and had no apparent relation to the vasculature. More intensely reactive clusters of these were found in upper layer 2 in V1; these clusters bore no consistent relation to the CO "blobs" in layers 2 and 3. Within layer 4, relatively dense NOS-ir was also seen in fine caliber processes and puncta. Variation in the density of NOS-ir in layer 4C has been found to be activity-dependent (Aoki et al., this volume). In Area V2, the same overall laminar distribution of NOS-ir was seen, but the most dense labeling of both neurons and neuropil occurred in register with both the thick and thin CO-dense stripes. NADPH-d histochemistry produced labeling that was almost indistinguishable from the NOS-ir. A possible exception was that the large NADPH-d neurons in the white matter adjacent to V2 were clustered among the more plentiful NOS neurons. These results suggest that NO may influence neuronal activity in V1 and V2 by distinct mechanisms: 1) control of blood flow via large neurons that may also contain NPY, and 2) direct synaptic modulation by a separate class of smaller, more locally restricted neurons. (Supported by EY08055 & 2SO7RR07062-26 to C.A.; EY02017 & HHMI Investigatorship to J.A. Movshon.)

99.11

ACTIVITY-DEPENDENT ALTERATION OF NITRIC OXIDE SYNTHASE IMMUNOREACTIVITY IN CORTICAL AREA V1 OF MONOCULAR MONKEYS. C. Aoki[1*], S. Fenstemaker[1,2], C-G Go[1], T.M. Dawson[3] & S.H. Snyder[3]. [1]Center for Neural Science & [2]Howard Hughes Med. Inst., New York Univ., NY, NY, 10003 & [3]Dept. of Neurol., Pharmacol. & Mol. Sci., The Johns Hopkins Univ. Schl. of Med., Baltimore, Md, 21205.

NO is a neuronal messenger with vasodilatory and synapse-strengthening properties (Bredt & Snyder, '92, Neuron 8:3). We examined the distribution of NO synthase (NOS) in visual cortical area V1 of monkey cerebral cortex using a previously characterized anti-NOS antiserum. Adjacent sections were reacted for cytochrome oxidase histochemistry to delineate cortical laminae and, in the case of monocular monkeys, ocular dominance columns (ODC). Two years following removal of one eye, NO synthase (NOS)-immunoreactivity was detectably less in layer 4C of the enucleated eye's ODC compared to that of the intact eye. Light microscopy revealed that NOS is concentrated in non-pyramidal neurons of supra- and infragranular layers whose processes are juxtaposed to large parenchymal blood vessels. These darkly stained neurons showed no detectable intercolumnar difference and were absent in lamina 4C of either ODC. The difference in NOS levels of 4C was, instead, due to the greater prevalence of short and punctate profiles containing NOS. Electron microscopy revealed that almost all of these profiles correspond to axons of passage and terminals that form synaptic junctions onto dendrites without contacting blood vessels. The observations suggest that the level of NOS in lamina 4C is regulated by local synaptic activity. These processes may be modulating synapse strength rather than cerebral blood flow. (Supported by EY08055 & 2SO7RR07062-26 to C.A.;EY02017 &HHMI Investigatorship to J. A. Movshon)

99.12

ROLE OF NITRIC OXIDE IN NMDA-RECEPTOR MEDIATED RELEASE OF NEUROTRANSMITTER IN VISUAL CORTEX. M.J. Friedlander, P.R. Montague, F. Hester* and R.B. Marchase. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294 USA.

Nitric oxide (NO) is a non-polar gas made in the mammalian brain by a calcium/calmodulin dependent enzyme upon N-methyl-D-aspartate (NMDA) receptor activation. NO may serve as a diffusible signal that communicates the status of postsynaptic sites at excitatory amino acid (EAA) synapses to neighboring pre- and postsynaptic sites. We investigated the role of NO in synaptosomes prepared from anesthetized adult guinea pig and cat visual cortex. The release of exogenously applied neurotransmitter (^{3}H-D-aspartate or ^{3}H-L-norepinephrine) or endogenous neurotransmitter (L-glutamate) was measured by scintillation counting and luminometry, respectively. NMDA caused release of all three neurotransmitters (EC_{50} = 10-30 μM, n=25). This effect was blocked in zero calcium or by APV (n=7). Preincubation of synaptosomes with competitive blockers of NO production (100 μM N^G-monomethyl-L-arginine [LMMA] or N^G-nitro-L-arginine [LNNA] (n=19)) reversibly blocked the NMDA mediated release of neurotransmitter as did 20 μM Hemoglobin (Hb, n=10). The D-isomers of the NO blockers were ineffective. The block of NO production did not affect K^+-induced release of neurotransmitter. Activation of the NMDA receptors on a second nearby group of synaptosomes induced neurotransmitter release from the first group. This effect was also blocked by Hb. Thus, NMDA receptor activation can cause neurotransmitter release from neighboring synaptic elements via NO production, and can link the EAA and catecholamine pathways in visual cortex.

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MOLECULAR BIOLOGY OF SEROTONIN RECEPTORS

100.1

MOLECULAR CLONING OF A NOVEL HUMAN SEROTONIN RECEPTOR (5-HT_{1E}) **G.McAllister , A.Charlesworth, M.S.Beer, A.J.Noble, D.N.Middlemiss and P.Whiting*. Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR.**

A novel human serotonin receptor (5-HT_{1E}) cDNA(AC1) was isolated from a hippocampal cDNA library using polymerase chain reaction (PCR) techniques. When expressed transiently in HEK293 cells the AC1 encoded receptor displayed high affinity for [^{3}H]5-HT(K_d=15nM) but low affinity for 5-CT. The pharmacology of the receptor expressed both transiently and stably in HEK293 cells correlated well with the observed binding of the putative 5-HT_{1E} receptor in human cortex. The AC1 encoded 5-HT_{1E} receptor is functionally coupled in stably transfected HEK293 cells and has been shown to mediate the inhibition of adenylate cyclase activity.

100.2

THE HUMAN GENE S31 ENCODES THE PHARMACOLOGICALLY-DEFINED SEROTONIN 5-HT_{1E} RECEPTOR. J.M. Zgombick*, L.E. Schechter, M. Macchi, P.R. Hartig, R.L. Weinshank, and T. A. Branchek. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652

The gene encoding a human 5-HT_1 receptor subtype was isolated from a human placental genomic library using oligonucleotide probes derived from transmembrane (TM) regions of the cloned human 5-$HT_{1D\beta}$ receptor. The deduced amino acid sequence of the genomic clone hp75d is identical to the recently isolated, but uncharacterized, novel serotonin receptor gene S31. TM domain sequence comparison of clone hp75d to other guanine nucleotide-binding protein-coupled receptors (GPCR) revealed the highest degree of homology (64%) with the 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ subtypes and lower degrees of homology (35-52%) to other serotonergic and catecholaminergic receptors. A stable cell line expressing this gene was established using murine fibroblasts as the host cell for pharmacological evaluation. High affinity (K_d=9.7 nM), saturable (B_{max}=2.4 pmol/mg protein) [^{3}H]5-HT binding was detected using membranes derived from stable transfectants. Most compounds displayed low affinity (K_i > 100 nM) for the expressed gene with the exception of 5-HT (K_i = 10 nM). The rank order of potency of ligands to compete for the [^{3}H]5-HT-labelled site best matched the pharmacologically-defined 5-HT_{1E} binding site: 5-HT > methysergide > ergotamine > DPAT > 5-CT > ketanserin. 5'-Guanylylimidodiphosphate (Gpp(NH)p) decreased high affinity agonist ([^{3}H]5-HT) binding in a dose-dependent manner. 5-HT produced a dose-dependent inhibition of forskolin-stimulated cAMP accumulation in intact cells stably expressing the 5-HT_{1E} gene. The response was blocked by the nonselective 5-HT_1 receptor antagonist, methiothepin. The molecular biological and pharmacological data are consistent with the designation that clone hp75d encodes a functional 5-HT_{1E} receptor.

100.3

THR355→ASN SITE-DIRECTED MUTAGENESIS CONVERTS THE HUMAN 5-HT_{1B} (5-$HT_{1D\beta}$) SEROTONIN RECEPTOR TO THE RAT 5-HT_{1B} PHENOTYPE. M.W. Hamblin*, M.A. Metcalf and R.W. McGuffin. GRECC, Seattle VAMC, and the Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98108.

Despite 93% amino acid identity, the human and rat 5-HT_{1B} (5-$HT_{1D\beta}$) receptors display markedly different drug specificities. Based on the discrete species distribution of the two receptor phenotypes and mutagenesis work on adrenergic receptors by Kobilka and colleagues, we and others proposed that much of this pharmacological species-dimorphism is produced by a single (human Thr355/rat Asn351) residue difference in the seventh transmembrane region. We compared the drug specificity of a human 5-HT_{1B} Thr355Asn mutant with those of wild type human and rat 5-HT_{1B} receptors expressed in COS-7 cells. Drug IC50's in 2nM [^{3}H]5-HT competition binding assays were:

	HUMAN WILD TYPE	HUMAN Thr355Asn	RAT WILD TYPE
5-HT	10 nM	11 nM	9.1 nM
(±)CYP	190	2.1	1.5
RU24969	69	9.2	2.8
CGS12066B	4.2	37	64
YOHIMBINE	140	7400	3300
DPAT	750	>10000	>10000

These results demonstrate that much of the inter-species difference in 5-HT_{1B} drug selectivity is due to this single amino acid change and may provide further insight into the nature of 5-HT_{1B} receptor/ligand interactions. (Supported by the Department of Veterans Affairs.)

100.4

THE CLONED HUMAN SEROTONIN-1B RECEPTOR INDUCES PERTUSSIS TOXIN-SENSITIVE CALCIUM MOBILIZATION AND ADENYLYL CYCLASE INHIBITION IN FIBROBLAST CELLS. P.R. Albert*, D. Raquidan, H. Jin, B.F. O'Dowd. Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada H3G-1Y6 and Add. Res. Found., Ontario.

The cloned human serotonin-1B receptor (Jin, H. et al., J. Biol. Chem. 267: 5735, 1992) was stably-transfected into two serotonin receptor-negative murine fibroblast cell lines, Ltk- (clones L-1B) and Balb/c-3T3 (clones Balb/c-1B) cells, to investigate the transmembrane signaling of the receptor. Several clones were isolated, screened for receptor expression using RT-PCR and tested for responsiveness in an acute (20 min incubation) assay for cAMP levels. In both cell lines serotonin had no effect on basal levels of cAMP but inhibited forskolin-induced elevation by 50% maximally. In both cell lines, serotonin induced an immediate 3-fold increase in $[Ca^{++}]_i$ which decayed within a minute to a plateau level of 1.5-fold. Both the serotonin-induced increase in $[Ca^{++}]_i$ and the inhibition of cAMP accumulation were completely blocked by 24h pretreatment with 20 ng/ml pertussis toxin. We conclude that the serotonin-1B receptor, like the dopamine-D2 and serotonin-1A receptors, induces both calcium mobilization and inhibits cAMP accumulation by coupling to pertussis toxin-sensitive G proteins.These cells should prove useful for further characterization of the pharmacology and function of the human 5-HT1B receptor.

100.5

A SINGLE AMINO ACID ACCOUNTS FOR THE MAJOR PHARMACOLOGICAL DIFFERENCE BETWEEN THE HUMAN AND RAT 5-HT_{1B} RECEPTOR. D. Oksenberg*[1,2], H. Jin[3], B.F. O'Dowd[3], S.A. Marsters[1], A. Ashkenazi[1], and S.J. Peroutka[2]. Genentech, Inc., South San Francisco, CA 94080; Neurology, Stanford School of Medicine, Stanford, CA 94305; and Department of Pharmacology, University of Toronto, Ontario M5S 1A8, Canada.

Serotonin (5-HT) exerts its diverse actions via pharmacologically distinct receptors. Homologous human genes have been cloned recently that encode the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{S31} and 5-HT_2 receptors. The human 5-HT_{1B} receptor has 93% amino acid sequence identity with the rat 5-HT_{1B} receptor; however, while both receptors bind 5-HT with similarly high affinity, the rat, but not the human, receptor also exhibits high affinity for beta-adrenergic receptor (beta-AR) antagonists. Using site-directed mutagenesis, we replaced a threonine residue in the seventh transmembrane domain of the human receptor (position 355) with asparagine, found at the same position in the rat 5-HT_{1B} receptor, as well as in the 5-HT_{1A} and beta-AR. This single amino acid substitution, while not affecting the affinity for 5-HT, increases the affinity for alprenolol, DL- propanolol, and (-) pindolol by 670, 420 and 370-fold, respectively. These results indicate that the residue at position 355 in the seventh transmembrane domain plays a critical role in determining the ligand binding specificity of 5-HT_{1B} receptors, particularly with respect to drugs that also bind to beta-AR.

100.6

CLONING, EXPRESSION, AND FUNCTIONAL CHARACTERIZATION OF THE RAT STOMACH FUNDUS SEROTONIN RECEPTOR. M. Foguet, D. Hoyer*, L.A. Pardo, A. Pareck, H.O. Kalkman, W. Stühmer, and H. Lübbert. Preclinical Research, Sandoz Pharma Ltd., 4002 Basel, Switzerland, and Max-Planck-Institute for Biophysical Chemistry, 3400 Göttingen, Germany.

A segment of DNA with high homology to the third exons of the serotonin 1C and 2 receptor genes was isolated by low stringency screening of a mouse genomic library. The positions of the introns flanking these exons are conserved in the three genes. To examine whether the new fragment was part of an active gene, we used a quantitative PCR protocol to analyze rat RNAs from different tissues and ages. The gene was expressed in stomach fundus at an abundance of one in about $2x10^5$ mRNA molecules. This tissue contracts in response to 5-HT via a receptor that has previously refuted characterization. We next constructed a cDNA library from rat stomach fundus and, using oligonucleotides from the known sequence as probes, isolated positive clones containing 2000 bp inserts with open reading frames of 465 amino-acids comprising seven putative membrane-spanning regions. The protein was transiently expressed in COS cells and binding of serotonergic ligands to the membranes was analyzed. The pharmacological profile resembled that described for the serotonin-stimulated contraction of the stomach fundus. After expression of this receptor in *Xenopus* oocytes the application of serotonin triggered the typical chloride current which results from the activation of phospholipase C.

100.7

THE MOUSE 5HT1Bβ SEROTONIN RECEPTOR IS LOCALIZED PRESYNAPTICALLY ON AXON TERMINALS. R.Hen, U.Boschert, F.Saudou ,N.Amlaiky, J.L.Plassat ,D.°Ait Amara, J.°Lanoir* and L.°Segu , CNRS ,INSERM ,Faculté de Médecine , 11 rue Humann ,67085 Strasbourg - °CNRS, Laboratoire de Neurobiologie ,BP 71,13402 Marseille 09 -France.

5-HT is a neuromodulator which mediates a wide range of effects by interacting with multiple receptors. Using a strategy based on nucleotide sequence homology between genes encoding 5-HT receptors that interact with G proteins, we have isolated a mouse gene encoding a new serotonin receptor. When expressed in cultured cells, it displayed the pharmacological profile and negative coupling with adenylate cyclase characteristic of the 5HT1B receptor subtype. In 3T3 cells expressing this receptor, serotonin induced a decrease in forskolin stimulated cAMP levels. This effect was blocked by pertussis toxin indicating that the 5HT1Bβ receptor interacts with a pertussis toxin-sensitive G protein. We have analyzed the pattern of expression of the 5HT1Bβ receptor in the mouse brain by in situ hybridization and receptor autoradiography, using a 5HT1B/1D specific ligand, SCMG(^{125}I)TNH2 (Immunotech SA). Our results indicate that the 5HT1Bβ receptors are probably localized presynaptically on the axon terminals of striatal neurons projecting to the globus pallidus and substantia nigra , on the terminals of Purkinje neurons in the deep cerebellar nuclei and on the terminals of retinal ganglion cells in the colliculus. These receptors might therefore modulate the release of neurotransmitters such as GABA. The expression of the 5HT1Bβ receptor in the striatum, cerebellum and retina points to an involvement of this receptor in motor control and visual function.

100.8

CLONING OF THE MURINE SEROTONIN$_{1A}$ RECEPTOR cDNA AND FUNCTIONAL CHARACTERIZATION IN A SEPTAL CELL LINE. Alain Charest, Bruce H. Wainer*, and Paul R. Albert. Dept. of Pharm. and Therap., McGill University, Montreal, Canada, H3G 1Y6, Dept. of Pharm. and Physiol. Sci., University of Chicago, Chicago, IL, 60637.

We have cloned a 2.5 Kb cDNA encoding the serotonin$_{1A}$ (5-HT_{1A}) receptor from a mouse brain library. Analysis of the nucleotide sequence revealed striking homology with the rat and human counterparts (94% and 84% respectively). Ligand binding studies on stably transfected mouse Ltk$^-$ fibroblast membranes showed high affinity binding of ^{3}H-DPAT with a pharmacological profile corresponding to that of the 5-HT_{1A} subtype: DPAT> 5-HT> spiroxatrine> spiperone. In order to study the functional coupling of the receptor we have identified, using RT-PCR, a mouse septum X neuroblastoma fusion cell line (named SN-48) which endogenously express low amount of the receptor. Upon 48 hr treatment with 10 µM retinoic acid (RA), the cells morphologically differentiated into neurons and the level of expression of the receptor was greatly augmented as shown by northern blot analysis. In the undifferentiated SN-48, both VIP (200 nM) and PGE_2 (10 µM) induced a 5- to 10-fold enhancement of cAMP accumulation upon which DPAT (1-10 µM) had no inhibitory effect. However, in RA-differentiated cells, DPAT inhibited the VIP- and PGE_2-stimulated levels of cAMP by 50% and had no effect on basal cAMP levels. The DPAT-mediated inhibition of stimulated cAMP accumulation exhibited an EC_{50} of 10 nM and was abolished by 16h pretreatment with pertussis toxin (20 ng/ml), consistent with the known pharmacology of the 5-HT_{1A} receptor. We conclude that the SN-48 septal cells will provide an excellent model system for investigating the aspects of neuronal differentiation leading to the development of sensitivity to serotoninergic input. PRA was supported by the FRSQ and MRC, Canada, and BHW by US PHS NS-25787.

100.9

IDENTIFICATION OF DOMAINS WHICH MODIFY LIGAND BINDING TO 5-HT$_2$ AND 5-HT$_{1c}$ SEROTONIN RECEPTORS.

S. Choudhary*, S. Craigo and B.L. Roth. Department of Psychiatry, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

To define structural determinants essential for serotonin (5-hydroxytryptamine; 5-HT) pharmacology, we constructed and characterized chimeric 5-HT$_2$/5-HT$_{1c}$ serotonin receptors. After transient expression in COS-7 cells, high affinity and saturable [3H]-mesulergine binding was measured (Kd=1-2 nM; βmax=1.2-2.0 pmol/mg protein). For chimeric receptors in which the putative transmembrane (TM) domains V to VII were exchanged, a change in affinity of 5 to 10-fold for [^{3}H]-ketanserin toward that of the donated sequence was measured. Additionally, these same chimeras displayed altered affinities for a number of other serotonergic antagonists including spiperone, cinanserin, fluphenazine, risperidone and cinanserin. No changes in [^{3}H]-mesulergine, mianserin, ritanserin or LSD affinities were noted. One of the chimeras (Chimer2) displayed a 131-fold decrease in 5-HT's ability to activate phosphoinositide hydrolysis in stably transfected cells. Our results suggest that structural features essential for specifying the unique pharmacology of 5-HT$_2$ and 5-HT$_{1c}$ receptors reside in discrete transmembrane domains. (Supported by Scottish Rite Schizophrenia Research Foundation and the PMA Foundation to BLR).

100.10

LOCALIZATION AND DESCRIPTION OF 5-HT2 IMMUNOREACTIVE NEURONS IN THE RAT BRAIN. D. A. Morilak*, S.J. Garlow and R.D. Ciaranello, Nancy Pritzker Lab, Dept. Psychiatry, Stanford Univ., Stanford, CA 94305

5-HT2 receptors have been implicated in a variety of behavioral and physiological processes, as well as a number of neuropsychiatric disorders. To specify the brain regions and specific cell types possessing 5-HT2 receptors, we performed immunocytochemistry using a polyclonal 5-HT2 receptor antibody which we have previously characterized (Soc. Neurosci. Abstr. 17: 720, 1991). Rats were anesthetized and perfused with 4% paraformaldehyde. 50 µm sections were incubated in affinity-purified 5-HT2 antiserum, and immunoreactivity was visualized using an immunoperoxidase reaction. Numerous small round neurons were heavily labelled in the granular and periglomerular regions of the olfactory bulb. Heavy labelling of medium-sized multipolar and bipolar neurons was also seen in the ventral forebrain, including the nucleus accumbens, olfactory tubercle and ventral pallidum. A dense band of small round cells was stained in layer 2 of pyriform cortex. A moderate number of medium bipolar and multipolar cells were scattered throughout the neostriatum, and a moderate number of pyramidal and pyramidal-like cells were seen in the CA fields of the hippocampus. Other areas showing labelling were the medial habenula and anterior pretectal nucleus. In the hindbrain, two dense populations of multipolar cells were heavily labelled in the pedunculopontine and lateral dorsal tegmental nuclei, while lesser labelling was seen in the periacqueductal grey, spinal trigeminal and solitary tract nuclei. A sparse and even distribution of immunopositive cells were seen throughout layers 2-6 of cortex. This distribution suggests that central 5-HT2 processes are mediated by distinct populations of cells in the ventral forebrain and in the hindbrain. The localization and morphology of cells in many of these regions suggests that subpopulations of 5-HT2 receptor-bearing cells may be GABAergic interneurons or cholinergic neurons. We are currently in the process of testing these hypotheses using double-labelling with immunofluorescence.

100.11

BRAIN DISTRIBUTION OF 5-HT$_3$ RECEPTOR mRNA: AN IN SITU HYBRIDIZATION STUDY. L.H. Tecott*, A.V. Maricq and D. Julius. Depts. of Pharmacology and Psychiatry, University of California, San Francisco, CA 94143-0450.

5-HT$_3$ receptors have been implicated in a variety of central nervous system functions, and antagonists of this receptor have anxiolytic, antipsychotic and antiemetic properties in animal models. To determine with cellular resolution the anatomical distribution of 5-HT$_3$ receptors, a ^{35}S-labelled RNA probe was used for in situ hybridization in mouse brain sections. A highly restricted pattern of hybridizing cells was observed. Scattered positive cells were observed in all cortical areas, with highest densities in piriform, entorhinal and cingulate regions. Strongly hybridizing cells were seen in the hippocampal formation, where expression appeared restricted to interneurons. Positive cells were most abundant in the posteroventral hippocampal region, particularly in the lacunosum-moleculare layer of CA1. Scattered hybridizing cells were also seen in the major subdivisions of the amygdaloid complex, the subiculum, the olfactory bulb, the pontine dorsal raphe region, the dorsal tegmental nucleus, the nucleus of the spinal tract of the trigeminal nerve and in the spinal cord dorsal horn. Strongly hybridizing cells were also observed throughout the superior olivary complex. In the periphery, intense hybridization was observed within a subpopulation of dorsal root ganglion neurons. The above findings are in general agreement with prior radioligand binding and receptor autoradiographic studies (see review by Tyers, et. al. Pharmac. Ther. 47: 181, 1990). However, the results also suggest the presence of 5-HT$_3$ receptors in regions where they have not been previously seen, such as the superior olivary complex and the dorsal tegmental nucleus. In general, the observed distribution of signal is consistent with the involvement of 5-HT$_3$ receptors in the modulation of cognition and affect and in the modulation of sensory systems.

100.12

THE MOUSE 5HT5 AND 5HT6 RECEPTORS: TWO NEW "5HT1D- LIKE" SEROTONIN RECEPTORS: CLONING AND EXPRESSION. N. Amlaiky, J.L. Plassat, S. Ramboz, U. Boschert and R. Hen* LGME/CNRS-U184/INSERM 11 Rue Humann 67085 Strasbourg France.

Serotonin is a neuromodulator which mediates a wide range of effects by interacting with multiple receptors .The G protein-coupled 5-HT receptors which have been cloned so far, can be divided into two families : the 5HT1 family, including the 5HT1A, 5HT1B/1D and three Drosophila 5-HT receptors that modulate adenylate cyclase and the 5HT2 family containing the 5HT1C and 5HT2 receptors that activate phospholipase C. We took advantage of the amino-acid sequence homologies found in transmembrane domains III and VI of all these receptors to isolate mouse cDNAs that encode two new members of the G protein-coupled receptor family, 5HT5 and 5HT6. Amino-acid sequence comparisons revealed that the 5HT6 receptor belonged to the 5HT1 family while the 5HT5 receptor did not belong to either 5HT1 or 5HT2 families. When expressed transiently in Cos-7 cells both receptors had a high affinity for the serotonergic radioligand [^{125}I]-LSD. The 5HT5 receptor displayed the following pharmacological profile : ergotamine >5-CT >methysergide > 5-HT = RU24969 > yohimbine = bufotenine = 8OHDPAT, (ketanserine,sumatriptan and pindolol were inactive).The 5HT5 receptor might therefore correspond to previously reported "sumatriptan insensitive" 5HT1D- like binding sites. The 5HT6 receptor displayed also a "5HT1D- like" profile: methysergide > bufotenine > ergotamine = yohimbine = sumatriptan >5-HT= RU24969 (5-CT, ketanserine and cyanopindolol were inactive).The 5HT6 receptor might be related to "5-CT insensitive" 5HT1D-like sites. We have generated stable cell lines expressing these receptors and shown that the 5HT6 receptor is negatively coupled to adenylate cyclase.In situ hybridization experiments have revealed that the 5HT5 mRNA is expressed predominantly in the cerebral cortex ,hippocampus and granular layer of the cerebellum, while the 5HT6 mRNA is found only in the hippocampus.

SUBCORTICAL VISUAL PATHWAYS

101.1

ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF LGNd INTERNEURONS. D. A. McCormick [1], H.-C. Pape[2], Z. Kisvárday[2] and U.T. Eysel*[2]. Yale Univ. Med. Sch. New Haven, CT[1] and Ruhr Universitaet, Bochum, Germany[2].

The flow of visual information from retina to visual cortex is regulated by inhibitory interneurons located in the dorsal lateral geniculate nucleus (LGNd). Here we investigated the electrophysiological and pharmacological properties of cat LGNd interneurons using standard *in vitro* slice techniques. Intracellular recordings from morphologically identified interneurons revealed unique electrophysiological properties including short duration action potentials, a lack of sharp, rebound burst firing (although some cells could generate slower, rebound bursts), and the ability to generate high frequency (>500 Hz) trains of action potentials. Pharmacologically, putative LGNd interneurons responded to 1) ACh and GABA with marked inhibition of action potential generation, 2) Glutamate, kainate and quisqualate (and cortical and retinal afferents) with marked excitation, and 3) histamine and serotonin with weak excitation and with no response to noradrenaline. In addition, the occurrence of spontaneous IPSPs of all durations were inhibited in relay neurons by ACh. These results suggest that the excitability of intrageniculate interneurons is controlled largely by retinal, cortical, GABAergic (nRt?), and cholinergic (brainstem) afferents with less of a contribution by histaminergic, serotoninergic, and noradrenergic inputs.

101.2

A Nitric Oxide Synthetase Co-localizes with ACh in Brainstem Inputs to the Cat's LGN. A.E. Günlük*, M.E. Bickford, W. Guido, and S.M. Sherman. Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

The NADPH-diaphorase reaction stains neurons containing an enzyme used to produce citrulline and nitric oxide (NO) from arginine and NADPH. It has been suggested that NO acts retrogradely to affect the process of long term potentiation (LTP) in hippocampus. An LTP-like potentiation of retinal and cortical EPSPs has recently been demonstrated in the cat's lateral geniculate nucleus (LGN), raising the question of an NO contribution to LGN functioning. We found that, in the LGN, the NADPH-diaphorase reaction stains a subset of axons and terminals that closely resembles axons stained positively for choline acetyl transferase (CHAT). To determine the source of these axons positive for NADPH-diaphorase, we injected WGA-HRP into the LGN to retrogradely label afferent neurons and then employed the NADPH-diaphorase reaction on these neurons. The only area that contained a significant population of cells positive for both HRP and NADPH-diaphorase was the parabrachial region of the brainstem. In this region, 55% of the HRP-labeled neurons also contained NADPH-diaphorase. In experiments combining immunocytochemical and NADPH-diaphorase staining, no cells were double-labeled for NADPH-diaphorase and serotonin or tyrosine hydroxylase. However, 82% of the CHAT-positive cells also contained NADPH-diaphorase. Many of the axons innervating the LGN from the parabrachial region thus seem to contain both CHAT and NADPH-diaphorase. These terminals may influence LGN cells by release of both ACh and NO. Stimulation of the parabrachial region enhances visual responses in geniculate cells, presumably through the release of ACh. NO may complement ACh in increasing the visual responses of LGN cells, perhaps by enhancing the potentiation of retinal and cortical EPSPs. If so, our data also suggest that NO acts in an orthograde rather than retrograde manner. (Supported by USPHS grants EY03038 and EY06101.)

101.3

Activation of PBR switches responses of cat LGN cells from burst to relay mode during visual stimulation. S.-M. Lu*, W. Guido, and S.M. Sherman. Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

LGN relay cells respond in one of two distinct modes, *relay* and *burst*. The burst mode is due to a voltage-dependent Ca^{2+} conductance that can be activated from hyperpolarized V_m; this is the low threshold (LT) spike. When activated, the LT spike leads to a burst of action potentials. The LT spike is inactivated at depolarized V_m, leading to the relay mode during which the cell responds with a more tonic stream of action potentials. We recently demonstrated that both of these response modes participate in the transmission of visually-evoked responses to cortex. In the present study, we used an *in vivo* preparation with intracellular and extracellular recording to examine the effects of activating the parabrachial brainstem region (PBR) on switching between relay and burst response modes during visual stimulation by drifting sinusoidal gratings. The PBR provides a mostly cholinergic input to the cat's LGN. We found that PBR activation completely blocked LT spikes and switched all cells to the relay mode. Nearly all of our cell sample showed an increase in total response due to PBR activation, and this was due to an increase in the tonic response component that more than compensated for the loss of LT bursts. Because the tonic response components shows much more linear summation than do the LT bursts, overall response linearity was also enhanced by PBR activation. Interestingly, our intracellular records suggest that the slight, gradual depolarization of V_m caused by PBR activation (5-8 mV) was often insufficient by itself to explain the large increases in overall response. This may result from PBR inhibition of local inhibitory cells, which serves to disinhinbit the relay cells we recorded. (Supported by USPHS grants EY03038 and EY06082).

101.4

BRAINSTEM INFLUENCE ON THE RECEPTIVE FIELD STRUCTURE OF CELLS IN THE LATERAL GENICULATE NUCLEUS OF THE CAT. P. Heggelund*, E. Hartveit and S.I. Ramberg. Dept. of Neurophysiol., Univ. of Oslo, Oslo, Norway.

The visual response of cells in the lateral geniculate nucleus (LGN) can be markedly influenced by modulatory input originating from the peribrachial region (PBR) of the brainstem. Cholinergic afferents seem to play a major role. In the temporal domain increased PBR input increases mainly the sustained response component of non-lagged cells to a stationary flashing light spot. Thereby the visually driven response becomes more similar to that of the retinal input. The influence of the PBR input in the spatial domain is less well understood. We studied the influence of electrical PBR stimulation on the spatial response profile of single nonlagged LGN cells. The profiles were determined from the response to a stationary light slit presented in a series of positions across the receptive field. We also studied the effect of iontophoretic application of acetylcholine (ACh) on the LGN cells.

The most marked effect of PBR stimulation was an enhancement of the response in the centre of the receptive field. The strongest enhancement occurred around the response maximum. Thus, the slope at the flanks of the profiles became steeper. There was no marked change in the width of the receptive field centre, although a slight increase was found for some of the cells. The spontaneous activity increased, but less than the visually driven response. We found no marked change in the strength of the receptive field surround. The effects of ACh were generally similar to those of PBR stimulation.

We conclude that in the spatial domain a major effect of PBR stimulation and ACh on the retinogeniculate transmission is to enhance the response, in particular the response of the centre component of the receptive field. We found no evidence for any reduction of the spatial extent of the receptive field centre.

101.5

ROC Analysis of Cat LGN Cells during *Burst* and *Tonic* Response Modes. W. Guido*, S.-M. Lu, and S.M. Sherman. Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

Previous studies have demonstrated that relay cells of the cat's LGN can respond in one of two modes: *relay* and *burst*. These modes depend on membrane voltage, because the burst mode is due to activation of low threshold Ca^{2+} (LT) spikes, and these are activated only when the cell is relatively hyperpolarized. We wished to determine the ability of LGN cells to detect visual stimuli during both response modes. A powerful means of measuring such signal detectability is to construct receiver operating characteristic (ROC) curves, the area under the curve providing a reliable measure of detectability. We did this for a population of LGN cells, which we recorded, both extracellularly and intracellularly, in anesthetized, paralyzed cats. We analyzed neuronal responses to drifting sinusoidal gratings. We were able to distinguish the relay and burst response modes for these cells. Initially, we concentrated on responses to gratings of optimal spatial and temporal frequency, which thus provided the largest areas for the ROC curves. We found a small but consistent tendency for cells responding in the burst mode to display larger areas under the ROC curves than did cells responding in the relay mode. Furthermore, in a small cell sample, we were able to maintain recording while the cell switched response modes, and in each of these cases, the area under the ROC curve was larger when the cell responded in the burst mode than when in the relay mode. Our data from ROC analysis suggest that LT spiking and the burst response mode, while less linear and perhaps less useful in subserving a detailed analysis of visual stimuli, operate to enable a hyperpolarized cell to detect a visual stimulus. (Supported by USPHS grants EY03038 and EY06082).

101.6

RECEPTIVE FIELDS AND VISUAL RESPONSES OF GANGLION CELLS ACROSS THE PRIMATE RETINA.
Lisa J. Croner* and Ehud Kaplan. Rockefeller University, N.Y., N.Y. 10021.

The primate retina is inhomogeneous, varying in anatomy with distance from the fovea. In order to understand the effects and purpose of this architecture, we studied how receptive fields and visual responses of parvocellular-projecting (P) and magnocellular-projecting (M) ganglion cells vary with position on the primate retina.

Ganglion cell activity was recorded as synaptic (S) potentials in the LGN of anesthetized and paralyzed macaques. The receptive field organization, responses to contrast, and response variability of 149 cells in the central 80 (radius of 40) degrees of the retina were studied with drifting sinewave gratings. The effects of the eyes' optics on the stimuli appearing on the retina were determined. Thus, receptive field organization and contrast sensitivity in visual space ("visual" properties) and on the retina ("retinal" properties) were evaluated.

Spatial resolution decreases with increasing distance from the fovea, but other properties remain relatively constant. Contrast gains of P and M cells, to stimuli in the visual world, are constant and distinct from each other across the retina; in fact, P cells counteract the blur introduced by optical aberrations in the periphery by increasing the *retinal* contrast gain of peripheral cells in order to maintain constant *visual* gain across the retina. In addition, response noise of P and M cells is virtually constant across the retina, and color-selectivity of P cells persists into the periphery. Thus it appears that the primate eye maintains several important visual capacities of ganglion cells constant across the visual field. *Supported by EY 4888, EY 1428, and T32GM07524.*

101.7

SYNCHRONIZATION OF OSCILLATORY RESPONSES IN THE AVIAN OPTIC TECTUM. S. Neuenschwander# and F.J. Varela*. Institut des Neurosciences, CNRS, 9, quai St. Bernard, 75005 - Paris.

We have recorded multi-unit activity and local field potentials simultaneously from two spatially separate sites (1 mm) in the optic tectum of awake pigeons. The animal's head was fixed while a moving light bar was swept over the receptive fields of the two cell groups. The temporal structure of the responses was analysed by computing averaged auto- and cross-correlograms of the spike trains and local field potentials. As reported before[1] the autocorrelograms typically exhibited a pronounced periodic modulation, indicating that the responses were oscillatory. Oscillation frequency was in the gamma range, showed considerable variability and was centered around 35 Hz. Cross-correlation analysis revealed that the responses of the spatially separate cell groups were in most cases synchronized with close to zero phase lag. Cross-correlograms calculated from responses shuffled by one stimulus period (shift predictor) were flat. This indicates that the synchronization was not due to phase locking of the responses for the stimulus. Our results indicate that oscillatory responses int he gamma range and their synchronization across spatially segregated groups of neurons occur also in laminated structures other than the mammalian visual cortex, suggesting that response synchronization may serve as a binding mechanism in the optic tectum.

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1 Neuenschwander,S. and Varela, F.J. (1990), Soc. Neurosc. Abstr. 16:47.6.

102.1

BILATERAL PARIETAL INVOLVEMENT IN THE EXECUTION OF A SEQUENCE OF MEMORIZED SACCADES IN MAN. A. Berthoz*, B. Mazoyer, L. Petit, C. Orssaud, L. Raynaud, N. Tzourio. S.H.F. Joliot, C.E.A., Orsay, 91406, and L.P.N., C.N.R.S., Paris, France.

We measured regional cerebral blood flow (rCBF) using PET and [15O]-water in 5 young healthy volunteers (4 right-handed), during 3 or 4 runs of 2 different conditions : 1- fixation of an imagined central diode, 2 - repetition, at maximal frequency, of a sequence of 6 horizontal saccades (3 to both sides) that was memorized in the minutes preceding the rCBF measurement. All examinations were performed in total darkness and task performance was controlled by electro-oculogram recording. rCBF images were aligned with individual magnetic resonance images, and normalized CBF variations between pairs of measurements (N=18) were computed in individual anatomically defined regions of interest.

Cortical activations were found in the left precentral gyrus (2.1 ± 2.9, mean ± SD in %, p = 0.007, paired t-test), right supplementary motor area (SMA, 5.8 ± 5.7, p = 0.0005), both superior parietal lobes (LSP, 3.8 ± 4.8, p = 0.004, RSP 3.7 ± 7.6, p = 0.05), the left inferior parietal gyrus (LIP, 2.8 ± 3.1, p = 0.001), and in a region lying around the right intraparietal sulcus (4.6 ± 4.1, p = 0.006). Activated subcortical structures were the thalami (left: 2.7 ± 4.4, p = 0.02; right: 4.4 ± 4.1, p = 0.0003) and the left lenticular nucleus (3.1 ± 6.2, p = 0.047).

These results should be compared with our previous results obtained in subjects performing self-paced horizontal saccades in total darkness (Eur. J. Neurosc., suppl 4, pp. 169, 1991). In this paradigm, bilateral precentral gyri, SMA, lenticular nuclei, left median cingulate, right thalamus, and cerebellar vermis were activated.

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102.2

DIFFERENT MODES OF VISUALLY GUIDED SACCADES IN MAN DETERMINED BY REACTION TIME AND AMPLITUDE. H. Weber and B. Fischer *. Dept. Neurophysiology, Hansastr. 9a, D-7800 Freiburg

The saccadic eye movements of 39 naive subjects (age 10 to 50 yrs) were measured. Two single target (Tg) saccade tasks were used: In the gap task the fixation point (Fp) went off before the Tg occurred, in the overlap task it remained on throughout a trial. Tgs were randomly presented 4 deg to the left or right of the Fp. Subjects were instructed to look at the Tg when it appeared, not to "react as fast as possible" without any feed-back about their performance. Contrary to recent objections 31 naive subjects exhibited clearly separated peaks in the distribution of the saccadic reaction times (SRTs) in the gap task: at 100-135 ms (express saccades), at 140-180 ms (fast regular), and eventually at about 200 ms (slow regular). Five subjects, however, did not show clear signs of two modes between 100 and 180 ms, 3 did not produce any SRTs below 130 ms. In addition, many subjects produced three peaks with about the same modes also in overlap trials. The SRT data of individuals and of different age groups were fitted by the superposition of three gaussian functions. Analysis of over- and undershooting saccades show that express saccades almost never overshoot. Their mean size was smaller by 8% in gap and by 25% in overlap trials as compared with regular saccades. Therefore express saccades are not a phenomenon of exceptional subjects nor the result of lengthy training. Neither is the offset of the fixation point in the gap task a necessary condition for their occurrence. The neural mechansims for express saccades may be present in all subjects, but some may be unable to use this pathway due to their fixational/attentional habits. It is still unclear why in a given session some saccades are of the express and others of the regular type.

102.3

NONCONJUGATE ADAPTATION OF HUMAN SACCADES: FAST CHANGES IN BINOCULAR MOTOR PROGRAMMING. J. Van der Steen. Dept. of Physiology, Fac. of Medicine, Erasmus University, Rotterdam, 3000DR, The Netherlands. SPON: European Neuroscience Association.

Under normal conditions the oculomotor system programs saccades of approximately equal magnitude for both eyes. Nonconjugate saccades are made instantaneously in the presence of a vergence stimulus or can be made after an adaptation period in the situation where subjects are wearing anisometropic spectacles. The time course and limitations of the adaptation to such spectacles have been investigated in short-term (1-2 hrs) and in long-term (>2 hrs) experiments. In this study the early stages of the adaptation process resulting in differential saccadic motor programming for the two eyes were investigated using scleral search coils. Four subjects viewed two dichoptically presented, identical but aniseikonic, random checkerboard patterns (30°x30° visual angle). The size difference of all elements along both meridians or the horizontal or vertical meridian alone was 8%. Saccadic eye movements were made horizontally, vertically and diagonally symmetrical about the center in alternating trials and recorded before, during and after the adaptation period.

An adaptation period of 20 minutes was sufficient to obtain nonconjugate saccades. For nonconjugate horizontal saccades in the divergent direction the intrasaccadic adaptation was complete within this period. For convergent horizontal saccades the intrasaccadic adaptation amounted 80%. For vertical saccades the intrasaccadic differences were up to 60% for upward saccades and 40% for downward saccades, leaving a considerable postsaccadic vergence drift. The differences in saccadic metrics persisted in open-loop trials immediately after the adaptation period. The dynamics of vergence eye movements in response to steps before and after the adaptation remained unchanged. These results suggest that the nonconjugate adaptation is produced at the motor level by a differential strength of the motor commands to the muscles of both eyes.

102.4

IMPAIRED INITIATION OF PURSUIT EYE MOVEMENTS IN SCHIZOPHRENIA. J.A. Sweeney*, J.R. Carl, M.S. Keshavan, G.L. Haas. Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

Impairments of pursuit eye movements are well-established in schizophrenia. Investigation of pursuit initiation may help clarify the cause of pursuit impairment in such illnesses. IR recordings were obtained from 15 unmedicated schizophrenic patients and 14 age-matched controls during tracking of unpredictable Rashbass-style step-ramps moving in the horizontal plane. As expected, average pursuit velocity was reduced in schizophrenic patients when they tracked constant-velocity targets moving at 8, 16, 24 or 32 deg/sec.

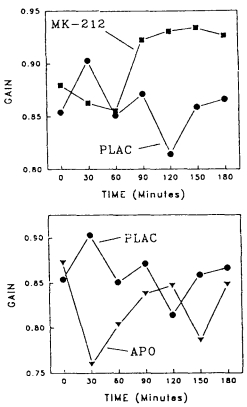
Several analyses indicate impaired pursuit initiation in schizophrenia. Latency to achieve a velocity of 2 deg/sec was increased across all target velocities. Eye velocity 175 msec after target motion onset, during the open loop period of pursuit, was significantly lower in patients. Peak eye velocity in the first 500 msec of target movement was lower in patients. This latter effect was greater at higher target velocities, suggesting that velocity saturation may occur at lower velocities in schizophrenia.

The findings indicate that the early phase of pursuit initiation is abnormal in unmedicated schizophrenic patients. Average pursuit gain was also impaired. Abnormalities in the open loop response and average pursuit gain were highly correlated. Supported by MH43613, MH42969 and MHCRC46745.

120.5

SEROTONERGIC STIMULATION INCREASES WHEREAS DOPAMINERGIC STIMULATION DECREASES SMOOTH PURSUIT GAIN IN NORMAL VOLUNTEERS. L. Friedman*, J.A. Jesberger, and H.Y. Meltzer. Lab. of Biological Psychiatry, Case Western Reserve University, Cleveland, OH 44106.

The effect of the direct-acting DA agonist apomorphine (0.01 mg/kg sc) or the direct-acting 5-HT agonist MK-212 (6-chloro-2-[1-piperazinyl]-pyrazine)(0.30 mg/kg po) on smooth pursuit gain was evaluated in 7 male normal volunteers. The target was a constant velocity (5 deg/sec) trapezoid. Eye movements were recorded with IR oculography. Apomorphine produced a statistically significant reduction (p=.001) in gain at 30 minutes post injection, whereas MK-212 produced a significant elevation in gain (p<.03) from 90 until 150 min. The results could not be attributed to side effects of the agonists, and are consistent with a role for 5-HT in motor facilitation. Pursuit gain may be a useful index of DA or 5-HT receptor sensitivity in clinical populations.



102.6

SACCADE-FACILITATED VERTICAL MOTOR FUSION, J. Ygge and D.S. Zee*, Department of Neurology, Johns Hopkins Hospital, Baltimore, MD, 21205.

We recorded movements of both eyes (search coil technique) in two emmetropic human subjects (S1,S2) during refixations between three vertically-aligned LEDs (0 ± 10 deg), located close to (about 11cm) and in front of the right eye. This near target array required about 29 deg of (horizontal) convergence and called for a change in relative vertical alignment (**ΔVA**) of about 1.15 deg for 0→ Up and 0→ Down saccades (right eye 10 deg, left eye 8.85 deg). Each eye was calibrated independently. First the eye was rotated into the same horizontal position as when viewing the near targets. Then, the subject refixated between vertically-aligned targets located at distance (125cm). Data were sampled at 500Hz.

For vertical refixations on the near array there was a vertical motor fusional response with two components. During the saccade, which lasted about 60ms for both Ss, there was a rapid **ΔVA** (peak vel 20 deg/s up and down for S1, and 18 deg/s up and 27 deg/s down for S2). Immediately following the saccade, a much lower velocity **ΔVA** ensued (in the first 160ms following the saccade the mean speed was 1.5 deg/s (range 1.0-1.7)). On average, 56% of the requisite **ΔVA** (range 29-87%) was completed within the saccade and 76% (range 52-100%) by 160ms following the saccade. As a control, subjects also refixated binocularly between vertically-displaced targets located at distance (125cm). There was little **ΔVA** (<0.11 deg for 10 deg saccades).

These results suggest that motor mechanisms can rapidly realign the eyes to assure vertical fusion when refixating between targets that create a vertical disparity under natural circumstances (ie, a target close to one eye). The speed of the change in vertical alignment during the saccade was markedly higher than that usually attributed to the vertical motor fusional system. This facilitation of vertical motor fusion by vertical saccades may be analogous to the facilitation of horizontal vergence by horizontal and vertical saccades.

102.7

FAST DISCONJUGATE ADAPTATIONS TO ANISEIKONIA. T. Eggert and Z. Kapoula*. Lab. de Physiologie Neurosensorielle, CNRS-UPR2, Paris, France.

Normal human subjects wearing anisometropic spectacles for 1-6 hr adapt to the resulting aniseikonia by making disconjugate eye movements (Lemij and Collewijn, 1991). We tested whether even faster adaptations to aniseikonia can occur.

In a dichoptic viewing condition, a grid of lines was viewed by each eye at a different magnification (1, 1.07). Three emmetropic subjects were asked to scan this grid with saccades for 10-25 min. Eye movements were recorded binocularly with search coils. In the presence of the adapting stimulus, all subjects exhibited saccade disconjugacies in the appropriate direction. For 2 subjects these effects persisted even for saccades in the dark, indicating adaptive changes. Subj YS was trained for only 10 min and showed a large saccade size disconjugacy: his pre-post change as estimated for saccades in the dark, was 10% for horizontal and 5% for vertical saccades. Subj SG trained for 25 min, showed smaller changes 4% (horizontal) and 2% (vertical). Subj SB exhibited post-saccadic eye drift disconjugacy but this effect did not persist in the dark.

These results indicate that disconjugate adaptations of saccades are possible within only a few minutes. Such adaptations seem to be larger for horizontal than for vertical saccades and may be produced by intra-saccadic, disparity driven vergence. Like disparity vergence, disconjugate changes in saccades could depend on the complexity of the image; in additional experiments we compare adaptations obtained with line grids vs random-dot patterns.

102.8

EGOCENTRIC LOCALIZATION OF A BRIEF PERISACCADIC FLASH BY MANUAL POINTING. Joel M Miller*. Smith-Kettlewell Eye Research Institute, 2232 Webster Street, San Francisco CA 94115

Reaching toward a visual object, without visual referents, requires information about position of eye in head supplied by an extraretinal signal (ERS). When a saccade occurs, ERS must be synchronized to the eye's rapidly changing position, or perisaccadic visual targets will be mislocalized.

We measured perisaccadic visual localization by presenting brief (250 μsec) monocular flashes in an otherwise dark field to normal human subjects instructed to point with unseen hand in the apparent direction of the flashes. Saccades were made toward auditory targets to eliminate intravisual comparison. Control trials, employing non-perisaccadic flashes and providing feedback of pointing errors, were randomly interspersed, to monitor and control drift of hand-eye coordination. The flash was presented randomly, on fovea, 10° nasal of fovea, or 10° temporal of fovea.

Saccades had mean magnitude 12.6°. For leftward saccades, loci in right hemiretina were updated faster (τ = 65 msec) than in left hemiretina (τ = 129). For rightward saccades, loci in left hemiretina were updated faster (τ = 62 msec) than in right hemiretina (τ = 90). Updating began within 1 msec of saccade initiation and was not a function of saccade direction or retinal locus.

Thus, there were no systematic mislocalizations preceding eye movement, and large errors during and after. Errors were maximal about 2/3 of the way through a saccade, and were about half the magnitude of the completed saccade. Stable post-saccadic localization was not achieved until about 150 - 350 msec after completion of a saccade; ERS was updated slowly, compared to eye position itself. The retina was not remapped uniformly: the hemiretina containing the target of a normal visually-evoked saccade (usually the target of a subsequent, corrective saccade) was updated with a shorter time constant. Updating began at about the time that saccade-related brainstem activity began, suggesting that collaterals of oculomotor signals drive egocentric localization. [Supported by NIH/NEI EY08399]

102.9

EFFECT OF LUMINANCE AND STIMULUS PREDICTABILITY ON HUMAN VERTICAL PURSUIT, OPTOKINETIC NYSTAGMUS (OKN), AND VISUAL-VESTIBULAR INTERACTION (VVI). J. L. Demer*. Jules Stein Eye Institute & Dept. of Neurology, University of California at Los Angeles, 90024.

To investigate the effects of predictability and large changes in target luminance, gain (eye velocity/stimulus velocity) and phase of vertical eye movements were measured in 5 normal adults using magnetic search coils. Projected targets were bright (185 Cd/m^2) as well as dimmed (2 log neutral density filter). Pursuit was tested using a small white spot, while OKN was tested using a 13° x 16° horizontal square wave grating (0.7 cycle/°, contrast 97%). The vestibulo-ocular reflex (VOR) was tested in darkness. VVI was tested during passive, whole-body, sinusoidal rotation while subjects viewed the stationary grating through 4× binocular telescopic spectacles (field diam. 10°). Predictable stimuli were single frequency sinusoids; unpredictable stimuli were sums of harmonics of 0.4 Hz (0.4-3.2 Hz).

For predictable motion, pursuit and OKN phase was always near 0° regardless of brightness. OKN gain consistently exceeded pursuit gain, although gains for both decreased with dimming, and with increasing stimulus frequency and velocity. For unpredictable motion, there were phase lags increasing with frequency, greater for pursuit than OKN. Target dimming reduced gain and increased phase lag.

For predictable head motion, enhancement of VOR gain by magnified vision was greatest at lower frequencies, and was reduced by target dimming. For predictable motion, phase was always compensatory regardless of brightness. For unpredictable motion, there was a phase lag increasing with frequency; target dimming reduced gain enhancement and introduced additional phase lag at high frequencies.

These data suggest that target brightness and extent may influence the speed of processing of visual afference to the human vertical ocular motor system, and may influence response gain even when motion is predictable.

Supported by EY-08656, NS-09823, and Research to Prevent Blindness.

102.10

EVIDENCE FOR DISTRIBUTED PARALLEL INTEGRATION IN THE INTERSTITIAL NUCLEUS OF CAJAL J.D. Crawford* and T. Vilis Depts. of Physiology and Ophthalmology, University of Western Ontario, London, Canada, N6A 5C1.

We have previously shown that the interstitial nucleus of Cajal (INC) is an essential part of the integrator for vertical and torsional eye movements. Here we examine the time course of the effects produced by injection of 0.3 μl of a 0.05% muscimol solution into a total of 40 INC sites in 5 alert monkeys (*Macaca fascicularis*) while recording eye movements in 3-D. After injection, saccades in the light were followed by torsional and vertical positional drift. In some cases, eye position drifted towards a unique vertical and torsional null position, at a time constant that progressively decreased. The torsional time constant was always lower than the vertical constant at intermediate stages. This can be simulated by single leaky vertical and torsional integrators. More often, eye position drifted rapidly immediately after muscimol injection (time constant ~ 200 ms), but not to a unique null position. Instead, this drift resembled a pulse-step mismatch, except that it only occurred after eccentric vertical and torsional saccades. This deficit progressed as if the step were getting smaller and smaller compared to the pulse. At intermediate stages of the deficit, the animal was still able to maintain a progressively narrowing band of vertical, and to a lesser extent, torsional positions. In some cases the drift clearly had more than one time constant. Similar observations were made when animals made spontaneous saccades in the dark. These results cannot be simulated by single integrator models. However, they were easily simulated by a model which incorporated a bank of parallel integrators in its velocity-to-position transformation.

102.11

THE ROLE OF OCULAR PROPRIOCEPTION IN DISCONJUGATE OCULAR MOTOR ADAPTATION. R.F. Lewis, D.S. Zee, and B.L Guthrie*, The Johns Hopkins Hospital, Baltimore, MD 21205.

We examined the role of ocular proprioception in disconjugate adaptation to a unilateral ocular muscle paresis. Two rhesus monkeys wore search coils and were trained to fix upon targets. A vertical muscle weakness was created in one eye (in the orbit, or by intracranial nerve section). Static alignment (phoria) and the ratio of the movements of the two eyes during saccades (Pulse-Pulse (P-P) ratio) were used as indices of yoking. Measurements were made after habitual (>7 days) monocular (nonparetic eye) viewing, and after habitual binocular viewing with a corrective prism.

Proprioceptive afference was eliminated from the paretic eye by intracranial section of the ophthalmic branch of the trigeminal nerve, and the animals were restudied. After deafferentation, the static misalignment and the P-P ratio significantly increased following both the habitual monocular and the binocular viewing conditions. For example, after habitual monocular (nonparetic) viewing the phoria value at primary position increased from 6.0 to 7.2 deg and the slope of the line relating the phoria to the position of the normal eye increased from 0.14 to 0.19. The mean P-P ratio increased from 1.13 before, to 1.21 after, deafferentation. Similar changes were observed after habitual binocular viewing.

We conclude that ocular proprioception contributes to disconjugate adaptation to asymmetrical ocular muscle weakness, under conditions of both monocular and binocular (disparity present) viewing. We hypothesize that proprioceptive information is used in the long-term calibration of the internal, efferent copy map of the position of the eyes in the orbit.

103.1

PRIMATE FRONTAL CORTEX: NEURONAL ACTIVITY FOLLOWING ATTENTIONAL VS. INTENTIONAL CUES D. Boussaoud* and S.P. Wise. Laboratory of Neurophysiology, NIMH, Poolesville, MD 20837

We examined neuronal activity in the dorsal (PMd) and ventral (PMv) premotor cortex and in the prefrontal (PF) cortex of two rhesus monkeys. Each monkey fixated a 0.2° white square in the center of a video display while depressing a switch located between two touch pads. Red or green squares (2°x2°) served as cues. On each trial, a spatial/attentional cue (SAC) was presented for 0.8 s. The SAC consisted of one square, red or green, and its location indicated where a relevant motor instructional cue (MIC) would appear 1.8 to 3.3 s later. The MIC consisted of either one square (red or green) or both a red and green square presented simultaneously. A green square at the previously cued location instructed a forelimb movement to the right touch pad; red instructed movement to the left. At the offset of the MIC (duration 1 to 3 s), the monkey had to execute a forelimb movement within 1 s (and could break fixation).

Because the SAC and MIC could be physically and spatially identical, the behavioral design allowed us to test the hypothesis that task-related activity reflects attentional (SAC) vs. intentional (MIC) processes.

Our results from a sample of 101 frontal cortex neurons show that the vast majority of cells in PMd (71%) have greater activity following intentional cues than after attentional cues. In most instances, there is no activity following SAC, although the identical stimulus causes profound modulation when presented as the MIC. In PMv and PF, by contrast, more cells showed comparable or greater activity following the attentional cue (50% and 61%, respectively). Responses to the SAC often showed spatial selectivity, but rarely if ever were affected by color.

103.2

MOTOR AND ATTENTIONAL COMPONENTS OF SENSORY EXTINCTION. R.K. Deuel*, Ped. Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Unilateral frontal and parietal lesions often result in extinction of stimuli in contralesional sensory fields. We tested responses on 10 trials each of bilateral simultaneous visual and somatosensory stimuli in macaques, removed frontal periarcuate (N=14) or posterior parietal (N=14) cortex, and retested within fourteen days. Ipsilesional fields were addressed first and with the ipsilesional hand in 91-100% of trials. The contralesional field was usually addressed second, but was sometimes omitted (30% of somatosensory trials and 7% of visual trials). In the 70% of trials where the second somatosensory stimulus was attended, the ipsilesional hand was used to grasp it. For the second visual stimulus, parietal subjects used the contralateral hand in 16% of trials and the frontals used it in 50%, as before operation, i.e. the motor performance of the contralesional hand was significantly different between the groups although they attended contralesional stimuli with equal frequency. Motor and attentional aspects of extinction are separable, and frontal subjects use the contralesional hand with normal frequency to grasp contralesional stimuli.

103.3

PARALLEL CORTICAL PATHWAYS FOR THE CONTROL OF MOVEMENT J.-O. Hahm*, M.F. Huerta, P.L. Strick°, I. Danielsson & T.P. Pons. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892, and °Research Services, VA Medical Center, SUNY, Syracuse, NY 13210.

Most current theories on the cortical control of movement hold that "premotor" (area 6) and "supplementary" motor cortex (SMA) act to program primary motor cortex (M-I) and that M-I corticospinal projections directly control movement. The discovery that cortical motor areas outside M-I also have direct corticospinal projections raises the possibility that the control of movement is mediated by multiple cortical pathways organized in parallel.

To test this hypothesis, we used microstimulation techniques to map primary and supplementary motor cortex in the relatively agyric owl monkey. After identifying various movement representations and determining current thresholds for stimulation sites, we ablated M-I representations of the forelimb (n=2) or vibrissae (n=1), or all movement representations in the SMA (n=1), and then remapped the regions of motor cortex left intact.

After ablation of either the forelimb or vibrissae region of M-I, forelimb and vibrissae movements could still be elicited by microstimulation in the respective regions of SMA. Current thresholds for stimulation sites were in the normal range. Similarly, ablating SMA had no detectable effect on the ability to drive movements in M-I. These results indicate that SMA is sufficient to drive movement, even in the absence of M-I, and likewise M-I is sufficient in the absence of SMA, providing strong support for the proposal that movement is under the control of at least two parallel corticospinal systems.

103.4

CORTICOSPINAL PROJECTIONS IN THE MATURE AND INFANT MACAQUE. Galea, M.P. & I. Darian-Smith*. Brain Research Lab., Dept. of Anatomy, Univ. of Melbourne, Parkville, Victoria, 3052. Australia.

Retrograde labelling of corticospinal (CST) neurons was achieved by the injection of up to four different fluorescent dyes, Fast Blue, Diamidino Yellow, Rhodamine and Green Latex Microspheres, into several defined zones in the cervical spinal cord of mature and infant monkeys. Maps of the CST projections resulting from these dye injections could then be compared in the one animal. A series of planar maps was generated to represent the 3-D spatial distributions of the different CST populations and to correlate them with their spinal target zones.

In the mature monkeys, CST somata labelled by a dye injection in the dorsolateral column were extensively distributed throughout the sensorimotor cortex, with peak densities in four cortical regions : frontal, parietal, mesial and perisylvian. The ipsilateral projection had a similar distribution but was less dense. When the projections to the cervical grey matter were analyzed, a shift in the relative weighting of pre- and post-central projections could be demonstrated according to the location of the injection site in the ventral or dorsal horn respectively.

The CST projection in the cervical dorsolateral column in the infant macaque had a similar distribution to that in the mature animal, but was *more dense*. Additional, transient projections were observed from the mesial, prefrontal, insular and posterior parietal areas, around the periphery of the mature distribution, and from regions that did not project to the spinal cord in the mature macaque. The changing postnatal corticospinal projection is a reflection of regressive changes which have also been observed in other central nervous system projections in infant macaques. Analysis of CST projections to the cervical grey matter in the infant showed that these were less than in the mature animal, suggesting that many CST terminals still had not formed synapses on spinal neurons and that this synaptogenesis was largely postnatal.

103.5

RECOVERY OF FORCE CAPACITY AFTER LESIONS OF THE FORELIMB PRIMARY MOTOR CORTEX IS DUE TO THE REORGANIZATION OF ADJACENT AREAS OF THE CORTEX. M.A. Castro-Alamancos, J. De Felipe* & J. Borrell.Cajal Institute (CSIC), Avenida Dr. Arce 37, 28002-Madrid, SPAIN.

We were interested in studying if reorganizational processes taking place in the cortex mediate recovery after cortical damage. Wistar rats were trained to press two independent isometric levers, each with one forelimb, in order to obtain a food reward. Three groups were formed. In group 1 the motor cortex was mapped bilaterally by using a microstimulation technique and lesions were placed in the primary motor cortex (MI) forelimb representations. Group 2 followed the same procedure as group 1 and an electrode was implanted in the ventral tegmental nucleus (VTN) unilaterally. Group 3 received sham surgery. The forelimb force and response velocity was evaluated as before surgery with the only difference that the animals with an electrode implanted in the VTN received an electrical stimulation in this nucleus each time they pressed the lever. The intensity of the current delivered increased with the force exerted on the levers. The results showed that after ablation of the MI forelimb representations (group 1) the animals showed a deficit in force capacity and in response velocity. In contrast, the animals that received VTN stimulation (group 2) recovered rapidly their force capacity and response velocity. The motor cortex of these animals was mapped again and forelimb movements were evoked posterior to the lesion in recovered animals. Finally, when the cortical area that evoked forelimb movements was ablated in recovered animals the behavioral deficit was reinstated. In conclusion, recovery after ablation of the MI forelimb representation of the rat seems to depend on the reorganization of adjacent areas of the motor cortex.

103.6

REORGANIZATION OF DISTAL FORELIMB REPRESENTATIONS IN PRIMARY MOTOR CORTEX OF ADULT SQUIRREL MONKEYS FOLLOWING FOCAL ISCHEMIC INFARCT. R.J. Nudo* & R. Grenda. Dept. of Neurobiol. & Anat., Univ. of Texas Med. Sch., Houston, TX 77030.

Early studies using surface stimulation techniques suggested that the hand representation in primary motor cortex of adult primates undergoes substantial reorganization following small lesions, and that cortical reorganization is correlated with functional recovery (Glees & Cole, 1950). In order to examine lesion-induced plasticity in primary motor cortex (area 4) of adult squirrel monkeys, we have employed intracortical microstimulation techniques to derive detailed maps of the hand representation before and a few months after a focal ischemic infarct. Representational maps were derived from hundreds of closely spaced microelectrode penetrations. Infarcts were made by bipolar electrocoagulation of a small vascular bed supplying a portion of the hand representation. To determine the extent of spontaneous recovery, no post-infarct training was conducted.

In sharp contrast to the results obtained by Glees & Cole, movements represented in the infarcted zone *did not* reappear in the cortical sector surrounding the infarct. Instead, relatively small lesions in representations of distal movements resulted in widespread reduction in the spared distal representations adjacent to the lesion, and apparent increases in adjacent proximal representations. Thus, it would appear that extensive reorganization occurs in primary motor cortex following focal ischemic infarct, but at least in the absence of post-infarct training, the movements formerly represented in the infarcted zone do not reappear in adjacent cortical regions. Supported by the Whitehall Foundation.

103.7

SLOW (0.3 Hz) SYNCHRONIZED OSCILLATION IN NEOCORTICAL CELLS. M. Steriade*, A. Nuñez and F. Amzica. Lab. of Neurophysiology, Laval Univ. Sch. of Med., Quebec, Canada G1K 7P4.

During the synchronization of electrical activity in the neocortex, various wave patterns are grouped in sequences that recur periodically at slow (<1 Hz) rhythms. To shed light on cellular bases of these rhythms, we have analyzed a slow oscillation of cortical neurons by means of intracellular recordings from association cortical areas 5 & 7 of cats under urethane anesthesia. Neurons were physiologically identified by ortho- and antidromic activation from LP and CL thalamic nuclei as well as from homotopic foci in the contralateral suprasylvian cortex. A group of representative elements were intracellularly stained.

In a majority (65%) of the 190 analyzed cells, the oscillation consisted of full action potentials or dendritic spikes riding on depolarizing envelopes that recurred rhythmically at 0.1-0.7 Hz, mostly around 0.3 Hz. The amplitude and/or duration of depolarizing envelopes were reduced at Vm more negative than -90 mV or by intracellular injection of QX-314. In the remaining neurons, the oscillation consisted of hyperpolarizations that periodically (≈0.3 Hz) sculptured the irregular firing or of rhythmic (≈0.3 Hz) sequences of IPSPs which could only be revealed by DC depolarization. All these slow oscillatory patterns were dramatically synchronous with EEG complexes at ≈0.3 Hz, recorded from a variety of cortical foci. Ten oscillatory neurons were intracellularly stained and all proved to be pyramidal-shaped cells located in layers II to VI. The slow oscillation survived in thalamectomized animals. Experiments in progress revealed the presence of the same type of slow rhythm in intracellularly recorded visual and motor cortical neurons. The discharges of slowly oscillating cells, simultaneously recorded from association and visual areas, displayed a time-lag around 90-120 ms, suggesting either slowly conducting corticocortical axons or, more probably, corticothalamocortical linkages. Supported by MRC of Canada (grant MT-3689).

103.8

FUNCTIONAL PROPERTIES OF BURST AND SINGLE SPIKE FIRING IN LAYER V CORTICAL BURST GENERATING NEURONS Z. Wang* and D.A. McCormick Sect. Neurobiology Yale Univ. Sch. Med. New Haven, CT

A subset of layer V pyramidal cells in the cerebral cortex can intrisically generate bursts or trains of action potentials in response to depolarization. Here we investigate the functional properties and the modulation by neurotransmitters of these two modes of action potential generation using standard *in vitro* slice techniques.

Recordings from cortical burst generating neurons revealed that a subset of these cells generated rhythmic spontaneous bursts at frequencies of around 0.2-2 Hz (n=20). Intracellular injection of repetitive inputs (e.g. sine waves) of varying frequencies into these cells revealed that they can faithfully generate repetitive bursts in the frequency range of only 1-4 Hz. Higher frequencies inhibited burst firing and could switch the cells to the single spike mode of action potential generation, where the cells fired predominately at 5-40 Hz with no evidence of spike frequency adaptation. Similar changes in firing mode and frequency could be induced by stimulation of α1-adrenergic, muscarinic or glutamate metabotropic receptors.

These results indicate that the burst firing mode enhances the appearance of slow oscillations or EPSPs which occur at low frequencies, while the single spike mode predominates during high frequency sustained activity.

103.9

CALCIUM CURRENTS IN THE APICAL DENDRITES OF NEOCORTICAL PYRAMIDAL NEURONS. H.G. Kim* and B.W. Connors. Department of Neuroscience, Brown University, Providence, RI 02912.

Pyramidal cells of neocortical layer V often have a long, elaborate apical dendrite that reaches layer I. This dendritic design allows the single neuron to combine synaptic inputs from deep and very superficial cortical layers. However, electrotonic considerations predict that the dendrites must have nonlinear membrane properties in order for the most distal inputs to be effective.

We have obtained whole-cell voltage- and current-clamp recordings directly from the apical dendrites of layer 5 pyramidal neurons, using a slice preparation of mature rat SI neocortex at 35°C. Recording sites were confirmed by measuring the electrode position and intracellular staining with biocytin. Stained dendrites often showed a distinct swelling at the presumed recording site. Recordings from apical dendrites were readily distinguishable from somatic recordings when K^+ gluconate-filled pipettes were used under control conditions. Dendrites showed three general responses to current injections: 1) small, broad, TTX-sensitive, Na^+-dependent action potentials, 2) complex, often clustered patterns of spikes, and 3) high threshold, long-lasting spikes and plateaus (no K^+ currents blocked). Co^{2+} (2 mM) and Ni^{2+} (2 mM) reduced complex spiking and blocked long-lasting spikes and plateaus, suggesting an involvement of regenerative, high-threshold Ca^{2+} currents. Calcium currents may amplify the effectiveness of distal synaptic inputs, and serve as a mediator or substrate for the modification of dendritic properties.

Supported by NS25983 from NIH, and ONR.

103.10

NMDA AS WELL AS NON-NMDA RECEPTORS ARE INVOLVED IN MOTOR TASK-RELATED NEURONAL ACTIVITY IN THE MOTOR CORTEX OF AWAKE MONKEYS. K. Shima and J. Tanji* Dept. of Physiology, Sch. of Med. Sendai, 980, JAPAN

The effects of iontophoretic application of agonists and antagonists for both NMDA and non-NMDA glutamate receptors on neuronal activity in the primary motor cortex of two monkeys (*Macaca fuscata*) was examined during performance of a trained motor task. Multi-barreled glass micropipettes were used for application of chemicals. A central pipette was equipped with a fine carbon fiber for extracellular unit recording. Motor task-related neuronal activity (both movement- and motor set-related), as well as background neuronal activity was quantitatively assessed before, during and after drug application. The following findings emerged from this study. 1) Both APV and CNQX reduced task-related activity with or without affecting background activity. NMDA and quisqualic acid (or kainic acid) had the opposite effects. 2) CNQX suppressed movement-related activity more often than APV. 3) APV suppressed background discharge more effectively than CNQX. 4) Unitary spikes of motor cortex neurons evoked by stimulation of the somatosensory cortex were suppressed more effectively by CNQX than by NMDA. These results indicate that both NMDA and non-NMDA glutamate receptors are involved in activating motor cortex neurons in relation to performance of the motor task. Differences in relative contribution of these two receptors among motor cortex neurons with different properties seem to exist.

103.11

EFFECTS OF BICUCULLINE ON LEARNING A HIDDEN OBJECT* PICK-UP TASK IN INFANT MONKEYS. K. Kubota*. Dept. of Neurophysiology, Primate Res. Inst., Kyoto Univ., Aichi 484, Japan.

Five newborn macaque monkeys were trained in a hidden object pick-up Task, comparable to a Delayed Response or AB Task. During the course of learning the Task, Bicuculline (BMI, 1-3μg in 0.5-1.0μl saline), was injected locally into the dorsolateral prefrontal cortex. While the monkey watched from a cage, a piece of apple was hidden under one of two opaque bottle caps (left or right)(11-12 w old). The transparent screen in front of the monkey was then raised and the monkey reached out to one of the bottle caps, picked up the piece of apple and ate it. When the monkeys performed correctly < 75% of the time, BMI did not influence the Task performance level (12 sites). However, when they had learned the Task, with the piece of apple hidden for 2-3 s (19-21 w old). BMI decreased the performance level (18 sites). The number of perseverative errors on the side contralateral to the hand used, increased, regardless of which side of the brain was injected. Apparently, $GABA_A$ inhibition in infant monkeys is involved in reducing the number of contralateral erroneous responses and promoting correct Task performance. Supported by the Human Frontier Science Program.

103.12

THE CONE ELECTRODE: CHRONIC RECORDING TECHNIQUES. **P.R.Kennedy*, A.Hopper, C.Linker, S.M.Sharpe and R.A.E.Bakay. Yerkes Res. Center of Emory Univ., & Neurosci. Lab., GA Tech, Atlanta, GA 30332.**

Previous studies (Kennedy, J. Neurosci. Methods, 29(1989)181-193) demonstrate chronic recording of multi-units via the Cone Electrode. Recent long-term studies demonstrate viable myelinated axons and supporting stroma inside the glass enclosure at the electrode tip (Neurosci. Letters, accepted 1992). Present studies focus on burying the electrode and electronics under the scalp to minimize infection and traumatic damage.

Following implantation, a headstage amplifier and FM transmitter are attached to the electrode pins. These electronic components are also linked to the secondary coil of a power induction system. They are protected by Elvax (moisture barrier), Silastic (mechanical barrier), Acrylic cement (insulator of electrode pins and mechanical barrier) and intact scalp (pathogenic barrier). The system is powered by placing a tuned primary coil near the secondary coil in the awake monkey for transcutaneous power transmission by RF induction. AC components are rectified to DC, and voltage is regulated to 3 volts.

The system is implanted long-term in a monkey and provides visual and auditory feedback of the firing rates of units recorded in motor areas.

104.1

INCREASED RATE OF SYMPATHETIC INGROWTH TO SMOOTH MUSCLE OF NEONATAL RAT. Jean M. Hiebert and Peter G. Smith*. Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66160.

Target tissue reinnervation is known to decrease as a function of age. One contributing factor may involve age related changes in the target tissue that prevent or deter ingrowth. The purpose of this study was to determine if sympathetic sprouting into smooth muscle targets is diminished in older animals. Superior tarsal smooth muscle from 6, 14, 30, and 48 PND donors was sympathetically denervated by performing a superior cervical ganglionectomy. Three days later, the muscle was removed from the donor and small pieces were implanted into the anterior chamber of host rats 84-90 days of age. Dopamine β-hydroxylase immunoreactivity was used to assess ingrowth of iris sympathetic nerves at 3, 6, and 10 days post implant.

Sympathetic ingrowth occurred in all four age groups. The rate of ingrowth was most rapid in tissue from the youngest donors; however, all age groups achieved similar innervation by day 10. These findings support the conclusion that while smooth muscle reinnervation can occur at all ages studied, it is facilitated in the neonatal animal. Supported by NS23502.

104.2

OUTGROWTH IS ACCELERATED BY ONE WEEK AFTER AXOTOMY. J.M. Jacob* and I.G. McQuarrie. Neural Regen. Ctr., Cleve. VA Med. Ctr., Cleveland, Oh. 44106.

In a conditioning lesion paradigm using rat sciatic motor neurons, we have previously shown that the rate of axonal outgrowth increases after a testing lesion. This is apparently due to an increase in the rate of structural protein transport (SCb) throughout the axon by 7 d after the initial (conditioning) axotomy. The acceleration of SCb can be assayed indirectly by radiolabeling fast axonal transport and measuring the rate of advance of growth cones. Using this assay without a conditioning axotomy, we now show that axonal outgrowth begins to accelerate at approximately 7 d after axotomy, i.e., when SCb begins to accelerate. Thus, between 3 and 6 d after axotomy the outgrowth rate was 3.6 ± 0.5 mm/d, whereas, between 9 and 12 d it was 5.1 ± 0.5 mm/d ($p < 0.001$). A non-axotomizing growth stimulus of L4 sciatic motor neurons (partial denervation of hindlimb muscles by excision of the L5 spinal nerve) did not accelerate SCb by the outgrowth rate assay. Our data confirm that a single axotomy is sufficient to induce an acceleration of SCb, and suggest that the formation of collateral sprouts by uninjured neurons is not sufficient to induce an acceleration of SCb.

Supported by a grant from DVA to IGM.

104.3

FUNCTIONAL AXON SPROUTING OF TETRODOTOXIN-INACTIVATED RAT PLANTARIS MOTOR UNITS AFTER PARTIAL DENERVATION OF THE MUSCLE. R.N. Michel*, and C. Boudreau. School of Human Movement, Laurentian University, Sudbury, Ontario, P3E 2C6.

Partial denervation (PD) of mammalian skeletal muscle causes surviving motor axons to sprout and reinnervate neighboring denervated muscle fibers. The purpose of this study was to determine whether there is an advantage for surviving motor units to be inactive (i.e. not propagating action potentials) during the short-term phase of this neuronal growth response. Sprague-Dawley rats had the sciatic nerve of both hindlimbs inactivated with tetrodotoxin (TTX) using an osmotic pump and cuff delivery system. After inactivation, approximately 80% of plantaris muscle fibers in one hindlimb were denervated by cutting radicular nerve L4. The extent of functional motor unit sprouting was assessed 10 days later by the ratio of force and electromyographic (EMG) responses evoked via stimulation of the sprouting L5 nerve to that produced by stimulation of its contralateral intact counterpart. Results were compared to plantaris from control animals that underwent PD only. TTX-inactivation alone caused plantaris mass (35%) fiber area (50%), and maximum force (70%) to be smaller than control. In spite of these changes, both TTX-inactivated and normally active sprouted motor units increased their functional size (force and EMG) more than 2-fold. The performance of the newly formed sprouts (i.e. maintenance of tension envelope and EMG profile at high stimulation frequencies) was not altered by TTX-inactivation. Our results demonstrate that motor units surviving a PD lesion but rendered inactive are comparably efficient at capturing and activating previously denervated muscle fibers. Neuromuscular denervation products seem to be a major prerequisite for robust and functional motor axon sprouting.

104.4

SYNAPTIC REMODELING REVEALED BY COMBINED VIDEO-ENHANCED FLUORESCENT MICROSCOPY AND ELECTRON MICROSCOPY OF IDENTIFIED FROG NEUROMUSCULAR JUNCTIONS. C. P. KO*, L. CHEN & A. THOMPSON. Dept. of Biol. Sci., Univ. of Southern California, Los Angeles, CA 90089.

Our previous studies using *in vivo* observations of identified frog neuromuscular junctions (NMJs) suggest that synaptic extracellular matrix (ECM) precedes nerve terminal during junctional extension and sprouting (J. Neurosci. 11:2920; Soc. Neurosci. Abstr. 17:735). The present work aims to examine at the ultrastructural level the sprouted region where synaptic ECM is longer than the nerve terminal. Sartorius muscles were double-labeled with 4-Di-2-Asp and rhodamine conjugated peanut agglutinin (PNA) to stain nerve terminals and synaptic ECM respectively. The identified NMJs were observed *in vivo* with video-enhanced fluorescent microscopy. Segments of contralateral sciatic nerve were then placed on the muscle surface to induce nerve sprouting. During the peak of sprouting (2-3 months later), the same NMJs were re-stained and re-examined as above. The identified NMJs which showed sprouting with synaptic ECM longer than the nerve terminal were then stained with HRP conjugated PNA and processed for electron microscopy. The result at the ultrastructural level showed the absence of nerve terminal in the extended PNA-stained region. In addition, Schwann cells surrounded by PNA-stained ECM were seen at this region. These findings confirm the validity of 4-Di-2-Asp staining for nerve endings seen with light microscopy and further support the suggestion that synaptic ECM precedes nerve terminal outgrowth during synaptic remodeling.

104.5

TEMPORAL PROGRESSION OF DESCENDING AXONS IN THE CHICKEN EMBRYO SPINAL CORD. I. C. Glover* and A.-L. Eide. Dept. of Physiology, Institute of Basic Medical Sciences, University of Oslo, 0317 Oslo, Norway.

The temporal pattern of growth of descending projections in the spinal cord of the chicken embryo has been inferred from retrograde labelling of supraspinal neurons after injections of WGA-HRP at different spinal levels (Okado & Oppenheim, J. Comp Neurol. 232:143-161, 1985). To provide a more complete description of the pattern of axon growth, we have labelled the descending axons anterogradely with the lipophilic tracer DiI in fixed spinal cords from embryonic day (E) 5 to E10. DiI was injected into a hemisection at the first cervical segment, with the aim of labelling all longitudinal axons traversing the hindbrain/spinal cord transition.

Labelled axons extended varying distances caudally in any given preparation. Typically we observed a wave of some tens of axons that were outpaced by a minority (<10) that extended 3-5 segments further. These few most caudally-projecting axons were restricted to cervical levels until E7-8, and had begun to sprout short primary collaterals in the cervical cord by E7. Some of the most caudally-projecting axons at these stages were descending branches of bifurcating axons that originated from spinal interneurons. By E10 the most caudally-projecting axons extended to mid-lumbar levels and had established more elaborate collaterals there, some crossing the midline in the ventral commissure.

Control experiments in which DiI was injected in mid-thoracic segments support the indication that axons originating from supraspinal levels do not reach the lumbar spinal cord until after E7-8. The progression of descending axon growth revealed by anterograde labelling is therefore about 2 days delayed relative to that suggested by retrograde labelling.

104.6

INITIAL DIFFERENCE IN THE GROWTH PATTERNS OF CUTANEOUS AND MUSCLE SENSORY AFFERENT COLLATERALS. A.-L. Eide* and I. C. Glover. Dept. of Physiology, Institute of Basic Medical Sciences, University of Oslo, 0317 Oslo, Norway.

Sensory neurons that innervate cutaneous versus muscle receptors in the periphery innervate different sets of central targets. In particular, muscle afferents project collaterals to the ventral horn where they innervate MNs, while cutaneous afferents project collaterals to the dorsal horn where they innervate interneurons. To investigate the development of these different patterns of collateral termination, the lipophilic tracer DiI was applied selectively to identified cutaneous and muscle nerves in the lumbar region of chicken embryos from embryonic day (E) 6 to E18. Tracer was applied to fixed preparations that were incubated for 4 to 400 days.

Sensory afferents in the lumbar region enter the cord on E4 and bifurcate to course rostrally and caudally. The longitudinal trajectory of muscle afferent axons is restricted more medially than that of cutaneous afferent axons. Ventrally-directed collaterals from both types of afferent begin to sprout on E6-E7. However, the cutaneous and muscle afferent collaterals differ in length already during the first day of growth. On E8, when cutaneous afferent collaterals have grown to a depth of about 50 μm, muscle afferent collaterals have grown about 250 μm, over halfway to the ventral limit of the gray matter. Cutaneous afferent collaterals are restricted to the dorsal region of the gray matter during subsequent stages. Thus, differences in the termination patterns of cutaneous and muscle afferents do not arise by equivalent growth followed by differential retraction, but through an initial difference in the extent to which the collaterals invade the gray matter.

104.7

CENTRAL PROJECTIONS OF SENSORY NEURONS IN ORGANOTYPIC CULTURES OF EMBRYONIC CHICK SPINAL CORD. Z. Korade* and E. Frank, Dept of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh Medical School, Pittsburgh, PA 15261.

During development, spinal sensory neurons establish an orderly set of central projections into the spinal cord. Cutaneous sensory neurons terminate within the dorsal horn, while muscle spindle afferent axons project nearly unbranched through the dorsal horn to arborize in the region of spinal motoneurons. Relatively few primary sensory fibers project across the midline. Although the first projections into the spinal cord occur shortly after sensory neurons are born, the characteristic central projection patterns are established only after sensory axons project to their targets in the periphery. In order to begin to understand the mechanisms responsible for the establishment of these orderly projections, we have developed an organotypic co-culture of embryonic chick spinal cord and dorsal root ganglia.

Slices of spinal cord are prepared from E6 chick embryos, a time at which some sensory axons have grown into the dorsal root entry zone but have not begun to arborize within the developing gray matter. Dorsal root ganglia, also from E6 embryos, are positioned next to the spinal cord slice or are left attached to it during the dissection. The explants are immobilized in a thrombin clot and cultured in normal medium with added muscle extract and NGF for 3-4 days. After fixation, DRG's are labeled with DiI to visualize the arborization of sensory fibers within the spinal cord.

Sensory axons growing from detached DRGs are normally excluded from the cord slices. Most fibers growing towards the slice turn to avoid it. Penetration into the cord is much more successful when DRGs are left attached and the axons enter the cord through the dorsal and, to a lesser extent, ventral roots. Arborizations of these fibers within the developing gray matter is virtually entirely limited to the dorsal horn; few project down to the level of motoneurons despite the fact that muscle afferent neurons survive in this medium. Another difference from the projection patterns seen in vivo is that many sensory fibers cross the midline into the contralateral dorsal horn. This co-culture system offers the possibility of studying factors that influence the development of these central projections.

Supported by NIH to EF.

104.8

VIDEO MICROSCOPY OF GROWTH CONES IN DECISION REGIONS OF THE CORPUS CALLOSUM IN LIVING CORTICAL BRAIN SLICES. M.C. Halloran* and K. Kalil, Neuroscience Training Program and Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

We have studied the behavior of growth cones extending in the corpus callosum of hamster cortical brain slices, using time lapse video microscopy under low light level conditions (Halloran and Kalil, Soc. Neurosci. Abstr., 1991). We found that DiI-labeled growth cones in the callosal pathway generally advanced steadily and rapidly and exhibited continual lamellipodial shape changes, whereas growth cones extending into the cortical target had smaller growth cones that maintained more constant morphologies. However, an important unanswered question is how growth cones make the decision to leave the callosal tract and enter the cortical target. We have therefore examined the behaviors of callosal growth cones in this transition region beneath their ultimate target area. We found that growth cones typically slowed down in these regions and repeatedly extended and retracted branches tipped with lammellipodia. In some cases the entire growth cone withdrew, leaving behind a filopodial extension, which then formed the pathway for the subsequent resurgence of the growth cone. In other cases the primary growth cone maintained its position while its numerous side branches extended and withdrew. This saltatory behavior can last for several hours, suggesting that the growth cone is undergoing a decision making process. We are currently investigating whether any of these transient branches ultimately become permanent axon collaterals extending into cortical targets.

Supported by NIH Grant NS14428 to K.K.

104.9

VISUALIZATION OF OPTIC ARBORS IN NORMAL AND REGENERATING LIVING ADULT GOLDFISH. A.M. Danks*, P. Kim, Z. Wang, and R.L. Meyer. Developmental Biology Center, Univ. of Calif., Irvine, CA 92717.

The fluorescent lipophilic membrane probe DiI (2.67 mM, 1.5 nl) was injected into the ganglion cell layer of the retinas of adult goldfish to allow in vivo visualization of axons and arborization of individual nerve cells as they project into the optic tectum. After allowing several days for dye transport, the tectum was exposed and the live fish were placed under a fluorescence microscope equipped with a cooled CCD camera. Individual axons and terminal arbors including many fine branches could be resolved.

Arbors were visualized repeatedly for as long as 5 hours. In addition, fish could be resuscitated and the arbor reexamined a day later. We found that the pattern of branching remains stable over time. No remodeling of fine processes could be detected. These data will be compared to regenerating goldfish that had been been subjected to optic nerve crush 2 to 5 weeks previously, and whose regenerating axons show a less developed arbor network.

Supported by NIH-EY 6746.

104.10

THE STRUCTURE AND FUNCTION OF *DROSOPHILA* NEURONAL CLUSTERS DEVELOPED *IN VITRO*. K. Ikeda* and J.H. Koenig. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

The structure and function of neuronal systems was studied with a primary culture of *Drosophila* neuroblasts. The neurons were developed from undifferentiated neuroblasts dissociated from *Drosophila* embryos at the early gastrula stage. The clusters of neurons in this culture developed into a structure reminiscent of an insect ganglion. This structure exhibited activities similar to an *in vivo* ganglion, such as spontaneous firing and patterned output in response to a simple train of input impulses. The structure of the cluster was strikingly similar in general architecture to a typical insect ganglion; i.e., the outer layer consisted of one or several layers of somata, while the inner space (core) consisted of a structure very similar to a typical neuropile. The neuropile was composed of many neurites which formed synapses on each other. The axons, which extended from the somata into the neuropile, made many dendritic branches before running toward the bottom of the cluster. There, they turned horizontally, forming several bundles which emerged from the cluster. The axon bundles coming out from a cluster eventually reached other clusters. Thus, various interconnected ganglion-like clusters were formed. The connections between these clusters were not only structural, but functional. Thus, stimulation with a train of electrical pulses of axons entering a cluster elicited variously patterned impulses in other bundles emerging from this cluster. Coordinated activity among separate ganglia was also observed. Furthermore, the activity pattern of a cluster was modulated by the input from the other cluster. Thus, a structure which functions similarly to a ganglion can develop *in vitro* without specific positional clues which are available during normal development *in vivo*.

Supported by NIH grant, NS-18856.

104.11

FORMATION OF NEURAL CONNECTIONS BETWEEN AGGREGATES OF RAT VISUAL CORTICAL CELLS CULTURED IN A MULTIELECTRODE DISH. H. Sugihara[1], T. Mitumata[1], N. Yamamoto[2], K. Toyama[2] *. [1]Central Research Lab., Matsushita Elec. Co., Moriguchi, Osaka, 570, Japan. [2]Kyoto Prefectural Univ. of Medicine, Kamigyo-ku, Kyoto, 602, Japan.

Aggregates of cortical cells dissected from embryonic (E18-17) rat visual cortex were cultured in a dish implanted with a 6 x 6 (or 8 x 8) multielectrode array made of indium-tin-oxide (interpolar distance, 300 um). Observed with ordinary light microscopy, neurites began to extend from each aggregate in 2-3 days *in vitro*, and neurite connectivity was formed among the aggregates within an additional few days. Anterograde labelling by fluorescent dye (DiI) at this stage indicated that a number of axons extended from the source aggregates to the target aggregates and formed axonal arborizations. However, establishment of synaptic transmission still required additional several days, studied by extracellular recording of the field potentials (FPs) evoked in the target aggregates by stimulation of the source aggregate. Development of synaptic transmission determined as the amplitude of FPs began at 7-9 days *in vitro* and completed at 11 days *in vitro*. The neural connections and synaptic transmission were maintained up to 1 month. A good parallelism was found during the later stage of development between the intensities of anterograde labelling in the target aggregates transferred from the source aggregates where the dye was injected and the amplitudes of FPs evoked in the target .

104.12

NEURITE OUTGROWTH FROM EMBRYONIC CORTICAL NEURONS ON ADULT OLIGODENDROCYTES IN CULTURE. March D. Ard*. Dept. of Anatomy, Univ. of Mississippi Med. Ctr., Jackson, MS 39216.

To test inhibition of neurite outgrowth by oligodendrocyte surfaces, co-cultures of oligodendrocytes and neurons were established and the areas occupied by each cell type measured. Oligodendrocytes were cultured from adult rat spinal cord under conditions prohibiting proliferation of precursor cells (addition of fluorodeoxyuridine, FDUR, to the medium). The primary cells were subcultured onto polylysine-coated coverslips, where they emitted branched processes labeled with anti-galactocerebroside monoclonal antibody but not with A2B5 antibody. After 5 days, dissociated cortical neurons from embryonic day 15 rat cerebral cortex were added, still with FDUR present. The MCID image analysis system of Imaging Research, Inc., was used to measure areas covered by immunostained processes of oligodendrocytes and of neurons in double-labeled cultures.

Neurite outgrowth after 2 days was vigorous. Neurites labeled with A2B5 extended onto both oligodendrocytes and polylysine. Oligodendrocyte processes covered about 19% of the surface in these cultures, and neuronal processes covered about 8%. In spite of the availability of unoccupied polylysine areas, neurites frequently overlapped and sometimes closely followed oligodendrocyte processes. The average area occupied by neurons and neurites was not reduced when oligodendrocytes were present. On the other hand, instances of exclusive territories where neighboring cells of different types refused to commingle indicated that neurite-inhibitory interactions did occur in some cell contacts. It appears likely that many of the oligodendrocytes in these culture conditions may lack neurite-inhibitory properties.

104.13

VIDEO MICROSCOPY OF CELL-CELL INTERACTIONS IN DISSOCIATED CULTURES OF DEVELOPING MAMMALIAN CEREBRAL CORTEX. M. Lu* and K. Kalil. Neuroscience Training Program and Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

During cortical development growth cones make cell specific choices to establish precise connectivity. To investigate the behaviors of growth cones during these cellular interactions, we have used video enhanced contrast-differential interference contrast (VEC-DIC) microscopy to observe attractive and inhibitory responses of neurites growing in dissociated cultures of embryonic (E-14) hamster cerebral cortex. The dissociated cultures, consisting primarily of cortical neurons and glia, were grown on coverslips coated with laminin alone to create a more natural substrate for axon outgrowth. Results from over 25 video recording sessions lasting about 5 hours for a single coverslip, in which images were taken every 5s, showed robust neurite outgrowth. Rates of extension ranged from 40-100 μm/hr, which is consistent with cortical axon outgrowth *in vivo*. The mode of axon elongation was similar to that previously described for *Aplysia* neurons by Goldberg and Burmeister (J. Cell Biol., 1986). Growth cones (~15μm) had large lamellae and extensive filopodia and were highly motile. Observations of individual cell-cell interactions during periods of 1-2 hours revealed that cell specific responses were mediated either by direct contact or by influences at a distance. Growth cones encountering other axons could either grow over them, turn and fasciculate upon them or collapse and retract away from them. Similarly, growth cones encountering other growth cones grew over one another or collapsed and retracted after brief filopodial contact. Glial cells could provided a favorable substrate for growth cone extension and in some cases glial cells responded to the approach of a growth cone by extending filopodia from the glial cell margin. However, growth cone contacts with glial cells were sometimes inhibitory, inducing the retraction of the growth cone as well as the glial cell. These results suggest that growth cones respond characteristically to specific neuronal and glial cell processes and thereby establish appropriate cortical circuitry during development *in vivo*.
Supported by NIH Grant NS 14428 (K.K.).

104.14

TRIGEMINAL GANGLION OUTGROWTH IN ORGANOTYPIC CO-CULTURES. R. Erzurumlu*, H. Takahashi, S. Jhaveri and R. McKay. Dept. Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Organotypic co-cultures of specific neuronal assemblies and their targets provide a powerful tool to address questions of axon-target interactions in developing nervous system. In serum-free medium isolated trigeminal ganglia from E15 rats were co-cultured for 6-8 days on Millicell wells (Brewer and Cotman, 1989; Molnár and Blakemore, 1990) with explants of vibrissa pads and hindbrain. The cultures were then fixed with 4% paraformaldehyde and the outgrowth of ganglion cell processes was labeled by implants of DiI crystals in the ganglia.

"Peripheral" and "central" projections from the trigeminal ganglion are strikingly different in their morphology. Peripherally directed axonal processes enter the vibrissa pad along a broad front and fan out towards vibrissa rows in thick fascicles. The elaborate branching of these processes in the vicinity of their targets, the vibrissa follicles, recapitulates their growth pattern *in vivo*. On the other hand, ganglion cell processes which enter the hindbrain explants consist of thin, beaded axons which are predominantly unfasciculated. Many of these axons form a distinct tract with an ascending and descending component. Other processes radiate into the brainstem. The presence of several retrogradely labeled cells in the hindbrain explants may represent the mesencephalic component of the trigeminal system.

These results demonstrate that many features of the trigeminal pathway can be replicated *in vitro*. The technique holds promise for future studies of brain development in a relatively simple culture system.
(Research supported by grants from N.I.H.)

104.15

MATHEMATICAL APPROACH TO MODELING DENDRITIC GROWTH *IN VITRO*. E. Uemura,* W. Kliemann, A. Carriquiry, J. Goodwin, and C. Martens. Neuroscience Program, Departments of Anatomy, Mathematics, and Statistics, Iowa State University, Ames, IA 50011.

A mathematical model that predicts the quantitative branching pattern of dendritic tree *in vitro* was evaluated. The apical dendrites of rat hippocampal neurons were measured at post-plating days 1, 2, 3, 4, 6, and 7. Cultured cells were stained with dil and pyramidal-shaped neurons that are physically separated from one another are photographed for digitizing apical dendrites. A Wald statistic for χ^2-test was developed for the branching pattern of dendritic trees and for the distribution of the maximal order of the tree. Using this statistic, we obtained an excellent fit of the mathematical model for the dendritic data. The apical dendrites of hippocampal neurons are characterized by a completely random splitting, i.e., splitting of each branch independent of all other branches. Being able to predict the form of a dendritic tree from a mathematical model implies that a mathematical model could be used for simulating the development of dendrites under various experimental conditions. Supported by NS28416.

104.16

DEVELOPMENT OF DORSAL ROOT AFFERENT ARBORIZATIONS IN HUMAN SPINAL CORD. A. Konstantinidou*, N. Flaris, I. Silos-Santiago and W.D. Snider. Dept. Neurology, Washington University Medical School, St. Louis, MO, 63110.

The primary sensory modalities are subserved by physiologically distinct classes of dorsal root ganglion (DRG) cells. Each of these DRG cell classes has a characteristic central arborization in the spinal cord. Although these arborizations have been well characterized in experimental mammals, no previous study has described spinal arborizations of DRG cells in humans. We have taken advantage of the ability of the lipid-soluble tracer, DiI, to reveal axonal arborizations in fixed tissue to study the devopment of primary sensory afferents in human spinal cord.

We have found that the DRGs are well developed by 6 weeks of age. By 9 weeks, some classes of dorsal root axons have entered the gray matter of the spinal cord and a few Ia axons have already reached the vicinity of motor neuron somata. By 14 weeks, fascicles of Ia axons traverse the dorsal horn along the entire mediolateral axis and project toward the ventral horn. Many ofthe fascicles converge near an area of the midline that presumably represents Clarke's column and then diverge in complex patterns to innervate motor neurons. Ia axons defasciculate in the ventral horn and elaborate boutons that appear to be in striking proximity to motor neuron cell bodies.

Several other classes of afferents have also entered the cord at this age. One major class enters the cord in a variety of mediolateral locations and projects in fascicles to lamina IV. These afferents have their major arborizations in the rostro-caudal axis. Between 14 and 16 weeks smaller afferents enter the cord and populate the superficial dorsal horn, primarily oriented rostro-caudally as well.

This is the first study to reveal the morphology of primary sensory afferents in humans. Supported by the American Paralysis Association.

104.17

TERMINAL AND SYNAPSE NUMBERS AND TYPES IN MEDULLARY DORSAL HORN LAYERS I-III IN NEWBORN RAT. J.P. Golden*, D.S. Zahm & M.F. Jacquin. Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

It is known that in adult rats subjected to infraorbital nerve section at birth, layers I and II of the medullary dorsal horn have a normal cytoarchitecture and at least normal #s of terminals and synapses, despite only a 26% survival of infraorbital axons. The selective sparing of inputs to layers I and II may reflect axon sprouting, a high survival rate for ganglion cells that normally project here, or maintenance of a synaptic organization that exists at the time of the lesion. As a first step in testing the latter hypothesis, total #s of synaptic and terminal profiles were determined in randomly selected transverse sections through the maxillary portions of layers I-III in 1 rat sacrificed at birth. Terminal and synaptic densities in the layer I-II region were 14.5 and 3.1 per 100 μm^2, respectively. A paucity of terminals and synapses at birth was also noted in a 2nd animal. Comparable values from an adult subjected to nerve section at birth were 37.6 and 10.3, respectively. On the intact side, comparable values were 28.0 and 9.5, respectively. These data suggest that there are far fewer terminals and synapses in layers I and II at birth than there are in the neonatally deafferented or normal adult. Thus, the presence of normal #s of terminals and synapses following deafferentation at birth does not reflect the preservation of an immature synaptic condition. In newborns, we also saw a significant # of terminals with large pleomorphic vesicles, some of which resembled growth cones, as well as dendrodendritic synapses. Higher terminal activity in more superficial, as opposed to deeper, laminae were also seen at birth: #s of terminals in layers I-III were 14, 15 and 8 per 100 μm^2; #s of synapses were 4, 2 and 2. DE07734, DE07662.

104.18

THE EXTENT OF AXONAL ARBORIZATION OF THE MESENCEPHALIC DOPAMINERGIC NEURON IS INDEPENDENT OF THE SIZE OF THE STRIATAL TARGET FIELD. H.K. Choi*, L.A. Won, B. Heller and A. Heller. Dept. of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637 and Dept. of Mathematics, Illinois Institute of Technology, Chicago, IL 60616

Using a three-dimensional reaggregate culture system we have previously demonstrated that the survival of mesencephalic dopaminergic (DA) neurons is dependent on the presence of striatal target tissue (Brain Res. 274:275, 1983). In the present study, we examined whether or not there is competition among DA neurons for a target field or whether the axonal arbor of such neurons grows to a given size, no matter what the extent of the available target field. Rostral mesencephalic tegmentum (RMT) containing DA neurons and corpus striatum (CS) were dissected from E14 mice and dissociated. Varying numbers of RMT cells ranging from 10,000 to 1 million were coaggregated with 5 million CS cells. After 3 weeks in culture, half of the aggregates in each flask were analysed for DA and protein content. The other half of the aggregates were sectioned and processed by tyrosine hydroxylase (TH) immunocytochemistry to allow identification and quantitation of DA neurons per flask as well as DA cell density within the aggregates. The results obtained demonstrated that there was a linear relationship between the number of RMT cells added and the DA content per flask. A linear relationship was also found between numbers of TH-positive cells within a flask and the number of RMT cells added. The percent of DA neurons remained constant (approximately 1%) over the entire range of RMT cells added. Similarly, there was a linear relationship between nanograms of DA content per flask and number of TH-positive cells per flask. From this linear relationship, it is evident that each DA neuron in these cultures contained approximately 2.8 pg of DA. Assuming that the DA content of a neuron is related to the extent of the axonal arborization, these results provide evidence that the DA neuron is intrinsically programmed to elaborate an axonal field of a given size even when presented with excess target field. Supported by MH-28942.

104.19

THE DISTRIBUTION OF A MYOSIN I mRNA IN THE DEVELOPING MOUSE BRAIN. M.P. Joyce*, E.H. Sherr, and L.A. Greene. Dept. of Pathology, Columbia University, Coll. of P & S, New York, NY 10032.

Myosin I is a one-headed version of the two-headed muscle myosin. Myosin I-immunoreactivity has been found at the leading edge of locomoting Dictyostelium, and may participate in generating propulsive movement in neuronal growth cones. We previously reported the identification of PCR products that corresponded to 3 novel myosin I genes. One of these, mouse myosin IC, showed strong expression in the developing brain. To map the expression of this gene, cDNA probes spanning different regions of the gene have been constructed. In situ hybridization using the digoxigenin-alkaline phosphatase method was performed on paraffinized tissue sections from the brains of 2-day-old, 12-day-old, and adult mice. Labeling was observed in many CNS sites including the cerebral cortex, hippocampus, cerebellum and brain stem. The 2-day-old and 12-day-old mice showed extensive labeling. In the adult animals, the intensity and extent of the distribution of the reaction product was significantly diminished. These results suggest that myosin I may participate in neuronal development.

104.20

DEVELOPMENT OF THE RAT SEPTOHIPPOCAMPAL PROJECTION: LIGHT AND ELECTRON MICROSCOPY OF IDENTIFIED GROWTH CONES. R. Linke* and M. Frotscher. Inst. Anat., Univ. Freiburg, D-7800 Freiburg, FRG.

We studied the ontogeny of the septohippocampal projection in rats from embryonic day (ED) 15 to postnatal day (PD) 10 by means of retrograde and anterograde transport of the fluorescent tracer DiI. In fixed material, injections of DiI (2% in abs. ethanol) were placed into the septal region, and horizontal sections were processed for fluorescent microscopy or for electron microscopy after photoconversion. We consistently found retrogradely labeled cells in the hippocampus at ED 17, but no anterogradely labeled fibers. At ED 18, the first septal axons arrived at the hippocampus proper but continued to grow to the subiculum and entorhinal cortex. The innervation of the hippocampus proper seemed to occurr mainly via collaterals of these passing axons. Septal axons in the dentate gyrus were not seen before PD 1. A nearly adult pattern was found at PD 15. Ultrastructural analysis of identified growth cones and their micromilieu in the fimbria and the hippocampus proper indicates that septal axons in the fimbria use other axons as guiding cues, probably those originating in the hippocampus. In the hippocampus contacts of the growth cones with all elements present were found.

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AXON GUIDANCE MECHANISMS AND PATHWAYS II

105.1

ABNORMAL NEURONAL DIFFERENTIATION IN THE SPINAL CORD OF A MUTANT ZEBRAFISH LACKING FLOOR PLATE CELLS. R.R. Bernhardt*, C.K. Patel, S.W. Wilson, and J.Y. Kuwada. Dept. Biology, University of Michigan, Ann Arbor, MI 48109-1048 and Neurobiology, ETH-Hoenggerberg, Zuerich.

We compared the differentiation of putative GABAergic neurons in wildtype zebrafish spinal cord and in *cyc-1* mutant embryos that are missing the midline floor plate cells (Hatta et al., '91). In wildtype embryos, an antibody against the neurotransmitter GABA labeled the cell bodies and axons of four classes of cells; DoLA, CoSA, VeLD, and Kolmer-Agduhr (KA) neurons. In the mutant, abnormal axons were observed in some, but not all, of the GABA reactive CoSA, VeLD, and KA neurons while the axonal trajectories of DoLA neurons were not affected. Furthermore, the number of KA cells was significantly reduced in the mutant while the numbers of the other GABAreactive cells were unperturbed. These observations corroborate the hypothesis that floor plate cells are one of several guidance cues that direct axonal outgrowth near the ventral midline of the spinal cord (Bernhardt et al., '92) and suggest a role of the floor plate in the determination of ventral cells in the zebrafish spinal cord. Supported by grants from NIH.

105.2

FORMATION OF THE DORSAL COMMISSURE IN RELATION TO INHIBITORY ECM MOLECULES IN THE SPINAL CORD ROOF PLATE. R. R. Pindzola*, M. de L. Gonzalez, and J. Silver. Dept. of Neurosciences, Case Western Reserve University, Cleveland, OH, 44106-4975.

It has been suggested that inhibitory ECM molecules in the roof plate form a barrier to all nearby axons during early development (Snow et al., Dev. Biol., '90). However, in late stage embryos the dorsal commissure does cross through the roof plate. How could the roof plate first restrict and later allow the passage of axons? We investigated the development of the rat dorsal commissure in relation to roof plate glia and the molecular environment in this region. The axons in the vicinity of the roof plate were identified with DiI or TUJ1 staining in a series of embryonic rats. The glia of the roof plate were analyzed with DiI labeling and staining for vimentin, in order to determine their geometry when the commissure first appears. The roof plate was also stained for the putative inhibitory matrix molecules, cytotactin/tenascin (CT), chondroitin sulfate (CS-PG) and keratan sulfate (KS-PG) proteoglycans to learn if the expression of these molecules decreases when the commissure forms.

From DiI and TUJ1 staining it appeared that the first dorsal commissural axons crossed the midline on E16. The vimentin labeled glia in the midline of the roof plate formed closely opposed processes, attached at the ventricular and pial surfaces, but which separated where commissural axons first traveled. It was clear that dorsal commissural axons crossed through the roof plate rather than through the more ventral area of ventricular fusion since measurements verify precisely where crossing first occurred. CT and CS-PG staining were no longer present at E16. KS -PG was still present, but only in a small area in the dorsal most part of the roof plate. The abundance of inhibitory molecules in the roof plate in early gestation and their disappearance when axons first crossed the dorsal midline is further evidence that these molecules may indeed act as barriers to elongating axons.

105.3

PRENATAL DEVELOPMENT OF CALLOSAL AXONS IN ACALLOSAL STRAIN MICE: A QUANTITATIVE STUDY WITH CARBOCYANINE DYES. H. S. Ozaki[1,2] and D. Wahlsten[1*]. [1]Dept. of Psychology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2E9, and [2]Dept. of Anatomy, Kagawa Medical School, Kagawa, Japan 761-07.

We have recently established a standard for normal callosal development in mice (Ozaki and Wahlsten, J. Comp. Neurol., in press). In the present study, callosal axons in acallosal 129/J and BALB/cWah1 mice were studied from frontal, parietal, temporal and occipital cortex using postmortem fluorescent tracers DiA and DiI.

The emergence and rate of growth of callosal axons are evidently normal in 129/J and BALB/cWah1 fetuses until the axons reach midplane, where there is a gap between the two hemispheres. Finding no bridge to the other side, the callosal axons loop back and form a large whorl, the Probst bundle. In some fetuses a few callosal axons cross over the dorsal surface of the hippocampal commissure. Growth cones of callosal axons in acallosal fetuses are very similar in size and morphology to normal fetuses.

These results indicate that the problem with callosal agenesis does not reside in the cells of origin or the axons themselves, but it does occur in the substrate of axon guidance at the midsagittal plane. Supported by grants from NSERC and AHFMR.

105.4

FORMATION OF THE HIPPOCAMPAL COMMISSURE IN NORMAL MOUSE EMBRYOS. Daniel Livy*. Dept. of Zoology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2E9.

The routing and timing of axon growth from the ventral hippocampal formation through the hippocampal commissure (HC) were studied in normal B6D2F2/J hybrid mouse embryos using fixation by perfusion and then insertion of crystals of the carbocyanine dyes DiI and DiA. Specimens ranged from 0.2 to 0.7 g body weight or 14 to 16 days of chronological age. In several embryos the corpus callosum (CC) or anterior commissure was also labelled with dye placements in the cerebral cortex.

The first crossing of HC axons between the hemispheres was seen in embryos weighing about 0.4 g, which occurs about a day earlier than the first crossing of the CC axons at body weights of 0.6 to 0.62 g. Prior to turning toward the midsagittal plane, the putative HC axons are part of the large bundle of fibres of the fornix, which appears in the septal region more than a day before the HC forms. The HC axons cross midplane at the base of a deep cleft in the septal region. Before they enter the opposite hemisphere, the growth cones of the HC axons in the vicinity of midplane are complex, having many filopodia. Once they have crossed and entered the fimbria on the opposite side, the growth cones adopt a much simpler morphology.

Supported by NSERC grants to S.K. Malhotra and D. Wahlsten.

105.5

ANTERIOR COMMISSURE PROJECTIONS IN DEVELOPING HAMSTERS. M.A. Pires-Neto*, R. Lent and A.L.C. Hartmann. Instituto de Biofísica Carlos Chagas Filho, U.F.R.J., 21941 Rio de Janeiro, Brasil.

We have studied the prenatal and postnatal development of anterior commissure (AC) projections in hamsters, employing fluorescent carbocyanines as neuronal tracers. Fixed brains of embryos from E12 through E16, and from P1 to P10 (E16 = P1 = day of birth), had crystals of DiI and/or DiA implanted into the paleocortex at different locations. The brains were vibratome-sectioned at 100 μm in the coronal, parasagittal and horizontal planes, and the sections were analyzed, charted and photographed using a fluorescent microscope coupled to a microcomputer. Anterogradely-labeled AC fibers tipped with growth cone-like swellings were seen approaching the midplane on E13, and crossing it on E14. On E14, a few (pioneer) axons took the lead and penetrated long into the opposite hemisphere, followed behind by the bulk of growing fibers. In some experiments, pioneer cell bodies could be identified by retrograde labeling in the paleocortex. A topographic organization was present from the start of AC formation: fibers from rostral paleocortex crossed through the rostral sector of the AC tract, while those originating more caudally took a caudal location. On E15 axons reached their targets, and initiated branching by interstitial budding. On E16 and later, a profuse terminal field was present at the olfactory peduncle and cortex, and in nuclei of the amygdaloid complex. The topography of crossing fibers was maintained throughout postnatal development.

Supported by FINEP and UFRJ.

105.6

CELLS OF THE OPTIC CHIASM MIDLINE INHIBIT UNCROSSED RETINAL FIBER OUTGROWTH *IN VITRO*. L.-C. Wang*, P. Godement and C.A. Mason. Dept. Pathology, Coll. Physicians and Surgeons, Columbia Univ., NY, N.Y., 10032 and CNRS, Gif-Sur-Yvette, France.

Our previous studies have shown that retinal axons diverge to each side of the brain near the midline of the optic chiasm (Godement et al., *Neuron* 5:173,1990). *In vitro*, whereas fibers from dorsal retina (crossed fibers) grow well on monolayers of cells dissociated from the chiasm midline, fibers from inferior temporal retina (uncrossed fibers) grow short neurites on such monolayers (Guillaume et al, *Soc. Neurosci. Abstr.* 17: 40, 1991). Within the monolayers are "islands" of neuronal cells settled around "spokes" of long glial processes, reminiscent of the radial glial palisade that spans the midline zone *in vivo* (Mason et al., Soc. Neurosci Abstr. 17: 39, 1991). To test whether the islands inhibit or permit retinal axon growth, chiasm cells were allowed to reaggregate before plating to preferentially form islands, and retinal axon growth monitored by dye labeling and video microscopy. Crossed fibers grew over islands. When uncrossed fibers met an island, their growth cones developed complex spread morphology along the border of the island, and continued to extend but at the outskirts of the island, resembling the behavior of uncrossed fibers *in vivo*. We are currently investigating whether the inhibitory properties of the midline require both neurons and glia, or reside in the radial glia themselves, by coculturing retinal explants with purified midline glia.

These studies provide further evidence that cells from the chiasm midline present cues for retinal axon divergence, and raise the issue of the role of cellular interactions between resident chiasm cells in the differential signalling for retinal axon divergence.

105.7

CORTICOSPINAL TARGET SPECIFICITY DEMONSTRATED IN EXPLANT CULTURES. R.Z.Kuang* M.Merline, and K.Kalil. Dept. of Anatomy and Neuroscience Training Program, University of Wisconsin, Madison, WI 53706.

Corticospinal axons develop arbors specific for their targets from the onset of innervation. (Kuang and Kalil, 1990 Neurosci. Abstr.). To test the degree of specificity in developing corticospinal connections we used an explant coculture system in which newborn hamster sensorimotor cortex was presented with choices of target tissue. Explants of P0-P1 cortex were cocutured in collagen gels with explants of P1-P3 cervical spinal cord (target) and cerebellar or olfactory bulb (control) placed on opposite sides of the cortical explant. After incubation in roller tubes for 4-7 days, the cultures were fixed and injected with DiI to label either cortical axons or cortical neurons. In over 200 successful experiments, cortical axons grew out into the collagen matrix ventrally and laterally showing no preferential attraction toward either the target or control explants. However, in all cases axons extending toward the spinal cord explant grew into it and established terminal branches resembling normal corticospinal arbors. By contrast, axons directly contacting the control explant were invariably deflected away from the edges of the tissue, even when cortical axons were presented only with control tissue. Axons extending randomly into the collagen matrix or toward the control tissue originated from neurons distributed throughout the cortical explant. By contrast, cortical axons growing into the spinal cord explant originated from pyramidal neurons that, in comparison with age-matched cortical explants retrogradely labeled *in vivo* from the spinal cord, resemble the distribution of layer V corticospinal neurons. We are now investigating whether axons from cortical explants can distinguish tissue from different levels of the spinal cord. The present results suggest that contact mediated inhibitory or permissive effects on growing axons may be important in determining target specificity during development. Supported by NIH Grant NS14428 (K.K.) and Training Grant T32 GM 07048 (M.M.).

105.8

THE DEVELOPMENT OF THE AXONAL PROJECTIONS FROM THE BASAL FOREBRAIN IN THE RAT. K.Murakami, M.Kuroda and K.Kishi* Dept.Anat., Toho Univ.- Sch. of Med. Tokyo 143, Japan

Development of the axonal trajectories from cells in the basal forebrain structures (BF) was studied by application of DiI to medial prefrontal cortices (ML), the olfactory bulb (OB) and the vertical or horizontal of the diagonal band (VDB or HDB) during ages from embryonic day 14 (E14) through postnatal day 6 (P6).

At E18, the axonal projections from BF did not reach OB and ML, but they were found to enter into the olfactory peduncle and medial septum. On E19, retrogradely labeled cells, after implantation of DiI into OB, were seen in HDB, VDB, the ventral pallidum, the lateral preoptic area (LPO), and the locus coeruleus (LC). On the other hand, DiI implantation in the cingulate cortex resulted in the finding that retrogradely labeled cells were detectable in VDB and HDB. Retrogradely labeled cells after DiI implantantion in the occipital cortex were not found in BF neurons. On P0, following placement of DiI in OB, retrogradely labeled neurons were found predominantly in HDB, LPO and LC. Additionally, some of cells in other BF structures (VDB, the substantia innominata, the bed nucleus of the stria terminalis, the lateral hypothalamic area) and in the orbital cortex were also labeled. Placement of DiI into OB resulted in the retrograde labeling of cells in BF, whose axon collaterals preferentially invaded the prelimbic and anterior cingulate cortex. These labeled collaterals traveled tangentially and rostrocaudally in the intermediate layer of the cingulate cortex, and gave off their branching into the subplate zone below the cingulate and prelimbic cortex. However, these fibers did not reached the occipital cortex. Retrogradely labeled cells after DiI implantation into ML (the prelimbic, anterior cingulate, occipital cortex) were scattered throughout BF. In conclusion, the first axons from VDB, HDB, LPO and LC begin to enter OB and ML on E19, and by P0 they further reach the occipital cortex. By P0, some of BF neurons have axon collaterals projecting to divergent forebrain area (OB and ML).

105.9

INHIBITORY INTERACTIONS MAY PROMOTE TARGET SELECTION BY RETINAL AXONS IN THE MAMMALIAN THALAMUS. R. Tuttle* and D.D.M. O'Leary. Molecular Neurobiology Lab, The Salk Institute, La Jolla, CA 92037.

We are using in vivo and in vitro approaches to characterize axon-target interactions and molecules that influence the development of retinal ganglion cell (RGC) connections to their principal thalamic target, the lateral geniculate nucleus (LGN). In rodents, RGC axons enroute to the superior colliculus (SC) have been reported to extend past the LGN and later extend side branches into LGN (Bhide and Frost, J. Neurosci. '91). We injected DiI intraocularly in E17 and E18 rats and perfused at E18 and E19. Thalamic nuclei were identified by retrograde fast blue labeling from cortex and acetylcholinesterase staining. Whole mounts reveal that RGC axons are tightly bundled in the optic tract in ventral diencephalon (vD). Upon entering the dorsal diencephalon (dD), however, RGC axons defasiculate and splay out dramatically over roughly a 3 mm rostral-caudal area of the dD. At these ages, many RGC axonal growth cones are apparent in this region of the dD, although others have entered the SC. Examination of sectioned material shows that while most RGC axons are on the surface of the diencephalon, some in the dD are in the parenchyma in both target and nontarget nuclei. In addition, a small number of side branches extend into target and nontarget nuclei of E18 and E19 dD, but by E19 branches appear to be more numerous and more complex in LGN. Collagen gel cocultures of retina and thalamus suggest that only subregions of diencephalon are stimulatory (or permissive) for RGC axon growth. While explants from most regions of diencephalon were inhibitory (or nonpermissive) for RGC axon growth, explants from a specific region, presumed to be LGN, were robustly invaded by RGC axons. Immunohistochemical detection of chondroitin sulfate proteoglycan (CSPG), a molecule shown to inhibit RGC axon growth in vitro (Snow et al., Develop. '91), reveals intense staining in the vD (optic tract and regions medial to it), and much lower staining in the dD. These findings raise the possibility that inhibitory interactions may contribute to the specificity of RGC innervation of thalamus, and that CSPG may contribute to this process. (Support: Spinal Cord Res. Fnd. and NIH EY07025)

105.10

QUANTITATIVE ANALYSIS OF TOPOGRAPHIC TARGETING OF RETINAL AXONS IN DEVELOPING RAT SUPERIOR COLLICULUS A.L. Roskies* and D.D.M. O'Leary. Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

Axons of retinal ganglion cells (RGCs) form a precise topographic projection to the adult mammalian superior colliculus (SC). Anterograde axon tracing using DiI shows that in E18-E19 rats RGC axons exhibit no tendency to target to the correct half of the SC (Simon & O'Leary, Soc Neurosci Abstr '91) and reveals a topographically diffuse retinocollicular projection in neonates (Simon & O'Leary, J Neurosci '92). However, retrograde tracing studies using Fast Blue (O'Leary et al J Neurosci '86) or rhodamine beads (Yhip & Kirby, Vis Neruosci '90) in neonates suggest that only a small proportion of temporal RGCs transiently project to the topographically inappropriate caudal SC. These tracers are less effective than DiI at labeling axons of passage. We have used DiI as a retrogarde tracer to reassess quantitatively the topographic targeting of RGC axons in neonatal rats. DiI was injected into various points along the rostrocaudal extent of the SC. The distribution of retrogradely labeled RGCs shows some bias toward the correct topographic region by P2; however, in all cases a large percentage of cells project to topographically incorrect locations. About 1/3 of all RGCs labeled with injections in far caudal SC are found in temporal retina, indicating that at least 1/3 of RGC axons topographically matched with rostral SC greatly overshoot their correct region at this age. This figure, though, is an underestimate, since injections in far rostral SC indicate that even DiI is not as effective at labeling axons of passage as it is axons topographcially matched with the injected region. Labeling patterns from injections made either immediately rostral to or caudal to the rostrocaudal midline of the SC are similar to each other; in both cases a large proportion of temporal RGCs are labeled and no drop-off in labeling density is apparent at the temporal-nasal retinal midline. These observations indicate that position dependent molecular cues hypothesized to guide or restrict the growth of RGC axons do not operate effectively *in vivo* in the developing rat SC. (Support: NIH grant RO1 EY07025 and training grant GM08107.)

105.11

REGIONAL POLARIZATION OF EARLY AXON EXTENSION BY CORTICAL NEURONS S.E. Koester*, C.A. Lucidi-Phillipi, D.D.M. O'Leary, Molecular Neurobiology Lab, The Salk Institute, La Jolla, CA 92037.

The two major cortical outputs are to subcortical targets via the internal capsule (IC) and to contralateral cortex via the corpus callosum (CC). In rats, axons from the neocortical preplate form the IC at E14 (DeCarlos & O'Leary J.Neurosci. 1992) while axons from the earliest generated neurons in medial (cingulate) cortex form the CC at E17 (Koester & O'Leary Soc.Neurosci.Abs. 1991). Neurons projecting subcortically send axons laterally through the intermediate zone (IZ) to the IC, and other cells later send axons medially through the IZ toward the CC, suggesting that initial cortical axon outgrowth must be polarized. We injected DiI in fixed rat cortex at various sites to determine when and where this polarization of axon extension breaks down. TuJ1 immunohistochemistry (Lee et al Cell Motil.Cytoskel 1990) was used to visualize the overall pattern of the cortical axon pathways. At E14, essentially all long axons extend toward the IC. As late as E16, all retrogradely labeled neurons are found medial to DiI injections in the lateral wall, but their distribution stops abruptly in dorsal cortex and no labeled cells are found more medially in cingulate cortex. Anterogradely labeled axons emerge from the DiI site and extend toward the IC. These findings indicate that all neocortical axon growth is still directed toward the IC at E16. TuJ1 staining shows that the axon pathway in the IZ thickens from dorsal cortex laterally, but not medially, confirming the addition of axons to the pathway in the lateral IZ. At E17, the medial border of subcortically projecting neurons persists. In lateral (more mature) regions of cortex, a large number of neurons lateral to the injection are now retrogradely labeled indicating that they have begun to extend axons along the callosal trajectory. Observations using TuJ1 are again consistent with this finding. These data demonstrate a strong polarity in the early extension of cortical axons; the most medially located cells extend axons medially, cells lateral to these extend axons laterally. The boundary representing the reversal in this polarity may correspond to the border between cingulate cortex and neocortex.

105.12

HIPPOCAMPAL GROWTH CONE ORIENTATION IN FOCAL ELECTRIC FIELDS C.D. McCaig1, L. Davis2*, R.W. Davenport2 [1]Department of Biomedical Sciences, University of Aberdeen, Aberdeen AB9 1AS, Scotland, [2]Program in Neuronal Growth and Development, Colorado State University, Fort Collins, Colorado 80523

A wide variety of cell types respond to electric fields in culture. Despite evidence for electric fields existing in the mammalian embryo, there are few studies testing the effects electric fields exert on neurons from the mammalian CNS. The present study demonstrates orientation responses to focally applied electric fields of embryonic rat hippocampal neurons isolated in culture. The most striking result is that different growth cones of the same neuron can show differential responsiveness to focally applied electric fields: growth cones on short, straight processes, the presumptive dendrites, oriented toward the cathode, while growth cones on the longest process, the presumptive axon, did not orient. Additionally, the present experiments bring a significant increase in resolution to the study of neuronal growth cone orientation by applied electric fields: a novel examination of the early events involved in orientation. A new method for quantitation of these events was devised. Growth cones on dendrites displayed a spectrum of orientation responses: directed lamellipodial extension, directed filopodial extension and/or re-orientation, cytoplasmic swelling of existing filopodia, consolidation of filopodia and rapid elongation of the entire process. Individual growth cones displayed only one or two of these responses. Additionally, not all growth cones on dendrites sustained their initial orientation response. Thirty-five percent of these growth cones adapted within 6 minutes. Thus, focal electric fields can orient mammalian CNS growth cones and ultimately may affect the final neuronal architecture.

105.13

EARLY DEVELOPMENT OF OLIVOCEREBELLAR PROJECTIONS IN THE FETAL RAT USING CGRP-IMMUNOCYTOCHEMISTRY. C. Sotelo* and A. Chédotal, INSERM U. 106, 75013 Paris (France).

We have shown that calcitonin gene-related peptide (CGRP) immunocytochemistry reveals a selective subpopulation of inferior olivary axons from embryonic day 17 (E17), as they start penetrating medially the rostral cerebellar surface. The expression of the labelling is transient and decreases progressively, although not completely, after postnatal day 6. During development : i) Olivocerebellar axons are not randomly distributed in the restiform bundle before they enter the cerebellum. ii) Just after their penetration, the olivary axon growth cones acquire more complex morphologies, and they abut in a region solely composed of migrating Pukinje cells (visualised on double-labelled sections by their Calbindin-D_{28K} immunoreactivity). iii) From E18 to E19, the labelled fibers are bypassed by migrating Purkinje cells,and a new vermal lateral stripe is formed. The distribution of CGRP-IR fibers in the vermal cortex coresponds to the known sites of axonal pojection of the observed CGRP olivay neurons.

The results suggest that there is neither a waiting period nor an initial phase of randomness in the formation of the olivocerebellar pojection map. This absence of chaotic cerebellar invasion, and the high selectivity of the entry points, let us suppose that the orientation of CGRP positive olivocerebellar fibers toward their targets is regulated on the basis of positionnal information shared between subsets of olivary neurons and clusters of Purkinje cells. This preliminary coarse topography would need further refinement.

105.14

LONG AXON GROWTH FROM EMBRYONIC NEURONS TRANSPLANTED INTO AN UNDAMAGED ADULT MYELINATED FIBRE TRACT. S.J.A. Davies, P.M. Field and G. Raisman*, Norman and Sadie Lee Research Centre, Laboratory of Neurobiology, National Institute for Medical Research, The Ridgeway, London NW7 1AA, UK.

A few thousand cells from embryonic (E14-E19) mouse hippocampi were microtransplanted into the fimbria of immunosuppressed adult rat hosts.

M6 and Thy-1.2 immunohistochemistry showed that the donor mouse neurons grew fine (<0.5μm diameter) axons which extended through the host tract at a rate of around 1-2mm per day, reaching their ipsilateral terminal field destinations (about 7mm) by about one week, and their contralateral destinations (at least 10mm) by about 2 weeks.

The axons ran singly or in fascicles and were closely aligned to the host tract axons and the interfascicular glial rows of the host tract.

These observations suggest that the microenvironment of unlesioned myelinated adult fibre tracts is permissive for an abundant and rapid growth of axons from transplanted mouse embryonic hippocampal cell suspensions. These axons can form collaterals and leave the host tracts to invade the appropriate terminal fields.

105.15

DEMONSTRATION OF SPECIFIC PROJECTIONS TO HIPPOCAMPUS IN ORGANOTYPIC SLICE CO-CULTURE. P.M. Field, D. Li and G. Raisman, (SPON: Brain Research Association*), Norman and Sadie Lee Research Centre, Laboratory of Neurobiology, NIMR, The Ridgeway, Mill Hill, London NW7 1AA, UK.

Species-specific markers (Thy-1 and M6) and anterograde axonal transport of biocytin have been used to demonstrate projections from embryonic mouse explants co-cultured with postnatal rat hippocampus.

Mouse entorhinal pyramidal cells send axons to rat hippocampal slices both in roller tube and static culture. The densest part of the projection was to the *stratum moleculare* of the dentate gyrus but the entorhinal axons also projected to the *stratum lacunosum-moleculare* and outer *stratum oriens* of the hippocampus, and in some cases perforated the pyramidal cell layer of the subiculum.

Projections from other brain areas that normally terminate in the hippocampus will be demonstrated using this co-culture technique.

105.16

REGENERATING AXONS OF THE ADULT RAT RETINA GROW PREFERENTIALLY TOWARDS TOPOGRAPHIC APPROPRIATE TARGET REGIONS *IN VITRO*. Mathias Bähr* and Andrea Wizenmann, Neurologische Universitätsklinik and Max-Planck-Institut für Entwicklungsbiologie, Tübingen,F.R.G.

We have recently described that adult rat retinal ganglion cell (RGC) axons are able to reconnect with target neurons *in vitro* (Bähr and Eschweiler, 1991). In the present study, we have asked the question whether regenerating RGC axons can recognize topographic cues within their main target region. During development, RGC fibers establish a topographic projection where temporal axons preferentially terminate in the anterior and nasal ones in the posterior part of the colliculus superior (SC).

We have explanted pieces of nasal and temporal retina 4-6 days after crushing the optic nerve. They were cocultured with midbrain slices from embryonic rats (E17-19), including the SC as a primary visual target region. Retinal explants were placed in between the anterior and posterior part of the SC. In all cases examined, a majority of fibers oriented towards one or the other end of the SC, only one third of the axons did not show a clear orientation. With temporal explants (n=46), the number of fibers growing towards the anterior pole of the SC exceeded that of axons orienting in the other, inappropriate direction by a factor of 2,49. For nasal explants (n=43), this factor was 2,64 for axons growing towards the posterior SC. In controls where retinal explants were placed at the border between SC and Colliculus inferior, only nasal fibers grew preferentially towards the posterior SC (55%) whereas temporal axons did not show an oriented growth. Thus, we conclude that also regenerating, adult RGC axons are able to recognize position specific cues from embryonic target regions.

Bähr and Eschweiler (1991), NeuroReport 2, 581-584.

105.17

AXONAL GROWTH AND BRANCH FORMATION OF GENICULATE FIBERS IN VISUAL CORTEX STUDIED IN COCULTURE PREPARATIONS.

N. Yamamoto*, S. Higashi, H. Sugihara and K. Toyama Department of Physiology, Kyoto Prefectural University of Medicine, Kyoto 602, Japan.

Our previous studies have demonstrated that geniculocortical connections are established with normal laminar specificity in cocultures of the lateral geniculate nucleus (LGN) and the visual cortex (VC) (Yamamoto et al. 1989, Science, 245, 192). To reveal cellular interactions which underlie the formation of the lamina-specific connections, axonal extension and branch formation of living LGN axons were studied in cocultures of the LGN and VC. A rat VC slice (P1-3) and a block of the LGN (E15-17) were cocultured so that the LGN faced the ventral side of the VC, and at 4-6 days *in vitro* a fluorescent dye (DiI) was placed on the LGN. A few days later, labeled LGN axons were observed for 1-2 days under a laser scanning confocal microscope at 0.01-2 hr intervals. All sampled LGN axons (n=7) were led by a growth cone (5-10 μm in length) and extended radially toward the pial surface of the VC. In 5 out of the 7 cases, the axons traveled through the infragranular layers at constant growth rates (20-60 μm/hr) and suddenly stopped when they reached around the granular layer. After a while (1-3 hr), some axons (3/5) began to branch behind the growth cone, while the other axons (2/5) began to regrow without forming any branches (one axon toward the pial surface, the other along the layer). The remaining 2 axons traveled through all cortical layers at constant speeds, and reached the surface without branching. These findings suggest that LGN axons stop and branch in response to local environments in the VC.

OTHER FACTORS AND TROPHIC AGENTS: BDNF, NT3, NT4

106.1

GENE CLONING AND EXPRESSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR.

Z.W.Hua, Y.K.Yu, S.Y.Cai, Y.S.Li and F.Z.Wang*. Lab. of Neuroscience, Institute of Basic Medical Sciences, Beijing, 100850, P.R. China.

Human and pig brain-derived neurotrophic factor(BDNF) genes were amplified by polymerase chain reaction (PCR). The primers used in both human and pig BDNF gene amplification were synthesized according to the sequence of pig BDNF gene (Leibrock, 1989). It was found that: (1) within the pig sequence, there was one nucleotide variation at position 288 (A→G) when comparing the present result with that from Leibrock (1989); (2) in the human sequence, only three nucleotide variations within the two synthesized primer areas were realized at position 6 (G→T), 12 (G→T) and 384 (G→A) when comparing with that reported by Jones (1990), and which could be attributed to the primers of pig sequence used in this work; (3)all the nucleotide variations reported above occured at the third base of amino acid codons and no amino acid alteration was resulted because of the degeneracy of codon.

The human BDNF gene was ligated to the fusion protein-expression vector pBG2, which contains the strong PL promoter of bacteriophage lamda and directs the synthesis of fusion proteins consisting of part of protein G. After transformation, positive clones were obtained by in situ hybridization. The induced products of several clones were further identified positive by Western blotting. Works on chemical treatment of the fusion protein is going on.

106.2

REGUALTION OF HUMAN BRAIN DERIVED NEUROTROPHIC FACTOR GENE EXPRESSION.

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Brain-derived neurotrophic factor (BDNF) is critical for the differentiation and survival of specific central neurons yet little is known about the regulation of BDNF gene expression in the human. We recently found that BDNF mRNA levels are increased by serum and the phorbol ester TPA were additive whereas they are known to be synergistic in stimulating NGF mRNA. To identify a suitable cell model for studies of human BDNF gene expression we examined WI38 cells. These extensively studied fibroblast-like cells were derived from human fetal lung by Hayflick and coworkers in 1968. A single BDNF mRNA of 1.6kb was observed on a Northern blot. PCR analysis did not detect NGF or NT-3 mRNAs. Vitamin D, TPA, and serum stimulated the secretion of biologically active BDNF. Co-treatment with corticosterone inhibited the stimulation. These results indicate that BDNF gene expression is regulated by multiple stimuli in mouse and human cells in culture. WI-38 cells represent a model system for analyses of the mechanisms that mediate the cell-specific and developmental regulation of human BDNF gene expression and its modulation by extracellular signals.

106.3

DETECTION OF BDNF mRNA IN THE DEVELOPING CHICKEN EMBRYO.

Daryn Kenny, Mike Cullen* and Marianne Bronner-Fraser, Developmental Biology Center, University of California, Irvine, Ca. 92717

Trophic factors regulate the survival of neurons and may be involved in neuronal differentiation. For example, Brain-Derived Neurotrophic Factor (BDNF) supports survival of dorsal root ganglion cells and some central neurons. To better understand the function of this neurotrophic factor, it is important to know its distribution in the embryo. Using a nuclease protection assay, we first detected BDNF mRNA in 2.5 day old embryos. This is just prior to the differentiation of dorsal root ganglia, which occurs at around 3 days of development. BDNF message was expressed continuously through 13 days, the last stage examined. Using *in situ* hybridization, we detected BDNF mRNA in individual cells in the developing embryo. In stage 24 embryos, high levels of BDNF mRNA were observed in a few cells near the lumenal portion of the trunk neural tube. This expression pattern was maintained at all subsequent stages. Interestingly, in the midbrain region of 9 day embryos, blood cells within endothelial vessels strongly expressed BDNF. Although we cannot rule out the possibility that other cells within the embryo express low levels of BDNF, below the limits of detection by *in situ* hybridization, our results suggest that BDNF mRNA is detectable in a few neural tube/spinal cord cells as well as selected blood cells close to regions which may contain BDNF responsive neurons. (Supported by USPHS HD-25138)

106.4

DOPAMINERGIC NEURONS EXPRESS NT-3 AND BDNF mRNAS IN RAT MESENCEPHALON.

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We recently demonstrated that in normal adult rat brain neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF) mRNAs are localized to specific ventral mesencephalic regions that contain dopaminergic cell bodies, including the ventral tegmental area (VTA) and medial substantia nigra (Gall et al., Mol. Cell. Neurosci. 3: 56-63, 1992). These data suggest that neurotrophic support for the dopaminergic neurons may arise from local synthesis. To determine whether the midbrain dopaminergic neurons, themselves, synthesize the neurotrophic factors we combined *in situ* hybridization of ^{35}S-labeled cRNA probes for the neurotrophic factor mRNAs with 6-hydroxydopamine (6-OHDA) lesions and with immunocytochemistry for the dopamine biosynthetic enzyme tyrosine hydroxylase (TH). Unilateral injection of the neurotoxin 6-OHDA into the ascending medial forebrain bundle or the ventral mesencephalon (which selectively destroys the mesotelencephalic dopamine cells), followed by a 1-5 week survival period, resulted in substantial depletion of NT-3 and BDNF mRNA-containing cells in the ipsilateral VTA and substantia nigra pars compacta. Combined *in situ* hybridization/TH immunocytochemistry revealed that the majority, but not all, of the NT-3 and BDNF mRNA-expressing cells in the VTA and medial substantia nigra pars compacta also contained TH. The present results indicate that the neurotrophins are indeed synthesized by midbrain dopamine neurons. These data also raise the possibility that aberrant expression of NT-3 and BDNF by the dopaminergic neurons, themselves, could contribute to the neuropathology of disorders such as Parkinson's disease and schizophrenia. Supported by the National Parkinson Foundation, Scottish Rite Schizophrenia Research Program and UK College of Medicine Research Fund (K.B.S.) and AG00538 (C.M.G.).

106.5

EXPRESSION OF BDNF mRNA IN THE DEVELOPING OPTIC TECTUM OF *XENOPUS LAEVIS*. S. Cohen-Cory* P. J. Isackson and S. E. Fraser. Division of Biology, Beckman Institute 139-74, California Institute of Technology, Pasadena, CA 91125, and Mayo Clinic, Jacksonville, FL 32224

The visual system of the frog provides a valuable system to study the formation and maintenance of topographically ordered connections between developing neurons and their targets. However, little is known about the trophic mechanisms that subserve it. Previous studies suggest that brain-derived neurotrophic factor (BDNF) plays an important role in the development of the visual system. BDNF promotes survival of retinal ganglion cells in culture, and is expressed in the optic tectum of higher vertebrates. To begin investigating the role of neurotrophic factors in the development of the visual system of the frog, the expression of BDNF message in the optic tectum of developing *Xenopus laevis* was analyzed. A *Xenopus laevis* specific, BDNF cRNA probe (Isackson et al., FEBS lett. 285; 260-264, 1991) was used to study BDNF gene expression by both Northern blot and RNAse protection assays. Two major BDNF transcripts highly expressed in the developing optic tectum were identified, and the time course of their expression was investigated. These results were compared with the expression of BDNF mRNA in various *Xenopus laevis* tissues of both neural and non-neural origin. (Supported by the Muscular Dystrophy Association).

106.6

EXPRESSION OF LOW-AFFINITY NGF-RECEPTOR-IMMUNOREACTIVITY (IR) AND BDNF AND NT-3 mRNAs IN DEVELOPING RAT RETINA AND SUPERIOR COLLICULUS. D. Rickman*, J. Lauterborn, N. Brecha and C. Gall. Depts. of Anat. & Cell Biol. & Med., UCLA & VAMC, LA, CA 90073; Dept. of Anat. & Neurobiol., UCI, Irvine, CA 92717.

In the rat, most retinal ganglion cells project to the superior colliculus (SC), where their axons terminate in a precise retinotopic pattern. Little is known about the molecular mechanisms which guide ganglion cell axons or determine the specificity and maintenance of their central connections. Using immunohistochemistry with an antibody to the rat low-affinity NGF-receptor (LNGF-R), which binds all neurotrophins, and *in situ* hybridization with ^{35}S-labeled RNA probes to brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), we examined the distribution of these molecules in the developing rat visual system. At postnatal day 0 (PD-0), LNGF-R-IR was present in retinal ganglion cells, especially in peripheral retinal regions. At PD-7, LNGF-R-IR was confined to far peripheral regions, and at PD-12 only rare staining was observed. In the SC, NT-3 mRNA was present at PD-1 in the superficial gray (retinorecipient) layer. The signal was less intense at PD-12, similar to adult levels. BDNF mRNA, however, was not observed in the SC until PD-6. The signal was higher at PD-12 and continued to increase until PD-25, when it reached adult levels. These data suggest that neurotrophins influence retinal ganglion cells and play a role in the establishment and maintenance of retinotectal connections.

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106.7

EXPRESSION OF THE BDNF GENE IN THE VISUAL SYSTEM OF THE CHICK EMBRYO. K.-H. HERZOG AND Y.-A. BARDE*. Dept. of Neurobiochemistry, Max Planck Inst. for Psychiatry, Am Klopferspitz 18a, 8033 Planegg-Martinsried, Germany

The in vitro survival of E11 chick retinal ganglion cells (RGC) has been shown to depend on the addition of brain-derived neurotrophic factor (BDNF). Partly because of the low levels of BDNF mRNA and the difficulties to recognize on Northern blots discrete mRNA transcripts, BDNF mRNA was measured with a PCR-based quantification method. This method includes the addition to the tissue homogenates of an artificial BDNF RNA standard containing a mutated nucleotide, allowing the introduction of a cleavage site not normally present in chick BDNF mRNA. This allows for the separate detection of the endogenous message from the standard. BDNF mRNA could be detected in tecta as early as E4, i.e. 2 days before the arrival of the earliest axons of the RGCs, and persisted until E17 (the last time-point investigated). While the BDNF mRNA levels were not changed after the in vivo application of NMDA antagonists, a reduction was observed after blockade of muscarinic receptors.

106.8

CO-EXPRESSION OF NGF, BDNF AND NT-3 IN THE DEVELOPING AND ADULT RAT CARDIOVASCULAR SYSTEM. I.A. Scarisbrick[*1], P.J. Isackson[2] and E.G. Jones.[1] Dept. of Anatomy and Neurobiology, University of California, Irvine,[1] and Dept. of Biochemistry and Molecular Biology, Mayo Clinic, Jacksonville.[2]

The neurotrophic factors nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) provide trophic support to select sets of peripheral neurons. We use *in situ* hybridization histochemistry to describe the expression of these factors within the embryonic, postnatal and adult cardiovascular system a major target of sensory, sympathetic and parasympathetic nerve fibers. Peripheral neurons of neural crest and epidermal placode origin innervating the great vessels have been examined using immunocytochemical localization of the cell surface markers, neural cell adhesion molecule (NCAM) and HNK-1.

Hybridization for the three riboprobes is robust within the tunica media of the major elastic arteries arising from the heart, from embryonic day 13 onwards, approximately one day following the earliest detectable innervation of these vessels. cRNA hybridization for each generally overlaps but is not co-extensive, and until PO, NT-3 is by far the most widespread. In each case hybridization is dense over the smooth muscle cells of the aorta, ductus arteriosus and pulmonary arteries and continues at a lesser intensity into their major branches, but in the ventricular wall is restricted to the bases of the aortic and pulmonary valves. Hybridization was not detected in the remainder of the ventricle or in the atria. In the postnatal period, mRNA encoding each neurotrophin also becomes detectable in the coronary arteries, but not in the heart proper. BDNF and NT-3 continue to be expressed at high levels by arterial smooth muscle cells of adult major elastic arteries, but the level of NGF is reduced.

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106.9

SYNTHESIS AND PURIFICATION OF BIOLOGICALLY ACTIVE RAT BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) FROM *E.COLI*. A. Negro, V. Corsa, , C. Moretto[2], S. D. Skaper[1] and L. Callegaro*. Advanced Technology Div. and Fidia Research Laboratories[1], Fidia S.p.a., Abano Terme and Dept. of Organic Chemistry, University of Padua[2], Italy.

The cDNA for rat BDNF was cloned as the prepro and mature sequences into two independent expression vectors under the control of the T7 promoter. When the vectors were transfected into *Escherichia coli*, the prepro and mature forms of BDNF accounted for about 20% and 25% of total *E.coli* proteins, respectively displaying molecular sizes of 26kDa and 15kDa. Mature BDNF was extracted from *E.coli* inclusion bodies, refolded in the presence of $CuCl_2$ and purified by HPLC. The resulting protein supported the survival of cultured embryonic dorsal root ganglion neurons with an ED_{50} of 3ng/ml. Although polyclonal antibodies against mature BDNF recognizd 26kDa and 15kDa bands on Western blots, but they failed to block its biological activity. This represents the first successful expression of biologically active BDNF in bacteria. The availability of pure recombinant BDNF will facilitate studies on the role of this neurotrophic protein in CNS physiology and pathology.

106.10

THE EFFECTS OF GRAFTING BDNF-PRODUCING FIBROBLASTS ON CHOLINERGIC NEURONS *IN VIVO*. CA Lucidi-Phillipi, UJ Kang*, CW Shults, KR Jones, LF Reichardt, and FH Gage. Depts Neurosci, UCSD, La Jolla, CA 92093 and UCSF, San Francisco, CA 94143.

Brain-derived neurotrophic factor (BDNF) is a member of a family of related neurotrophins. Nerve growth factor (NGF)-producing fibroblasts induce sprouting of cholinergic neurons when grafted into the intact adult rat striatum (Kawaja and Gage, 1991). We attempted to determine the effect of BDNF on central adult neurons by grafting putative BDNF-producing fibroblasts into the striatum of the adult rat.

We first inserted an expression cassette DNA encoding human BDNF into a retroviral vector to establish a producer line. Primary fibroblasts were then infected with the recombinant virus and selected for neomycin resistance. The evidence suggesting that BDNF is transcribed and translated by fibroblasts is fourfold: 1) Northern blot analysis 2) In situ hybridization at one and two weeks *in vivo* 3) Conditioned media from infected fibroblasts promote the survival of embryonic chick dorsal root ganglia and 4) fetal mesencephalic neurons in culture.

Unlike NGF-producing fibroblasts, grafts of BDNF-producing fibroblasts into the striatum did not induce the ingrowth of p^{75}LNGFR- or neurofilament-immunoreactive axons at time points of one, two, three, or eight weeks. There are, however, a variety of issues regarding the grafted cells that need to be addressed, including long term expression, immunological tolerance, and cell survival. In addition, we are currently investigating the role of BDNF in promoting survival of fetal cholinergic neurons by co-grafting fetal cholinergic neurons with BDNF-producing fibroblasts into the denervated hippocampus.

106.11

REGULATION OF PEPTIDE EXPRESSION IN CULTURED CORTICAL NEURONS BY BDNF. H. Nawa*(1,2), Y. Bessho (2), J. Carnahan (3), S. Nakanishi (2) and K. Mizuno (1). (1) Cold Spring Harbor Lab., Cold Spring Harbor, NY 11724, (2) Inst. Immunol., Kyoto Univ. Faculty of Medicine, Kyoto Japan, and (3) Amgen Center, Thousand Oaks, CA 91320

Brain-derived neurotrophic factor (BDNF) was recently shown to promote the survival of mesencephalic dopaminergic neurons and basal forebrain cholinergic neurons . The differentiation activity of BDNF, however, has been less clear. In this study, we examined the effects of the neurotrophic factors, BDNF, neurotrophin-3 (NT-3) and neurve growth factor (NGF) on neuropeptide expression in cultured cerebral cortical neurons. BDNF and NT-3 produced by COS cells and mouse NGF were added separately to glia-free cortical cultures. Only BDNF remarkably increased peptide contents of neuropeptide Y (10-fold) and somatostatin (5-fold) while CCK and GABA contents were modestly influenced (<2-fold) and neuronal viability was not affected. To determine whether the peptide elevation by BDNF resulted from increased synthesis of the peptides, we assayed their mRNA levels. The expression of those mRNAs was enhanced by BDNF to the same extent of the peptide increase. The elevation of the mRNA expression was fully dependent on the presence of BDNF in culture: Removal of BDNF during the culture decreased the mRNAs to a control level while delaying the addition of BDNF still elevated the mRNAs to the same maximum levels. This observation supports that the peptide-inducing activity of BDNF is independent of its activity in promoting neuronal survival reported previously. More than 90% of neuropeptide Y and somatostatin immunoreactivities are associated with GABAergic interneurons in the cortex. Therefore, our results suggest that in the cerebral cortex, the BDNF activity may regulate neuropeptide phenotype locally and could modify the mode of neurotransmission of GABAergic neurons.

106.12

BDNF INCREASES TH-IMMUNOREACTIVITY IN HUMAN FETAL VENTRAL MESENCEPHALIC NEURONS IN AN ORGANOTYPIC CULTURE. C.Spenger*, I.Stromberg+, L.Studer and R.Seiler. Dept. of Neurosurgery,Inselspital, CH-3010 Bern Switzerland;Dept. of Histology and Neurobiology, Karolinska Institute, S-10401 Stockholm, Sweden+.

There is evidence that BDNF has trophic actions on rat dopaminergic neurons *in vitro* (Hyman et al., 1991). Here we have studied the effects of BDNF on human fetal ventral mesencephalic tissue in an organotypic culture. The tissue was grown by the roller tube method for 12 to 31 days. The number of TH positive cells in cultures grown with 10 ng/ml BDNF was compared with that of cultures grown without BDNF. In cultures grown for 21 days with BDNF 11.1 ± 9.5 TH positive cells per 16000 μm^2 (n = 70) and in cultures grown for 21 days without BDNF 2.3 ± 2.6 TH positive cells per 16000 μm^2 (n = 45) ($p < 0.001$) were counted. The immunoreactivity of the pericaryon of the neurons of these cultures was measured by an image analysis system. Cells grown with BDNF showed a significantly ($p < 0.001$) higher TH-immunoreactivity compared with that of cells grown without BDNF. These results demonstrate a trophic action of BDNF on human fetal ventral mesencepahlic neurons *in vitro*. Supported by: SNF-grant No.31-32561.91 and the Swiss Parkinson Foundation. BDNF was kindly supplied by Regeneron Inc, NY.

106.13

TROPHIC EFFECTS OF NEUROTROPHIN-3 ON HIPPOCAMPAL NEURONS. D.L. Needels*, M.E. McGuire, S.B. Roberts, K.M. Ingalls, and J.P. Hammang. Cell and Molecular Neurobiology, Bristol-Myers Squibb, Wallingford, CT 06492.

Neurotrophin-3 (NT-3) is a member of the neurotrophic factor family that also includes nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) . We report here the effects of NT-3 on neuronal survival and neurite outgrowth in dissociated hippocampal cultures.

Dissociated E18 rat hippocampal cells were seeded in a defined serum free medium under one of three conditions: without growth factor, with 20 ng/ml of recombinant NT-3 (purified from a baculovirus expression system) or with 50 ng/ml NGF. After 2-7 days in culture, neuronal survival was assessed by counting process-bearing cells expressing either neurofilament-L or neurofilament-M by immunocytochemistry.

In these short term cultures, only a small percentage of cells with a neuronal morphology displayed unambiguous neurofilament protein immunoreactivity. NT-3 (but not NGF) resulted in a 2- to 5- fold increase in the number of neurofilament-M labeled cells. There was also a concomitant increase in both the extent and complexity of neuritic outgrowth. Neurofilament-positive cells did not show the intense nuclear labeling characteristic of dividing cells for up to 6 days following a pulse of ^{3}H-thymidine. Thus, NT-3 was found to display neurotrophic activity for hippocampal neurons, as assessed by increased survival and differentiation of neurofilament-positive cells.

106.14

TROPHIC EFFECTS OF NT-3 ON ASTROCYTES IN MIXED CELL CULTURES FROM FETAL RAT HIPPOCAMPUS. M.E. McGuire*, D.L. Needels, S.B. Roberts, K.M. Ingalls and J.P. Hammang. Cell and Molecular Neurobiology, Bristol-Myers Squibb, Wallingford, CT 06492.

Neurotrophic factors have been shown to promote cell survival, differentiation and proliferation in a variety of cell types. We report here the effects of two structurally related neurotrophic factors, NT-3 and NGF, on glial cell proliferation in dissociated rat E18 hippocampal cultures.

Dissociated fetal rat hippocampal cells were seeded in a defined serum free media under one of three conditions: without growth factor, with 20 ng/ml of recombinant NT-3 (purified from a baculovirus expression system) or with 50 ng/ml NGF. An overnight pulse of 1 µCi/ml ^{3}H-thymidine was added to the cultures either 2 or 24 hours after plating. Cultures were fixed after 2, 4 and 7 days, then labeled with antibodies against glial fibrillary acidic protein (GFAP) and prepared for autoradiography.

Virtually all ^{3}H-thymidine incorporating cells also expressed GFAP, independent of growth factor treatment. In the NT-3 treated cultures the total number of double labeled cells increased 2- to 5-fold over control, depending upon days in culture and length of exposure to NT-3. NGF treated cultures did not differ significantly from control. These results suggest that NT-3 may play a unique role in the proliferation and/or differentiation of hippocampal astrocytes.

106.15

NEUROTROPHIN-4 (NT-4) INCREASES THE NUMBER OF POSTNATAL CHOLINERGIC BASAL FOREBRAIN NEURONS IN CULTURE. M. Yokoyama*, L.D. Cain, W.J. Friedman, J.B. Black, & C.F. Dreyfus, Dept. Neuroscience & Cell Biology, Robert Wood Johnson Medical School, UMDNJ, Piscataway, NJ 08854.

Previous work in our laboratory has demonstrated that nerve growth factor (NGF) and related trophic factors affect embryonic day 17 (E17) basal forebrain (bf) cholinergic neurons. To define the role of these agents on postnatal bf cholinergic neurons, we established bf cultures from older rats (postnatal day 1: P1). E17 bf served as control. Cholinergic neurons were studied by acetylcholinesterase staining (AChE) and CAT activity. With time in culture, the number of cholinergic neurons in E17 and P1 cultures decreased. This loss was most dramatic in P1 cultures. To determine whether NGF can elicit an increase in postnatal cholinergic cell number, we grew E17 and P1 cultures in the presence of NGF, which increased CAT activity, but did not affect the number of cholinergic neurons surving in either. In contrast to the lack of effect of NGF on cholinergic neuronal number, NT-4, a related neurotrophin produced in transfected COS cells, elicited a significant 5-7-fold increase in the number of cholinergic neurons in cultures grown in COS cell medium. These data indicate the selectivity of distinct trophic molecules in the regulation of survival. Supported by NIH grant HD23315 and NS10259.

106.16

THE NEUROTROPHINS *BDNF* AND *NT-3* RAPIDLY STIMULATE THE PHOSPHORYLATION OF PHOSPHOLIPASE-Cγ1 IN CORTICAL CULTURES BY THE *trk*PROTONCOGENE PRODUCT ASSOCIATED KINASE. Hans R. Widmer*, +David R. Kaplan, Beat Knusel, Klaus D. Beck, and Franz Hefti, Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089 and +NCI-Cancer Research and Developmental Center, Frederik, MD 21701

Phospholipase-Cγ1 (PLC) is involved at an early step in signal transduction of many hormones and growth factors. PLC catalyzed hydrolysis of phosphatidyl 4,5 bisphosphate (PI) produces two potent intracellular second messengers molecules. We recently reported that the neurotrophins BDNF and NT-3 stimulate PI-hydrolysis in primary cell cultures of fetal brain neurons. We now present evidence that BDNF and NT-3 stimulate the phosphorylation of PLC by activating *trk*-type protein kinase receptors in these cultures. The stimulation was very rapid i.e. within 20 seconds after addition of the neurotrophins. The effect lasted up to 30 min with a peak after 4 min. ED_{50} values were similar for BDNF and NT-3 with 25-50 ng/ml. As earlier shown for PI hydrolysis, phosphorylation of PLC by neurotrophins was found in cultures from all major brain areas. K-252b which has been shown to block the neurotrophin actions by inhibiting *trk*-type receptor proteins prevented the BDNF and NT-3 stimulated phosphorylation of PLC. The presence of *trk*B mRNA in our culture systems was substantiated by northern blot analysis. The action of BDNF and NT-3 seems to be neuron specific since no phosphorylation was observed in preparations from non-neuronal cultures. The results suggests that the BDNF and NT-3 dependent increase in PI hydrolysis in primary cell cultures from rat brain is due to selective phosphorylation of PLC by the *trk* receptor protein and that cortical neurons are functionally responsive to BDNF and NT-3 during early development.

106.17

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) PREVENTS FUNCTIONAL SENSORY LOSS FOLLOWING CAPSAICIN TREATMENT IN MICE. L. M. Liu*, L. B. Fu, V. Wong, R. F. Alderson, P. S. DiStefano, S. J. Wiegand, R. M. Lindsay Regeneron Pharmaceuticals, 777 Old Saw Mill River Road, Tarrytown, NY 10591.

The excitotoxin capsaicin selectively damages most of the small and some medium sized primary sensory fibers that are involved in the response to noxious stimuli. The neurotrophin, BDNF, has been shown both *in vitro* and *in vivo* to protect survival of dorsal root ganglion sensory neurons. In seeking to develop an animal model for small fiber sensory neuropathy, we have examined the effects of BDNF in protecting or restoring sensory functions following capsaicin treatment *in vivo*. The degree of capsaicin neurotoxicity can be conveniently measured by testing the thermal nociceptive response in experimental animals, using tail flick latency test. Adult CD 1 female mice were injected with 3 doses of capsaicin over a 2 day period. Prior to and on day 12 post capsaicin treatment, tail flick latency was recorded in anesthetized mice to the nearest 0.1 second. Tail flick latency in normal animals was about 4 seconds. After capsaicin treatment (85 mg/kg), it was increased by 3-fold or greater. A cut off time of 15 seconds was used to prevent injury. Treating animals with BDNF was found to improve the tail flick response in a dose dependent manner. When injected daily at 10 mg/kg *s.c.* for 12 days, BDNF was found to totally restore tail flick latency to normal levels. Doses of 5 mg/kg or 1 mg/kg had significant but smaller effects. BDNF is effective when administered before or during, but not after the capsaicin treatment. We conclude that BDNF can prevent/reduce the functional sensory loss from capsaicin insults in mice.

106.18

AGE-RELATED CHANGES IN EXPRESSION OF NEUROTROPHINS AND TRKS IN THE SENESCENCE-ACCELERATED MOUSE (SAM P/8). H. S. Phillips*, M. P. Armanini, G. R. Laramee, J. W. Winslow, J. F. Flood, J. E. Morley, & R. Strong. Dept. of Neuroscience, Genentech, S.S.F., CA 94080 and Dept. of Internal Medicine., St. Louis U. School. of Med. & VA Med. Ctr., GRECC, St. Louis MO 63125.

SAM P/8 mice display early onset changes in learning and memory (Yagi *et al*, 1988; Flood *et al*, 1992). Recent obsevtions of an age-related decline in hippocampal ChAT activity (Strong, *et al* these proceedings) led us to examine the distribution of mRNA for neurotrophins and their receptors in the septo-hippocampal system of these animals. In situ hybridization was employed to examine age-related changes in expression of mRNAs for the neurotrophins NGF, BDNF, and NT3 and for the receptors trkA, trkB, and trkC in basal forebrain and hippocampus of SAM P/8 mice. Optical density readings from sheet film autoradiographs and visual inspection of emulsion autoradiography revealed no age-related changes in expression of NGF, BDNF, or NT3 in the hippocampal formation of SAM P/8 animals. TrkA expressing cells were observed in the medial septum, diagonal band, nucleus basalis, and striatum. Visual inspection of emulsion autoradiographs from young (4 month) and old (12 month) SAM P8 and R1 animals revealed no substantial differences in number of trkA expressing cells in medial septum and diagonal band between any of the groups. Trk B expression was examined in the hippocampus and found to exhibit age-related changes in the P/8 animals that were not apparent in the R/1 controls. These findings raise the possibility that BDNF receptor function may be altered in the SAM P/8 mouse.

106.19

REGULATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR mRNA AND PROTEIN AT THE CELLULAR LEVEL IN PENTYLENETETRAZOL-INDUCED EPILEPTIC SEIZURES. C. Humpel*, C. Wetmore and L. Olson. Dep.Histology and Neurobiology, Karolinska Institute, POB60400, S-10401 Stockholm, Sweden

Brain-derived neurotrophic factor (BDNF) mRNA has been reported to increase in the hippocampal formation and in some other brain areas after seizures induced by lesions, electrical kindling or kainic acid injection. Pentylenetetrazol (PTZ) acts at the picrotoxin site of the $GABA_A$ receptor and induces seizures by decreasing the inhibitory activity. The effects of a single acute convulsive dose (50 mg/kg) of PTZ on BDNF mRNA and protein were analyzed at different time points by in situ hybridization or immunohisto-chemistry, respectively. Kindling was induced by daily subconvulsive injections (30 mg/kg) of PTZ. At different time points during the kindling process, BDNF mRNA and BDNF protein were measured. We show that BDNF mRNA is dramatically increased in the dentate gyrus, piriform cortex and amygdala 3h but not 6h after an acute high dose of PTZ, while BDNF protein levels are decreased in dentate gyrus and hilar region. In fully kindled rats, BDNF mRNA is slightly increased in the hippocampal formation 3h but not 24h after the last PTZ injection. BDNF protein is markedly increased in the hippocampal formation 3h after the last PTZ injection, and still increased after 10 days. These changes suggest that BDNF may be involved in protection mechanisms after damage during seizures and in sprouting responses.

OTHER FACTORS AND TROPHIC AGENTS: TRKS

107.1

COMPARISON OF TRK C mRNA EXPRESSION WITH NT-3 RECEPTOR BINDING AUTORADIOGRAPHY IN BRAIN J.W. Winslow*, M.P. Armanini, G.R. Laramee, H.S. Phillips, A. Shih. Dept. of Neuroscience, Genentech, Inc. South San Francisco, CA. 94080.

Significant levels of specific [125-I]NT3 binding sites have been observed by receptor binding autoradiography in neocortex, hippocampus, and cerebellar granule cell layer of 1 month old rat brain. To gain further insight into the cellular localization of NT3 receptors in the brain, in situ mRNA hybridization of trkC, a putative NT3 receptor, was compared with [125-I]NT3 binding in 1 month old rat brain. In situ hybridization was performed with a 600 bp antisense riboprobe encoding a portion of the mouse trk C extracellular domain sharing minimal homology with other trk genes. Prominent trkC hybridization was observed within hippocampal pyramidal and granule neurons, neuronal layers of neocortex, and the granule cell layer of cerebellum. Although the pattern and resolution of [125-I]NT3 binding does not allow absolute cellular assignments, the autoradiographic distribution of NT3 binding sites is consistent with the trkC expression pattern within these regions. In particular, the significant [125-I]NT3 binding within the molecular, oriens, and radiatum layers of the hippocampal pyramidal neurons, and the molecular layer of dentate gyrus granule neurons, may partly result from binding to trkC receptors on dendrites of these neurons. Additional sites of trkC mRNA expression will be compared to the distribution of NT3 binding within other brain regions.

107.2

DISTRIBUTION OF TRKB TYROSINE KINASE IMMUNO-REACTIVITY IN THE RAT CENTRAL NERVOUS SYSTEM. R.A. Rush*, X-F. Zhou, L. Parada. Centre for Neuroscience, Flinders University, Adelaide, South Australia, 5042 and Molecular Embryology, NCL-FCRF, Frederick, MD 21701

Recent evidence suggests that TrkB tyrosine kinase is a high affinity receptor for brain-derived neurotrophic factor (BDNF). BDNF can act as a survival factor for several neuronal subgroups and its mRNA is distributed widely throughout the central nervous system. However, the functional targets of BDNF are poorly defined. We have used immunochemical and immunohistochemical techniques to determine the regional distribution and cellular localization of TrkB tyrosine kinase-like immunoreactivity. The staining pattern indicates that the TrkB-like antigen is widely distributed and present within both glia and neurons. Astrocytes were the most intensively labelled but many neuronal populations were also detected. In some regions including the brain stem, spinal cord, hippocampus, and diagonal band of the Broca, neurons were stained with varying intensities. In other areas such as the cortex of the forebrain and amygdaloid nucleus the stain was intense but diffuse preventing positive identification of the cell types involved. Immunoblot results indicated two bands, in all the brain and spinal cord regions examined, of molecular weights 145 and 85 kDa respectively. These findings aid the definition of neuronal and glial subpopulations of the central nervous system that utilise BDNF.

107.3

DEVELOPMENTAL REGULATION OF NEUROTROPHIC FACTOR RECEPTORS IN THE MAMMALIAN VISUAL SYSTEM. K.L. Allendoerfer*, R.J. Cabelli, E. Escandon#, K. Nikolics#, and C.J. Shatz. Dept. of Molecular and Cell Biology, U.C. Berkeley, CA 94720 and #Genentech Inc., S.S.F., CA 94080.

The development of specific patterns of connections in the CNS is characterized by the selective loss of neurons and by remodelling of axonal projections. To address the possibility that neurotrophic factors may play a critical role in these events, we have begun to identify the neurotrophic factor receptors that are present in the ferret visual system using EDAC-mediated cross-linking of iodinated BDNF and NT-3 to their receptors (Escandon et al., Soc. Neuro. Abs. 1991, 362.4). Bands corresponding to BDNF cross-linked to p75, to a truncated form of trkB that lacks the tyrosine kinase domain, and to a molecule that most likely is full-length trkB have been positively identified by immunoprecipitation and competition analysis. At the earliest developmental age examined (E30), the ratio of full-length to truncated trkB is >>1 in the retinal target tissues, lateral geniculate nucleus (LGN) and superior colliculus (SC). During the ensuing period of retinal ganglion cell death and segregation into eye-specific layers, the amount of truncated trkB increases markedly relative to full-length trkB. By P27 truncated trkB is the predominant receptor for BDNF in the retinal target tissues and this pattern is maintained into adulthood. In visual cortex, a similar profile of bands is observed, but the developmental increase in abundance of truncated trkB relative to full-length occurs later, with the major increase between P27 and P50. This delay correlates with the later maturation of connections and onset of cell death in the cortex. Using iodinated NT-3, a band most likely corresponding to full-length trkC was observed in cortex, LGN, and SC at all developmental ages studied. We have also observed cross-linking of neurotrophic factors to p75 within the retina and to a lesser extent within the other tissues. These data demonstrate that receptors for BDNF and NT-3 are present in the visual system. The correspondence between changes in the developmental profile of neurotrophic factor receptors and the critical periods for cell death and axon remodelling suggests a role for neurotrophic factors in the development of mature connections in the ferret visual system.[Supported by EY02858 and the Alzheimer's Association (CJS), EY06327 (RJC), and NSO7158 (KLA)].

107.4

DIFFERENT *TRK B* TRANSCRIPTS IN THE RAT RETINA AND OPTIC NERVE. T.N. Jelsma, M. Berkelaar, G.M. Bray, and A.J. Aguayo*. McGill University Centre for Research in Neuroscience, Montreal General Hospital Research Institute, 1650 Cedar Avenue, Montreal, Quebec, H3G 1A4.

We have investigated the expression of the neurotrophin receptor *trkB* in the retina and optic nerve of adult and developing rats.

In adult and newborn (P1) rats, Northern analysis of total brain RNA showed two major bands of approximately 8-10 kb hybridising to the full length *trkB* probe (provided by Regeneron). The upper band alone was observed in the retinal RNA while the RNA obtained from adult optic nerves showed only the lower band. The origin of this band was also investigated in cultured astrocytes where again only the lower band was detected. Furthermore, a probe specific for the internal tyrosine kinase domain of *trkB* hybridised only to the upper band in brain RNA and to neither band in optic nerve RNA.

In situ hybridisation of adult retina with probes corresponding to extracellular and intracellular portions of *trkB* gave the strongest signal in the photoreceptor layer.

In summary, while *trkB* transcripts are expressed in both the retina and optic nerve, different forms are produced in these two regions of the CNS. The *trkB* transcript found in the retina, expressed primarily in the photoreceptor layer, contains the internal tyrosine kinase domain, while that expressed in the optic nerve and cultured astrocytes lacks this domain.

107.5

NT3 AND *trkB* mRNA EXPRESSION IN THE DEVELOPING RAT BRAIN FOLLOWING KAINIC ACID-INDUCED SEIZURES E. Ohsawa*, R. W. Rodgers, K. D. Beck, F. Hefti, and M. M. Dugich-Djordjevic. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

We have previously reported that increases in BDNF mRNA expression in the rat brain following kainic acid-induced seizures is developmentally dependent, with the onset of stimulus-transcription coupling in response to seizure occuring after P13 (Dugich-Djordjevic et al., Neuron, 1992, in press). In the present study we used Northern blot and in situ hybridization analyses to investigate the expression of NT3 and *trkB* mRNAs during development and following kainic acid-induced seizure activity. *Trk*B expression was evaluated with probes specific for (1) the full receptor with the tyrosine kinase domain (*trk*B) and (2) the truncated receptor lacking the intracellular protein kinase domain (*trk*B (-)). Regional patterns of expression of *trk*B and *trk*B *(-)* were similar. From P4 to P13, high levels of *trk*B and *trk*B (-) mRNA expression were evident in cortex and dorsal thalamic nuclei, subiculum, CA1 and CA3, with low levels in DG. From P13 to adult, cortical and thalamic levels were decreased, while hippocampal levels were slightly increased. Following KA-induced seizure activity NT3 mRNA expression in the hippocampus was decreased after P17. *Trk*B and *trk*B (-) mRNA expression was increased in hippocampal subregions from P17 to adult; however, the time course and localization of the increases in response to seizure activity differed among the full length and truncated *trk*B receptors.

These data indicate that, similar to BDNF, the regulation of NT3 and *trk*B mRNA expression in response to seizure activity is developmentally dependent. However, the onset of stimulus-transcription coupling for NT3 and *trk*B mRNAs is observed at a later stage of development compared to the onset of changes in BDNF mRNA following seizure. The delayed developmental onset of transcriptional regulation of *trk*B mRNA compared to BDNF mRNA following seizure activation and the differential time course and distribution of *trk*B mRNA expression following seizure activity suggests that the regulation of neurotrophin receptor transcription differs from that of their neurotrophin ligand.

107.6

Urine levels of NGF Receptor-truncated are Elevated in Diabetic Neuropathy. R.E. Hruska*, M.M. Chertack, and D. Kravis. Dept. 90J, Abbott Labs, Abbott Park, IL and 64 Old Orchard Rd., Skokie, IL.

NGF receptor-truncated (NGFR-t) is the cleaved extracellular portion of NGFR and contains the NGF binding domain. NGFR-t is present in the urine and (corrected for creatinine) is elevated in infants, in patients with ALS, and in an animal model with a sciatic nerve lesion. In order to reproducibly measure NGFR-t, a two-site EIA was developed with sensitivity of <5 ng/ml and capacity of at least 800 ng/ml. In multiple studies, the variation between analyses was less than 10%. Reagents, including the recombinant standard, were stable in the appropriate matrix for at least 30 days at 5°C. For accuracy, urine specimens were pH normalized with a 0.15 M phosphate buffer. Urine creatinine levels were quantitated on the TDx analyzer. Using these methods, the level of NGFR-t (ng/μg creatinine) in normals is 0.085 ± 0.022 (mean $\pm$ SD, N=45), in diabetics is 0.105 ± 0.034 (N=63), and in diabetics with neuropathy is 0.144 ± 0.046 (N=98). These results show a significant elevation of NGFR-t ($P < 0.001$) in patients with neuropathy, 66% having levels more than 2-SD above the mean of normals. Proteinuria, as measured by microalbumin, does not alter these results. These changes were found in males and females and in Type I and Type II diabetics. Interestingly, a few females over the age of 60 and with Type II diabetes also have elevated levels. NGFR-t is easily measured in urine specimens, is stable under normal storage conditions, and is elevated in patients with diabetic neuropathy. The elevation in NGFR-t urine levels could be associated with increased neuronal damage and neurotrophic factor turnover. In conclusion, measurement of NGFR-t provides an independent assessment of a biochemical change associated with neuropathy.

HORMONES AND DEVELOPMENT: NON-GONADAL HORMONES

108.1

MORPHOMETRY REVEALS MARKED RECOVERY OF RAT BRAIN HIPPOCAMPUS AND DENTATE GYRUS FROM EARLY HYPOTHYROID GROWTH RETARDATION. Arash Farahvar and Esmail Meisami*, Physiology Dept., Univ. of Illinois, Urbana, IL 61801.

Early thyroid deficiency causes major retardation in brain growth, behavior and learning ability. Less is known about the capacity of the brain to recover from this cretinoid retardation. Growth of hippocampus (HC) and dentate gyrus (DG) of the rat brain was used as a model system. Sprague-Dawley rats were made hypothyroid by administration of the reversible goitrogen PTU (propylthiouracil, 0.1% w/v) from birth. Some of the hypothyroid rats were allowed to recover by withdrawal of PTU at 25d (weaning). Control, hypothyroid and recovery rats were compared for surface area and volume of HC and DG at 25d & 90d postnatal. At 25d, DG measures in hypothyroid rats were significantly less than normal (surface area 16%, volume 23%, $p<0.01$). The retarding effects on HC were less severe. In normal rats, between 25d & 90d, surface area and volume of DG increased by 37% & 51%, reaching mean values of 24.5 mm^2 & 14.2 mm^3; in HC, surface area and volume increased by 44% & 35%, reaching mean values of 42.9 mm^2 & 23.5 mm^3. In the 90d hypothyroid rats, growth was retarded, resulting in mean values that were significantly (20-30%) below normal. In contrast, the growth of DG & HC in recovery rats was more robust than normal, compensating for the earlier hypothyroid deficits, so that by 90d the mean values of normal and recovery rats were not significantly different. Supported by University of Illinois Research Funds.

108.2

OUABAIN INHIBITION OF ADULT HYPOTHYROID MOUSE BRAIN Na,K-ATPase. J.M. Bertoni* and P.M. Sprenkle. Neurology Department, Creighton University, Omaha, NE 68131.

Na,K-ATPase activity is regionally reduced in the brain of the adult genetically hypothyroid mouse *hyt/hyt*. These local reductions in enzyme activity may be accompanied by altered ratios of Na,K-ATPase isoforms. Since Na,K-ATPase isoforms show characteristic affinities for binding by ouabain, we examined the effects of ouabain on K-pNPPase activity (a partial reaction of Na,K-ATPase) in subcortical and hindbrain homogenates from *hyt/hyt* (hypothyroid) and *hyt/+* (euthyroid) mice. K-pNPPase activity (V_{max}) was only slightly depressed in *hyt/hyt* hindbrain relative to *hyt/+* controls (175 ± 3.68 and 188 ± 1.63 nmol/mg/min, respectively), while subcortical K-pNPPase activity was 28% lower ($p<0.001$) in *hyt/hyt* mice (108 ± 11.1 vs 150 ± 6.74 nmol/mg/min). Both hindbrain and subcortical data were best fit to one-site, rather than two-site, ouabain binding models with no significant differences in *hyt/hyt* vs *hyt/+* K_i's. This suggestion of single-site ouabain binding models for both thyroid hormone-sensitive and -insensitive brain regions does not support the hypothesis that reductions in Na,K-ATPase activity are related to altered isoform ratios in the hypothyroid state.

108.3

THE DISTRIBUTION OF THYROID HORMONE RECEPTOR MRNAS IN THE CNS OF *XENOPUS LAEVIS* DURING METAMORPHOSIS. M.S. Beattie*¶§, J.C. Bresnahan¶ and J.N. Masters¶◆ Department of Cell Biology, Neurobiology and Anatomy, § Division of Neurosurgery, ◆Ohio State Biotechnology Center, The Ohio State University, Columbus, OH 43210

The metamorphosis from larval to juvenile form in anuran amphibians encompasses a radical transformation from swimming to quadrupedal locomotion and includes the addition and innervation of limb musculature, additions and deletions of central neuronal elements and connections, and tail resorption. This process is dependent on thyroid hormones (TH) which rise dramatically during metamorphic climax and fall to very low levels immediately after metamorphosis. The alpha and beta forms of the *Xenopus* thyroid hormone receptor (TR) mRNAs have been recently shown to be independently regulated during development (Yaiota et al., Genes Dev. 4:1917 (1990), Kawahara et al., Development 112:933 (1991)). The TRα transcript is present relatively early in development while TRβ expression correlates with metamorphosis. Thus we hypothesize that TRα may mediate generative processes while TRβ mediates the degenerative components during Xenopus development. As a test of this hypothesis, we used *in situ* hybridization with probes specific for the TRα and TRβ mRNA (provided by D. Brown) to localize expression of the receptor subtypes during Xenopus development. The TRα mRNA is easily observed by *in situ* hybridization and is expressed in highest amounts in the developing central nervous system. Its expression in the brain or spinal cord follows the relative abundance previously reported by others using isolated RNA measurements; namely a peak at stage 58 which declines rapidly by stage 62. We were not able to localize expression of the TRβ mRNA in any of the developing frogs, in agreement with others. This result most probably reflects the detection limits of *in situ* hybridization with relatively rare transcripts. (NS10165)

108.4

$5HT_3$ RECEPTOR MEDIATION OF TRH INDUCED INCREASES IN INTESTINAL TRANSIT IN DEVELOPING RATS. M. Heitkemper, E. Bond, K. Gruver, and A. Horita*. Departments of Nursing and Pharmacology, Univ. of Washington, Seattle, WA 98195.

Intracisternal (ic) injection of thyrotropin releasing hormone (TRH) increases gastric contractile activity and small intestinal transit. Both responses are mediated primarily by a vagal cholinergic mechanism. However, the $5HT_3$ antagonist MDL-72222 blocked TRH induced increases in gastric motility in young (<14 day [D]) but not older rats, suggesting a serotonergic component is involved early in development. Serotonergic contribution to TRH stimulation of intestinal transit is not described. This study examined the effects of $5HT_2$ antagonists (ketanserin, xylamidine) and $5HT_3$ antagonists (MDL-72222, ICS 205-930) on TRH stimulated transit of a charcoal bolus in rats 7, 10, 14 and >50D. Rats were anesthetized with urethane (1.25mg/kg). One of the following agents was administered intraperitoneal: ICS 205-930 (1mg/kg), MDL-72222 (1mg/kg), ketanserin (5mg/kg), xylamidine (10mg/kg). A midline incision was made, the gastroduodenal junction identified and 50μl (7 and 10D), 100μl (14D) or 200μl (adult) of a 20% charcoal in saline solution injected into the duodenum. At 25 min post charcoal instillation, TRH (15μg) in saline or saline alone was administered via ic injection (0.6μl). Two hrs post TRH/saline injection transit (distance travelled by charcoal expressed as a percentage of total small intestinal length) was measured. ICS 205-930 effectively blocked TRH induced intestinal transit in all age groups while MDL-72222 blocked in 7 and 10D rats. $5HT_2$ antagonists did not block TRH induced transit in 7D but did in older rats. These results suggest that while serotonin may mediate TRH induced transit increases, receptor expression is dynamic during development. (Supported by K07 NR00007)

108.5

Effects of Adrenalectomy on the Dentate Gyrus of Rats and Hamsters in Both Sexes. D. Leone, C.D. Condon, V. Doppalapudi, and E.J. Roy*. Neuroscience Program & Dept. Psychology, Univ. of Illinois, Champaign, IL 61820.

Adrenalectomy (ADX) has been shown to cause specific degeneration in the hippocampus of male rats. Given that progesterone can bind to corticosteroid receptors in the hippocampus, we determined whether females show degeneration similar to males. We also determined whether a species that differs from rats in its primary glucocorticoid and in characteristics of corticoid receptors would show the same degeneration. We therefore quantified the volume of the dentate gyrus of rats and hamsters in both sexes using stereological estimates.

Long-Evans rats and Syrian hamsters were approximately 10 weeks old at the time of the ADX/SHAM operation. Rats and hamsters were sacrificed 6 and 12 weeks after ADX respectively. Both female and male ADX rats showed significant reduction ($p<0.001$) in volume of the ventral and dorsal blades of the dentate gyrus. These results are the first to show that female rats are susceptible to ADX-induced degeneration in the dentate gyrus. The hamster dentate gyrus results were complex, with sex differences and regional differences in the effects of ADX. Supported by MH18412 and Univ. of Illinois Neuroscience Prog.

108.6

BRIEF ASPHYXIA DURING BIRTH PRODUCES PERMANENT CHANGES IN STRESS-INDUCED GLUCOCORTICOID SECRETION. W. Brooks*, K. Betito, S. Bhatnagar, M.J. Meaney and P. Boksa. McGill University, Dept. of Psychiatry, Douglas Hospital Research Centre, Montreal, Quebec, Canada, H4H 1R3.

Little is known about the specific long-term functional consequences, to the CNS, of periods of asphyxia during the birth process. Morphological studies indicate that the hippocampus is particularly vulnerable to damage resulting from perinatal asphyxia. The hippocampus plays an important role in feedback regulation of glucocorticoid (GC) secretion in response to stress. Thus the current study examined consequences of brief asphyxia during birth on subsequent regulation of GC secretion in the adult rat. Birth asphyxia was produced as described by Bjelke et al (Brain Res., 1991, 543, 1); in this model the entire uterus was removed on the day of birth and immersed in a water bath for 10-20 min before delivery. Control animals were either born vaginally or delivered by Caesarian section without delay. At 3 months of age, asphytic and control rats underwent 20 min of restraint stress and corticosterone was measured in tail vein samples taken before and after the stressor. Adult rats exposed to a period of asphyxia during birth exhibited a prolonged hypersecretion of corticosterone following termination of the acute stressor. Alterations (hypersecretion) in the diurnal pattern of basal corticosterone levels were also observed in adults exposed to perinatal asphyxia. Long-term exposure to elevated GC levels has been previously associated with neuronal damage in a number of paradigms. It is possible that the permanent alterations in GC secretion we observed in asphytic animals may contribute to the production of long-term CNS damage following a brief period of asphyxia during birth.

108.7

HYDROCORTISONE (HC) INCREASES DYSTROPHIN IMMUNOREACTIVITY AND CAUSES DYSTROPHIN AGGREGATION IN ANEURALLY CULTURED HUMAN MUSCLE. J McFerrin, V Askanas* and WK Engel. USC Neuromuscular Center, Los Angeles, CA 90017-1969.

The glucocorticoid (GC) prednisone is widely used as an immunosuppressant for treating myasthenia gravis and polymyositis. Recently, its beneficial effect in Duchenne muscular dystrophy (DMD) has been reported. Nevertheless, a direct influence of GC on NMJs and on muscle fibers themselves is not established. In aneurally cultured human muscle, GCs increase the number of non-junctional acetylcholine receptors (AChRs) and the number and size of AChR clusters (Askanas et al., 1986); and in innervated cultured human muscle, GCs increase accumulation of junctional-AChRs (Braun et al., 1991). We have now studied the influence of HC on dystrophin immunoreactivity (Dys-IR), using a monoclonal antibody against the C-terminus region of dystrophin. (Novocastra). In seven experiments, each from a different patient's biopsy, treatment with 20 uM HC, a GC, was initiated on 8-day-old aneurally cultured normal human muscle and was continued for 2,3 and 4 weeks. In both HC-treated cultures and controls, Dys-IR was increased in more mature cultures and it had a more distinct sarcolemmal localization. In the HC-treated muscle, Dys-IR was quantitatively increased on immunoblot. In contrast to the uniform distribution of Dys-IR in cultured control muscle, in all experiments (evaluated blind), 80% of the HC-treated muscle fibers contained large patchy aggregates of strikingly increased Dys-IR. Our study demonstrates that in addition to AChRs, treatment with GC influences Dys and perhaps other proteins of human muscle plasmalemma.

108.8

PRENATAL STRESS REDUCES T-LYMPHOCYTE PROLIFERATION IN RATS. M. Flory, S. Klein[1] & C.H. Kinsley*. Dept. of Psych., Univ. of Richmond, VA 23173 and [1]Randolph-Macon College, Ashland, VA, 23005.

Both sexual differentiation and immune system development occur largely prenatally. Prenatal stress (PS) disrupts the former, and we have begun to investigate its effects on the latter, process. Adult offspring from mothers stressed on days 15-22 of pregnancy (thrice-daily exposures to heat, light and restaint) were stressed for 60-min in a restraint tube. One ml trunk blood samples were obtained and all subclasses of IgG T-lymphocytes were extracted through affinity chromatography with immunologically-coated, magnetic beads (Dynabeads, Inc.) and visually quantified. PS animals displayed a marked and significant decrease in the number of T-lymphocytes, relative to controls (1.7×10^6 v. 4.0×10^6, respectively). These data, in combination with our work on thymus and on skin graft transplantation immunity, suggest that PS reduces the integrity and effectiveness of the immune system. Prenatal events, therefore, may be considered a significant force in shaping the immune system. (Supported by UR research funds.)

108.9

THE COMPARISON OF ANGIOTENSIN LEVELS IN THE BRAINS OF SHR AND WKY RATS DURING PERINATAL DEVELOPMENT. V.I. Cook,* V. Puig, D. Lu and M.I. Phillips. Dept. of Physiology, Univ. of Florida, Gainesville, FL 32610.

Our previous work has shown that during perinatal development SHR and WKY rats exhibit some significant differences in the number of angiotensin receptors and the timing of receptor appearance in specific brain areas. Because the SHR's appear to be on a different developmental schedule than the WKY, as exhibited by angiotensin receptors in the brain, we decided to examine the levels of angiotensin to see if the same developmental differences would be seen.

Utilizing radioimmunoassay (RIA), we determined the levels of angiotensin in the brains of each strain at different perinatal time points. By the 21st day of gestation, SHR's already show significantly more brain angiotensin than WKY rats (227.7±14.68, 125.8±37.5 pg/g of tissue), respectively. By the 5th postnatal day, the difference is even greater (SHR = 323.3±69.1 pg/g tissue and WKY rats = 162.5±22.6 pg/g tissue).

These data support the idea that abnormalities of the renin-angiotensin system, as early as the fetal stage of development, may be associated with subsequent hypertension.

108.10

EFFECT OF THE STEROID HORMONE 20-HYDROXYECDYSONE ON THE BRANCHING PATTERNS OF CULTURED NEURONS FROM THE DEVELOPING OLFACTORY LOBE OF THE MOTH. Lynne A. Oland*, Jon H. Hayashi, and Leslie P. Tolbert. ARL Div.of Neurobiology, Univ. Arizona, Tucson, 85721.

During metamorphic adult development in *Manduca sexta*, 20-hydroxyecdysone (20-HE) titers rise rapidly during the same period in which the developing antennal lobe (AL) neurons undergo extensive process outgrowth and branching. While patterning of the shape of the arbors has been shown (Tolbert and Oland, 1990) to be dependent upon an interplay among ingrowing antennal sensory axons, AL glia, and the AL neurons, the role of 20-HE on growth has not been examined. Using a well-established culture system, we examined the effect of 20-HE on the number of branches and total neurite length of several morphological types of neurons. Neurons were grown for two weeks in 0.2, 1, or 10 µg 20-HE/ml medium, values that span the physiological range in the blood. Solitary neurons were photographed and their arbors traced on a digitizing pad for subsequent morphometric analysis. In all cases, the pattern of changes in values for number of branches mirrored changes for total length. Among the 6 cell types examined, the response within a stage to increasing hormone (increase, decrease, or no response) depended upon the type as well as on the stage of ALs from which the neurons were dissociated. Several types showed significantly greater outgrowth when the neurons had been dissociated from stage-5 ALs, which have begun to receive sensory innervation, compared with those from stage-2 ALs, which have not yet received sensory innervation. In addition, outgrowth was significantly greater in neurons from stage-5 ALs than in stage-5 ALs that had developed in the chronic absence of sensory axons. Taken together, these results suggest that sensory input promotes neurite outgrowth and branching, but that, where 20-HE has an effect on outgrowth, the polarity of the effect is cell-type specific. [Supported by NS 28495.]

108.11

THE DEVELOPMENT OF POTASSIUM CURRENTS IN CULTURED INSECT OLFACTORY NEURONS. J.H. Hayashi*, L.A. Oland, and J.G. Hildebrand. ARL Div. of Neurobiology, Univ. of AZ., Tucson, AZ, 85721.

During metamorphic adult development in *Manduca sexta* neurons in the developing antennal lobe undergo extensive process outgrowth and are contacted by ingrowing sensory axons from the antenna. In addition, the neurons develop in a background of a rapidly rising titer of the steroid hormone 20-hydroxyecdysone (20-HE). To assess the role of sensory input and 20-HE on the biophysical development of antennal lobe neurons, we dissociated neurons from the developing antennal lobe and grew them in primary culture where several types can be identified on the basis of their characteristic morphologies. Neurons were dissociated from antennal lobes at stage 2, when antennal sensory axons have not yet arrived at the lobe, and at stage 5, when the sensory axons have just begun to exert their influence over the morphological development of the lobe. We controlled the titer of 20-HE to mimic either the low (0.2 µg/ml) stage-2 level or the elevated (1µg/ml) stage-5 levels. We used whole-cell patch-clamp technique to study the expression of specific voltage-gated currents in four neuronal types. We found that both morphological cell type and developmental stage affected neuronal responses to 20-HE by altering their level of A-type and delayed rectifier potassium current expression. The responses of the individual neuronal types to a given protocol differed suggesting to us that the response to ecdysone is a specific developmental cue. Finally, we compared the expression of currents in neurons derived from normal stage-5 antennal lobes with those that had developed *in vivo* in the chronic absence of antennal sensory axons. Within a neuronal type, a history of exposure to ingrowing sensory axons *in vivo* led to decreased expression *in vitro* of A-type currents and increased expression of inward currents. [Supported by NIH grant NS 28495 to JHH, LAO, and JGH]

HORMONES AND DEVELOPMENT: GONADAL HORMONES I

109.1

MECHANISMS OF ESTROGEN-INDUCED NEURON ADDITION TO AN AVIAN SONG CONTROL NUCLEUS. M.J. Burek*, K.W. Nordeen, and E.J. Nordeen. Dept. Psych. and Neurosci. Program, U. of Rochester, Rochester, N.Y. 14627.

In zebra finches, only males sing and most brain regions controlling song contain more neurons in adult males than females. In one region, the higher vocal center (HVC), neurogenesis extends throughout sexual differentiation and the addition of new neurons is greater in young males than in females. Estradiol (E2) can masculinize (increase) the incorporation of HVC neurons in females and render them capable of male-typical song. Using 3[H]-thymidine autoradiography to label HVC neurons born on days 15 and 16 post-hatch, we established previously that these neurons are sexually dimorphic in number within days after their production, implying that sexual differentiation affects their production or early survival, rather than long-term retention.

To further distinguish between these possibilities, we determined if E2 could increase the incorporation of HVC neurons born before hormone exposure. Females were given 3H-thymidine on days15-16, and on day 17 received either E2-containing or blank subcutaneous silastic implants. On day 35, birds were sacrificed and autoradiograms through HVC were analyzed to determine neuron number and the incidence of thymidine-labeling. Heavily-labeled neurons (HL) had grain densities exceeding one-half the maximum observed and were presumed to have undergone their last division on day 15 or 16 (i.e. before E2 exposure). Lightly-labeled neurons (LL) had grain densities below one-half maximum, but still exceeding 5X background, and were believed to consist mostly of cells born after the thymidine regime, (i.e during E2 exposure). As expected, E2 treatment significantly increased neuron number in HVC. E2 also significantly increased the incidence of LL neurons, but did not affect the proportion of HL cells. Insofar as neurons born during E2 exposure were disproportionately affected by the hormone, our results support the hypothesis that estrogenic regulation of neurogenesis contributes to the sexual differentiation of HVC neuron number.

109.2

LACK OF SYNERGISTIC EFFECT OF DIHYDROTESTOSTERONE AND ESTRADIOL ON MASCULINIZATION OF THE SONG SYSTEM OF THE ZEBRA FINCH. E.C. Jacobs' and A.P. Arnold. UCLA Brain Research Inst. and Dept. Psychol., Los Angeles, CA 90024-1563.

Previous studies have suggested that both major active metabolites of testosterone, estradiol (E2) and dihydrotestosterone (DHT), are needed for complete masculinization of the brain regions controlling song in passerine birds. However, DHT treatment of hatchling female zebra finches (ZFs) has only small masculinizing effects on the song system.

We examined the effects of DHT given to female ZFs in combination with E2. Female ZFs were given one of the following hormone treatments: 1) E2 at day (D)1; 2) E2 + DHT at D1; 3) E2 at D1 + DHT at D14; 4) E2 at D1 + DHT at D70; 5) no treatment (n=4-6 per group). Control males were untreated. Hormone treatments at D1 consisted of Silastic pellets mixed with 50µg of each steroid. Treatments at D14 or D70 consisted of 5mm Silastic tubes filled with DHT, left in place for 35 days. Each bird was perfused at D105-110 and the brain was sectioned at 30µm and stained with thionin. Volumes of HVC in E2+DHT birds were 114-117% of volumes of E2-alone birds (not statistically significant). Volumes of RA in E2+DHT birds were 69-104% of volumes of E2-alone birds (not statistically significant). Volumes of HVC in E2+DHT birds were 56-64% of male values (not statistically significant), and volumes of RA in E2+DHT birds were 44-67% of male values (some significantly smaller than males). These results suggest that androgens play a relatively minor role in the sexual differentiation of these attributes of the ZF song system.

Supported by NIH grant DC00217 and an NSF Graduate Fellowship.

109.3

GLIA EXPRESS HIGH LEVELS OF AROMATASE IN CULTURES OF DEVELOPING ZEBRA FINCH TELENCEPHALON. A.P. Arnold*, S.Amur-Umarjee, A. Campagnoni and B.A. Schlinger. Depts. of Psychology and Psychiatry, BRI, UCLA, Los Angeles, CA 90024.

Conversion of androgen to estrogen (aromatization) in brain regulates effects of circulating androgen on neural development and behavior in males of many vertebrate species. Previous studies indicate that aromatase, the enzyme that catalyzes this conversion, is expressed most abundantly in *neurons* present in *limbic* brain regions. Songbirds are unique in that aromatase is expressed at high levels in the telencephalon (TEL) of both males and females. Because estrogens masculinize TEL brain regions that control song, we are interested in whether the brain itself synthesizes the estrogen responsible for masculinizing the song system. Accordingly, we have established primary cultures using TELs from 1-6 day old zebra finches. Immunohistochemical analysis of these cultures 1-3 weeks *in vitro* indicate the presence of neurons and glia. Aromatase activity (^{3}H-androstenedione conversion to ^{3}H-estrone plus ^{3}H-estradiol) was abundant in these cultures (up to 6.7 pmoles/min/mg protein). To identify the cell type responsible for aromatization, cultures were treated with the neurotoxin kainic acid (KA, 10^{-2} M) for 4-7 days. Although KA reduced neuronal numbers by as much as 90%, it had no effect on aromatase activity. We conclude that aromatase in the TEL of the developing zebra finch is present in non-neuronal elements, probably astrocytes. This unique cellular localization may account for some of the unusual properties of brain aromatase in this species, including the capacity to secrete estrogen into the general circulation of adults. Supported by NSF and NIH grants BNS9020953, DC00217 and NS23022.

109.4

3β-HYDROXYSTEROID DEHYDROGENASE/ISOMERASE ACTIVITY IN PRIMARY CULTURES FROM DEVELOPING ZEBRA FINCH TELENCEPHALON. B.A. Schlinger*, A.M. Vanson, S. Amur-Umarjee, A. Campagnoni and A.P. Arnold. Depts. of Psychology and Psychiatry, Brain Res. Inst., UCLA, Los Angeles, CA 90024-1563.

Sex steroid metabolism and synthesis are important properties of brain tissue. Steroid synthesis is present in glia, whereas neurons and glia metabolize circulating sex steroids to active or inactive products. We have recently found that aromatase, the enzyme that catalyzes the synthesis of estrogen from androgen, is expressed at extremely high levels in primary cultures derived from the telencephalon (TEL) (1-3 weeks *in vitro*) of zebra finches (1-4 days post hatching). Thus, we are interested in whether other steroidogenic enzymes might also be expressed at high levels in these cultures. 3β-Hydroxysteroid dehydrogenase/isomerase (3β-HSD) is an essential enzyme in steroidogenic tissues and synthesizes active sex steroids from pregenenolone (Preg) and dehydroepiandrosterone (DHEA). 3β-HSD activity was identified in these cultures since ^{3}H-progesterone or ^{3}H-androstenedione (AE) was present in the media (products verified by recrystallization to constant specific activity) after incubating cultures (2.5 to 24 hrs) with 130-430 nM [7-^{3}H]-Preg or 62.5-130 nM [1,2,6,7-^{3}H]-DHEA, respectively. ^{3}H-Estrogens were also identified (and recrystallized) in media after incubation with ^{3}H-DHEA, presumably derived from ^{3}H-AE or ^{3}H-testosterone. These data further identify glia as important modulators of the steroidal environment in the zebra finch TEL and indicate that DHEA can serve as a precursor for brain synthesis of active androgen and estrogen. Supported by NSF and NIH grants BNS9020953, DC00217 and NS23022.

109.5

INTRAUTERINE POSITION MODULATES SACCHARIN PREFERENCE IN ADULT MALE MICE. M.E. Bushong and M.A. Mann*. The University of Texas at Arlington, Arlington, TX 76019.

Saccharin preference is a sexually dimorphic trait in rats in that adult females exhibit a greater preference relative to males. To establish a preference profile for adult mice, separate groups of males and females received tap water and one of five concentrations of saccharin solution (either 0.1, 0.25, 0.5, 0.75 or 1.0%). A significantly higher preference score was obtained for females vs. males that received the .25% solution; other concentrations did not yield a reliable sex difference.

To examine if the preference patterns of adult males are organized during prenatal life, mice were delivered by C section to determine their position relative to same- or opposite-sex fetuses. Males were classified as having resided between two male (2M), two female (0M) or one male and one female (1M) fetuses. As adults, saccharin preference was examined in separate groups that received one of two preferred solutions, 0.25 (which in Experiment 1 yielded a significant sex difference) or 0.75% (which yielded no sex difference). A significantly higher preference score was obtained for 0M vs. 2M males that received the 0.25 but not the 0.75% solution. Since 0M males are exposed prenatally to higher titers of estradiol than 2M males, estradiol may be responsible for organizing a pattern of saccharin preference that resembles that reported for females.

109.6

ASYMMETRY AND SEXUAL DIMORPHISM INDUCED BY CORTICAL DAMAGE IN RATS: EFFECT OF NEONATAL EXPOSURE TO TESTOSTERONE PROPIONATE IN FEMALES. C.H. Woodworth* and R.G. Robinson. Dept. Psychiatry, Univ. of Iowa Coll. Med., Iowa City, IA 52242.

Previous work in this laboratory has shown that female rats differ from male rats in their lack of a lateralized behavioral response to focal cortical damage. Although right frontocortical suction lesions induce hyperactivity in male rats, neither right nor left lesions produce hyperactivity in female rats, regardless of whether the females are intact, ovariectomized prior to puberty, or exposed to the activating effects of testosterone proprionate (TP) in adulthood. The present experiment investigates whether female rats exposed as neonates to the organizing effects of TP will display male-pattern hyperactivity to cortical suction lesions as adults.

Subjects were F1 Sprague-Dawley females born to commercially-obtained breeders mated in our colony. Pups were either ovariectomized or sham-operated within 24 hours after birth (Postnatal Day 0), and injected with either TP or vehicle on Postnatal Days 1 and 5. At 90 days of age, females were subjected to either right or left cortical suction lesions and implanted with either empty or TP-filled silastic capsules. Subjects were housed in running-wheels, and activity was monitored by computer for 21 days preceding, and for 30 days following surgery.

Results indicate that baseline running-wheel activity was significantly lower in ovariectomized females and in all females exposed neonatally to TP. TP implants produced hyperactivity in ovariectomized but not sham-operated females. Lesions were found to have a complex effect, depending both on neonatal treatment and on availability of circulating TP. These data support the conclusion that behavioral lateralization depends on the hormonal environment of the developing brain.

109.7

CORPUS CALLOSUM: INTERACTIVE EFFECTS OF HANDLING AND OVARIECTOMY IN THE RAT. C. M. Mack*, P. E. Cowell, and V. H. Denenberg. Biobehavioral Sciences Graduate Degree Program, University of Connecticut, Storrs, CT 06269.

Both testosterone treatment and ovariectomy in early postnatal development have independently been shown to increase the midsagittal area of the corpus callosum (CC) of female rats handled in infancy. This effect of testosterone on the CC is not observed in nonhandled females. This raised the possibility of interactive effects of handling and ovariectomy on the female callosum. Whole litters (N=6 females, 2 males) of rats were handled or nonhandled between Days 1-21. On Day 12, three females within each litter were ovariectomized and the other three received sham surgery. The CC was examined at 110 days of age. Midsagittal callosal area and seven regional width factors, derived from a prior factor analysis of the rat callosum, were used to analyze group differences. Both handled and nonhandled ovariectomized females had increased callosal area compared to their respective controls. Analysis of the width factors revealed that handled ovariectomized females were significantly larger in the genu and body of the callosum, whereas the nonhandled group was significantly larger in the body and splenial regions. Thus although infantile handling had no effect on overall callosal area, there is regional specificity of the handling effect in ovariectomized females.

109.8

WHAT IS THE INFLUENCE OF TESTOSTERONE ON RAT VISUAL CORTEX DENDRITIC PLASTICITY? P. Seymoure*, J. Jang, J. Pluskwa and J. M. Juraska. Dept. of Psychology, University of Illinois, Champaign, IL 61820.

Sex differences in dendritic morphology of pyramidal neurons occur in rat visual cortex layer III in response to differential housing. Males show robust increases in dendritic field size, but females do not (Juraska, Brain Res., 295:27, 1984). The present study investigates whether testosterone influences this plasticity. Six littermate sets of Long-Evans male rats were used. Within 6 hours of birth two rats from each litter were castrated and the other two rats received a sham operation. At 25 days of age, castrated and sham rats from each litter were housed in either a complex environment (EC) or in individual lab cages (IC). After 30 days of housing the rats were sacrificed and 16 Golgi-Cox stained pyramidal cells were quantified from the monocular and binocular regions of the visual cortex. Sholl ring analysis of the basilar dendrites revealed that EC rats had larger dendritic fields than IC rats ($p < 0.02$). However, there was no difference between sham and castrated males. This result indicates that if testosterone has an influence on this plasticity, postnatal testosterone is not required. We are currently examining the size of the apical field and analyzing branch number and length. Supported by NSF BNS 89-09164.

109.9

ESTROGEN RECEPTOR OCCUPATION IN THE CEREBRAL CORTEX OF THE DEVELOPING RAT. N. J. MacLusky*, T.J. Brown and R.B. Hochberg Division of Reproductive Science, The Toronto Hospital, Toronto, Canada M5G 1L7, and Dept. of Obstetrics and Gynecology Yale University New Haven, CT06510

Previous studies have demonstrated the presence of aromatase activity and estrogen receptors (ER) in regions of the developing mammalian cerebral cortex, raising the possibility that local conversion of testosterone to estradiol may play a role in the sexual differentiation of higher cognitive functions. Attempts to demonstrate occupation of ER in the developing brain have, however, been unsuccessful. Using *in vitro* exchange autoradiography (Walters *et al.*, Soc. for Neurosci., 17:1410, 1991), we re-examined the distribution of occupied ER in the brain of developing male and female rats, throughout the first two weeks of postnatal life. Cryostat sections (10μm) through the brain were incubated with either 11β-methoxy 16α-[^{125}I] iodoestradiol or [^{3}H] Moxestrol, then washed, dried and exposed against Hyperfilm (Amersham). Regional distribution of estrogen binding was assessed from the autoradiograms by computerized densitometry. ER ocupation was lower in the brain of the female than in the corresponding regions of the male. In males, particularly high ER occupation was observed in the periventricular preoptic area and ventromedial nucleus. Weaker labelling was observed in the hippocampus, as well as in the anterior cingulate and suprarhinal cortices. In all regions of the neocortex, ER occupation declined with age. In the hippocampus, however, estrogen binding remained high until postnatal d10, paralleling the elevated levels of aromatase activity observed in this region of the brain throughout the first two weeks of life. These results support the view that local estrogen biosynthesis may contribute to cerebral cortical differentiation during early life, and suggest that the hippocampus may be a particularly important target site for the developmental effects of aromatizable androgens [Supported by grants MT-11235 (to TJB) and PG 11115 (to NJM) from MRC Canada, and CA 37799 from NIH (to RBH)].

109.10

ESTROGEN RECEPTOR MESSENGER RNA LEVELS ARE TRANSIENTLY ELEVATED IN THE RAT HIPPOCAMPUS DURING THE EARLY POSTNATAL PERIOD. J. A. O'Keefe and R.J. Handa, Dept. of Cell Biology, Neurobiology and Anatomy, Loyola University School of Medicine, Maywood, IL. 60153

We previously reported a transient elevation in estrogen receptor (ER) protein levels in the postnatal rat hippocampus. This suggests that this limbic cortical structure may be sensitive to the trophic and organizational influence of gonadal steroids during a critical period of sexual differentiation. In order to examine whether alterations in ER gene expression underlie the ontogenetic pattern of the hippocampal ER, we examined steady-state ER mRNA levels over the early postnatal period and in adult male rats. ER mRNA was measured with a highly sensitive RNAse protection assay. Hippocampal ER mRNA levels increased significantly ($p < 0.005$) between birth and postnatal day (PND) 4 when peak concentrations were found (0.1848 ± 0.014 fmoles/mg total RNA) and declined by PND-10 (0.1206 ± 0.017). Adult male hippocampal ER mRNA values were similar to those found in newborn and PND-10 animals but were significantly less ($p < 0.05$) than those observed on PND-4. Thus, the temporal pattern in steady-state ER mRNA levels in the developing hippocampus correlates with our previous developmental profile of the ER protein. These results suggest that the ontogeny of ER protein in the hippocampus may be regulated by alterations in ER gene expression.

109.11

THE SEXUALLY DIMORPHIC VOLUME OF THE MEDIAL AMYGDALA IS RESISTANT TO THE DEMASCULINIZING EFFECT OF PRENATAL STRESS. M. Kerchner[1]* and I. L. Ward[2]. *Department of Psychology, [1]Washington College, Chestertown, MD 21620, and [2]Villanova University, Villanova, PA 19085.*

Prenatal stress diminishes levels of plasma testosterone and diencephalic aromatase activity in male rats on the 18th and 19th day of gestation. As a result, many sexually dimorphic behaviors (e.g., play and copulatory patterns) and CNS structures (SDN-MPOA, DLN, SNB) are incompletely masculinized. In the present study we examined the effect of prenatal stress on a dimorphic feature of the amygdala (Mizukami, et al., *Exp. Neurol.*, 1983, 79, 569). Using a computer-assisted image analysis technique, the volume of the medial amygdala was measured unilaterally in adult male rats (N=43) whose mothers had been exposed to a combination of heat and restraint stress during days 14-21 of pregnancy and compared it to that in the male (N=52) and female (N=8) offspring of nonstressed mothers. The medial amygdala in nonstressed males was nearly twice as large as in females (mean = 8.45 vs 4.59 $mm^3 \times 10^{-1}$, $p < 0.01$). There was no significant difference in the volume of this nucleus between prenatally stressed (mean = 8.58) and nonstressed males. While the abnormal androgenic milieu produced in male fetuses by maternal stress alters the sexual differentiation of some brain and spinal nuclei, the present findings indicate that it does not influence the development of all dimorphic CNS structures. (Supported by grants HD-04688 from NICHHD and RSA 2K-05-MH-00049 from the NIMH to I.L.W.)

109.12

TIMING OF PRENATAL TESTOSTERONE EXPOSURE AND NEURON NUMBER IN THE SNB OF FEMALE RATS. O.B. Ward*, J.R. Carlucci and I.L. Ward. Dept. of Psychology, Villanova University, Villanova, PA 19085.

Ward & Weisz (Science, 207, 328, 1980) suggested that the surge in plasma testosterone (T) found in fetal male rats on days 18 and 19 of gestation sensitizes the developing nervous system to the lower levels of T which circulate at later stages. The present study supports this hypothesis in that the number of neurons in one sexually dimorphic nucleus of the lumbar spinal cord (the spinal nucleus of the bulbocavernosus [SNB]) was maximized in female rats that were exposed to testosterone propionate (TP) during two stages of ontogeny; on days 17.5 & 18.5 of gestation (2 mg daily to the dam) and again on day 25 (5 μg/pup) postconception (2 to 3 days postpartum).

TP on fetal days 15.5 & 16.5 (early) or 17.5 & 18.5 (mid) without postnatal TP caused a 50% increase in the number of SNB neurons. Animals exposed to TP only on days 19.5 & 20.5 (late) or only postnatally did not differ from control females. However, the combination of either early, mid, or late prenatal TP together with the low dose of postnatal TP significantly increased the number of SNB neurons above the corresponding prenatal alone condition. Mid prenatal plus postnatal TP produced a 120% increase, yielding a significantly higher number of SNB neurons than any other condition. (Supported by grants HD-04688 from NICHHD and RSA 2K-05-MH-00049 from NIMH to I.L.W.).

109.13

PRENATAL GONADAL STEROIDS AFFECT DEVELOPMENT OF THE GERBIL SNB. T. B. Decker*, C. Ulibarri, and T. R. Akesson. Dept of Vet & Comp Anat, Pharm, & Phys, Wash State Univ, Pullman, WA 99164-6520.

In gerbils the spinal nucleus of the bulbocavernosus (SNB) is located above the central canal in lamina X. The SNB of male gerbils contains about 200 motoneurons that innervate the bulbocavernosus muscle (BC), levator ani (LA), and anal sphincter (AS). Female gerbils lack a BC and LA and have an SNB that contains about 30 motoneurons that innervate only the AS. Previous work demonstrated that postnatal estrogens and androgens were important in differentiation of SNB motoneuron number, size, and placement. However, these studies did not completely masculinize the SNB. This suggested that prenatal steroids may also be important in the development of the gerbil SNB.

To test this hypothesis, timed-pregnant female gerbils (n = 8/group) were injected daily from embryonic day 16 until birth (postnatal day 1, PND1) with either testosterone propionate (TP, 4mg), dihydrotestosterone P (DHTP, 4mg), androst-1,4,6-triene-3,17-dione (ATD, 6mg), or safflower oil. At birth pups were fostered to untreated mothers. As adults gerbils were castrated and implanted with Silastic capsules containing T (10mm). Five weeks later, gerbils were anesthetized and injected bilaterally with 0.5 μl of cholera toxin conjugated to HRP (CT-HRP) in the BC or AS. One week later gerbils were anesthetized and aldehyde perfused. Lumbosacral spinal cords were removed, sectioned. Alternate sections were processed for CT-HRP and thionin staining.

Control males had about 200 SNB motoneurons. TP- and DHTP-treated females had about 180 and 150 SNB motoneurons respectively. ATD-treated males had about 250 SNB motoneurons. Control females had about 30 SNB motoneurons. Few of the BC-projecting motoneurons were displaced from the SNB. Prenatal steroids seem to be important in development of the number of SNB motoneurons but not migration. BSN9112097 (CU) & HD22869 (TRA).

109.14

ROLE OF PRENATAL GONADAL STEROIDS IN SEXUAL DIFFERENTIATION OF THE GERBIL SDApc. C. Ulibarri*. Dept of Vet & Comp Anat, Pharm, & Phys, Wash State Univ, Pullman, WA 99164.

The sexually dimorphic area of the gerbil hypothalamus (SDA) is a complex of cell groups found between the preoptic area and anterior hypothalamus. One of these cell groups, the SDA *pars compacta* (SDApc), typically exists only in males. Adult female gerbils do not develop an SDApc even after testosterone (T) therapy. Ulibarri and Yahr (*Behav. Neural Biol. 49*:27) showed that gonadal steroids present postnatally affect the development of the SDApc. Female gerbil pups given T shortly after birth develop SDApcs. The SDApcs of these androgenized females do not become as large of those of normal males, even after adult T treatment. Further, neonatal castration does not prevent the development of the SDApc. These data suggest that sexual differentiation of the SDApc begins prenatally and continues into the postnatal period. This research investigated the role of prenatal gonadal steroids in development of the SDApc.

Beginning on embryonic day 16 (E16) timed-pregnant females (n = 8/group) received daily injections of one of the following compounds, T propionate (TP; 4 mg), dihydrotestosterone P (DHTP; 4mg), androst-1,4,6-triene-3,17-dione (ATD; 6mg), or safflower oil vehicle. Injections continued until the day of birth (E25/PND1). At birth pups were fostered to untreated mothers. On PND30 pups were weaned into same-sex, same-treatment pairs. As adults gerbils were castrated and implanted with Silastic capsules containing TP (10mm). Six weeks later, the gerbils were anesthetized and aldehyde perfused. Brains were removed and sectioned coronally at 60 μm. Sections were examined for SDApcs.

All oil-treated males had bilateral SDApcs. Only 50% of ATD-treated males had SDApcs. All DHTP- and TP-treated females had SDApcs. Oil-treated females rarely (20%) had SDApcs. The data suggest that prenatal estrogens and androgens are important for development of the SDApc. Support BSN9112097.

109.15

DEVELOPMENT OF ALTERED MOTONEURON-TO-MUSCLE SPECIFICITY IN ANDROGEN-SENSITIVE RAT SPINAL NUCLEI. A.E. Kalkbrenner and D.R. Sengelaub*. Program in Neural Science, Department of Psychology, Indiana University, Bloomington, IN 47405.

In male rats, the spinal nucleus of the bulbocavernosus (SNB) projects to the bulbocavernosus muscle (BC) and the dorsolateral nucleus (DLN) projects to the ischiocavernosus muscle (IC). Development of this system is androgen-dependent, and the motoneurons and muscles are normally present in females but degenerate perinatally. However, females treated prenatally with dihydrotestosterone propionate (DHTP) lose SNB motoneurons but retain the BC muscle which is now anomalously innervated by motoneurons in the DLN (Breedlove, '85; Kurz et al., '90). To examine the factors which influence motoneuron specificity, we compared the development of normal and hormonally altered connectivity in this system.

Motoneuron projections to the BC muscle were examined in normal males and in females who were treated with DHTP (4 mg/day, embryonic days 17-22). Counts of HRP labeled motoneurons in the SNB and DLN were made following unilateral injections of cholera toxin-HRP (BHRP) into the BC on postnatal days 4, 10, 28 and 90. At P4, labeled motoneurons (mean=73) were found almost exclusively (95%) in the SNB of normal males; DHTP females did not differ from normal males in either the number (mean=78) or location of labeled motoneurons (92% in SNB). Following the postnatal SNB motoneuron death in DHTP females, increasing percentages of labeled motoneurons were located in the DLN after BC injection, and at adulthood about 40% of labeled motoneurons were located in the DLN. Injections of Fluorogold and BHRP into the IC and BC muscles indicated that anomalously projecting DLN motoneurons projected exclusively to the BC. Thus, the anomalous DLN-BC projection results from the death of SNB motoneurons and a subsequent BC invasion by DLN axons. (Supported by NIH NS24877)

109.16

TIME COURSE OF REGRESSIVE CHANGES IN AN AGING ANDROGEN-SENSITIVE RAT SPINAL NUCLEUS. M.C. Clark*, C.L. Iwema, E.M. Kurz, and D.R. Sengelaub. Program in Neural Science, Department of Psychology, Indiana University, Bloomington, IN 47405.

Motoneurons in the spinal nucleus of the bulbocavernosus (SNB) in rats are sensitive to androgens during development, at adulthood, and in old age. In adults, castration significantly reduces SNB soma area and dendritic length, which can be restored with androgen treatment. Androgen titers decline with normal aging in male rats, and we have previously reported reductions in both SNB motoneuron soma area and dendritic length at 22 months of age. In this experiment we examine the time course of these changes.

SNB motoneuron number and morphology were assessed over a period which spans normal changes in testosterone titers in male rats (2, 9, 16, and 22 months of age). Counts of SNB motoneurons (cresylecht violet stained, 10 μm paraffin sections) did not vary with age (n=42). SNB morphology was assessed after retrograde labeling following unilateral muscle injection with cholera-toxin HRP (n=19). Labeling was comparable across groups, and no reductions in arbor area or radial dendritic extent were observed through 22 months of age. However, SNB dendritic length regressed 29% by 16 months of age, and continued to decline thereafter (54% overall). SNB soma area was reduced 14% by 22 months of age. The weight of the androgen-sensitive SNB target muscles also decreased by 9 months of age (bulbocavernosus/levator ani, 18%; ischiocavernosus, 32%) and continued to decline thereafter (overall decrease approximately 45%). Because the timing of these changes closely parallels declines in testosterone titers, age-related regressive changes in SNB motoneurons and their target muscles may result from declining androgen levels.
(Supported by NIH AG09309)

109.17

EFFECT OF UNILATERAL DORSAL RHIZOTOMY ON MOTONEURON NUMBER IN A SEXUALLY DIMORPHIC RAT SPINAL NUCLEUS. A. Mills* and D.R. Sengelaub. Program in Neural Science, Indiana University, Bloomington, IN 47405.

Normally occurring motoneuron death in the sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) is regulated by androgens. It is unclear if this steroid-induced rescue of SNB motoneurons is a direct effect, is due to retention of target muscle, or if androgens act at another site. Neuron number in the dorsal root ganglia (DRG) serving the SNB decline during the same perinatal period as SNB motoneuron number, and this decline in DRG number is also androgen-sensitive (Mills and Sengelaub, '91). As primary afferent input is important to the survival of spinal motoneurons (Davis et al., '83; Okado and Oppenheim, '84), it is possible that afferents are important in the androgen regulation of SNB motoneuron survival.

Primary afferent input to the SNB was reduced by left dorsal rhizotomy (L3-S2) of male rats on the day of birth (P1). Since SNB primary afferents are thought to project bilaterally, this manipulation should deprive both sides of the SNB. At P10, SNB motoneurons were counted after rhizotomy (n=6) and in intact males (n=4). In intact rats, there was no difference in average motoneuron number between the left and right sides of the SNB (127 vs. 123). SNB counts from the unoperated side of rhizotomized animals (131) were not different from counts in intact rats, but there were significantly fewer motoneurons in the operated side of the SNB (57). Failure of rhizotomy to affect SNB motoneuron number on the unoperated side could indicate that either the degree of deafferentation was insufficient, or that primary afferents are not critical for SNB survival. Loss of SNB motoneurons on the operated side could indicate an effect of deafferentation or inadvertent axotomy of ventral roots. (Supported by NIH NS08917 to AM.)

109.18

NEURON SPECIFIC ENOLASE (NSE)-IMMUNOREACTIVITY IS PRESENT IN CELLS ON THE MIGRATION ROUTE OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) NEURONS ORIGINATING FROM THE OLFACTORY PLACODE IN MICE. M. Schwanzel-Fukuda* and D.W. Pfaff, The Rockefeller University, N.Y., N.Y.

LHRH-immunoreactive (ir) neurons originate in the epithelium of the olfactory pit on day 11 of embryogenesis (E) and migrate into the brain along branches of the terminal and vomeronasal nerves. Neural cell adhesion molecule (NCAM)-ir "pioneer" cells migrate out of the epithelium of the olfactory pit, form an aggregate between the olfactory pit and the developing forebrain, and receive the ingrowing NCAM-ir central processes of the olfactory, vomeronasal and terminal nerves. By E11, the NCAM cellular aggregate contacts the rostral forebrain, and NCAM-ir central processes form a scaffold along which the LHRH neurons migrate into the brain [Schwanzel-Fukuda et al.(1992) J. Comp. Neurol. 320:1-18]. Among cells which come from the olfactory placode, which are neurons? Our results show NSE-ir in cells in the epithelium of the olfactory pit, in clumps and cords along nerve fibers on the nasal septum, and in cells limited to the medial edge of the cellular aggregate below the forebrain from day 12. Double-label immunocytochemistry indicates that LHRH-ir neurons show NSE-ir. We speculate that a population of NCAM-expressing glial cells necessary for normal migration [Schwanzel-Fukuda et al. Soc. Neurosci. Abstr.(1991) 17:427] emerge early from the placode and lay down the NCAM scaffolding, later followed by processes of the vomeronasal and terminal nerves, and the LHRH neurons themselves. Supported by NIH grant DC 00880 (M.S.-F).

109.19

SEX DIFFERENCES IN SYNAPTIC EFFICACY: PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERIZATION IN *XENOPUS LAEVIS* LARYNX. M.T. Tobias*, M.H. Ellisman† and D.B. Kelley. Dept. Biol. Sci., Columbia Univ., NY, NY. 10027, †San Diego Microscopy and Imaging Resource, Dept. Neurosci., Univ. Calif. at San Diego, LaJolla, CA. 92093

In *Xenopus laevis* the response of laryngeal muscle fibers to nerve stimulation is sexually dimorphic: males produce subthreshold events while females produce action potentials (Tobias and Kelley, J. Neurosci., 1988). These data suggested an underlying sex difference in synaptic efficacy at laryngeal neuromuscular junctions. To further investigate the mechanism for weak synapses in males and strong synapses in females, we performed a quantal analysis in adult male and female frogs using the method of synaptic failures. At a Ca++/Mg++ ion concentration sufficient to block visible muscle contractions in female larynx, quantal content was significantly lower in males than in females ($p<.0005$). Sex differences in quantal content are Ca++ dependent; at a higher Ca++/lower Mg++ concentration, quantal content in male larynx can be increased to values in the female range. Sex differences in quantal content are due to the probability of transmitter release rather than to the amount released. Analysis of spontaneous miniature endplate potentials revealed no significant sex difference in amplitude or frequency.

Calcium entry and subsequent transmitter release occur at active zones in the motor nerve terminal. We measured the number, length and density of active zones in freeze fracture micrographs and produced 3-D reconstructions of male and female laryngeal motor terminals. The number of active zones/ terminal does not differ in males and females. However, length of active zones ($p<.0005$) and length of active zone/unit length of terminal ($p<.01$) are significantly greater in females than in males. Thus, female laryngeal motor terminals probably contain more sites for Ca++ entry than do male terminals, a factor which may contribute to the greater probability of transmitter release in this sex.

110.1

CALCITONIN GENE-RELATED PEPTIDE- AND METHIONINE-ENKEPHALIN-IMMUNOREACTIVE NEURONS WERE INFLUENCED BY ESTRADIOL-17β TREATMENT IN THE ESTROGEN SENSITIVE REGION. K. Yuri* and M. Kawata, Dept. of Anatomy, Kyoto Pref. Univ. of Med., Kyoto 602, Japan.

Estrogen receptor-immunoreactive cells were distributed in the periventricular preoptic nucleus (Pe), the medial preoptic nucleus (MPN) and around the third ventricle in the female rat, and these were regarded as estrogen sensitive nuclei. In the present study, female rats were ovariectomized and administered with estradiol-17β (E2)-contained silastic capsule or a blank. After 28days of E2 exposure, the rats were sacrificed and the brain sections were immunohistochemically examined using anti-calcitonin gene-related peptide (CGRP)- and anti-methionine-enkephalin (Met-Enk)-antisera. Colchicine was also injected into the lateral ventricle 48hr before fixation. Semi-quantitative analysis showed that the number of CGRP- and Met-Enk-immunoreactive fibers was increased by the E2 treament in the Pe and the MPN. In the colchicine treated rats, a large number of CGRP- immunoreactive cell bodies was recognized in the Pe and the MPN; numerous Met-Enk-immunoreactive cells were observed in the MPN. These findings suggest that estrogen sensitive neurons make an increase of CGRP- and Met-Enk-immunoreactive materials in their perikarya as well as processes.

110.2

SEX DIFFERENCES IN ANDROGEN AND ESTROGEN ACTIONS ON VASOPRESSIN mRNA LEVELS IN THE BED NUCLEUS OF THE STRIA TERMINALIS OF RATS. G.J. De Vries*, Z.X. Wang, N.A. Bullock and S.Numan. Prog in Neurosci. and Behav., Univ. of Mass., Amherst, MA 01003

The vasopressin-immunoreactive (AVP-IR) neurons of the bed nucleus of the stria terminalis (BST) lose their immunoreactivity and their AVP mRNA content after castration. They are also sexually dimorphic: male rats have more AVP-IR cells than females even if they are given similar testosterone treatment. To analyze the cellular basis of this sex difference, we compared the effects of testosterone metabolites on AVP mRNA expression in BST cells in both sexes.

Males and females were gonadectomized at 3 months of age and divided into 4 groups receiving either empty tubing, dihydrotestosterone (DHT), estradiol (E), or E plus DHT. Four weeks later, all subjects were decapitated and their brains were processed for in situ hybridization with a ^{35}S-labeled oligonucleotide. There were hardly any labeled cells in rats with empty implants or implants with DHT. The results of the remaining steroid treatments indicated that there are at least three sex differences in AVP cells of the BST. First our results confirm that there is an absolute sex difference in the number of cells that produce AVP mRNA under equal steroid treatment. Second, E significantly increases AVP mRNA levels in both sexes, but more so in males than in females: the peak number of labeled cells per section was 37.8 in males and 29.1 in females. The number of grains per cell in these animals was 87.6 in males and 71.7 in females. Third, DHT when given in combination with E significantly increases AVP mRNA levels in males only: E plus DHT-treated males and females had 52.4 and 28.8 cells per section with a density of 124.5 and 76.5 grains per cell, respectively. The frequency distribution of cells with different numbers of grains suggests that DHT not only boosts AVP mRNA levels per cell, but also recruits new cells that were not synthesizing AVP mRNA before.

110.3

SEXUAL DIFFERENTIATION OF VASOPRESSIN CELLS OF THE BED NUCLEUS OF THE STRIA TERMINALIS AND MEDIAL AMYGDALOID NUCLEUS IN RATS. Z.X. Wang, G.J. De Vries, N.A. Bullock, N.R. Carlson* and E. Rabin. Prog. in Neurosci. and Behav., U. of Mass., Amherst, MA 01003

The vasopressin-immunoreactive (AVP-IR) projections of the bed nucleus of the stria terminalis (BST) and medial amygdaloid nucleus (MA) are much denser in male than in female rats even if they are treated with similar amounts of testosterone. Previous studies have indicated that testosterone influences AVP-IR projections during development, but they did not indicate whether these effects of testosterone were permanent. This study tests the effects of various hormonal manipulations during development on the ability of testosterone to influence the AVP immunostaining in cells of the BST and MA and of fibers in the lateral septum during adulthood.

In the first experiment, male and female rats were gonadectomized or sham-operated at the day of birth. Three months later, the subjects that had been sham-operated were gonadectomized and all subjects were implanted with testosterone capsules for four weeks before their brains were processed for AVP immunocytochemistry. Males castrated at three month of age had more AVP-IR cells in the BST and a higher density of AVP-IR fibers in the lateral septum. This suggests that androgens influence sexual differentiation of AVP pathways after birth. The second experiment showed that male rats castrated at the day of birth or day 7 had less AVP-IR cells in the BST and MA and a lower AVP-IR fiber density in the lateral septum after four weeks of testosterone treatment in adulthood than similarly treated males castrated on days 21 and 90. This suggests that testicular secretions influence the differentiation of AVP-IR cells around day 7. This was confirmed in a third experiment, in which male and female rats that were neonatally gonadectomized and treated with testosterone at day 7 showed a higher AVP-IR fiber density in the lateral septum than those treated with vehicle.

110.4

EXPRESSION OF pCCK mRNA IS DEPENDENT ON ADULT GONADAL STEROIDS NOT NEONATAL STEROID ENVIRONMENT. P. E Micevych*, C. Ulibarri, L. Abelson, and H. Fok. Dept. of Anatomy & Cell Biology, Laboratory of Neuroendocrinology, UCLA School of Medicine, Los Angeles, CA 90024 and the Dept. VCAPP, Washington State University, Pullman, WA 99164.

The expression of cholecystokinin (CCK) in the medial amygdala, the bed nucleus of the stria terminalis and the medial preoptic nucleus is highly dependent on the gonadal steroid environment of the animal. Estrogen, in females and males, increases the levels of CCK peptide, the number of CCK immunoreactive cells and levels of preproCCK (pCKK) mRNA in these limbic-hypothalamic nuclei. Microinjections of CCK alter female reproductive behavior. Neonatal gonadal steroids determine the adult behavioral response to exogenous CCK. This experiment tested whether alteration of the neonatal environment influences the expression of pCCK mRNA. Neonatal rats were gonadectomized and/or treated with Silastic™ capsules filled with testosterone (4 mm), estrogen (1 mm) or 1,4,6-androstrien-3,17-dione (ATD, 4 mm). At 90 days of age, the animals were gonadectomized as needed, implanted with an estrogen, testosterone or blank Silastic™ capsule (see above), and allowed to survive 4 weeks. All animals were killed by transcardial perfusion under pentobarbital anesthesia and processed for pCCK *in situ* hybridization using a ^{35}S-labeled riboprobe. Both male and female rats treated with estrogen or testosterone in adulthood, regardless of the neonatal treatment, had large numbers of pCCK cells and the highest levels of hybridization (measured by the density of silver grains/cell) in the limbic-hypothalamic nuclei. These results suggest that reproductively relevant circuits of both male and female brains expressing high levels of pCCK are dependent on levels of sex steroids in adulthood and not during neonatal development. Supported by NS 21220.

110.5

CALCITONIN GENE-RELATED PEPTIDE (CGRP) IS SEXUALLY DIMORPHIC IN THE DEVELOPING SNB. L.L. Hodges, N.G. Forger, S.M. Breedlove, and R.B. Fishman*, Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

Motoneurons of the spinal nucleus of the bulbocavernosus (SNB) innervate sexually dimorphic perineal muscles which rely on androgen for development. Last year we reported that CGRP expression in the SNB of males is delayed relative to that in other lumbar motoneurons. We now report the proportion of motoneurons positive for CGRP in the three major motoneuron groups of the lumbar spinal cord of male and female rats from postnatal days 1-55. CGRP was expressed by most motoneurons outside of the SNB at all ages examined. CGRP-LI was again low in the SNB of neonatal males, but was virtually undetectable in the female SNB prior to day 27, then rapidly approached male levels:

Proportion of CGRP-positive SNB cells on postnatal day:

	1-3	6	9-10	16	27	33	55
Females	.009	.009	.016	.033	.288	.593	.464
Males	.064	.197	.424	.676	.532	.693	.645

Because androgen inhibits CGRP expression in adult male rats (Popper & Micevych, '89), the finding of lower expression in females was somewhat unexpected. We are now examining the effect of exogenous androgen on CGRP expression of neonates to test the possibility that androgen regulates CGRP expression differently in developing and adult motoneurons.

Supported by NS28421.

110.6

GONADAL STEROIDS MODULATE THE GROWTH-ASSOCIATED PROTEIN GAP-43 mRNA IN POSTNATAL RAT BRAIN. P.J. Shughrue* and D.M. Dorsa, University of Washington, Department of Pharmacology, and GRECC, VA Medical. Center., Seattle, WA 98108.

The growth-associated protein GAP-43 is concentrated in axonal growth cones and has been implicated in axonal elongation and synaptogenesis. The results of recent studies indicate that estrogen modulates GAP-43 mRNA in adult rodent hypothalamus. Since gonadal steroid hormone action during early postnatal life determines the growth and connectivity of certain neuronal populations in hypothalamus, the present study investigated the effect of various gonadal hormonal conditions on GAP-43 mRNA levels in postnatal rat brain. Postnatal male rats were castrated at birth and sc injected daily with 50µl sesame oil. Intact male and female postnates were also injected daily with oil, while additional females were administered 50µg testosterone propionate (TP). On postnatal day 6, brains were frozen and 16µm cryostat sections processed and hybridized with a ^{35}S-labeled antisense riboprobe for GAP-43 mRNA. Section-mounted slides were apposed to x-ray film and then dipped in liquid emulsion. After exposure, slides were developed and the regional silver grain count determined with a computer assisted analysis system. Film autoradiograms revealed an extensive presence of GAP-43 mRNA in the medial preoptic area, bed nucleus of the stria terminalis, basal hypothalamus, and cerebral cortex. In the central aspect of the medial preoptic nucleus, GAP-43 gene expression was elevated in intact male, attenuated after male castration, low in intact females, and augmented in TP treated females. These changes were positively correlated with changes in serum testosterone levels as determined by RIA. The results of these studies indicate that GAP-43 mRNA levels in the medial preoptic area are modulated by changes in gonadal steroid hormone levels. The differential regulation of GAP-43 mRNA by sex steroids in the male and female postnatal hypothalamus may influence the phenotype of neuronal circuitry and thereby determine the pattern of adult hypothalamic function. Supported by NS20311 and NS07332.

110.7

ESTROGEN RECEPTOR mRNA IN THE PREOPTIC AREA OF NEONATAL RATS. L.L. DonCarlos*, R.J. Handa , C.A. Lisciotto & C.K. Wagner. Dept. Cell Biol., Neurobiol., & Anat., Loyola Univ. Stritch Sch. Medicine, Maywood, IL 60153, & Inst. Animal Behav., Rutgers Univ., Newark NJ 07102.

Exposure to the estrogenic metabolites of testosterone during the early postnatal period has permanent effects on rodent brain development. We used in situ hybridization to chart the development of ER mRNA expression in the rat preoptic area, a brain region for which sexual dimorphisms and the effects of estrogen on development are particularly well-documented. Neonatal male and female rats were sacrificed by perfusion fixation on selected postnatal days (PND; day of birth is PND 0). ER mRNA was detected using an 850 base 35S-labeled riboprobe (ER cDNA gift of M. Muramatsu); autoradiograms were exposed 4 weeks. Many ER mRNA-labeled cells were detected in the periventricular preoptic area and medial preoptic nucleus at the three time points examined so far: PND 4, which corresponds to the period of maximal sensitivity to the masculinizing effects of estrogenic metabolites of testosterone, PND 7 and PND 10. The distribution of ER mRNA containing cells was identical in both sexes although the intensity of label was consistently higher in females compared with males. In adult rats, estrogen down regulates ER mRNA (Simerly and Young, 1991). Aromatization of androgens in the preoptic area may provide sufficient local estrogen to down-regulate ER mRNA in the neonatal male preoptic area. Further analyses of the distribution and relative abundance of ER mRNA in males and females of earlier ages are in progress. Supported by the Potts Foundation.

110.8

DEVELOPMENTAL EXPRESSION OF 3-ALPHA HYDROXYSTEROID OXIDOREDUCTASE IN RAT CNS M.S. Ward, N.S. Nadi, A.E. Schaffner*, and J.L. Barker. Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

This study examines the developmental expression of 3α-hydroxysteroid oxidoreductase (3αOHSOR), an enzyme responsible for reduction of A-ring reduced 3-keto steroids such as dihydrocortisone, dihydrotestosterone, and dihydroprogesterone. This enzyme converts 5α-pregnane-20-one to 3α-hydroxy-5α-pregnane-20-one (3α-THP), a ligand that has been found to be active at the embryonic GABA-A receptor. Preliminary results from enzymatic analysis using newly developed chromatographic techniques for the separation of tritium labeled pregnane steroids indicate that the enzyme is active early in development and that embryonic brain is one of the first tissues to produce 3α-THP. Enzymatic activity first appears in a craniocaudal gradient. In later stages there is little difference among brain regions but significant differences are seen between the sexes. These differences are believed to be due to estradiol regulation of enzyme expression.

These results indicate that local brain metabolism may be an important part of producing steroid ligands for the GABA-A receptor in development. Current investigation focuses on regulation of enzyme expression and the relationship of 3α-THP to other GABA-A ligands throughout development.

110.9

EARLY POSTNATAL ADMINISTRATION OF THE PHYTOESTROGEN COUMESTROL DOES NOT INDUCE C-FOS IN BRAIN OR UTERUS IN ADULTHOOD. F. A. Caputo*, K. L. Medlock and A. C. Scallet. National Center for Toxicological Research, Jefferson, AR 72079.

Phytoestrogens are estrogens which occur naturally in plants and are commonly found in the human diet in foods such as grains, soya and other fiberous plants. It has been shown that prenatal and postnatal exposure to sex steroid hormones have profound effects on the anatomy of the brain in the developing rodent fetus. Female rats were injected with the phytoestrogen coumestrol (100 ug/10 ul/pup) on postnatal days 1-10. Control animals received no injection. At 60 days of age, animals were sacrificed and brain and uterus examined for c-fos immunoreactivity. Results suggest that early phytoestrogen exposure does not induce c-fos immunoreactivity in estrogen concentrating areas of the brain or in the uterus in adult female rats.

110.10

FACILITATION COMPENSATES FOR LOWERED SYNAPTIC EFFICACY IN A SEXUALLY DIMORPHIC MUSCLE. N. Nagaya* and A.A. Herrera, Neurobiology Section, Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-2520.

The flexor carpi radialis (FCR) is a sexually dimorphic forelimb muscle used by male frogs during mating. The FCR is innervated by motor neurons in spinal segments 2 and 3. Studies comparing castrates given testosterone implants (CT) with castrates given blank implants (C) have shown that FCRs from CT males have more junctions that are subthreshold in response to single nerve stimuli. This effect of testosterone is most pronounced in spinal nerve 2 (SN2) motor units. In CT muscles, 28 % (n=213) of fibers in SN2 motor units were subthreshold compared to 4 % (n=133) in C muscles ($p<0.01$). The lowered synaptic efficacy in CT muscles may result from significant decreases in input resistance (0.28 MΩ, n=214 vs. 0.44 MΩ, n=109) and quantal content in low Ca^{2+} (1.63, n=24 vs. 3.42, n=31).

Muscles were bathed in Ringer with 10-12 μM curare and intracellular recording was used to study facilitation of transmitter release in the FCR in response to 30 nerve stimuli at 70 Hz. Facilitation was measured as the ratio of the 5th endplate potential (EPP) to the 1st. There were no significant differences between junctions of SN2 and spinal nerve 3 (SN3) motor units or between CT and C muscles. All junctions facilitated greatly (median=2.74, interquartile range: 1.99-3.57) and EPPs at the end of the stimulus train did not fall below the level of the 1st EPP. The magnitude of facilitation in FCR junctions suggests that repetitive nerve stimulation can recruit otherwise subthreshold junctions. Our data indicate that FCR junctions can be active and can maintain high levels of transmitter release even though testosterone reduces synaptic efficacy.

Supported by NIH grant NS 27209.

110.11

EFFECTS OF TESTOSTERONE ON MUSCLE FIBER SIZE: REPEATED *IN VIVO* OBSERVATION OF A FORELIMB FLEXOR MUSCLE IN *XENOPUS LAEVIS*. M. Dorlöchter, A.A. Herrera*, and T. Guyette. Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-2520.

The flexor carpi radialis (FCR) is a sexually dimorphic muscle used by male frogs to clasp females during mating. A previous study from our laboratory revealed differences in fiber size between FCR muscles of castrated (C) frogs compared with castrated but testosterone provided ones (CT). As measured from frozen sections 8 weeks after the surgery, the average fiber cross sectional area was 45% smaller in C than in CT frogs (Regnier & Herrera, Soc. Neurosci. Abstr. 16, 329, 1990).

To define the time course of fiber size changes we studied FCR muscles of C and CT frogs *in vivo* at the time of castration and 2, 4, and 8 weeks thereafter. Surgically exposed muscles in anaesthetized frogs were stained with the vital mitochondrial dye DIOC (1 μM) and fibers recorded on videotape using a fluorescence microscope (x50/1.0 W objective) and a SIT camera. Already at 2 weeks the apparent muscle fiber diameter was increased by 9 % in CT and decreased by 15 % in C frogs (n = 5 each). After 8 weeks muscle fibers in CT animals were on average 21 % larger and in C animals 30 % smaller than at the day of castration (n = 3 each). For comparison, fiber diameter was measured in sartorius muscles in some of the frogs. It was found to steadily decline within 4 weeks to about 80 % of the value on the day of castration; no difference was detected between C and CT frogs. Since body weight in these animals decreased similarly, we conclude that this was the cause of sartorius muscle fiber atrophy. Our data suggest that manipulation of the hormonal status in male *Xenopus* leads to a fast regulation of fiber size in the FCR muscle.

Supported by NIH grant NS27209.

111.1

LAMPREY NEUROFILAMENTS LACK THE 'KSP' REPEATS FOUND IN OTHER VERTEBRATE NEUROFILAMENTS. AJ Jacobs, MA Peck, ME Selzer*, Department of Neurology and David Mahoney Institute of Neuroscience, University of Pennsylvania, Philadelphia, PA 19104

Spinal axons of the sea lamprey regenerate following transection and unlike cultured mammalian neurons, their growth cones are packed with neurofilaments (NF). In contrast to the triplet proteins making up mammalian neurofilaments (NF-L, NF-M, NF-H), lamprey neurofilaments are homopolymers of a single 180 kDa subunit (NF180). We have cloned and sequenced two overlapping cDNAs (identified by hybridization to mammalian NF cDNA probes and expression screening with NF specific mAbs) that encompass the entire coding region for NF180. Deduced amino acid sequence shows NF180 is most homologous to NF-M of mouse (40% overall identity and 61% in the core). However, there is a conservative substitution of aspartate for glutamate* in the highly conserved KLLE*GEE sequence found at the carboxy terminus of the core in all intermediate filaments. The carboxy sidearm of NF180 is rich in glutamate and contains multiple tandem EEAEAEE repeats. However, KSP and KSP-like repeats are conspicuously absent. It has been postulated that the phosphoserine repeats in the sidearm of mammalian NFs extend the sidearm away from the filament core by electrostatic repulsion, and thereby regulate axon diameter. The glutamate rich sidearm of NF180 may serve the same function in the lamprey, but with a fixed charge would not be under the regulatory control of NF kinases and phosphatases. Chou and Fasman analysis of NF180 predicts an alpha helical structure within the core similar to that described by Geisler and Webber (1982), except one additional non-helical spacer may be present in each of the three core coils (total of 6 coils). An extended alpha helical structure is also predicted for the long carboxy sidearm of NF180. The extreme carboxy termini of human, rat and mouse NFM are 98% identical and contain two amino acid repeats. Lamprey NF is weakly homologous to NFM in this region, but does contain two repeated sequences which align with the terminal NFM repeats. These sites may mediate NF cross-linking, other NF-protein interactions or have a role in NF transport. (NIH Grant # NS14837)

111.2

A NEURAL KERATAN SULFATE PROTEOGLYCAN, CLAUSTRIN, IS RECOGNIZED BY ANTIBODIES TO MAP1B. Michael Burg and Gregory J. Cole*, Dept. Anat. & Cell Biol., Med. Univ. of South Carolina, Charleston, SC 29425.

Our laboratory has recently described the identification of a chick nervous system keratan sulfate proteoglycan (KSPG), named claustrin, that inhibits neural cell adhesion and neurite outgrowth. Polyclonal antisera raised against the intact proteoglycan (PG) and the major core protein, a 70 kDa polypeptide, were used to screen an E9 chick Uni-Zap cDNA library, with eight overlapping partial cDNAs isolated that reacted with both antisera. Initial sequence analysis indicated that the cDNAs encoded a protein homologous to MAP1B, although the claustrin cDNAs represent a 5' fragment of MAP1B, with the 3' MAP1B coding region missing from these cDNAs. Since previous studies of MAP1B have identified in rat a 3' fragment termed neuraxin, encoding a 94 kDa protein, and a similar clone was isolated from our chick library upon screening with an antiserum to chick brain PGs, this raised the possibility that MAP1B is a PG(s), specifically a KSPG. Our present studies show that the MAP5 MAb recognizes only claustrin in chick, and that antisera to chick claustrin react only with MAP1B in rat. In addition, a MAb (5D4) to human cartilage keratan sulfate reacts with MAP1B in rat and claustrin in the chick. Keratanase treatment eliminates the immunoreactive protein (MAP1B) in rat that is recognized by both the MAP5 and MAP1B-4 MAbs. In addition, in immunohistochemical studies we show that MAP5 MAb recognizes midline glial structures in E20 rat spinal cord, hindbrain and midbrain, which are putative barrier structures known to express claustrin in the chick. Interestingly, the rat midbrain midline expression does not extend into the decussation of the superior cerebellar peduncle, raising the possibility that claustrin may regulate the midline crossing of these axons. These data also suggest that MAP1B is a KSPG, and that the two core proteins of claustrin, with molecular weights of 100 and 70 kDa, may be encoded by distinct cDNAs previously suggested to identify MAP1B.
Supported by NIH Grant NS26087

111.3

Viral Latency and GAP-43: Changing Enrichment of a Growth Associated Protein in Wound Healing and Viral Infection. Rex E. Martin[1]*, Jim M. Hill, and Nicolas G. Bazan[1]. L.S.U. Eye Center and Neuroscience Center[1]. New Orleans, LA, 70112.

Trigeminal nerves are linked to the wound healing response of cornea, and the GAP-43 in these nerve fibers may be involved. Using a rabbit model, the effect of latent viral infection on GAP-43 content in cornea was investigated. Corneas were infected with Herpes simplex virus, type 1 (HSV-1). At 52 days post-infection (viral latency), the relative GAP-43 content of uninfected and infected corneas was quantified. In nerve endings of uninfected corneal epithelium, GAP-43 appeared equally enriched in membrane and cytosolic fractions. Viral latency resulted in 29% enrichment in membrane associated GAP-43 and 3-4 fold overall enrichment of the protein in comparison to uninfected rabbits. Within 12h of mild alkali burn to corneal epithelium, the relative enrichment of GAP-43 between membrane and cytoplasm was reversed. Membrane-associated GAP-43 was most enriched 48h post-burn and decreased in parallel with cytoplasmic GAP-43 over the next 48h. Higher levels of GAP-43 expression may be induced in the trigeminal ganglia by HSV-1 and modify plasticity or intracellular trafficking in corneal nerves. Relationships between HSV-1 latency and modifications in wound healing are being explored. Supported by EYO6635, EYO6311.

111.4

FACTORS REGULATING PROTEOLYSIS AND CALMODULIN BINDING OF B-50/GAP-43. **P.J.Coggins, and H. Zwiers*Departments of Medical Physiol/ Medical Biochem. University of Calgary, Calgary, Canada, T2N 4N1.**

The neuronal growth-associated protein B-50/GAP-43 is a substrate for Protein kinase C, binds to Calmodulin in a calcium independent manner, and is subject to hydrolysis adjacent to the single kinase C phosphorylation site. All of these processes can be inhibited by corticotrophin (ACTH). We have investigated the structural requirements for ACTH-mediated inhibition of B-50 proteolysis by an endogenous protease that co-purifies with B-50 in a detergent extract of synaptosomal plasma membranes. The results indicated that inhibition of proteolysis is related to the presence of a basic amphiphilic helix in those ACTH fragments and analogues that were inhibitory, and moreover, the presence of this motif in other peptides appears to confer inhibitory activity. Chymotryptic digestion of B-50 in the presence and absence of a non-ionic detergent, and ACTH, demonstrated that hydrolysis is also influenced by a lipid-like environment that primarily effects the protein rather than the protease or the peptide. Furthermore this lipid dependency appears to extend to the binding of dephosphorylated-B-50 to Calmodulin which appears to occur only in the presence of non-ionic detergent and the absence of calcium. The results are discussed with reference to the putative secondary structure of B-50 and changes that may take place in the presence of membrane lipids or non-ionic detergents. The conclusions of this study suggest that B-50 is subject to regulation by post-translational enzymes and binding proteins as a consequence of its structural conformation at the cytoplasmic side of the plasma membrane.

111.5

LOCALIZATION OF CALMODULIN (CaM) AND THE MULTIPLE CaM mRNAs IN DIFFERENTIATED PC12 CELLS. S-P. Zhang*, N. Natsukari, R.A. Nichols and B. Weiss. Div. of Neuropsychopharmacology, Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Calmodulin (CaM) is encoded by three genes from which five CaM mRNAs are transcribed. Earlier studies showed that all five CaM mRNAs are present in the PC12 pheochromocytoma cell line, and that both nerve growth factor (NGF) and dibutyryl cyclic AMP (db-cAMP), which induce neurite outgrowth in these cells, increase the levels of CaM and the CaM gene mRNAs. In the present study we examined the subcellular distribution of CaM and its mRNAs in PC12 cells differentiated with NGF (50 ng/ml) and db-cAMP (1 mM). We determined the levels of CaM by Western blot analysis and its subcellular distribution using immunocytochemistry. We measured the cellular distribution of the CaM mRNAs by *in situ* hybridization cytochemistry, using radiolabeled oligodeoxynucleotide probes. Intense, patch-like staining for CaM was associated with the nucleus; a diffuse, weaker signal was found in the cytoplasm; and significant staining for CaM was detected along some of the neurites. Results of experiments using an oligodeoxynucleotide probe which detects all the CaM transcripts showed that the CaM mRNAs were distributed throughout the PC12 cell bodies; significant amounts of the CaM mRNAs were also found in some, but not all, neurites. Using separate probes specific for the CaM mRNAs for each CaM gene, we found a distinct pattern of localization for the mRNAs of each CaM gene. The mRNAs transcribed from CaM genes I and II were found in neurites as well as in cell bodies. By contrast, CaM gene III mRNAs were in relatively low abundance in the PC12 cells, and no significant signal was found in neurites. That different CaM mRNAs are localized in different compartments in PC12 cells suggests that the various CaM mRNAs might synthesize CaM in different cellular locations and, therefore, may have different functional roles. (Supported by MH42148).

111.6

ALTERATION IN THE LEVELS OF CALMODULIN-BINDING PROTEINS DURING DIFFERENTIATION OF PC12 CELLS. N. Natsukari, S-P. Zhang, R.A. Nichols* and B. Weiss. Div. of Neuropsychopharmacology, Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Calmodulin (CaM) plays a pivotal role in numerous cellular processes by interacting with its target proteins, CaM-binding proteins (CaMBPs). To elucidate the role of these CaMBPs in neuronal differentiation and their possible role in determining the compartmentation of CaM within cells, we examined the expression and subcellular distribution of CaMBPs during differentiation of the PC12 pheochromocytoma cell line. Undifferentiated cells and those differentiated with either nerve growth factor (NGF), dibutyryl cyclic AMP (db-cAMP) or both agents were subfractionated at various times, and their CaMBPs were separated by SDS gel electrophoresis and assayed using ^{125} I-CaM gel overlay autoradiography. In membrane fractions from untreated cells, proteins having apparent MWs of >200, 110, 52, and 47 kDa exhibited Ca^{2+}-dependent binding to CaM, while many smaller proteins (20, 17 and <15 kDa) displayed predominantly Ca^{2+}-independent binding. Many protein bands in the molecular weight range of 25-37 kDa bound CaM equally in the presence and absence of Ca^{2+}. Most of the CaMBPs of the Ca^{2+}-independent type were relatively small and were present in the particulate fractions, consistent with previous findings obtained with fractions from brain. Upon treatment with NGF and/or db-cAMP, the pattern of CaM-binding changed dramatically. The 20 kDa CaMBP of the Ca^{2+}-independent type increased after 4 days of treatment with db-cAMP. NGF itself had only a small effect on this protein, while simultaneous treatment of cells with both NGF and db-cAMP led to a marked increase in this 20 kDa CaMBP. In contrast, other Ca^{2+}-independent CaMBPs (17 and <15 kDa) decreased during differentiation. These results show that CaMBPs are differentially altered by NGF and db-cAMP, suggesting that they may play a role in neuronal differentiation. (Supported by MH42148).

111.7

CHANGES IN THE DISTRIBUTION OF RYANODINE RECEPTOR ISOFORMS IN THE CNS DURING DEVELOPMENT. Y. Ouyang*+, T. J. Deerinck+, J. A. Airey◊, J. L. Sutko◊, and M. H. Ellisman+. San Diego Microscopy and Imaging Resource, +Dept. of Neurosciences, Univ. of Calif. San Diego, La Jolla, CA.92093; ◊Dept. of Pharmacology, Univ. of NV., Reno, NV. 89557.

We recently identified two ryanodine binding proteins (α and β ryanodine receptors, RR's) in the chicken CNS using antibodies against the purified skeletal muscle RR (Ellisman et al., Neuron, 5:135-146, 1990). The RR's are caffeine sensitive calcium activated calcium release channels of intracellular membrane systems. Well characterized isoform specific monoclonal antibodies (mab) 110F and 110E (Airey et al., J. Biol. Chem. 265:14187-14194, 1990) discriminate between skeletal and cardiac muscle species as well as two species found in the CNS of the chick. Mab 110F recognizes only the skeltal muscle α form while mab 110E is specific for skeletal muscle β and the cardiac muscle form. The pattern of appearance of α and β/**cardiac** isoforms has been investigated during development of the chick CNS using histochemical methods. The α form of the RR is in highest concentration in cerebellar Purkinje cells (PC's). All PC's are strongly labeled for α as are deep cerebellar nuclei and some large neurons of the pons. The α form appears relatively early in these neurons before hatching and persists thereafter through adulthood. In contrast, strong β/**cardiac** form-like immunoreactivity is found throughout the brain, including the deep cerebellar nuclei and large neurons of the pons. The distribution of β/**cardiac** immunoreactivity changes markedly in the cerebellum during the first few weeks of development. Weak β/**cardiac** labeling was observed in all PC's of hatchlings but restricted to clusters of PC's at 1 week post hatch (PH). At this time weak β/**cardiac**-like immunoreactivity appears in the granule cell layer which is more strongly labeled at 4 wks. PH. This increase in β/**cardiac** immunoreactivity corresponds with an overall increase in precipitable protein in the cerebellum. In contrast, few PC's are immunoreactive for the β/**cardiac** form at 4 wks. PH. The developmental changes in the distribution of the β/**cardiac** form of the RR suggests involvement in the maturation of neuronal properties.

111.8

OPPOSITE TRANSITION OF CHICK BRAIN CREATINE KINASE ISO-ENZYMES DURING EMBRYONIC AND POST-NATAL DEVELOPMENT. O.C. Ramírez*, E. Jiménez and A.M. Cibrián. Departamento de Bioquímica, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional.México,D.F.07000.

Owing to its dimeric structure, cytosolic creatine kinase (cCK) exists as three main isoenzymes: skeletal muscle (MM), heart(MB) and brain (BB). During ontogeny of heart and skeletal muscles it is believed that cCK isoenzyme transition is similar among vertebrates. As in skeletal muscle cultures, during differentiation the appearing of BB-CK type is followed by the MB-CK type and concludes with the adult MM-CK isoenzyme. It is currently accepted that BB-CK is the only type present in brain tissue during the entire life of vertebrates and a faint trace of MM-CK may appear only in older rats. However, evidence was presented that, as in muscle tissues, post-natal rat brain synthesizes abundant catalytic forms of MM and MB-CK in addition to the brain type (Ramírez O. & Licea, G. Exptl. Neurol. 77:225 1982).

In this work it is shown that transition of chick brain cCK isoenzymes follows a pattern opposite to the universally accepted one. In the absence of creatine, developing chick brain initiates with catalytic MM-CK isoforms (from 46 h through the 7th day of incubation); with creatine in brain, the MB-CK types are present from the 7th to the 14th day; from hatching to adulthood (1.5 years), the BB-CK isoforms are synthesized, coinciding with higher brain energy demands.

111.9

MODIFICATION OF PHOSPHOLIPID COMPOSITION IN BRAIN SYNAPTOSOMES ISOLATED FROM RATS TREATED WITH GUANIDINOETHANESULFONATE. P.-L. Lleu, R.J. Bowers and R.J. Huxtable*, Department of Pharmacology, College of Medicine, University of Arizona, Tucson, Arizona 85724

Recently, developmental decreases in taurine concentration in rat brain synaptosomes (P_2B fraction) have been shown to be correlated with: (1) an increase in the phosphatidylethanolamine/phosphatidylcholine (PE/PC) ratio and (2) a decrease of [^{3}H-methyl]methionine incorporation into synaptosomal phospholipids (Huxtable *et al., Neurochem Int.* 15:233; 1989, and Lleu and Huxtable, *Neurochem Int.*, in press, 1992). The relationship between taurine and phospholipid methylation has been explored further in rats depleted of taurine during development by treatment of the mothers during pregnancy and lactation with 1% guanidinoethane sulfonate (GES) in drinking water. P_2B fraction was isolated from brain hemispheres of rats at 7, 14, 21, 28, and 56 days of age, and the phospholipid composition determined. The PE/PC ratio was systematically decreased at all time points, the largest decrease (15%) being seen in 56 day-old animals. These findings suggest that taurine and its analog, GES, are directly influencing the alteration in neutral phospholipid ratio and the marked decrease in phospholipid methylation seen in rat brain synaptosomes during development.

111.10

PKC ACTIVITY AND SUBCELLULAR LOCALIZATION DURING NEURONAL DIFFERENTIATION OF SH-SY5Y HUMAN NEUROBLASTOMA CELLS. V. Parrow*, G. Meyerson, E. Nånberg and S. Påhlman Department of Pathology, University Hospital, S-751 85 Uppsala, Sweden.

SH-SY5Y neuroblastoma cells can be induced to differentiate into neuron-like cells by treatment with nanomolar concentrations of the phorbolester 12-O-tetradecanoyl-phorbol-13-acetate (TPA), in the presence of serum or growth factors. The optimal concentration of TPA for neuronal differentiation is 16 nM. To evaluate the possible role of a sustained protein kinase C (PKC) activation during the neuronal differentiation, PKC protein and RNA levels as well as the PKC activity were determined during 4 days of TPA treatment. PKC activity, measured by the phosphorylation of the 80 kDa PKC substrate, was not downregulated when the cells were treated with this low concentration of TPA. In contrast, high concentrations of TPA (1.6 μM) resulted in downregulation of PKC and a poor neuronal differentiation. Translocation of PKC to the membrane compartment could be measured within one hour after stimulation with 16 nM TPA. However, Western blots showed that the PKC-α protein was present after four days of TPA treatment and subcellular fractionation revealed that the PKC-α protein, as well as the 80 kDa PKC substrate, could be found in the growth cones and in the nuclear fractions, as well as in the cytosolic compartment.

111.11

DIFFERENTIATION-INDUCED CHANGES IN SECOND MESSENGER-DEPENDENT PROTEIN KINASE ACTIVITIES IN A RAT NEURONAL CELL LINE. H.B. Rind*[1], J.T. Neary[3], and S.R. Whittemore[1,2]. The Miami Project[1], Depts. Physiology and Biophysics[1] and Neurological Surgery[2], Univ. Miami and V.A. Medical Center[3], Miami, FL 33136

Protein phosphorylation is an important mechanism by which neuronal differentiation may be transduced intracellularly. Elucidation of both the dynamics and substrates of the kinase pathways activated during neuronal differentiation may provide insight into the specific effector molecules involved. Our studies focused on changes in Ca^{2+}/calmodulin, cAMP, and Ca^{2+}/phospholipid dependent protein kinases (CaMK, PKA, and PKC, respectively) in RN33B cells. RN33B is a temperature sensitive, neuronally differentiating cell line derived from E12.5 rat medullary raphe. Significant levels of all 3 enzymes were detected in undifferentiated RN33B cells. While the total CaMK activity in membrane and soluble fractions did not change upon differentiation, increased phosphorylation of a number of CaMK substrates was observed. One of these substrates was an approximately 67 kDa phosphoprotein suspected to be the autophosphorylated subunit of Ca^{2+}/CaM Kinase II. Differentiated cells also exhibited a significant decrease in PKA activities in both cytosolic (83%) and membrane (48%) fractions. Differentiation, however, did not appear to induce an activation of PKC or its translocation from cytosol to membrane. Instead, soluble PKC activities decreased by 54% with a more conservative decrease in the particulate fraction upon differentiation. Current studies are aimed at examining the role of these second messenger-dependent protein kinases in neurotrophic factor-induced differentiation of RN33B cells. Supported by the Miami Project and NS26887.

111.12

PHORBOL ESTER- AND GLUTAMATE-SENSITIVE PHOSPHORYLATION OF HIPPOCAMPAL MEMBRANE PROTEINS FROM ADULT AND NEONATAL RATS. L. M. Shaffer* and L. A. Dokas+. Departments of Biochemistry & Molecular Biology, and Neurology+, Medical College of Ohio, Toledo, OH 43699.

The phosphoinositide (PI) system in the neonatal rat brain is differentially stimulated as compared to that of the adult by glutamate. Hydrolysis of PI generates diacylglycerol (DAG), which activates protein kinase C (PKC). Phorbol esters directly activate PKC and inhibit agonist-stimulated PI hydrolysis, presumably via PKC substrate proteins. This study compared membrane protein phosphorylation under conditions that affect PI hydrolysis in neonatal and adult rat hippocampus. Basal phosphorylation of two membrane proteins (M_r: 46,000, and 80,000, pI: 4.4 and 4.2, respectively) is enhanced when compared to the adult. The former protein is found only in neonates. Phosphorylation of a 48,000 M, protein (pI of 4.5) is prominent in both neonatal and adult membranes. Incubation of hippocampal slices with 12-O-Tetradecanoyl phorbol-13-acetate (TPA, 10μM) decreased post-hoc phosphorylation of all three proteins. Glutamate (10^{-3}M) did not alter phosphorylation of neonatal proteins, while increasing that of the adult 80,000 M, protein, implying that phosphorylation in neonates may not be as efficiently coupled to receptor stimulation as in the adult. Immunoblot analysis identified the 48,000 M, protein as B-50/GAP-43, the 80,000 M, protein as a membrane-associated form of MARCKS, and showed that MARCKS is expressed at higher levels in the neonatal rat hippocampus. The proteins differentially phosphorylated in neonates as compared to adults may mediate developmental differences in PI metabolism.

111.13

NEUROGRANIN (RC3): DEVELOPMENTAL EXPRESSION OF PKC SUBSTRATE IN THE HUMAN VISUAL CORTEX; Y.H.Yücel*+, J.Baudier # and L.E. Becker +. + Dep. Path. /Neuropath., Hospital for Sick Children, U. of Toronto, CANADA, # Lab. de BMCC, INSERM U 309 Grenoble, FRANCE.

The developmental expression of Neurogranin (NG), a brain specific protein kinase C (PKC) substrate, was investigated in human calcarine cortex (autopsy material without neuropathological findings at macroscopic and microscopic examination) with immunocytochemical techniques using a specific anti-NG polyclonal antibody. NG and GAP-43 bind calmodulin in the absence of calcium and share a 17 aminoacid sequence that corresponds to the PKC phosphorylation site and to the calmodulin binding domain. Immunoreactivity was localized in cell bodies and occasionally in dendrites. In adult human cortex, NG immunoreactivity (NGI) was distributed mainly in layer II and in layer VI. The superficial layer II showed the highest number of cells labelled and greatest intensity of NGI. Layer VI also demonstrated marked NGI relative to the other layers. Few NGI cells were detected in layer III and layer V. In layer II, pyramidal cells were the main cells identified by NG. In younger visual cortex, there was a similar pattern of NGI observed in all cases older than 3 years (n=5). In cases younger than 12 months (m) and older than 3 m, NGI was found only in deep layer VI or was negligible (n=5). In all cases younger than 3 m, no immunoreactivity was observed in the cortical layers (n=6). These preliminary results suggest that the developmental pattern of NG in human visual cortex shows an "inside first outside last" spatio-temporal distribution, similar to what we described in cat visual cortex (21st SFN Meeting, 1, p366, 1991). Its postnatal developmental expression suggests a role for this substrate of PKC in human visual cortex development.

111.14

CLONING AND CHARACTERIZATION OF A NOVEL PUTATIVE PROTEIN TYROSINE PHOSPHATASE FROM THE NEONATAL RAT CORTEX. Mustafa Sahin* and Susan Hockfield. Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510.

Regulation of protein function through tyrosine phosphorylation is critical for control of cellular processes, including proliferation and differentiation. The state of tyrosine phosphorylation of a protein is determined by the opposing actions of kinases and phosphatases. Recently, a growing number of protein tyrosine phosphatases (PTPases) have been identified from various species and tissues, including the mammalian nervous system. These enzymes share a conserved catalytic domain and can be divided into two groups based on subcellular location: intracellular vs. transmembrane. PTPases are thought to play roles in such divergent functions as cell cycle regulation, tumor suppression, cell adhesion and axon guidance.

Given these observations and our interest in cellular diversity in the mammalian CNS, we sought to identify PTPases in the developing rat cortex. A cDNA library from neonatal rat neocortex was used as a template for PCR and amplified with degenerate primers to a conserved region of PTPases. PCR products were subcloned and sequenced. One of the products encodes a novel PTPase. The predicted amino acid sequence shows highest homology to previously described human transmembrane PTPases. Analysis by Northern blotting demonstrates a predominant mRNA species at 5.5 kb. The message is enriched in the brain compared to the liver or kidney. Furthermore, the expression of the novel PTPase is dramatically down-regulated in adult cortex compared to postnatal day 1 cortex. *In-situ* hybridization reveals that the mRNA is present in the gray, but not white matter of the cortex, suggesting expression by neurons not by glia.

Further characterization of this developmentally-regulated PTPase will provide insight into its functions during CNS development. (Supported by NS22807.)

111.15

THE RECEPTOR PROTEIN-TYROSINE KINASE, TYRO-3, EXHIBITS AMINO ACID SEQUENCE SIMILARITY WITH NEURAL ADHESION MOLECULES. C. Lai[1] and G. Lemke*. Molecular Neurobiology Laboratory, The Salk Institute and Department of Neuropharmacology, The Scripps Research Institute[1], La Jolla, CA 92037.

We have isolated and characterized full-length cDNA clones encoding the novel neural protein-tyrosine kinase (PTK), tyro-3 [Neuron 6, 691-704 (1991)]. The deduced amino acid sequence reveals a putative transmembrane (PTK) receptor. The extracellular region is composed of two immunoglobulin-like domains and two fibronectin type III repeat motifs. The tyro-3 extracellular region shows sequence similarity to neural adhesion molecules including N-CAM, L1, TAG-1 and fasciclin II. It also exhibits similarity to protein-tyrosine phosphatases including the leukocyte common antigen-related protein (LAR). These observations suggest that tyro-3, which has been observed in large neurons in cortex and CA1 hippocampus, is involved in cell-cell interactions. (Supported by grants from the N.I.H. and the National Multiple Sclerosis Society)

GENE STRUCTURE AND FUNCTION II

112.1

ASTROCYTE HETEROGENEITY IDENTIFIED BY EXPRESSION OF GFAP-LACZ TRANSGENES. M. Brenner, Y. Su, W.C. Kisseberth, F. Besnard, & A. Messing. University of Wisconsin-Madison, Madison, WI; NIH, Bethesda, MD

Glial fibrillary acidic protein (GFAP) is an intermediate filament expressed in nearly all astrocytes of the central nervous system. Cell transfection studies (J Biol Chem 266:18877, 1991) found that astrocyte-specific expression can be directed by a 2 kb 5'-flanking segment of the human GFAP (*hgfa*) gene. In addition, two subregions were identified, one adjacent to the basal promoter and one about 1500 bp upstream, that when juxtaposed to form a synthetic (400 bp) promoter produced increased levels of astrocyte-specific expression in cell lines. Transgenic mice carrying the bacterial β-galactosidase (*lacZ*) reporter under the control of the full length (2 kb) or synthetic (400 bp) promoters have now been examined for specificity of expression. The 2 kb promoter drives *lacZ* expression in astrocytes throughout the central nervous system, whereas the 400 bp promoter is active primarily in cerebral astrocytes. These studies demonstrate heterogeneity among astrocytes in their use of regulatory elements to control expression of GFAP.

112.2

IDENTIFICATION OF PUTATIVE TRANSCRIPTIONAL REGULATORY ELEMENTS IN THE OLFACTORY MARKER PROTEIN (OMP) PROMOTER. K. Kudrycki, C. Behn, C. Stein-Izsak* and F.L. Margolis. Dept. of Neurosciences, Roche Inst. of Molec. Biol., Nutley, NJ 07110.

OMP is found almost exclusively in cytoplasm of mature olfactory receptor neurons and its expression must be tightly controlled by tissue specific and age dependent factors. In order to understand the mechanism of OMP transcriptional regulation we have initiated a study aimed at identification of possible cis-regulatory elements within the rat OMP promoter and their corresponding transcriptional factors. Studies with transgenic mice previously conducted in our laboratory indicated that at least 800 nucleotides upstream of the transcriptional start site are required for correct expression of OMP. DNase I footprinting analysis and gel mobility shift assays with nuclear protein enriched extracts from the olfactory mucosa and other neuronal and nonneuronal tissues have allowed us to identify several regions within this promoter area that may be involved in transcriptional regulation of the OMP gene (C. Stein-Izsak *et al.* Soc. for Neurosci., Abstr. 513.5, 1991, New Orleans). Two of these regions contain the sequence motif TCCCC(A/T)PuGGAG and interact only with nuclear protein enriched extracts from the olfactory mucosa. The activity binding to these sites was designated Olf-1. Gel mobility shift assays with a number of synthetic oligonucleotides demonstrated that Olf-1 binding activity requires both the five purines and the TCCCC sequence. Another putative regulatory element located between the two Olf-1 binding sites interacts with nuclear protein enriched extracts from a number of tissues resulting in various gel mobility shifts and DNase I footprinting patterns. In addition, we have identified sequence motifs adjacent to the OMP transcriptional start site that bind factors present in nuclear protein extracts from the olfactory mucosa and some other tissues that we have tested. These results demonstrate existence of several putative transcriptional regulatory elements in the OMP promoter, two of which seem to be controlled by the newly defined olfactory specific binding activity, Olf-1. These elements may also play a role in transcriptional regulation of other olfactory specific genes.

112.3

TRANSGENIC ANALYSIS OF OMP PROMOTER USING TWO REPORTER GENES. M. Grillo*, R. Morris[1], R. Akeson[2], M. Sakai, R.J. Smeyne, C. Stein-Izsak, C. Bocchiaro, J. Corbin, A. Phillips and F.L. Margolis. Dept. of Neurosciences, Roche Inst. of Molec. Biol., Nutley, NJ 07110. [1]National Inst. for Med. Research, The Ridgeway, Mill Hill, London, England. [2]Inst. for Developmental Res., Children's Hosp. Res. Foundation, Cincinnati, OH 45229.

Olfactory chemoreceptor neurons express a unique, phylogenetically conserved, developmentally regulated protein, the olfactory marker protein (OMP). The highly specific cell and tissue distribution of the protein implies that the expression of the OMP gene is spatially and temporally controlled at the level of transcription. To study the elements regulating OMP expression *in vivo* we have utilized transgenic mice. Chimeric gene constructs have been generated that combine varying amounts of the rat OMP 5' upstream genomic sequence with the coding region of Thy 1.1 or LacZ as reporters. Immunocytochemistry, reverse transcriptase/PCR and RIA have shown that consistent, robust tissue-specific expression of the Thy 1.1 reporter is seen if 0.8 Kb or more of the 5' OMP sequence is present in the transgene. However, 0.3 Kb of 5' upstream sequence gives, at best, variable, borderline Thy 1.1 expression in several independently derived lines. In contrast, results obtained from histochemical (Xgal) and immuno-enzyme assay of tissues from mice bearing similar constructs directing the E. coli LacZ gene indicate that unambiguous, tissue-specific expression of reporter gene activity can be seen with as little as 0.3 Kb of 5' OMP sequence. These observations indicate that genomic motifs for olfactory specific gene expression reside within 0.8 and possibly 0.3 Kb of 5' upstream OMP sequence. Further, these data are consistent with our report of two copies of a novel DNA sequence motif (Olf-1) that are located 5' of the OMP transcription start site (Kudrycki et al., Soc. Neurosci. 1992). Evaluation of the minimal amount of promoter sequence required to determine timing and tissue-specific regulation of gene expression may depend on the choice of reporter gene and on the sensitivity of its assay.

112.4

3' ENHANCER ELEMENTS FROM THE HUMAN TH GENE DIRECT HIGH LEVELS OF GENE EXPRESSION IN ADRENALS OF TRANSGENIC MICE. S. C. Wong*, M. A. Moffat, J. Merlie†, and K. L. O'Malley. Departments of Anatomy and Neurobiology, and †Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

Our previous studies indicated tissue specific elements involved in human tyrosine hydroxylase (TH) gene expression were 3' of the last exon. A maximal response element was defined within 261 bp of exon 13 which averaged 120-fold increased activity over basal level controls. To extend these results *in vivo*, we generated 9 lines of transgenic mice with a vector containing the 261 bp fragment cloned in front of a thymidine kinase promoter driving a chloramphenicol acetyl transferase (CAT) reporter. We have analyzed 4 lines of mice using reverse-transcription-polymerase chain reaction (RT-PCR) with CAT specific primers. In all cases CAT mRNA was expressed primarily in the adrenal gland followed by pancreas (26% of adrenal gland expression), cerebellum (8%), midbrain (6%), heart (3%), Pons (3%), lung (2%), and spleen (1%). CAT mRNA could not be detected in cerebral cortex, liver, spleen and thymus. Despite abundant CAT mRNA, CAT enzyme activity could not be detected in any of these tissues. Preliminary RT-PCR data indicated cryptic splicing within the CAT transcript generated a truncated CAT gene product. These data suggest that the human TH 3' enhancer can function in vivo but that additional sequences are required for total tissue specific responses.

112.5

TRANSGENIC CONSTRUCTS FOR ASSESSING THE ROLE OF SNAP-25 EXPRESSION IN THE PHENOTYPE OF THE HYPERACTIVE MOUSE MUTANT *COLOBOMA*. E.J. Hess*, J.B. Bergsman, T.M. Slater and M.C. Wilson, Dept. of Neurosci. & Anat., Penn State Univ., M.S. Hershey Medical Center, Hershey, PA 17033 and Dept. of Neuropharm., The Scripps Research Institute, La Jolla, CA 92037.

SNAP-25 is a neuron-specific synaptosomal associated protein that contributes to synapse formation. In characterizing SNAP-25 expression and function, we have identified the mouse mutant coloboma (*Cm/+*) as carrying a chromosome 2 deletion mutation that includes the *Snap* gene. Coloboma mice are heterozygous neurological mouse mutants whose phenotype includes opthalmic deformation, stereotypic head shaking and extreme hyperactivity. Although it is clear that the coloboma mutation is a deletion of less than 5 cM, it is not known if other genes surrounding *Snap* are also deleted. To determine the specific contribution of SNAP-25 expression to the coloboma phenotype, we have assembled *Snap* constructs for generating transgenic mice. Both constructs consist of a *Snap* promoter that includes ~11 kb of undefined, untranslated genomic DNA 5' to the first *Snap* exon. One construct, designed to test the specificity of the *Snap* promoter *in vivo* and *in vitro*, consists of the promoter, an SV40 splice site, the ß-galactosidase cDNA and an SV40 polyadenylation signal. This reporter construct was transfected into PC12 cells, which express low levels of SNAP-25, and promoter efficacy was tested. *In situ* X-gal staining and a quantitative ß-galactosidase enzyme assay revealed that the 11 kb *Snap* promoter was effective in neuronal cells and is a good candidate for driving SNAP-25 expression in neurons of transgenic mice. Therefore, this promoter was used to assemble a *Snap* mini-gene to rescue the coloboma phenotype; this construct contains a rat insulin intron, the SNAP-25 cDNA and an SV40 polyadenylation signal in addition to the promoter. Both the *Snap* mini-gene and the *Snap*/ß-galactosidase constructs are currently being used to generate transgenic mice. Supported by PHS MH48989.

112.6

REGULATION OF TYROSINE HYDROXYLASE GENE EXPRESSION DURING BRAIN DEVELOPMENT IN TRANSGENIC MICE. T.H.Joh,* K.S.Kim, H.Baker, J.H.Son Lab. of Molec. Neurobiol., Cornell Univ. Med. Coll., Burke Med. Res. Inst., White Plains, NY 10605

Tyrosine hydroxylase (TH) enzyme is specifically expressed in the catecholaminergic neurons in the brain and plays a key role in the regulation of catecholamine biosynthesis. The expression of catechoaminergic phenotype is developmentally regulated in the different brain regions including the dopaminergic neurons of the substantia nigra, ventral tegmental area and olfactory bulb and the noradrenergic neurons of the locus ceruleus. For example, TH immunoreactivity was first detected at rat embryonic day (E) 12.5 in the substantia nigra, but in locus ceruleus it became visible on E14. In substantia nigra an overshoot of TH activity was seen by postnatal day (PD) 15 and gradually decreased to adult level by PD 28.

To investigate the tissue-specific expression of TH gene during prenatal and postnatal development, we generated a series of lacZ fusion DNA constructs using various amounts of the 5' flanking promoter region of the rat TH gene. With these fusion DNA constructs we established a total of 19 different transgenic mouse lines. Among them, the transgenic lines harboring the 2.4 kb of 5' flanking TH sequence showed the correct expression in most brain regions, which correlates with our results from in vitro functional analysis of the upstream promoter region of TH gene. To further investigate whether the expression of the lacZ reporter gene is colocalized with the endogenous expression of TH gene during the development of murine central nervous system, these and the other transgenic animals are being examined.

112.7

PRODUCTION OF TRANSGENIC MICE MODEL FOR THE SPATIOTEMPORAL EXPRESSION STUDY OF CHOLINE ACETYLTRANSFERASE GENE. J.H.Son,* T.Wessel, H.Baker, C.Peng, T.H.Joh Lab. of Molec. Neurobiol., Cornell Univ. Med. Coll., Burke Med. Res. Inst., White Plains, NY 10605

Progressive cell loss or degeneration of particular central cholinergic neurons is observed in normal aging and several fatal neurological disorders, such as Alzheimer's and Huntington's diseases. Choline acetyltransferase (ChAT) has served as a specific phenotypic marker for cholinergic neurons due to its selective expression in these neurons. Expression of ChAT mRNA and ChAT enzyme activity in the central nervous system has been known to be developmentally regulated in different regions of rat brain, such as basal forebrain, cerebral cortex, hippocampus and corpus striatum. Furthermore various growth factors, such as nerve growth factor (NSF), brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF), interleukin 3 (IL-3) or leukemia inhibitory factor (LIF), affect the cholinergic phenotype by inducing or enhancing ChAT activity. We cloned and determined the genomic organization of rat ChAT gene in order to study the differential and developmental expression of the ChAT gene in the CNS using transgenic mice. The 15 exons of the ChAT gene are distributed over a distance at least 64 kb in the rat genome. Immediate upstream from the putative transcription start site, we found several potential known cis-acting DNA sequences. This allowed us to make several lacZ fusion DNA constructs with various amount of the upstream DNA sequence and/or some intron sequence to generate transgenic animals. Preliminary analysis of the animals containing the upstream 1.9 kb/lacZ fusion construct showed that this construct was not fully sufficient to give correct expression in mouse brain. Results from further analyses of other transgenic animals will be presented.

112.8

SOMATIC DNA RECOMBINATION IN THE TRANSGENIC MOUSE BRAIN: CONTRASTING AND COMPLEMENTARY PATTERNS REVEALED BY COMPARISON OF TWO FOUNDERS. L. Kingsbury, M. Matsuoka, D.T. Larue[1], J.A. Winer[1]* and H. Sakano. Divisions of Immunology and [1]Neurobiology, Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

DNA recombination enables lymphocytes to generate the genetic diversity required to produce immunoglobulins against billions of potential antigens. We recently reported (Matsuoka et al., Science [1991] 254:81-86) evidence that somatic DNA rearrangement also occurs in the brain of a transgenic mouse. The transgene construct contained a reporter gene, *lacZ*, in reverse orientation with respect to a promotor, such that rearrangement results in ß-galactosidase expression. Treatment of fixed brain sections with X-gal, a chromogenic substrate for ß-galactosidase, yields a blue reaction product which labels areas where the reporter gene rearranges. A polymerase chain reaction procedure (PCR) which amplifies only rearranged genes showed that the *LacZ* expression is due to actual DNA rearrangement rather than integration of the transgene near an endogenous tissue-specific promotor. We described the distribution of ß-gal activity in the brain of one founder mouse that produced progeny. Here we compare the staining of the second (infertile) founder and offer speculation about the functional significance of DNA recombination in the brain.

The patterns of ß-gal activity in founders No. 1 and No. 2 were similar but not identical. In general, the staining in No. 2 was less intense but more widespread than in No.1. For example, in No. 1, cerebellar X-gal staining was strong, but restricted to lobes IX and X, while in No. 2, the ß-gal activity was lower but was distributed over the entire cerebellum. Likewise, staining in sensory and motor cortex, which was concentrated in layers III-IV in No. 1, was spread over layers II-VI in No. 2. Overall, most regions of the brain showed some ß-gal activity in one or both founders.

The different distribution of ß-gal activity in the two founders may reflect different sites of transgene integration which affect its accessibility to putative recombination enzyme(s). Thus, it is possible that all central neuronal populations have DNA recombination capability but that only a subset might have access to the reporter gene in each transgenic founder. Why central neurons have the capacity to rearrange DNA is unclear. However, there is a postnatal progression of ß-gal expression which suggests a role in some developmental process in the maturing brain; the regulation of gene "superfamilies" is one possibility. Whether DNA rearrangement in the brain is closely related to that in lymphocytes, and which, if any, endogenous genes undergo recombination in the brain, remain to be determined. Supported by U.S.P.H.S. grant AI18790 and American Cancer Society grant IM-366 (H.S.) and U.S.P.H.S. grant NS16832-13 (J.A.W.).

112.9

TRANSFECTION OF CEREBELLAR GRANULE NEURONS AND ASTROCYTES BY ELECTROPORATION.

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Several techniques for gene transfer into dividing cells have been devised (Biotechniques 1988, Vol.6, No. 7). Gene transfer into post-mitotic cells has proven very difficult. Few methods have been successful in transferring genes into a variety of post-mitotic cells. We investigated the use of electroporation to transfer the RSV-ß galactosidase (ß-gal) and RSV chloramphenicol acetyltransferase (CAT) genes into primary cultured cerebellar granule neurons and astrocytes. We found that the efficiency of gene transfer is about 3-to 5-fold greater in astrocytes than neurons. Assays of these cultures gives an easily detectable signal for CAT activity. Preliminary results comparing electroporation and liposomal techniques have shown them to be comparable. We have successfully used electroporation to transfer genes into primary neurons and astrocytes. Our results suggest that electroporation is an easy and rapid method to transfer genes into post-mitotic cells such as neurons.

112.10

MOLECULAR MECHANISMS OF SYNAPTIC REGULATION OF TYROSINE HYDROXYLASE GENE EXPRESSION. A. Goc*, E. Puchacz, M.K. Stachowiak. Barrow Neurological Institute, Phoenix AZ 85013.

Synaptic stimulation of TH gene expression in catecholaminergic cells is mediated via depolarization of cellular membrane and influx of Na^+ ions. The present study was undertaken to elucidate molecular mechanisms underlying this regulation. Incubation of bovine adrenal medullary cells (BAMC) with depolarizing agent veratridine increased mRNA levels of TH and another catecholamine biosynthetic enzyme, PNMT. The increases in PNMT mRNA were prevented by Ca^{++} channel antagonist, nifedipine, and by down-regulation of protein kinase C (PKC) with PMA, indicating mediation through Ca^{++}/PKC. In contrast, induction of TH mRNA was not affected by nifedipine, down-regulation of PKC, or calmodulin antagonist calmidazolium. It was also not affected by TMB-8 an inhibitor of intracellular Ca^{++} mobilization. This suggested induction via Ca^{++}, PKC-, and calmodulin-independent mechanisms. To elucidate nuclear mechanisms of TH gene activation we transiently expressed chimeric TH promoter(-428/+21 bp)-luciferase genes in cultured BAMC. Veratridine produced 3-fold increase in LUC activity (normalized to amount of intracellular plasmid DNA), but had no effect on expression of LUC from promoterless pOLUC plasmid. Deletions of sequences from -194 to -54 or from -269 to -54 had no significant effect on stimulation of TH promoter activity. This indicated that POU/OCT and AP-1 elements contained within deleted regions may not participate in depolarization induced activation of TH gene, and that remaining elements including proximal CRE and further upstream sequences may be sufficient. In contrast, in parallel studies we showed that Ca^{++}/PKC-dependent activation of TH gene expression by angiotensin II receptors was mediated by multiple regions of the TH promoter including those upstream from -54 nt (Stachowiak, abstract). Thus, different signaling pathways and promoter mechanisms participate in synaptic and hormonal induction of the TH gene.

112.11

***K*B Enhancer Binding Complexes in Mammalian Brain.** K. Cauley*, L. Chen, F. H. Gage, and I. Verma. Dept. of Neurosciences, 0624, U.C.S.D., and The Salk Institute, La Jolla, CA. 92037.

*K*B enhancer binding proteins have been shown to play key roles in the regulation of tissue specific gene expression, primarily in lymphoid cells. Very little is known about the role of these factors in the regulation of gene expression in the mammalian nervous system. Utilizing the electrophoretic mobility-shift assay, we have identified several complexes from extracts of rat brain which specifically bind to the *K*B site of the HIV-LTR. We have identified a factor indistinguishable from NF-*K*B, and another factor which includes a protein identical with, or highly related to, the nuclear oncoprotein cRel. Both of these *K*B binding complexes are found throughout the brain. Considerable levels of the NF-*K*B-like factor is present in the inactive form in the adult brain; presumably the appropriate input signal would activate this transcription complex.

112.12

TISSUE-SPECIFIC DNA METHYLATION AND DNASE HYPERSENSITIVE SITES IN THE PROMOTER AND TRANSCRIBED REGIONS OF THE RAT PREPROENKEPHALIN GENE

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To examine the mechanisms involved in tissue-specific regulation of preproenkephalin (PPE) gene expression, we have studied the methylation state of C-residues in the promoter and intron A regions of the PPE gene. Rat genomic DNA was digested with the methylation sensitive restriction enzyme HpaII, followed by southern blot analysis using single stranded DNA probes produced by linear PCR (Soc. Neurosci. Abst. 17:219.5,1991). We found that two sites in the promoter region are partially methylated in all tissues examined (liver, testis, brain and adrenal gland), and that the methylation pattern of the sites varies significantly between tissues. In contrast to the promoter region, two sites within intron A were found to be completely unmethylated in all tissues. We have also investigated the chromatin structure of the PPE gene using the DNase hypersensitivity (DH) assay. Crude nuclei isolated from fresh tissues were digested with DNase and DH's were mapped by indirect end-labeling with single stranded DNA probes. Several DH sites, which are located near the TATA box and within transcribed region, have been identified in brain, including the caudate, cortex, ventromedial hypothalamus (VMH), and adrenal gland. In liver, only one site is present, suggesting that the identified sites in brain and adrenal gland correlate with PPE gene expression. Since estrogen increases PPE mRNA in the VMH, we are presently determining whether estrogen can induce any additional DH sites in VMH nuclei.

112.13

Cell-Specific Regulation of Preproenkephalin Intron-A Derived Heteronuclear RNA in the Rat Basal Forebrain, Thalamic Reticular Nucleus, and Testis. P.J. Brooks*, T. Funabashi, S.P. Kleopoulos, C.V. Mobbs, and D.W. Pfaff. The Rockefeller University, Box 275, New York, NY 10021

Using *in situ* hybridization with single-stranded DNA probes to different regions of the rat preproenkephalin (PPE) gene, we have identified a population of neurons in the rat reticular thalamic nucleus (RT) and basal forebrain (BF) which utilize a novel mechanism for the control of preproenkephalin gene expression. These cells contain very high levels of heteronuclear preproenkephalin RNA derived from downstream of a previously described (Mol. Cell Biol. 10:3717) germ-cell specific transcription initiation site within somatic intron A, without detectable PPE RNA derived from exon 2. Sites of hybridization in these neurons appear as 1-3 intranuclear foci of silver grains using tritium labeled probes. Similar foci of hybridization are also observed with the same probes in a population of testicular germ cells. The data indicate that in cells in which PPE gene transcription initiates from the intron A initiation site, PPE gene expression can also be regulated by an additional mechanism involving transcriptional termination prior to the 5' end of exon 2, which results in the accumulation of high levels of nuclear PPE intron A-derived heteronuclear RNA. Weisinger et al (Soc. Neurosci. Abst. 16:71.12, 1990) have previously proposed a transcriptional down-regulation process regulating PPE transcripts initiating from exon 1 in the striatum.

Neurons in the horizontal and vertical limbs of the diagonal band of Broca, the ventral pallidum, and medial septum contain high levels of PPE intron A derived heteronuclear RNA, as do GABAergic neurons of the RT. A population of BF neurons project to the RT (JCN 262:105) indicating that neurons utilizing this mechanism may be connected, and that many of them are importantly involved in the control of cerebral cortical function.

112.14

CHARACTERIZATION OF BRAIN MEF-2 ACTIVITY. D. Krainc*, D. Leifer, Y.-T. Yu, R. E. Breitbart, R. L. Neve, B. Nadal-Ginard, and S. A. Lipton. Depts. of Neurology, Cardiology, and Psychiatry, Harvard Medical School, Boston, MA.

We have cloned a novel transcription factor that belongs to the MADS (MCM1-AGAMOUS-DEF A-serum response factor) family and is expressed in brain and muscle. Our transcription factor has several isoforms and binds specifically to the myocyte-specific enhancer binding factor-2 (MEF-2) regulatory element and to a variant MEF-2 site present in the promoter region of the brain creatine kinase gene (Leifer et al., this meeting). A nuclear factor that has previously been identified only in muscle binds to the MEF-2 site ([C/T]T[A/T]AAATA[A/G]), and MEF-2 activity in muscle correlates with muscle differentiation.

Here, we show that a factor present in nuclear extracts from rat brain (P2) and from human fetal cerebral cortex also binds to the MEF-2 site. The binding is specific since it is competed by excess unlabeled probe in gel-shift assays but not by certain double-stranded oligonucleotides with mutated MEF-2 sites. An antiserum against our cloned proteins specifically recognizes the MEF-2 binding activity in both extracts. The endogenous brain activity and *in vitro*-translated proteins derived from our cDNA clones have indistinguishable properties both in terms of their electrophoretic mobility and their target specificity as determined by gel-shift assays. In addition, there is no MEF-2 binding activity in extracts of human fetal brainstem, where we have not detected RNA transcripts of our clones by Northern blotting. Our findings raise the possibility that the MEF-2 activity that we have identified in brain has a specific role in neural development.

112.15

CHARACTERIZATION OF THE RAT B-50/GAP-43 PROMOTER.
B.J.L. Eggen*, L.H. Schrama, E.R.A. Jap Tjoen San and W.H. Gispen. Rudolf Magnus Inst., Lab. Physiol. Chem., Inst. Molec. Biol. & Med. Biotechnol., Univ. of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

In an attempt to determine regulatory elements controlling transcription of the neural-specific phosphoprotein B-50 (GAP-43) we have characterized a B-50 promoter and modulatory regions located within the first 1016 basepairs (bp) upstream (5') of the ORF. B-50 promoter constructs are driving the firefly-luciferase gene as a reporter, and their activities are assayed via lipofection in P19 EC cells. Transfection efficiency and cell number are corrected for by co-transfecting the B-50 constructs with the Rous Sarcoma Virus (RSV) promoter fused to the LacZ gene. The RSV promoter fused to luciferase is used as an internal reference for B-50 promoter activity. The murine embryonal carcinoma cell (EC) line P19 and its differentiated derivatives are used as a model system for a neural and non-neural specific cell line. Previous experiments (1) have shown an increase in B-50 mRNA and protein levels after differentiation by aggregation in the presence 10^{-6}M retinoic acid (RA). In these cells B-50 immunofluorescence is restricted to neurons. Transfections were carried out in P19 EC cells which were either aggregated in the presence of 10^{-6}M RA, resulting in a heterogenous culture consisting of neurons, glia and fibroblasts, or in the absence of RA, yielding a non-neuronal phenotype thereby generating a system in which possible neural-specific element(s) in the B-50 promoter can be determined within one cell line.

Deletion of the stretch of 80 alternating AG residues shows a decrease (±50%) in promoter activity as compared to the 1016 bp construct, only when the first 115 bp 5' of the ORF are present. The 1016 bp construct itself reaches up to 15% of the activity of the RSV promoter. Studies of truncated promoter contructs, to determine modulatory elements are currently under investigation.

1) Jap Tjoen San *et al.*, *Abst. Soc. Neurosci.* 17, 222.9: 1991.

112.16

DIFFERENTIAL EXPRESSION OF NEUROTRANSMITTER TRANSPORTER GENES IN WILD TYPE PC12 CELLS AND IN WNT1-EXPRESSING FLAT CELL VARIANTS.
B. Ramachandran, K. Houben and B. D. Howard*. Dept. of Biol. Chem. UCLA Sch. of Med. Los Angeles, CA 90024

Wild type PC12 cells exhibit several sodium-dependent transporters for various catecholamine and amino acid substrates exclusive of aspartate. Flat cell variants, including those induced by expressing the Wnt-1 oncogene, lack catecholamine uptake but do take up aspartate. This difference in aspartate transport makes the flat cells more sensitive to alanosine, a toxic aspartate analog. Using PCR and degenerate oligonucleotides corresponding to the conserved regions of previously cloned transporters, we obtained partial clones for several distinct transporter cDNAs from PC12 wild type and flat cell variant cells. These partial clones were subsequently used to screen cDNA libraries to obtain full length clones. One of these clones is almost identical in sequence to the human norepinephrine transporter. The other clones are homologous to, but distinct from all previously cloned neurotransmitter transporters. Northern blot analysis revealed that these clones are expressed differentially by wild type and flat cell variant PC12 cells and by rat brain and liver. These results suggest that transporter expression can be used to study the signalling pathway induced by Wnt-1.

GENE STRUCTURE AND FUNCTION III

113.1

CHARACTERIZATION OF ENHANCER, SILENCER, AND GROWTH FACTOR RESPONSIVE REGULATORY SEQUENCES IN THE PROMOTER FOR THE MOUSE CHOLINE ACETYLTRANSFERASE GENE
Haifeng Pu*, Ping Zhai, and Mark Gurney Department of Cell, Molecular & Structural Biology, Northwestern University, 303 East Chicago Ave. Chicago, IL 60611.

A genomic clone containing the upstream flanking region of the mouse choline acetyltransferase gene has been isolated and functionally characterized. The transcription start-site was determined by RNA primer extension. ChAT promoter-lacZ fusion genes have been constructed and transfected into various cell lines. Transient expression of β-galactosidase in transfected cells showed that the ChAT DNA spanning -930 to +335 confers promoter activity in cholinergic neuronal PC12 cells, but not in the noncholinergic neuronal cell lines B103 and F11, or in the myoblast cell line L6. Cholinergic-cell type specific promoter activity was lost by deleting the region from -935 to -558. Both NGF and bFGF potentiated promoter activity of the -935 to +335 construct in PC12 cells. Growth factor responsiveness was lost when the region from -935 to -499 was deleted from the constructs. Enhancer activity was localized to the region -430 to -115 by its ability to activate by more than 10-fold transcription from a heterologous, minimal SV40 promoter and addition of the region -825 to -430 to the construct conferred responsiveness to NGF. Thus, the region from -925 to -558 upstream of the ChAT gene transcription start-site contains a probable silencer which restricts expression to cholinergic PC12 cells, NGF and bFGF responsive regulatory elements reside in the region -825 to -430 and enhancer elements have been mapped to the region -430 to -115.

113.2

POSITIVE TRANSCRIPTIONAL ACTIVITY OF AN AP-1-LIKE ELEMENT IN THE PROMOTER REGION OF THE DYNORPHIN GENE.
D.J. Messersmith*, J. Gu, R. Dubner, J. Douglass, M.J. Iadarola. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

During experimental nerve injury and inflammation, spinal cord dynorphin gene expression is markedly increased. While multiple protein-DNA interactions likely regulate such transcription, we have focused on a promoter element located at -1546 which has sequence similarity to the AP-1 element and homology with the ENKCRE2 enhancer. The dynorphin AP-1-like site (DAP), TGCGTCA, differs from the AP-1 consensus sequence (TGAGTCA) by a one base-pair substitution. Our previous studies demonstrated that a Fos and non-Fos-containing protein-DNA complex formed with DAP-containing oligonucleotides using nuclear extracts of CNS regions or HeLa cells. To examine the functional significance of the DAP site, constructs of 100 and 350 base pairs containing the DAP site and a non-DAP containing 250 base pair region were subcloned into the pCAT-promoter plasmid vector with a 200 base pair region containing the minimal dynorphin promoter. HeLa or PC-12 cells were transiently transfected with these plasmids and levels of the CAT reporter gene were measured in cell extracts. In cells transfected with constructs containing the DAP site, CAT conversion levels were approximately four-fold greater than in cells transfected with the non-DAP containing construct. These data support the role of this site as a positive transcriptional regulatory element of the dynorphin gene.

113.3

CHARACTERIZATION OF HORMONE RESPONSE ELEMENTS IN THE RAT OXYTOCIN PROMOTER
Roger A.H. Adan, Sophia F. Lopes da Silva, Joke J. Cox, H. Rigter* and J. Peter H. Burbach Rudolf Magnus Institute, Department of Pharmacology, Medical Faculty, University of Utrecht; *Gezondheidsraad, Den Haag, The Netherlands.

The oxytocin (OT) promoter has several TGACC motifs suggesting that it is regulated by members of the ER/TR subfamily of nuclear receptors. The transactivation of the OT gene by estrogen, retinoic acid (RA) and thyroid hormone is demonstrated in transient heterologous expression systems using luciferase as reporter enzyme. One region confering RA-responsiveness was located between nucleotides -172 and -148 and another was located between nucleotides -112 and -77 from the start site of transcription. The same regions were involved in estrogen transactivation of the OT gene. Both these regions were bound by the estrogen receptor (ER) and the DNA-binding domain of RARß as shown by gel retardation and footprint analysis. This profile of RA- and ER-responsive elements in the rat OT promoter was different from that of thyroid hormone-responsiveness, but overlap was found in the -172/-148 element. Another member of this nuclear receptor family for which thusfar no ligand has been found and which is known as the COUP factor suppressed the transactivation by estrogen via the same element. This element can be considered as a composite hormone response element, forming an important enhancer for the regulation of OT gene expression. The core sequence of this element GGTGACCTTGACC was analyzed in more detail by site directed mutagenesis. The sequence motifs within this element necessary for optimal transactivation by the different receptors differed. The organization of the regulatory regions in the OT promoter suggests that the rat OT gene can integrate multiple stimuli to be able to exert an adequate transcriptional response. The composite hormone response element renders the OT gene to a potential target for multiple members of the ER/TR/RAR subclass of receptors in the brain.

113.4

A CLONE ENCODING A PROTEIN THAT BINDS TO AN UPSTREAM REGULATORY ELEMENT IN THE PROMOTER REGION OF THE RAT PRODYNORPHIN GENE.
J.Gu, D.J.Messersmith, R.Dubner* and M.J.Iadarola. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda MD 20892.

An upstream regulatory element (URE) in the promoter region of the rat prodynorphin gene was identified by gel mobility shift and footprinting assays. The URE contains a pyrimidine-rich, initiator (Inr) consensus sequence YAYTCYYY at -209 from the transcription start site. Transient transfection assays of PC12 cells suggest that the URE is important for optimal expression of a chloramphenicol acetyl transferase reporter gene. Labeled URE was used for southwestern screening of a rat brain cDNA library. Two clones UreB1 and UreB2 were isolated. One of them, UreB1, has been characterized further. It contains a 1.9 kb cDNA insert with an open reading frame that encodes a 309 amino acid polypeptide. Fusion protein, induced by IPTG from the UreB1 clone, was capable of forming a complex with a labeled Ure-containing oligonucleotide in a gel mobility shift assay. In comparison to sequence data bases, UreB1 is a novel molecule at both the DNA and protein levels. UreB1 does not bear structural motifs for known DNA binding proteins. Northern blots showed that transcripts of the UreB1 gene were expressed in a tissue specific fashion and widely expressed in regions of the CNS. The results suggest that UreB1 is a novel transcription factor involved in the control of expression of the prodynorphin gene, and possibly other genes, in the CNS.

113.5

REGULATION OF PNMT GENE PROMOTER CONSTRUCTS TRANSFECTED INTO MEDULLOBLASTOMA AND PRIMARY ASTROCYTE CELL LINES. L.M. Hemmick*~, M.E. Ross+, E.F. LaGamma~, and M.J. Evinger~ ~Depts. of Neurobiol.& Behav., Pediatrics, SUNY Stony Brook, +Dept. Neurol., Univ. Minn.

Promoter constructs for phenylethanolamine N-methyltransferase (PNMT), exhibit considerable cell specificity of expression in transient transfection assays. Studies of gene expression in terminally differentiated cells of neuronal origin have generally been limited by low efficiencies of transfection and reproducibility. We sought to identify replicating cell lines capable of expressing PNMT gene constructs that also share regulatory features common to adrenal medullary PNMT expression. We report that the human medulloblastoma line TE 671 (provided by R.J. Lukas), and primary rat striatal astrocytes each possess features suitable for investigating neural regulation of the PNMT gene.

TE 671 cells express nicotinic plus M3 and M4 muscarinic AChRs. When TE 671 cells were electroporated with constructs containing the proximal 900 bp of the rat PNMT promoter, both nicotine (50 uM) and carbachol (200 uM) increased CAT and luciferase reporter gene expression. Hexamethonium (50 uM) blocks the nicotine response, while the muscarinic antagonist atropine (20 uM) partially inhibits the carbachol increase, thus indicating that nicotinic and muscarinic AChRs are coupled to PNMT expression in these cells.

Primary rat astrocytes likewise support basal expression of the 100 and 900 bp PNMT-CAT constructs. Muscarine increases CAT expression ~3 fold for both constructs. However, nicotine fails to increase CAT production, consistent with the reported lack of nAChRs on astrocytes. Consequently, astrocytes provide a suitable system in which to examine cholinergic regulation of the PNMT gene exclusively by muscarine. Therefore, TE 671 cells in conjunction with primary striatal astrocytes are two convenient alternatives to primary cultures for the study of cholinergic regulation of the PNMT gene.

113.6

PROMOTER ORGANIZATION OF HUMAN MONOAMINE OXIDASE (MAO) A AND B GENES. Q.S. Zhu, J. Grimsby and J.C. Shih*. Dept. of Mol. Pharm. and Tox., Sch. of Pharmacy, Univ. of Southern California, Los Angeles, CA 90033.

The promoter regions for human MAO A and B genes have been characterized using a series of 5' flanking sequences linked to a human growth hormone reporter gene and transfected into NIH3T3, SHSY-5Y and COS7 cells. The highest promoter activity is found in a 0.14 kb PvuII/DraII fragment (A0.14) for MAO A and a 0.15 kb PstI/NaeI fragment (B0.15) for MAO B. Both fragments are GC rich, contain potential Sp1 binding sites and are in the region where the MAO A and B 5' flanking sequences share the highest identity. However, the organization of the transcription elements are different between these two promoters. Fragment A0.14 consists of three Sp1 sites, all in reversed orientations and lacks a TATA box. Fragment B0.15 contains a Sp1-CACCC-Sp1-TATA structure. Additional transcription elements are found in further upstream sequences of both genes, inclusion of these sequences decreases promoter activity. The different promoter organization of MAO A and B genes may provide the basis for their different tissue and cell specific expression. (Supported by NIMH grants R37 MH39085 (Merit Award), K05 MH00796 (Research Scientist Award), R01 MH37020 and Welin professorship)

113.7

IDENTIFICATION OF A BIFUNCTIONAL GENETIC REGULATORY ELEMENT IN THE RAT DOPAMINE ß-HYDROXYLASE GENE. E.J. Lewis*, D. Greco, L. Asnani and J. Shaskus. Dept. Biochemistry and Molecular Biology, Oregon Health Sci. Univ., Portland, OR 97201

Dopamine ß-hydroxylase (DBH), the enzyme which converts dopamine to norepinephrine, is expressed in a cell-type restricted pattern in neuroendocrine tissue. The molecular mechanisms which contribute to specificity of expression of this enzyme were investigated by an analysis of the genetic regulatory elements present on the rat gene. A segment of the gene containing 395 bases of 5'-flanking sequence was cloned adjacent to a reporter gene. When transfected into mammalian cells, this construct is expressed in a cell-type specific pattern, with the highest level of expression observed in the human neuroblastoma cell line SHSY-5Y. Progressive deletion of the 5'-flanking sequence led to the identification of a 30 base genetic regulatory element, designated DB1. Recombinants containing multiple copies of DB1 cloned adjacent to a heterologous promoter exhibit a 5-20 fold elevation in reporter activity when transfected into neuroendocrine cell types, but do not enhance transcription in C6 glioma or CV1 kidney cells. DB1-specific DNA-protein complexes are found in nuclear extracts from all cell lines examined, and the migration pattern differs between cell lines.

The 5'-flanking region of the DBH gene also contains genetic regulatory elements responsive to cyclic AMP and phorbol ester stimulated second messenger pathways. DBH mRNA and reporter gene activity are increased in neuroblastoma cells treated with these effectors, and simultaneous treatment with both agents results in a synergistic response. Deletion analysis has localized the second messenger response site to the region containing the DB1 element. Reporter plasmids containing the DB1 element respond to treatment with inducers with an increase in reporter gene activity. The results of this study identify a *cis*-acting element in the dopamine ß-hydroxylase gene which acts as a dual function transcriptional enhancer, influencing both cell type specificity and the response to second messengers.

113.8

DISCORDANT ESTIMATES OF TPA-INDUCED CMV PROMOTER ACTIVITY AS DETERMINED BY REPORTER GENE mRNA LEVELS AND ENZYMATIC ACTIVITY. D.M. Kovacs*and B.B. Kaplan, Dept. of Psychiatry, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15213

Transient cell transfection methodology is widely employed in the analysis of neural-specific gene expression. To augment the sensitivity of the transfection assay in neural clonal cell lines, we have employed quantitative PCR to assess reporter gene activity. Advantage was taken of the AP1 elements present in the human cytomegalovirus (CMV) promoter to compare both basal and stimulated transcription activity by PCR and standard enzymatic assay. The CMV promoter fused to the β-gal reporter gene, was introduced into human neuroblastoma SK-N-BE(2)-C cells, and cultured in the presence TPA (0.1 μM), for various time periods. Surprisingly, significant differences were observed in the TPA induction profile of CMV promoter activity, as judged by β-gal mRNA levels and activity. For example, 24 h post-transfection β-gal mRNA levels increased 2.4 fold in TPA-treated cells, whereas 7.3 fold increases were observed in β-gal activity. This apparent discrepancy could derive from TPA-induced alterations in (1) β-gal mRNA translation efficiency, (2) post-translational modification of the enzyme, or (3) half-life of the protein. Regardless of the mechanism, these findings raise a cautionary note on the reliance of reporter gene activity to estimate the transcriptional activity of heterologous promoters.

113.9

RAT GAD_{65} CONTAINS TWO TRANSCRIPTION START SITES. C.S. Pinal* and A.J. Tobin, Department of Biology, University of California, Los Angeles, California, 90024.

Our laboratory has cloned the human and rat genes encoding the 67,000 and 65,000 Mr forms of glutamic acid decarboxylase (GAD_{67} and GAD_{65}). The human GAD_{67} gene contains at least two transcriptional start sites in human adult and fetal brain (Erlander and Tobin, J. Neurochem., in press). The rat GAD_{65} gene also contains at least two transcription start sites. We are using ribonuclease protection assays to determine whether the two start sites in adult rat brain are used in a region-specific or developmentally regulated manner. Computer analysis of the genomic sequence upstream of the proposed transcription start sites for rat GAD_{65} contains sequence similarities to the consensus sequences for several transcription factors, including pancreas-specific transcription factors, whose significance we are evaluating in transient expression assays. (supported by NS22256)

113.10

cAMP-MEDIATED TRANSCRIPTIONAL REGULATION OF GENE EXPRESSION IN C6 CELLS. G.M. Lawless*[1], J. Segovia[1], M. Brenner[2] and A.J. Tobin[1,3,4]. [1]Department of Biology, [3]Brain Research Institute, and [4]Molecular Biology Institute, University of California, Los Angeles, CA 90024, and [2]Lab. of Molec. Biol., NINDS-NIH, Bethesda, MD 20892.

The C6 rat neuroglioma cell line is known to exhibit strong biochemical responses to increased intracellular cAMP, which are accompanied by major morphological changes. We have previously reported that such increases in cAMP result in a decrease in both GAD_{67} mRNA and GAD activity (Segovia, J. et al. *Neurosci. Abs.*, 17:159.11, 1991). These decreases appear to represent a shift from a neuronal to glial phenotype. Using forskolin, which directly stimulates adenylate cyclase, we have observed increases in GFAP mRNA levels. In order to determine if these changes in gene expression occur at the transcriptional level, we have obtained the DNA sequences of the human GFAP gene, 5' to the transcriptional start site, linked to the CAT reporter gene. This construct was transiently transfected into C6 cells, and the transfected cells were treated with forskolin and assayed for CAT activity. Preliminary results show a 300% increase of CAT activity. This result suggests that at least part of the observed increase in GFAP mRNA results from an increase in transcription. We have linked 5' upstream DNA sequences of the human GAD_{67} gene, previously cloned in our laboratory, to a CAT reporter vector. We are currently studying the transcriptional regulation of the GAD_{67} gene in forskolin- treated C6 cells. (Supported by NS22256 and The Scottish Rite Schizophrenia Research Program)

113.11

VARIABLE MODES OF HUMAN GONADOTROPIN-RELEASING HORMONE(GNRH) GENE TRANSCRIPTION IN JEG AND MDA CELL LINE.
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Previous studies have shown GnRH gene expression in the human hypothalamus with a transcriptional start site similar to that observed in rodent hypothalamus, however GnRH expression in non-brain tissue appeared to have additional 5' promoter starts. Since several non-brain cell lines are being used to study GnRH promoter structure, we characterized the transcriptional start sites in these cells. By using ribonuclease protection assay and primer extension, we identified an upstream transcriptional start site in a human placental tumor cell line (JEG) and a human breast tumor cell line (MDA). The downstream start site has a typical TATA box (-25 to-31) and CAAT box (-49 to -52) while the upstream start site lacks the TATA and CAAT box. However, the upstream start site contains the sequence 5'-GTCTTGCT-3' (84 bases upstream)that is similar to the sequence 5'-CTCCCTGCT-3' located in the 5'-flanking region of the murine brain expressed *Thy*-1 gene, which also lacks TATA and CAAT boxes and has multiple transciption start sites. In JEG cells about 68% and in MDA cells about 81% of the GnRH mRNA is transcribed from the upstream start site in both cytoplasm and nucleus. The mRNA encoded from this upstream transcriptional start site still encodes the GnRH precursor protein. These results indicate that human GnRH gene actively uses an upstream start site for mRNA transcription in the tissues outside the brain.

113.12

FUNCTIONAL ANALYSIS OF THYMOSIN BETA-10 GENE PROMOTER REVEALS ELEMENTS SENSITIVE TO RETINOIC ACID AND AT LEAST ONE NUCLEAR RETINOIC ACID RECEPTOR.
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The 5Kda putative G-actin binding protein, namely,thymosin beta-10 has been identified as a retinoid-responsive gene expressed during the early phases of neuroembryogenesis [see Hall(1991)Molecular Brain Res 9:175-177]. To identify possible regulatory elements (RARE) in the beta-10 gene we constructed a chimeric expression vector harboring a Taq 1 restriction fragment containing approximately 900bp (which encompass the human beta-10 gene promoter and 5'flanking region) cloned upstream from the bacterial CAT reporter gene. Retinoic acid (RA) failed to induce CAT activity in rat NB cells but did induce a 3-5 fold stimulation of CAT in human osteosarcoma cells cotransfected with the hRAR-alpha gene. These findings in concert with DNA sequence analysis favor the likely presence of elements responsive to at least one class of nuclear retinoic acid receptor in the human thymosin beta-10 gene promoter.
[AKH is supported by NCI CA 49422-03]

113.13

FUNCTIONAL ANALYSIS OF THE cis-ACTING ELEMENTS INVOLVED IN EXPRESSION AND REGULATION OF TRYPTOPHAN HYDROXYLASE GENE
S. O. Huh*, D.H. Park, T. H. Joh, and J. H. Son Lab. of Molecular Neurobiol. Cornell Univ. Med. Coll., The Burke Med. Res. Inst.,White Plains, NY 10605

Tryptophan hydroxylase (TPH), the rate-limiting enzyme in the biosynthesis of serotonin, is expressed in a distinct cell-type specific manner. To identify the cis-acting regulatory elements of the mouse TPH gene that direct this tissue specific expression in both neuronal and non-neuronal tissue types, we carried out both in vitro and in vivo functional analyses. A mouse mastocytoma cell line that expresses TPH was used to test TPH promoter activity in a transient transfection assay. A series of constructs containing TPH upstream sequences of varying lengths fused to a firefly luciferase (Luc) reporter gene were constructed and examined for their ability to confer expression. The 1.1kb-Luc construct showed at least 20 fold more activity than that of promoter-less Luc plasmid. In addition, this construct represented 20% of the activity of a positive control vector, pRSVLuc, suggesting that the functional promoter/enhancer elements for appropriate expression of the mouse TPH gene in this cell type were located in the 1.1 kb 5'-flanking region. In order to corroborate our results from the transfection experiments, we also generated transgenic lines containing two different TPH-lacZ fusion constructs, pWH6.4-LacZ and pWH1.1-LacZ, containing TPH promoter fragments with 5' endpoints at -6.4 kb and -1.1 kb, respectively. Four transgenic lines containing pWH1.1-LacZ and 5 lines with pWH6.4-lacZ transgene were established. The expression pattern of these gene constructs in transgenic mice will permit us to determine if 1.1 kb is sufficient for tissue specific expression in vivo.

113.14

THE 5' UPSTREAM REGION OF THE HUMAN DOPAMINE-β-HYDROXYLASE GENE DRIVES TRANSCRIPTION IN A CELL-TYPE SPECIFIC MANNER.
H. Ishiguro*, T.H. Joh and K.S. Kim. Cornell Univ. Med. Coll. at the Burke Med. Res. Inst., White Plains, NY 10605.

Dopamine-β-hydroxylase (DBH) catalyzes the conversion of dopamine to norepinephrine. *In vivo*, DBH expression is limited to adrenergic and noradrenergic neurons as well as chromaffin cells of the adrenal gland, indicating the existence of tight tissue-specific regulation of this gene. In order to gain a better understanding of the underlying molecular mechanisms involved in transcriptional control of the human DBH gene, a genomic clone containing 4.3 kb upstream region of the DBH gene was isolated from a human placenta genomic library. A series of deletion constructs fused to the chloramphenicol acetyltransferase (CAT) gene were prepared. Transient transfections in DBH-expressing human neuroblastoma SK-N-BE(2)C and DBH-nonexpressing HeLa cell lines indicated that the upstream region of the human DBH gene directs transcription in a cell-type specific manner. Furthermore, when the upstream sequence was deleted to -1.0 kb or -262 bp, CAT activity increased 2-fold and 4-fold, respectively, indicating the presence of some negative elements in this upstream region. When the upstream region was further trimmed up to -114 bp, >90% of the CAT activity disappeared. These data suggest that there is an important positive element(s) in a region ranging from -262 to -114 bp. Supported by MH24285.

113.15

DISCOORDINATE REGULATION OF α-2 AND β-3 nAChR GENE EXPRESSION IN THE DEVELOPING RETINA: CLONING AND CHARACTERIZATION OF THE α-2 AND β-3 nAChR GENE PROMOTERS
F. Hoover*, F. Locklear and D. Goldman. Department of Biological Chemistry and Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

We have previously shown that the nicotinic acetylcholine receptor (nAChR) α-3, α-4, β-2, β-3 and β-4 subunit genes are induced in developing rat retinal cells around E13-14 (Exp. Eye Res., in press). Retrograde labelling and the anatomical position of these cells indicated that they were recently differentiated ganglion cells. We have now identified a member of the nAChR gene family (α-2) whose expression in the developing retina is not temporally correlated with the above nAChR subunit genes. RNase protection assays show the α-2 gene is induced in the postnatal retina. In situ hybridizations are being performed to determine the retinal cell type(s) expressing the α-2 gene.

In order to determine the molecular mechanisms by which nAChR genes are induced in the retina, we have cloned the α-2 (induced postnatally) and β-3 (induced at E13-14) gene promoter regions. We have mapped the 5' ends of the mRNAs encoding the α-2 and β-3 genes and report that both genes use multiple transcriptional start sites. The DNA sequence has been determined for 1354 nucleotides and 570 nucleotides 5' to the initiator methionine of the α-2 and β-3 genes, respectively. Canonical promoter CCAAT and TATAA sequences were not present in the α-2 gene. However, the β-3 gene contained a CAAAAAT sequence and a TAATAA sequence 168 nucleotides and 109 nucleotides upstream from the most 5' start site, respectively. In order to identify cis-acting DNA sequences conferring tissue and developmental stage-specific expression, we have constructed expression vectors with the α-2 or β-3 promoter driving the expression of a luciferase structural gene. Introduction of these expression vectors into retinal, neuronal, muscle, and fibroblast-like cell indicates the α-2 gene is promiscuously expressed, while the β-3 gene is preferentially expressed in retinal and muscle cells.

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113.16

REGULATION OF TYROSINE HYDROXYLASE GENE EXPRESSION BY CYCLIC AMP AND PHORBOL ESTER.
Y. Chen*, J.A. Best and A.W. Tank. Department of Pharmacology, University of Rochester, Rochester, NY 14642.

The specific intracellular signal transduction pathways that mediate the regulation of tyrosine hydroxylase (TH) gene transcription rate are not known. Receptors that are linked to the elevation of cyclic AMP and the activation of phosphatidylinositol turnover have been shown to regulate TH gene expression in vivo. We have used transiently transfected rat pheochromocytoma cell lines to test the effects of cyclic AMP analogs and phorbol esters on the TH gene promoter-driven expression of the reporter gene, chloramphenicol acetyltransferase (CAT). The wild-type pTH-CAT construct (pTH.3-CAT) contains the region from -272 to +27 of the TH gene promoter linked upstream to the coding region of CAT. A second construct (pTH.3mCRE-CAT) contains this same promoter region in which the cyclic AMP response element (CRE) is mutated. Both these constructs are kind gifts from Dr. Dona Chikaraishi, Tufts University. Both the cyclic AMP analog, 8-CPT-cyclic AMP and the phorbol ester, TPA, increase the expression of CAT in all tested rat pheochromocytoma cell lines transfected with pTH.3-CAT. As expected, 8-CPT-cyclic AMP does not increase CAT expression in cells transfected with pTH.3mCRE-CAT. Surprisingly, in rat pheochromocytoma PC18 cells the effect of TPA on CAT expression is totally blocked by mutating the CRE. However, this dependence of the TPA effect on an intact CRE is cell-line dependent, because the response to TPA is only partially inhibited using the mutated CRE construct in two other cell lines. In all tested cell lines, the basal expression of CAT is diminished, when the CRE is mutated compared to that observed using the wild-type promoter. These results suggest that the TH gene CRE participates in the response of the gene to receptors that elevate cyclic AMP and in some cell types to receptors that activate protein kinase C. The TH gene CRE may also play a role in maintaining the basal transcription rate of the TH gene. (Supported by DA 05014 and DE 00159).

113.17

PROTEIN INTERACTIONS WITH A PUTATIVE AP1 ELEMENT IN THE RAT TYROSINE HYDROXYLASE GENE PROMOTER. K.M. Piech*, J.A. Best and A.W. Tank, Department of Pharmacology and Department of Dental Research, University of Rochester, Rochester, NY 14642.

Using gel shift assays rat pheochromocytoma PC12 cell extracts were used to examine protein binding to a portion of the rat TH gene promoter (-192 to -212) that contains a consensus AP1 binding sequence (GTGATTCAGA). The affinity and/or level of proteins binding to the TH gene AP1 element was increased in nuclear extracts isolated from PC12 cells treated for 3 hr either with a cAMP analog or with phorbol 12-myristate 13-acetate (PMA). The binding of proteins to the AP1 element was shown to be specific in gel shift assays utilizing competitor DNAs. Formation of the TH gene AP1-protein complex was inhibited, when an antibody to c-fos was preincubated with the PC12 cell nuclear extracts. These results suggest that Fos and/or Fos related antigens may participate in the formation of this complex. TH gene AP1-protein binding was also examined in nuclear protein extracts isolated from Sprague-Dawley rat adrenal medullary cells. The protein extracts were isolated 3 hrs. after repeated injections with either saline, bethanechol (5mg/kg) or nicotine (2.3mg/kg). Formation of a protein-DNA complex was apparent using adrenal medullary nuclear extracts isolated from rats treated with bethanechol or nicotine, but not saline. No TH gene AP1-protein complex was formed using rat adrenal medullary nuclear extracts isolated 20 minutes after one injection of saline, bethanechol (10mg/kg) or nicotine (2.3mg/kg). These results suggest that the activation of either cyclic AMP-dependent protein kinase or protein kinase C in PC12 cells may lead to the regulation of the TH gene via the AP1 response element. Furthermore, these data indicate that the TH gene may be regulated by AP1 binding factors in vivo in the adrenal medulla when rats are administered cholinergic agonists repeatedly. (Supported by NIDA grants DA 05014, DA 07232, and DE 00159).

113.18

EVIDENCE THAT RETINOIC ACID, GLUCOCORTICOIDS AND CYCLIC AMP MAY REGULATE GENE EXPRESSION AT A COMMON REGULATORY SEQUENCE IN C6 GLIOMA CELLS. Wayne A. Schreier*. Andrus Gerontology Center, Univ. Southern California, Los Angeles, CA 90089-0191.

The upstream regulatory region of the rat glutamine synthetase (GS) gene contains a potential GRE/CRE consensus sequence, 5'-GGTAAAATTCTCT (Mill et al., Mol. Brain Res. 9:197, 1991), with similarity to previously identified glucocorticoid/cAMP regulatory elements. We examined the effects of the combined treatment of hydrocortisone (HC) and forskolin (F) with retinoic acid (RA) on GS activity in C6-2BD cells. HC and F both induce an increase in GS activity. RA reduces both the HC- and F-induced increase in GS activity by ≈ 50%. RA also reduces the basal levels of GS activity to a similar extent. It is possible that HC (via the glucocorticoid receptor) and forskolin (via activation of an ATF/CREB transcription factor) may stimulate GS mRNA transcription by binding at this common site. RA, on the other hand, may inhibit GS mRNA transcription via the retinoic acid receptor binding to this same site in a competitive manner. (This work was supported by NIH grant HD-06576 to Jean deVellis, UCLA Mental Retardation Research Center).

113.19

NUCLEAR FACTORS INTERACTING WITH THE CHICK MUSCLE ACETYLCHOLINE RECEPTOR δ-SUBUNIT GENE DURING CHICK EMBRYO DEVELOPMENT. Y. E. Lee* and J. Schmidt. Dept. of Neurobiol. & Behavior and Dept. of Biochem. & Cell Biol., State Univ. of New York at Stony Brook, Stony Brook, NY 11794.

Transacting factors that interact with the acetylcholine receptor (AChR) δ-subunit enhancer region were studied by EMSA. Nuclear extracts from embryonic hind limb muscle were examined. Two major protein-DNA complexes were observed with a δ-subunit enhancer-derived probe. Using point-mutated DNA fragments as competitors in EMSA and DNAse I footprinting, the specific binding sites were identified as an E-box or MEF1 site and a CG rich element.

Among these complexes, the E-box binding activity is developmentally regulated, with the highest intensity in embryonic day 11 hind limb muscle. The migration of this E-box binding complex can be retarded when a polyclonal antibody specific for human E12/E47 proteins is applied, suggesting that the complex contains a chick E12/E47-like protein.

The mRNA levels of the five bHLH proteins MyoD, myogenin, Myf5, herculin, and CTF4, which have the ability to bind and transactivate E box-containing promoters, were also measured and compared with the mRNA levels of the AChR subunit genes during embryonic hind limb muscle development. Preliminary results indicate that the mRNA expression pattern of MyoD, myogenin, Myf5 and CTF4, but not of herculin, positively correlates with the expression of AChR subunit mRNAs.

PRESYNAPTIC MECHANISMS I

114.1

Ca^{++}-INDUCED DOCKING OF SYNAPTIC VESICLES AT THE ACTIVE SITE. J.H. Koenig*, K. Yamaoka and K. Ikeda. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

The involvement of Ca^{++} in the transmitter release mechanism, while recognized many years ago, is still poorly understood. One process in the release mechanism in which Ca^{++} may be involved is the translocation and docking of vesicles at the release site, possibly mediated by such vesicle-associated proteins as fodrin, synapsin or actin. In this study, the effect of raising $[Ca^{++}]_o$ on the docking of vesicles at the active site was investigated using the coxal neuromuscular junction of *Drosophila*. In the resting coxal synapse in normal saline (1. 8 mM Ca^{++}) only a small percentage active sites possess vesicles in a docked position under the dense body adjacent to the plasma membrane. After 10 m exposure to 18 mM Ca^{++} saline, however, a dramatic increase occurs in the number of sites possessing docked vesicles, as well as in the number of docked vesicles per site. Intracellular recordings reveal that 18 mM Ca^{++} saline induces both an increase in the frequency of spontaneous release and an increase in the frequency of multiquantal MEJPs. Thus, an increase in the number of sites possessing docked vesicles is accompanied by an increase in spontaneously released MEJPs. Also, an increase in the number of quanta/MEJP is accompanied by an increase in the number of docked vesicles/site.

114.2

USE OF ANTISENSE OLIGONUCLEOTIDES WITH THE PATCH-CLAMP TECHNIQUE REVEALS A SMALL GTP-BINDING PROTEIN IN THE CONTROL OF EXOCYTOSIS. P.-M. Lledo*, R. Zorec[1], P. Vernier[2] and W. T. Mason, Dept. of Neurobiology, AFRC, Babraham, Cambridge CB2 4AT, U.K.; [1]Inst of Pathophysiol, School of Medicine, 61105 Ljubljana, Slovenia and [2]CNRS, Lab de Neurobiol., 91198 Gif-sur-Yvette, France.

Low-molecular-weight GTP-binding proteins (G-proteins) are strong candidates for regulators of membrane traffic. Recently, we have demonstrated that GTP-γ-S regulates secretory processes in anterior pituitary cells. To identify the G-protein, we screened G-proteins with Northern blotting and found a high content of a small G-protein of the $p21^{ras}$ superfamily (rab3B protein) in anterior pituitary cells. To determine whether this rab3B protein could have a physiological function, we developed a new approach using patch-clamp recordings and intracellular perfusion of antisense (AS) and sense (S) oligodeoxynucleotides designed to block specifically the translation of rab3B. The whole-cell recording configuration with a dual-phase lock-in amplifier was employed to measure cell membrane capacitance (C_m) to monitor secretory activity, and to load rat pituitary cells with AS or S. Cells were bathed with 5 mM $CaCl_2$ and loaded for 2 min with intracellular solution containing 1 μM Ca^{2+} and about 50,000 molecules AS or S. Effects on Ca^{2+}-induced exocytosis were determined in the same cells 48-52h after loading with oligonucleotides. We found that the calcium-induced increase in membrane capacitance was dramatically reduced by the injection into cells of AS but not S. Endocytotic processes were not altered. These results provide evidence for the involvement and identification of a small G-protein which directly modulates the Ca^{2+}-induced exocytosis in anterior pituitary cells.

114.3

GTP-BINDING PROTEINS REGULATE SYNAPTIC VESICLE CYCLING. P.A. Doroshenko and G.J. Augustine*. Dept. Neurobiology, Duke Univ. Medical Center and MBL, Woods Hole.

The non-hydrolyzable GTP analog, GTPγS, inhibits neurotransmitter release when injected into squid giant presynaptic terminals (*Biol. Bull.* 181, 320,1991). Because this inhibition is not associated with any change in presynaptic Ca signalling, it is possible that the synaptic vesicle cycling pathway may be a target of the nucleotide. We have tested this possibility by using electron microscopy to examine the distribution of synaptic vesicles in terminals. Active zones of control terminals were surrounded by a dense cloud of synaptic vesicles, including a sub-set apparently docked at the presynaptic membrane. Terminals injected with GTPγS had a dramatically reduced total number of vesicles associated with the active zone. Comparison of the distribution of vesicles in control and GTPγS injected terminals revealed a gradient of vesicle depletion within the active zone, with virtually no depletion of docked vesicles but a 60% loss of vesicles found 0.5 μm away from the membrane. The observation that GTPγS inhibits transmitter release without reducing the number of docked vesicles indicates that a GTP-binding protein is involved in a step of the vesicle life cycle that is at or near the docking step. The depletion of upstream vesicles suggests that one or more additional GTP-binding proteins are involved at other steps in the cycle. Supported by NIH grant NS-21624.

114.4

INHIBITION OF EXOCYTOSIS BY MYOSIN LIGHT CHAIN KINASE INHIBITOR IN ADRENAL CHROMAFFIN CELLS. M. O.-Imaizumi[1], T. Sakurai[2], S. Nakamura[2], Y. Nonomura[2], and K. Kumakura[1]*. [1]Life Science Inst. Sophia Univ., Tokyo 102, and [2]Dept. Pharmacol., Univ. of Tokyo Sch. Med., Tokyo 113, Japan

To elucidate the possible involvement of myosin light chain kinase (MLCK) in the mechanism of exocytosis, we studied the effects of wortmannin, a specific MLCK inhibitor, on the secretory function of bovine adrenal chromaffin cells. Wortmannin inhibited both acetylcholine-evoked and high K^+-evoked release of catecholamines (CA). It also blocked the Ca^{2+}-induced release of CA from the digitonin-permeabilized cells. The inhibiton of both high K^+-evoked and Ca^{2+}-induced CA release was dose-dependent (IC_{50} = 1 μM), and there was complete inhibition at 10 μM. Thus, MLCK seems to be involved in the mechanism of exocytosis at downstream of Ca^{2+} increase in adrenal chromaffin cells, although it has been debatable.

114.5

THE PRESYNAPTIC EFFECT OF ω-CONOTOXIN ON IPSPS IN RAT HIPPOCAMPUS INVOLVES A PERTUSSIS TOXIN (PTX)-SENSITIVE G-PROTEIN. P. Dutar*, B. Potier and Y. Lamour. INSERM U161, 75014 Paris France.

The nature of the coupling mechanism of presynaptic calcium channels involved in the release of neurotransmitters in the mammalian central nervous system is unknown. Omega-conotoxin (ω–CgTx) blocks partially the excitatory (EPSP) and totally the inhibitory (IPSP) synaptic transmission in CA1 hippocampal pyramidal neurons through an action on N-type calcium channels. We show in this study that the inhibitory effect of ω–CgTx on IPSP is blocked by the intrahippocampal injection of PTX. PTX (Sigma) (or vehicle for control rats) was injected into str. radiatum (s.r.) of hippocampus (1.5-2μg) of male Sprague-Dawley rats, 3 days before electrophysiological experiment. Intracellular recordings from CA1 neurons in the rat hippocampal slice preparation were performed using standard procedures. EPSPs and IPSPs were induced by electrical stimulation of CA1 afferents in s.r. ω–CgTx was applied at 3 different concentrations: 0.1, 0.5 and 1μM. In control rats, ω–CgTx depressed EPSP and IPSP respectively by 23.1 (n=7) and 48% (n=6) at 0.1μM, 49.5 and 78% at 0.5μM (n=11), 65.7 (n=4) and 91.6% (n=3) at 1μM. In rats pretreated with PTX, percentages of inhibition were respectively 24.2 (n=5) and 20.3% (n=4) at 0.1μM, 40.1 (n=10) and 31.3% (n=9) at 0.5μM, 57.1 (n=5) and 57.9% (n=4) at 1μM.

These results show that 1) IPSPs are more sensitive to the effect of ω–CgTx than EPSPs in control rats; 2) The inhibitory effect of ω–CgTx on IPSPs is blocked (at least in part) by PTX while the effect on EPSP is not. The results suggest that the nature or the regulation of calcium channels might be different depending on their location on excitatory or inhibitory terminals. *Supported by grants from Bayer Pharma France and MGEN.*

114.6

REAL-TIME CONFOCAL MICROSCOPY OF DYNAMIC CALCIUM CHANGES IN SINGLE PRESYNAPTIC BOUTONS. W.M. DeBello, G.J. Augustine and J.W. Moore*. Dept. Neurobiology, Duke University Medical Center, Durham, NC 27710.

We have used a Noran confocal laser scanning microscope to measure dynamic changes in calcium concentration ([Ca]) within presynaptic terminals of the lizard *(Anolis carolinensis)* neuromuscular synapse. The high resolution of this system allows measurement of changes in presynaptic [Ca] in single boutons loaded with the fluorescent Ca indicator, fluo-3-AM. In response to brief trains of action potentials (1-50 Hz, 1-10 s), presynaptic [Ca] rose transiently and subsequently decayed over many seconds. The magnitude and time-course of this Ca signal varied with the number of action potentials and with fluo-3 concentration. In optimum conditions, responses to single action potentials could be detected and the measured changes in [Ca] were approximately 100 nM per action potential. We saw no evidence for independent activation of single boutons; rather, action potentials elevated [Ca] simultaneously in all boutons within a given presynaptic terminal. Additionally, stimulation frequently evoked a damped [Ca] oscillation that superimposed on the decay phase of the response. In summary, we have demonstrated that it is possible to make high-resolution measurements of presynaptic [Ca] dynamics in single boutons. Supported by a NIH grants NS-21624 and NS-03437.

114.7

PRESYNAPTIC CALCIUM AND SHORT TERM SYNAPTIC ENHANCEMENT AT HIPPOCAMPAL MOSSY FIBER SYNAPSES: EXPERIMENTS AND KINETIC MODELING. W.G. Regehr*, K.R. Delaney and D.W. Tank, Biological Computation Research Dept., AT&T Bell Labs, Murray Hill, NJ 07974.

Calcium in presynaptic terminals ([Ca]) and post synaptic field potentials were simultaneously measured at hippocampal mossy fiber synapses to examine the role of presynaptic [Ca] in short-term synaptic enhancement.

Following the onset of moderate frequency stimulation (.1-5 Hz) both synaptic enhancement and presynaptic [Ca] buildup and reach a steady state plateau. During the plateau phase, a linear relationship between presynaptic [Ca] and synaptic enhancement is observed. However, during the initial buildup, the presynaptic calcium levels rise more rapidly than synaptic enhancement. Similarly, during the decay phase immediately following termination of the stimulus train, the Ca decay rate exceeded the decay rate of synaptic enhancement.

High concentrations of intracellular EGTA greatly slow the buildup and decay of presynaptic [Ca] produced by moderate frequency trains. In the presence of this elevated calcium buffering, the time course of the buildup and decay of synaptic enhancement and presynaptic [Ca] are the same. This suggests that despite the mismatch in time courses observed under normal Ca buffering conditions, increases in presynaptic [Ca] play a causal role in synaptic enhancement.

A simple kinetic model that assumes that synaptic enhancement is controlled by the concentration of a Ca-dependent first order reaction product can account for the temporal mismatch between [Ca] and enhancement. Accordingly, the enhancement is produced by presynaptic calcium, but the biochemical reactions implement a temporal filtering.

114.8

CALCIUM CHANNELS ON HIPPOCAMPAL MOSSY-FIBER NERVE TERMINALS. H.J. Markram, K.E. Krebs* and E.F. Stanley. NINDS Biophysics Lab, NIH Bethesda Md 20892.

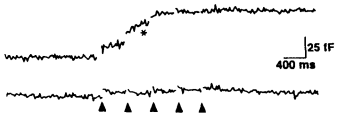
The direct examination of calcium influx through calcium channels in presynaptic nerve terminals in the vertebrate CNS has been greatly limited by the general small size and inaccessibility of these structures. We have used the patch clamp technique to record calcium currents in the unusually large mossy-fiber terminals of the rat hippocampus.

The CA3 region of the hippocampus was dissected and acutely dissociated. Nerve terminals were identified with a specific fluorescent stain and were whole-cell patched using the nystatin perforation technique. Voltage steps were applied from a negative holding potential of -90 mV. A calcium current was recruited at -50 mV that peaked at -40 mV and exhibited significant inactivation during a 200 ms pulse that was tentatively identified as T-like. In some terminals a second non-inactivating component was recruited at more depolarized voltages (>0 mV).

114.9

CALCIUM ENTRY, BUFFERING AND EXOCYTOSIS IN ISOLATED PEPTIDERGIC NERVE TERMINALS. E.P. Seward,* N. Chernevskaya, and M.C. Nowycky. Dept. Anat. & Neurobiol. Med. Coll. PA, Philadelphia, PA 19129.

Ca^{2+}-secretion coupling in neurohypophysial terminals was studied with the capacitance (C_m) recording technique (preceeding abst.). Trains of depolarizing pulses (20-200 msec duration; 300-700 msec intervals; delivered every ~1-2 min) elicited Ca^{2+} entry and C_m increases. Ca^{2+} buffering was manipulated by adding exogenous buffers to the pipette. With 0.5 mM EGTA, the first pulse of a train increased C_m by ~10 fF. This step size occured over a broad range of pulse durations and Ca^{2+} entry rates. Pulses later in the train can elicit larger C_m steps (15 to 40 fF), which are often followed by slow, post-stimulus increases (*, Fig.). With low EGTA (0.1 mM), the first pulse produced a large step. High EGTA (10 mM) abolished all C_m increases in the train except for the first step (Fig). With BAPTA (0.5 mM), both the first and many of the later pulses elicited small steps; larger steps and "slow" increases were not observed. The effects of buffers in peptidergic terminals are strikingly different from those at a fast synapse (Adler et al., J. Neurosci. 11: 1496, 1991) and will be interpreted using Ca^{2+} transients predicted from computer modeling (next abst.).



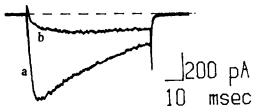
C_m traces from two terminals, stimulated with 5 pulses, 40 ms duration. $[EGTA]_i$ = 0.5 mM (top), 10 mM (bottom). * slow secretion. Total Ca^{2+} entry ~ equal for 2 terminals.

114.10

GTP-γ-S INHIBITS CALCIUM CURRENTS BUT NOT EXOCYTOSIS IN ISOLATED PEPTIDERGIC NERVE TERMINALS. N. Chernevskaya and M.C. Nowycky*. Dept. Anatomy & Neurobiol., Med. Coll. Penn., Philadelphia, PA, 19129.

Axons of hypothalamic magnocellular neurons project to the neurohypophysis (NHP) where they form numerous large endings that secrete vasopressin or oxytocin in response to bursts of action potentials. Secretion of the neuropeptides is evoked by Ca^{2+} entry through voltage-dependent Ca^{2+} channels. Nerve endings were isolated, and whole-"terminal" patch clamp recordings were used to monitor Ca^{2+} currents. NHP terminals contain both N- and L-type Ca^{2+} channels (Lemos & Nowycky, Neuron 2: 1419, 1989). Inclusion of 50 or 100 μM GTP-γ-S in the pipette decreased the peak Ca^{2+} current. The remaining Ca^{2+} current had slow kinetics of activation (figure). This suggests that Ca^{2+} channels in peptidergic terminals undergo inhibitory G-protein modulation as previously characterized in neuronal cell bodies.

Time-resolved capacitance measurements were used to monitor changes in surface membrane area (ΔC_m) arising from exocytosis. C_m increased in response to trains of depolarizations (see next abst.). Ca^{2+} currents remaining in the presence of GTP-γ-S were able to trigger C_m increases. Thus, GTP-γ-S does not disrupt Ca^{2+}-secretion coupling in terminals.



Ca^{2+} current traces from 2 terminals. Trace (a): typical control. Trace(b): current remaining in presence of 100 μM GTP-γ-S.

114.11

THE INFLUENCE OF BUFFER KINETICS ON CALCIUM TRANSIENTS IN A MODEL CELL. M.C. Nowycky, A.G. Romano,* and M.J. Pinter. Depts. Anatomy & Neurobiology and Pharmacology*, Med. Coll. Penn., Philadelphia, PA 19129.

The spatial and temporal profiles of free $[Ca^{2+}]$ following Ca^{2+} entry through voltage-gated ion channels are shaped by Ca^{2+} diffusion and by intracellular Ca^{2+} buffers. We have used a model that incorporates Ca^{2+} diffusion and both fixed and diffusible buffers in a spherical cell to calculate levels of free Ca^{2+} and of Ca^{2+}-bound buffer in a series of concentric shells. The interaction of buffer affinity, binding rate, and concentration and Ca^{2+} current amplitude were explored for a 15 micron diameter cell.

Fixed buffers tend to restrict Ca^{2+} elevation to the outer shells and slow Ca^{2+} equilibration. Bound fixed buffer in the outer shells acts a Ca^{2+} source after termination of Ca^{2+} influx. Diffusible buffers speed Ca^{2+} equilibration throughout the cell. The relative contribution of fixed vs. diffusible buffers to shaping the Ca^{2+} transient is determined to a large extent by the forward binding rate of each buffer, with the diffusible buffer dominating at equal binding rates. Buffer affinity has little effect on Ca^{2+} transient amplitude in superficial shells, despite large effects in deeper shells and at equilibrium. The results of calculations are considered from two viewpoints: 1) the use of various exogenous buffers as tools to experimentally manipulate $[Ca^{2+}]$ and 2) limitations on the use of mobile Ca^{2+} dyes as indicators of free Ca^{2+}.

114.12

A POSSIBLE ROLE OF CALCIUM BUFFERING IN LONG-TERM ADAPTATION OF NEURONS. J. C. McMahon and A. J. Mercier*. Dept. of Biol. Sci., Brock University, St. Catharines, Ont., L2S 3A1.

Increasing the impulse activity of neurons in vivo over 3 or more days causes a reduction in transmitter release that persists for days to weeks (eg. Mercier & Atwood, J. exp. Biol. 145: 9-22, 1990). This effect is usually accompanied by decreased synaptic fatigue. These two changes involve presynaptic mechanisms and indicate "long-term adaptation" (LTA) of nerve terminals. Previous experiments indicate that LTA requires extracellular calcium and protein synthesis (eg. Hong & Lnenicka, Soc. Neurosci. Abstr. 17: 1322) and appears to involve communication between the cell body and the nerve terminals. The present study examines the possibility that the reduction in transmitter release is caused by an increase in the calcium buffering ability within the nerve terminals. It examines the responses of adapted and control nerve terminals to the exogenously applied calcium buffer, BAPTA-AM. If LTA increases intrinsic Ca^{2+}-buffering, the membrane permeant form of BAPTA should have less of an effect on adapted nerve terminals than on controls.

Experiments are performed on the phasic abdominal extensor motor neurons of the crayfish, Procambarus clarkii. BAPTA-AM decreases excitatory postsynaptic potentials (EPSP's) of the phasic extensors in a dose-dependent manner between 5 and 50 μM. LTA is elicited by in vivo stimulation at 2.5 Hz for 2 h per day over 3 days, which reduces EPSP's by over 50%. Experiments to date (n=4) indicate that BAPTA-AM produces a smaller change in EPSP's in adapted neurons than in controls. These preliminary results support the hypothesis that impulse activity alters intrinsic calcium buffering.

Supported by NSERC Canada.

114.13

CALCIUM IONOPHORE A23187 ACTIVATES REPRESSED SYNAPSES IN THE CRAYFISH. E.R.Smith and S.J.Velez* Dept. of Biological Sciences, Dartmouth College, Hanover, N. H. 03755.

Neuromuscular synapses in the superficial flexor muscle system of the crayfish Procambarus clarkii become physiologically repressed (no junction potentials are detected under normal physiological conditions) after cutting the nerve in the middle of the muscle field and removing the lateral fibers (Prosser & Velez, Soc. Neurosci. Abstr. 12: 1575, 1986). These repressed synapses do not respond to changes in calcium concentrations of the Ringers bathing the preparation: junction potential (jp) sizes of control synapses increase significantly in size while those few detected from repressed synapses remain essentially constant (Gupta & Velez, Soc. Neurosci. Abstr. 16: 1162, 1990). We suggested then that calcium was involved in the mechanisms underlying repression in this system. In the present experiments we recorded jp's from identifiable control and repressed synapses that were bathed in Ringers containing normal and 200% calcium concentrations and Ringers containing the calcium ionophore A23187 dissolved in DMSO. Control synapses showed a 1.3x-1.5x increase in jp sizes in 200% calcium and a 2x-3x increase in jp sizes in the presence of the ionophore. Repressed synapses had no change in jp sizes in 200% calcium but showed a 4x-5x increase in jp sizes in the presence of the ionophore. These results suggest that in repressed synapses the presynaptic calcium channels are not working properly, i.e. they are blocked or have become less voltage sensitive.

114.14

DIFFERENTIAL EFFECTS OF CALCIUM ENTRY ON PHASIC AND TONIC MOTOR AXONS OF CRAYFISH. H.L. Atwood* and G.A. Lnenicka. Department of Physiology, University of Toronto, Toronto, Canada; and Department of Biological Sciences, State University of New York, Albany, New York 12222.

The closer muscle of the crayfish claw is innervated by two motor axons: a phasic axon which fires only when maximal muscle activity is required, and a tonic axon which is active much of the time and which regulates normal ongoing muscle activity. The phasic axons shows more rapid synaptic depression and is more susceptible to the actions of metabolic inhibitors. We examined these axons with video-enhanced contrast DIC microscopy during exposure to calcium ionophores to observe the effects of calcium entry on axonal transport and mitochondrial morphology. A bundle containing the two axons was mounted in a small perfusion chamber and different concentrations of the ionophore A-23187, in combination with various concentrations of external calcium, were delivered. In zero-calcium solutions, axonal transport persisted for over 2 hours. In calcium-containing solutions, axonal transport was blocked in ionophore concentrations of 2 μm or higher; mitochondrial morphology was also affected. These changes always occurred first in the phasic axon. The tonic axon apparently is better adapted to handle a calcium load; this may be linked to its normal activity pattern.

(Supported by NSF grant BNS-9121757, and by grants from NCE and MRC Canada.)

114.15

RESIDUAL FREE CALCIUM IS NOT RESPONSIBLE FOR FACILITATION OF NEUROTRANSMITTER RELEASE. J. Blundon*, S. Wright, M. Brodwick, & G. Bittner. Dept. Zoology, Univ. TX, Austin, TX 78712; Dept. Physiology & Biophysics, Univ. TX Medical Branch, Galveston, TX 77550.

Using a two electrode current clamp of the presynaptic terminal and a single microelectrode impalement of the subsynaptic muscle fiber of the crayfish opener neuromuscular junction, we have previously reported that measures of Ca^{2+}-activated K^+ conductance (gK_{Ca}) can be used as a bioassay for submembrane levels of free Ca^{2+} within the presynaptic terminal (Sivaramakrishnan *et al.*, 1991, *J. Gen. Physiol.* 98:1181). We now demonstrate that the time constant (τ) of gK_{Ca} decay ranges from 2 to 6 msec. In contrast, facilitation of transmitter release evoked with depolarizing pulses in the same terminals has a τ of decay of 10 to 35 msec. If the activation of gK_{Ca} and facilitation of transmitter release both require free Ca^{2+}, then differences in the time constants of these two phenomena might be due to a lower affinity or stoichiometry of gK_{Ca}. Using a conditioning pulse - test pulse paradigm in which conditioning pulse amplitude and interpulse interval can vary, we now report that two different test pulses which generate *identical* gK_{Ca}'s and membrane voltages at different time intervals release markedly *different* amounts of transmitter. We conclude that the residual free Ca^{2+} hypothesis does not explain facilitation at this synapse. Rather, facilitation of transmitter release is better explained by residual Ca^{2+} in the presynaptic terminal bound to the transmitter release machinery.

114.16

NON-SINGLE STEP CALCIUM CHANNEL OPENING AND THE TIME COURSE OF TRANSMITTER RELEASE. D. M. J. Quastel*, J. Zhai, and G. Polyakov. Dept. of Pharmacology & Therapeutics, Univ. of British Columbia, Vancouver, Canada V6T 1Z3

In mouse diaphragm brief (0.2ms) depolarizations of end-plates in the presence of tetrodotoxin (and TEA and 4-AP to suppress K currents) elicit EPPs in the presence of Ca^{2+} or Sr^{2+}. Repetitive stimulation (10-60 Hz) in the presence of Sr^{2+} causes increase in MEPP frequency which (from previous work) is attributable to temporary accumulation of Sr^{2+} within the nerve terminal and provides a measure of Ca-channel opening per pulse. When a depolarizing pulse is followed at short intervals (0-0.5 ms) by a relatively small brief depolarizing pulse (25% the amplitude of the first pulse and 0.1 or 0.2ms duration), which alone causes little detectable quantal release, it substantially increases the EPP quantal content, per pulse Sr^{2+} entry, and mean latency of quantal components of the EPP. The opposite occurs with hyperpolarizing postpulses. At low temperature (16° C) latency histograms show changes (with Sr^{2+} or Ca^{2+}) mainly in components delayed from the start of release by the interpulse interval - early components alter as expected from modification by the postpulse of ion entry through channels already opened. We conclude that changes in the time course and magnitude of transmitter release by postpulses reflect changes in Ca^{2+} or Sr^{2+} entry, rather than any "direct" action of voltage on release. Taking into account the effective time constant of the terminal (obtained from the effect of hyperpolarizing prepulses) and the relation between release and stimulation intensity, calculations show the data to be incompatible with a Hodgkin-Huxley type scheme in which Ca-channel opening occurs with one voltage-sesensitive step, but easily explained by a scheme with three sequential voltage-sensitive steps.

(Supported by the Muscular Dystrophy Association of Canada.)

114.17

ω-CONOTOXIN-SENSITIVE CALCIUM CURRENT MEDIATES PRESYNAPTIC INHIBITION AT THE SENSORY NEURON-SPINAL CORD SYNAPSE. W. Gruner, L.R. Silva * and K. Dunlap, Dept. of Physiology, Tufts Medical School, 136 Harrison Ave., Boston, MA 02111.

We have studied synapses between individual dorsal root ganglion (DRG) neurons and spinal neurons grown together in primary culture. DRGs and spinal cords were dissected from chick embryos, dissociated, and plated onto dishes containing small collagen "islands" (Segal and Furshpan '90, J. Neurophys. **64**: 1390). Dual recordings from DRG-spinal cell pairs were made using whole cell patch clamp techniques.

DRG action potentials evoked short-latency EPSPs in spinal neurons. EPSPs were rapidly and reversibly blocked by CNQX (1 μM). ω-conotoxin (ωCgTX; 1 μM), which blocks N-type Ca^{2+} currents in chick DRG neurons (Cox and Dunlap 1992, J. Neurosci. **12**: 906-914), irreversibly blocked EPSPs in spinal neurons without altering responses to focally applied L-glutamate. Baclofen (10-100 μM), which inhibits ωCgTX-sensitive Ca^{2+} current in chick DRG neurons (Cox and Dunlap '92), reversibly abolished EPSPs without altering responses to focally applied L-glutamate. Baclofen effects were blocked by pre-treatment with pertussis toxin (170 ng/ml).

These data support the hypotheses: 1) that N-type Ca^{2+} currents mediate transmitter release during fast excitatory transmission at the primary afferent synapse; 2)that presynaptic inhibition of primary afferents is mediated via modulation of these same N-type calcium currents. (Supported by the NIH.)

LIGAND-GATED ION CHANNELS

115.1

MUTAGENESIS OF HOMOMERIC NMDA-R1 RECEPTOR IN PUTATIVE CHANNEL REGION. **S. Kawajiri*, G.-C. Yeh and R. Dingledine, Dept. Pharmacol., Univ. North Carolina, Chapel Hill, NC 27599.**

A single amino acid position in the GluR1-GluR4 family of glutamate receptor subunits controls the degree of divalent ion permeability of the activated receptor channel. A glutamine (Q) or asparagine (N) in this position allows high divalent ion permeability, whereas an arginine (R) in heteromeric assemblies with Q-containing subunits greatly reduces divalent ion permeability. An arginine-containing homomeric receptor channel carries very little current. The homologous position in the NMDA-R1 subunit contains an asparagine. To explore whether the same "rules" may apply for control of permeation through NMDA receptor channels, we used site-directed mutagenesis to convert this asparagine of NMDA-R1 to an arginine. RNA transcribed from plasmids harboring NMDA-R1 or the N615R mutant was injected into Xenopus oocytes and voltage-clamp recordings obtained 2-4 days later in solutions containing 1 uM TPA. Substitution of Ba for Na in the external solution caused a large right-shift in the reversal potential for NMDA-activated currents in NMDA-R1, confirming the high divalent ion permeability of these homomeric receptors. Homomeric N615R receptors carried little NMDA current in Na-solution; in Ba-solution the reversal potential appeared to be very negative indicative of low Ba permeability. Heteromeric NMDA-R1 + N615R receptors (1:1 molar ratio) exhibited no consistent shift in reversal potential upon changing from Na to Ba solution. These results suggest NMDA and AMPA receptors may use similar structural determinants for control of divalent ion permeability. NMDA-R1 cDNA was a gift from Dr. S. Nakanishi.

115.2

KINETICS STUDY OF NMDA RECEPTOR DESENSITIZATION

Fan Lin* and Charles F. Stevens
The Salk Institute, MNL, La Jolla, CA 92038

We have studied NMDA receptor desensitization with patch clamp techniques in hippocampus CA region neuron. Under our experimental condition, the NMDA receptor desensitization is membrane potential and extracellular Ca^{2+} concentration insensitive, but is an increasing function of NMDA concentration. The desensitizing time course, measured as the decay phase of NMDA current, has to be described as a multiple exponential process. The recovery rate from the desensitized state is in range of seconds. From our results, it appears that NMDA receptor need not open in order to reach the desensitized state.

115.3

MULTIPLE EFFECTS OF SPERMINE ON NMDA RECEPTOR RESPONSES OF RAT CULTURED HIPPOCAMPAL NEURONS.

M. Benveniste* and M. L. Mayer, Lab. Cellular and Molecular Neurophysiology, NICHD, NIH, Bethesda, MD 20892.

Concentration jump responses to 100 μM NMDA in saturating glycine (10 μM) revealed potentiation by 3 mM spermine under whole cell voltage clamp at +60 mV, but depression at -120 mV. Dose-response analysis of spermine-evoked potentiation of responses to NMDA with 10 μM glycine at +60 mV revealed a half maximal effect at 126 μM. In addition, dose-response analysis for glycine yielded a 3.5- and 5.4-fold decrease in peak and steady state EC_{50} values in the presence of 1 mM spermine. This increase in potency for glycine was accompanied by a 3.3-fold decrease in the rate of glycine-sensitive desensitization, and a 2.4-fold decrease in the rate of dissociation of glycine from NMDA receptors.

The block of responses to NMDA by spermine was highly voltage-dependent ($z\delta$ = 1.13) with a K_d at -60 mV of 1.7 mM. Arcaine, DET and DA10 also produced voltage-dependent block with K_d values at 0 mV of 0.75, 2.93 and 4.79 mM, respectively. These compounds did not block spermine-induced potentiation or the spermine-induced decrease in the rate of glycine-sensitive desensitization.

The time course of potentiation at +60 mV induced by concentration jump application of 1 mM spermine in the presence of 100 μM NMDA developed with a slow exponential component, the amplitude and time constant of which decreased with increasing glycine concentration (30 nM glycine: $\tau = 780 \pm 79$ ms; 3 μM glycine: $\tau = 45 \pm 13$ ms), and a faster exponential component ($\tau < 20$ ms at all concentrations of glycine) the amplitude of which appeared to be proportional to the number of active NMDA receptor channels. The decrease in NMDA receptor current measured upon removal of spermine also showed double exponential kinetics with a similar dependence on glycine concentration.

Our results suggest that spermine acts at a minimum of 2 and more likely 3 distinct sites on NMDA receptors to produce voltage-dependent block; potentiation via an increase in affinity for glycine; and potentiation via a glycine-independent mechanism.

115.4

GLUTAMATE AND GLYCINE DECREASE [^{3}H]MK-801 BINDING IN THE PRESENCE OF MAGNESIUM

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In order to elucidate the complex actions of Mg^{2+} on the NMDA receptor-coupled ion channel, we have investigated the effects of glutamate and glycine on [^{3}H]MK-801 binding in cerebrocortical membrane preparations from male rats in the presence of various concentrations of Mg^{2+}. The affinity of [^{3}H]MK-801 binding correlates to the activation of the NMDA receptor-coupled ion channel.

Glutamate and glycine synergistically decreased specific [^{3}H]MK-801 binding in the presence of 4 mM Mg^{2+} or in Krebs solution. The potencies of glutamate and glycine alone or together to decrease [^{3}H]MK-801 binding in the presence of Mg^{2+} were similar to their respective potencies to increase [^{3}H]MK-801 binding in the absence of Mg^{2+}. The glutamate- and glycine-induced decreases of [^{3}H]MK-801 binding were due to a reduced affinity of [^{3}H]MK-801 binding without affecting the number of [^{3}H]MK-801 binding sites, and could be reversed by the competitive NMDA-receptor antagonist D-AP7 and the glycine antagonist 5-chloroindole-2-carboxylic acid.

These results indicate that glutamate and glycine may inactivate the NMDA receptor-coupled ion channel in the presence of physiological concentrations of Mg^{2+}. Since electrophysiological experiments have shown that Mg^{2+} only affects NMDA receptor-coupled ion channels at negative membrane potentials, it is suggested that glutamate and glycine inactivate the NMDA receptor-coupled ion channel during resting membrane potentials and activate the channel during action potentials.

115.5

INHIBITION OF EXCITATORY AMINO ACID-ACTIVATED ION CURRENTS BY INHALATIONAL ANESTHETICS.

Robert W. Peoples* and Forrest F. Weight. Section of Physiology, Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

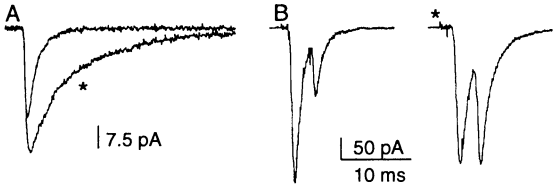
Previous studies in this and other laboratories have shown that several substances with general anesthetic activity, including aliphatic alcohols, diethyl ether, and barbiturates, inhibit excitatory amino acid-activated ion currents in mammalian neurons at physiologically relevant concentrations. We have investigated the actions of inhalational anesthetics on currents activated by kainate (KA), N-methyl-D-aspartate (NMDA), and quisqualate (QUIS) in voltage-clamped mouse hippocampal neurons in culture using the whole-cell patch-clamp technique. Halothane inhibited currents activated by each of the agonists tested in a concentration-dependent manner with the order of potency KA > QUIS ≥ NMDA. Isoflurane inhibited currents activated by each of the agonists tested with the order of potency KA > NMDA ≥ QUIS, but was less potent than halothane. We are currently evaluating the effects of other anesthetic agents on excitatory amino acid-activated currents. Inhibition of excitatory amino acid-mediated neurotransmission in the CNS by general anesthetics may contribute to their behavioral effects. (This work was supported in part by a National Research Council-NIH Research Associateship to R.W.P.).

115.6

CYCLOTHIAZIDE MODULATES EXCITATORY SYNAPTIC TRANSMISSION AND AMPA/KAINATE RECEPTOR DESENSITIZATION IN HIPPOCAMPAL CULTURES.

D. K. Patneau*, L. Vyklicky, Jr., and M. L. Mayer. Laboratory of Cellular and Molecular Neurophysiology, NICHD, NIH, Bethesda, MD 20892.

To determine whether desensitization of AMPA/kainate receptors contributes to the rate of decay of excitatory synaptic responses, we analyzed the effects of cyclothiazide, a novel modulator of desensitization. A saturating dose of cyclothiazide (100 μM) fully blocked desensitization of the response to L-glutamate in whole-cell and outside-out patch recordings and dramatically slowed the decay time constant of monosynaptic EPSCs evoked between pairs of neurons in low-density hippocampal cultures. The decay of control EPSCs was well fit with a single exponential, τ=3.5±1.0 ms, but in the presence of cyclothiazide (*) the decay exhibited double exponential kinetics, τ_{fast}=10.1±3.1 ms (61%), τ_{slow}=43.6±13.2 ms (39%). Cyclothiazide also increased both the amplitude and decay time constant of sucrose-evoked miniature EPSCs (Fig. A), and similarly slowed the decay time constant of responses to 1 ms applications of 1 mM L-glutamate in outside-out patches. The amount of desensitization was examined with a twin-pulse paradigm. Responses separated by an interval of 3-8 ms showed 40-60% desensitization; this was completely abolished by cyclothiazide (*) (Fig. B). Similar to the results of Trussell and Fischbach (1989) and Colquhoun, Jonas and Sakmann (1992), our experiments suggest that AMPA/kainate receptor desensitization occurs during single synaptic responses and in addition that this may contribute to the rate of decay of EPSCs.



115.7

INSURMOUNTABLE INHIBITION OF AMPA INDUCED INCREASE OF INTRACELLULAR Ca^{++} IN RAT CORTICAL NEURONS BY EMOPAMIL.

T. J. Mangano*, M. Britt, L.M. Pullan, C. Thompson and R. A. Keith. Dept. of Pharmacology, ICI Americas, Inc. Wilmington, De. 19897.

The effects of emopamil on α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrobromide (AMPA), kainic acid (KA) and N-methyl-D-aspartate (NMDA) induced increase in intracellular calcium ($[Ca^{++}]_i$) in 14-21 day old rat cortical neurons in primary cultures were examined using fura-2. Emopamil caused a concentration-dependent and virtually complete inhibition of 10 μM AMPA induced increase in $[Ca^{++}]_i$ ($IC_{50} \cong 5$ μM). Nitrendipine (1 μM) and ω-conotoxin GVIA (0.1 μM), inhibitors of L- and N-type voltage-sensitive calcium channels (VSCC), respectively, had no effect on 10 μM AMPA induced increase in $[Ca^{++}]_i$. At 30 μM, emopamil had little effect on 100 μM NMDA or 100 μM KA induced increases in $[Ca^{++}]_i$. NBQX (1 μM) caused > 90% inhibition of 10 μM AMPA and 100 μM KA induced increase in $[Ca^{++}]_i$, while MK-801 (3 μM) caused > 90% inhibition of 100 μM NMDA-induced increase in $[Ca^{++}]_i$. NBQX (1 μM) caused a parallel displacement of the AMPA concentration-response curve, whereas emopamil (30 μM) inhibition was insurmountable at all concentrations of AMPA. Emopamil (up to 300 μM) had no effect on NBQX sensitive (IC_{50}=0.14 μM) ^{3}H-AMPA binding to brain membrane fragments. The results suggest that emopamil inhibits AMPA-induced increase of $[Ca^{++}]_i$ by a mechanism that is distinct from direct AMPA receptor interaction. Emopamil also inhibited cortical neuron VSCC responses, as indicated by its ability to inhibit K^+-induced increase in $[Ca^{++}]_i$ ($IC50 \cong$ 2 uM). Thus, AMPA receptor stimulation may activate, either directly or indirectly, VSCC which are sensitive to emopamil.

115.8

IDENTIFICATION OF CYCLIC NUCLEOTIDE-GATED CHANNEL VARIANTS BY PCR.

Peter J. McKinnon and Robert F. Margolskee*, Department of Neurosciences, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

The cyclic nucleotide-gated ion channels are a class of cation channels activated directly by intracellular cAMP or cGMP. The channels from the retina and olfactory epithelium are well characterized, and have recently been cloned. The molecular structure of the cyclic nucleotide-gated channels is distinct from other known ligand gated channels. However, there is some homology between this class of channel and the transmembrane topography and voltage sensor motif of the voltage-gated channels. Recently, cyclic nucleotide-gated channels have been identified in heart (the pacemaker current), kidney, inner ear, pineal gland, bipolar cells of the retina and Drosophila larval muscle. The wide distribution of these channels suggests the possibility of a multigene family. However, presently only the genes for the olfactory (rat and catfish) and retinal (bovine) cyclic nucleotide-gated channels have been isolated. These genes show a high degree of conservation at the amino acid level, particularly in the transmembrane and cyclic nucleotide binding regions. To identify and characterize other cyclic nucleotide-gated ion channel genes, we have used PCR with degenerate primers designed against various conserved regions of the olfactory and retinal genes. Using this aproach, we have found a number of new genes closely related to the previously known cyclic nucleotide-gated ion channels. These new sequences were identified in various rat cDNA libraries, and rat genomic DNA and show a high degree of relatedness at the amino acid level to the olfactory and retinal channels.

115.9

ACTIVATION AND DEACTIVATION KINETICS OF CYCLIC NUCLEOTIDE GATED CHANNELS FROM OLFACTORY NEURONS STUDIED WITH CONCENTRATION JUMP TECHNIQUES. F. Zufall, H. Hatt+ and S. Firestein*. Section of Neurobiology, Yale Univ. Medical School, New Haven CT 06510 and +Physiological Institute, Technical University, D-8000 Munich 40, Germany.

A critical step in vertebrate olfactory transduction is the gating of a cyclic nucleotide-activated cation channel. Gating kinetics of this channel have been studied under steady state conditions, i.e. in the continuous presence of cAMP or cGMP. It is known, however, that in response to odors cAMP production is transient, with a peak (in vivo) around 50 msec.

Here we have studied the gating kinetics of recombinant cyclic nucleotide channels from rat olfactory neurons (expressed in a kidney cell line) and native channels from salamander olfactory neurons with fast application and removal of cyclic nucleotides. Agonist pulses were delivered by a piezo-driven 'liquid filament' that allowed solution exchange at excised membrane patches in less than 200 us. Upon application of a step of cyclic nucleotide containing solution the current rose to plateau along a time course that was dependent on agonist concentration. With 1 mM cGMP the rise time could be as short as 2 ms, under optimal conditions. Upon rapid removal of the stimulus (i.e. stepping back to control solution) the current decay was much slower. Typically after removal of agonist (1 mM cGMP) several hundred milliseconds were required to reach 50% of the plateau value. Several tests were undertaken to assure that kinetic components were due to channel gating and not to restricted diffusion in the patch. These included tests of the voltage sensitivity of kinetic states and the use of solutions with different ionic compositions. Supported by NIH DC 00086 and Deutsche Forschungsgemeinschaft

115.10

FUNCTIONAL EXPRESSION OF $GABA_A$-Cl^- CHANNELS AND BENZODIAZEPINE BINDING SITES IN BACULOVIRUS INFECTED INSECT CELLS. D.B. Carter, D.R. Thomsen, W.B. Im*, D.J. Lennon, D.M. Ngo, W. Gale, H.K. Im, P.H. Seeburg and M.W. Smith. CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Recent cloning of the cDNAs coding for the subunits comprising the $GABA_A$-benzodiazepine receptors has afforded the opportunity to study this complex receptor-channel system in transient expression systems. However to date high level production of this receptor-channel complex with an intact benzodiazepine (BZD) binding site has not been possible in animal cells. Since it was recently shown that functional multi subunit proteins and ion channels could be expressed in the baculovirus expression system (BVS) we investigated the BVS for the high level production of the $GABA_A$-BZD Cl^- channel complex. Membranes from cells infected with baculoviruses harboring subunits encoding cDNAs of the rat $GABA_A$-BZD receptors were tested with a panel of well characterized small molecule ligands and found to have the appropriate binding pharmacology. The $GABA_A$ receptor subunit combinations investigated are $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$ and $\alpha_6\beta_2\gamma_2$. Several novel non-benzodiazepine ligands having low to high affinity for the $\alpha_6\beta_2\gamma_2$ are described. In addition, whole-cell patch clamp of infected cells indicates the formation of GABA-gated Cl^- channels having the appropriate responses to GABA and diazepam.

115.11

POTENTIATION OF GABA-INDUCED CHLORIDE CURRENT BY LANTHANIDES. M.Yan* and T.Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Lanthanides, or rare-earth metals, constitute a series of fifteen metals starting with lanthanum (atomic number, 57) and ending with lutetium (atomic number, 71). We have previously reported that lanthanum reversibly potentiates the GABA-induced chloride current by acting at a distinct site on the $GABA_A$ receptor-channel complex (Yan and Narahashi, Soc. Neurosci. Abstr. 17:1333, 1991). We now report that in addition to lanthanum, all other lanthanides tested potentiate the GABA-induced chloride current with greater efficiencies. The currents were recorded from the rat dorsal root ganglion neurons in primary culture by the whole cell patch clamp technique. At a concentration of 1 mM, lanthanides reversibly potentiated GABA response (times of control): lutetium (Lu^{3+}) 12.64 > erbium (Er^{3+}) 11.87 > terbium (Tb^{3+}) 10.28 > europium (Eu^{3+}) 6.15 > neodymium (Nd^{3+}) 4.32 > cerium (Ce^{3+}) 3.70 > lanthanum (La^{3+}) 2.41. This order of potentiation exactly follows the atomic number: the larger the atomic number, the greater the potentiation. The enhancing effect of Tb^{3+} was dose dependent with an EC_{50} around 350 μM and weakly voltage dependent increasing with hyperpolarization. Tb^{3+} shifted the GABA dose-response curve in the direction of lower concentrations without significantly changing the maximum response. These data strongly support the existence of a lanthanide regulatory site at the $GABA_A$ receptor-channel complex.

115.12

GLYCINERGIC INHIBITION IN THE MAMMALIAN OLFACTORY BULB. Paul Q. Trombley*& Gordon M. Shepherd. Section of Neurobiology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510.

Glycine is considered the principal inhibitory transmitter in the brainstem and spinal cord, whereas GABA is generally believed to dominate inhibition in the brain. In the olfactory bulb (OB) GABA localization to periglomerular and granule cells supports the notion that GABA contributes to the inhibition mediated by these cells. However, several laboratories have observed some components of inhibition in the OB that were resistant to GABA antagonists. In addition, recent immunohistochemical experiments have demonstrated localization of glycine receptors in the OB. These results have led us to hypothesize that glycine may contribute to synaptic inhibition in the OB.

We examined the responses of OB neurons to glycine using primary cell culture and whole cell recording techniques combined with a rapid flow drug application system. Olfactory bulb neuron cultures from E18-P2 rat pups contained both mitral/tufted cells and periglomerular/granule cells. All neurons tested (n=20) responded to glycine with an apparent EC50 of 125 μM. When intracellular [Cl^-] was altered, the membrane current reversed at the Nernst value for Cl^-, consistent with activation of the glycine gated Cl^- channel. The glycine evoked current was blocked by the glycine antagonist strychnine but not by the GABA antagonist bicuculline. GABA evoked a distinct Cl^- current which was antagonized by bicuculline. All neurons responded to both glycine and GABA although the ratio of current amplitudes evoked by glycine vs GABA was not the same for all cells.

We propose that glycine as well as GABA may contribute to inhibitory mechanisms in the olfactory bulb. The use of more than one inhibitory transmitter could allow for differential inhibition through selective modulation. Glycine receptors for example, are not thought to be sensitive to substances that modulate GABA receptors. Our proposal is supported by immunohistochemical evidence demonstrating that glycine receptor immunoreactivity is localized in the external plexiform layer, whereas GABA immunoreactivity is highest in the glomerular layer.
Supported in part by an NRSA to PQT and an NIDCD research grant to GMS.

115.13

Cloning and Expression of the Rat $5HT_3$ Receptor Reveals Species-Specific Sensitivity to Curare Antagonism. David S. Johnson* and Stephen F. Heinemann. The Salk Institute, La Jolla, CA 92037; UCSD, Group in Neurosciences, San Diego, CA 92037.

We have cloned the rat homolog of the $5HT_3$ receptor cloned in mouse by Maricq et al. last year. The rat clone was isolated from a superior cervical ganglion cDNA library. The cDNA is 2230 bp and encodes a protein of 461 amino acids. The mature protein, after removal of the predicted signal peptide, exhibits 94% identity with the mouse receptor.

Voltage clamp studies of Xenopus oocytes injected with RNA transcripts of this clone demonstrate a serotonin-gated current which is sensitive to external Ca^{2+} concentrations and exhibits a region of negative-slope conductance in the IV curve. Preliminary pharmacological studies reveal similarities to the mouse $5HT_3$ receptor. One exception to the similarities is the sensitivity of the rat receptor to curare antagonism. Wheras the current induced by 10 μM 5HT in oocytes expressing the mouse $5HT_3$ receptor is completely blocked by 5 nM curare, currents in oocytes expressing the rat $5HT_3$ receptor are attenuated only about 20% by 100 nM curare. This is consistent with previous studies from other laboratories indicating murine neurons and cell lines which possess $5HT_3$ receptors are extremely sensitive to curare block, wheras $5HT_3$ receptors in other species (rat, rabbit, guinea pig) are roughly three orders of magnitide less sensitive to curare antagonism. We are conducting site-directed mutagenesis to determine which of the few amino acids which differ between the rat and mouse sequences confer the extreme sensitivity to curare.

115.14

SINGLE CHANNEL CONDUCTANCE UNDERLYING SEROTONIN DEPENDENT DEPOLARIZATION OF AN IDENTIFIED NEURON FROM HELISOMA. C. J. Price* and J. I. Goldberg. Department of Zoology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9.

The neurotransmitter serotonin (5-HT) regulates neurite outgrowth in an identified neuron from the snail *Helisoma trivolvis*. Membrane depolarization, involving gating of a sodium conducting channel, is necessary for this response to occur. To gain an understanding of the nature of the ion channel responsible, single channel studies have been initiated. Cell-attached patch recordings were made from the soma of neuron B19 isolated in cell culture (pipette solution: 70mM NaCl and 5mM Hepes; pH 7.4). These recordings revealed an inwardly directed current, with a conductance of approximately 5pS, that appeared following bath application of 5-HT (final concentration: 25μM). Such single channel events were, however, rather rare in occurrence, since less than 20% of patches contained channel activity. Channel openings were generally burst-like in nature, involving flickering between open and closed states. Moreover, channel activity was typically interrupted by variable periods of inactivity, that ranged from several seconds to minutes in duration. Owing to the nature of the recordings performed and the method of 5-HT application, a diffusible second messenger is likely involved in the gating of this channel. This is consistent with findings from whole cell recordings which implicated a role for cAMP in the generation of serotonin dependent depolarization.

Supported by Natural Sciences and Engineering Research Council of Canada.

115.15

ATOMIC FORCE MICROSCOPY IMAGING OF CLONED NEUROTRANSMITTER RECEPTORS. R. Lal[1]*, J. Liu[2], J. Hurley[2], L. J. Bloem[2] and L. Yu[2]. [1]Dept. of Med., Univ. of Chicago, Chicago, IL 60637; [2]Dept. of Med/Mol Genetics, Indiana Univ. Sch. Med., Indnpls, IN 46202.

Atomic force microscopy (AFM) is a powerful tool that allows high resolution imaging of the surface topography with nondestructive scanning probes under physiological conditions. A large numbers of membrane proteins, such as receptors, ion channels, and transporters, have been molecularly cloned. The structural information of most of these proteins, however, is lacking. In an attempt to develop a convenient method for obtaining such information, we used two expression systems, *Xenopus* oocytes and COS cells, for AFM imaging of cloned receptors. Two neurotransmitter receptors were studied: the nicotinic acetylcholine receptor (AChR), an ion channel, and the serotonin 1c (5-HT_{1C}) receptor, a G protein-coupled receptor. The AChR was expressed in *Xenopus* oocytes by microinjection of the mRNAs synthesized *in vitro* from the cDNA clones for the mouse muscle AChR α, β, γ, and δ subunits. Recording of these oocytes by two-electrode voltage clamp showed an ACh-induced slow depolarizing current, indicating the functional expression of the AChR. AFM imaging of these oocytes revealed a pentameric structure of ~ 7 - 9 nm in diameter with a central pore, consistent with the proposed AChR topography. The 5-HT_{1C} receptor was expressed in COS cells from a mouse 5-HT_{1C} receptor cDNA clone, as confirmed by membrane binding to radiolabeled mesulurgine. AFM imaging showed a protein protrusion of ~ 5 - 7 nm in diameter without a central pore, consistent with the calculated molecular size of the receptor. (Supported by grants from NIH, Whitaker Foundation, and Dept. of Medicine.)

115.16

WITHDRAWN

115.17

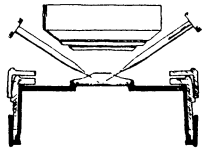
PROTON-GATED SODIUM CURRENT IN MESECENPHALIC DOPAMINERGIC NEURONS.
Andrew Bullen* & Martin Morad. Dept. of Physiology. University of Pennsylvania. Philadelphia, Pa 19104-6085

Small acid shifts (to pH = 6.7) in external solution were used to activate a fast inward Na^+ current from mesecenphalic neurons in primary culture. Currents were measured from all neurons present (after 5 days) but predominantly from dopaminergic cells. Dopaminergic neurons were identified by their uptake of the autofluorescent marker, 5,7-DHT (Silva et al, 1988). Currents were measured in both the whole cell and perforated patch configuration of the patch clamp technique (Hamill et al, 1981). Solutions bathing the patched cell were changed using a rapid perfusion system. In a control solution, a shift to pH = 6.7 elicited a fast activating (τ = 0.2 s) inward current of 0.5 - 3 nA. This current inactivated in the continued presence of an activating pH and rapidly deactivated if the bathing solution was returned to pH = 7.4. The size of this current was graded with proton concentration reaching a maximum at pH = 6.5. The current reversed close to sodium equilibrium potential suggesting Na was the primary charge carrier. However, the relative selectivity of this channel to Ca^{++} and K^+ remains to be quantified. Although this current was somewhat similar to other proton-gated currents seen in peripheral neurons (Krishtal & Pidoplichko, 1981; Konnerth et al, 1987) it had significantly different inactivation kinetics (τ= 2.4 s vs 0.3 s). The physiological role of this proton-gated current remains to be determined but it appears some form of this current is expressed in many neuron types.

115.18

PATCH-CLAMP RECORDING IN HIPPOCAMPAL SLICES USING DRY OBJECTIVE. P. Bregestovski, A. Kurchikov and Y. Ben-Ari*INSERM, Unité 29, Hopital Port-Royal, 75014, Paris, FRANCE

High-resolution patch -clamp recording from slices provides a wide range of possibilities for the analysis of molecular mechanisms of mammalian CNS functioning. In the method originally developed by Edwards et al. (Pflugers Arch, **414**: 600, 1989) a water immersion objective was used to identify single neurons in thin slices . However, this approach has two inconveniences: (i) limited working distance (less than 1,6 mm with a x40 objective) and restricted space electrode manipulation; (ii) high probability of damage of the expensive water immersion objective by leak curren via small scratches of the insulation. We have now developed a new chamber with a cover glass support system. This allows the use of an upright microscope with a dry long distance (5 mm) objective (Plan x40 NIKON). The slice is located in the water stream with about 4 mm of free space between the cover glass and the surface of the slice. In contrast to the system of Edwards et al., we can bring the recording and the stimulation electrodes to the preparation at a high angle ($\gtrsim$ 45°). With an ordinary TV-camera one can readily visualize pyramidal cells and interneurones lying on the surface of the slice. Using this chamber, whole-cell and single channel ionic currents from neurones in neonatal hippocampal slices have been recorded.



115.19

SK&F 96365 INHIBITS PHOSPHOINOSITIDE HYDROLYSIS BY BLOCKING RECEPTOR-MEDIATED CALCIUM INFLUX. W.C. Moore*, H.H. Hargrove, and J. Patel, Dept. of Pharmacology, ICI Americas Inc., Wilmington, DE 19897.

Calcium response to agonist-stimulated phosphoinositide (PI) hydrolysis is typically biphasic. It is composed of an initial transient increase, corresponding to the release of calcium from intracellular stores, followed by a sustained elevation of calcium due to influx. In fura-2 loaded SH-SY5Y neuroblastoma cells, SK&F 96365 (30μM) was shown to selectively block carbachol- (CB) mediated Ca^{2+} influx without effecting the initial intracellular Ca^{2+} release. Similar results were observed with La^{3+} (10μM), however, nitrendipine (10μM) and ω-conotoxin (10nM) were without effect. These data demonstrate that the CB-stimulated Ca^{2+} entry in neuronal cells is via a SK&F 96365-sensitive, non-voltage-operated calcium channel.

Previously it has been demonstrated that sustained PI hydrolysis in neuronal cells requires continued influx of extracellular Ca^{2+}. In the present study, SK&F 96365 inhibited CB-stimulated PI turnover in SH-SY5Y cells with an apparent IC50 of 29.1 μM. No inhibition of PI hydrolysis was observed in the presence of maximal concentrations of nitrendipine or ω-conotoxin. Inhibition of PI hydrolysis was not the result of muscarinic receptor antagonism as SK&F 96365 showed very weak inhibition of ^{3}H-QNB binding. Additionally, it was determined that SK&F 96365 was able to inhibit quisqualate- and CB-stimulated PI hydrolysis in primary cultures of cortical neurons with a potency similar to that found in SH-SY5Y cells. Taken together, these data suggest that SK&F 96365 inhibits PI hydrolysis in neuronal cells by inhibiting receptor-mediated calcium influx.

116.1

PHYSIOLOGICAL RELEASE OF ACETYLCHOLINE IN RAT STRIATUM MONITORED *IN VIVO*: MODULATION BY DOPAMINERGIC DRUGS.

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Striatal acetylcholine (ACh) release, as measured with *in vivo* microdialysis, is stimulated by systemic administration of dopamine (DA) D-1 receptor agonists and is inhibited by systemic or local administration of DA D-2 receptor agonists. When indirect DA agonists like amphetamine (AMPH) are given systemically, the stimulatory effect of DA on *in vivo* striatal ACh output predominates. Tissue levels of striatal ACh, however, are found to increase after such treatments, suggesting a decrease in ACh utilization. To determine whether the presence of an inhibitor of acetylcholinesterase (AChE) might have altered the pharmacological responsiveness of striatal ACh in previous *in vivo* studies, the effect of DA-ergic drugs on the output of striatal ACh was studied using *in vivo* microdialysis in the absence of AChE inhibition. ACh output was 2.7 **fmol**/sample under physiological conditions vs. 1.44 **pmol**/sample in the presence of the AChE inhibitor neostigmine (NEO; 300 nmol/l). Systemic AMPH (2 mg/kg) increased the output of ACh by 116% under physiological conditions. This increase was greater (180%) and more prolonged in the presence of NEO. Increases in striatal ACh output after AMPH (without NEO) most closely paralleled increases in the output of DA in frontal cortex and substantia nigra rather than DA changes in striatum. Further pharmacological characterization of striatal ACh output in the absence of AChE inhibition revealed an increased output after systemic administration of the D-1 agonist CY 208-243 (2 mg/kg; 180% of control) and a decreased output after systemic administration of the D-2 agonist quinpirole (3 mg/kg; 48% of control). These data show that 1) DA D-1 receptors stimulate and DA D-2 receptors inhibit striatal ACh output measured under physiologically relevant conditions, 2) AChE inhibition alters pharmacological responsivity of striatal cholinergic neurons, and 3) changes in tissue levels of neurotransmitters do not neccesarily correlate with release. (Supported in part by USPHS grant NS19608, American Parkinson's Disease Association (EDA) and Tourette Syndrome Association (PDB)

116.2

ACETYLCHOLINE MICRODIALYSIS IN THE FREELY MOVING RAT USING HPLC-ED: METHODOLOGICAL ASPECTS.

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Microdialysis is a well established technique which permits direct *in vivo* sampling of neurotransmitters from specific brain regions in the conscious animal. High performance liquid chromatography with electrochemical detection (HPLC-ED) is a sensitive method for the measurement of acetylcholine (ACh). Enzyme catalysis is necessary to detect ACh and its metabolite choline (Ch) which are not electroactive and do not absorb U.V. light under normal conditions. The method consists of the separation of ACh and Ch by reverse phase HPLC followed by the detection of hydrogen peroxide (H_2O_2) produced enzymatically by the reaction of the column effluent with acetylcholinesterase (AChE) and choline oxidase (ChO) which are immobilised on a small column. AChE converts ACh to Ch and acetate, the former is then broken down to betaine with the production of H_2O_2 In previous studies where the enzymes are immobilised on a post column reactor (IMER-Immobilised Enzyme Reactor) the detection of ACh in dialysate samples from freely moving Hooded Lister rats was limited both by inadequate chromatographic separation of ACh from the solvent front and Ch peak, and baseline noise. In current studies the use of a novel double IMER system where a second IMER, containing ChO only, is placed before the analytical column and a new prototype platinum electrode developed by Antec Analytical Systems has led to improved chromatographic separation with reduced baseline noise. The system is currently being used to investigate the effects of the benzodiazepine inverse agonists, S 135 and RU 33965 on ACh release in the hippocampus of Hooded Lister rats using microdialysis. Full metholodological details of the system and drug effects will be presented.

R.S.K is sponsored by Roussel Laborarories.

116.3

ULTRASENSITIVE MEASUREMENT OF ACETYLCHOLINE RELEASE IN THE CONSCIOUS HUMAN HIPPOCAMPUS AND ANESTHETIZED RAT STRIATUM USING MICRODIALYSIS.

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Due to the low basal levels of acetylcholine (ACh) present in brain microdialysis samples, the use of a highly sensitive and selective mode of detection is necessary. We have improved on current methodologies for the simultaneous analysis of ACh and choline (Ch) using electrochemical detection. The limit of detection for both ACh and Ch is below 50 femtomoles. With this method, the analysis of ACh in rat and human microdialysis samples is possible using ultralow levels of an acetylcholinesterase (AChE) inhibitor. Use of high levels of AChE inhibitors significantly interferes with the responsiveness of muscarinic autoreceptors. Thus, lower amounts of inhibitor should minimize the degree to which physiological conditions are altered.

ACh and Ch release was monitored in the striatum of anesthetized rat over a range of AChE inhibitor concentrations, 10nM to 10μM Neostigmine, in the artificial CSF perfusate. At the lowest inhibitor level (10nM Neostigmine), basal ACh and Ch levels were determined to be 36.7±7.2fmol and 21.8±2.7pmol per 10μL (mean±S.E.M.,n=6) respectively.

Intrahippocampal human microdialysis samples from an awake epileptic patient were also monitored for ACh and Ch. With 150nM Neostigmine in the artificial CSF perfusate, basal levels were determined to be 2.6±0.2pmol ACh and 65.3±6.4pmol Ch per 10μL (mean±S.E.M.,n=10). Since these human ACh values do not approach the sensitivity limits of this system, Neostigmine concentrations lower than 150nM can easily be used or temporal resolution can be enhanced.

116.4

LESIONS OF THE MESOTELENCEPHALIC DOPAMINE SYSTEM ENHANCE THE EFFECTS OF SELECTIVE D1 AND D2 DOPAMINE RECEPTOR AGONISTS ON STRIATAL ACETYLCHOLINE RELEASE.

G.W. HUBERT, G.S. ROBERTSON*, C.-S. THAM, AND H.C. FIBIGER. Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3

In vivo microdialysis was used to determine the effects of 6-hydroxydopamine (6-OHDA) lesions of the mesotelencephalic dopamine system on dopamine receptor agonist induced changes in extracellular acetylcholine (ACh) concentrations in the striatum. Such lesions increased the inhibitory effect of a low dose of the D2 receptor agonist quinpirole on striatal ACh release. In addition, 6-OHDA lesions enhanced the facilitatory effect of the selective D1 receptor agonist CY 208-243 on striatal ACh release, enabling a subthreshold dose to increase striatal dialysate concentrations of ACh by over 60%. These results indicate that denervation supersensitivity potentiates both the facilitatory effects of D1 receptor agonists and the inhibitory effects of D2 receptor agonists on striatal cholinergic activity. It was also found that the 6-OHDA lesions reduced basal interstitial ACh concentrations by 75% in the ipsilateral striatum. The latter results are consistent with the hypothesis that the prepotent action of dopamine in the forebrain is to enhance striatal ACh release via a D1 mechanism. Experiments are underway to identify the locus of the D1 mediated enhancement of striatal ACh release.

116.5

COCAINE RELEASES ACETYLCHOLINE VIA AN ACTION OF DOPAMINE ON D1 RECEPTORS.

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The effect of cocaine on dopamine (DA) and acetylcholine (ACh) release in the striatum was compared using brain microdialysis in freely moving rats. Cocaine (10 and 20 mg/kg i.p.) produced a parallel increase in the dialysate content of DA (by 350 and 500%) and ACh (by 55 and 80%). Release of DA and ACh by cocaine was markedly enhanced by the blockade of D2 receptors with (-) sulpiride (10 mg/kg s.c.). On the other hand, blockade of D1 receptors with SCh 23390 (0.050 mg/kg s.c.) prevented cocaine-induced ACh release but not that of DA. These results suggest that ACh release stimulation after cocaine results from two opposite effects mediated by DA on D1 and D2 receptors, and that the effect of DA on D1 is predominant in respect to that exerted on D2 receptors. To further clarify the role of endogenous DA on ACh output, rats were treated with reserpine (3 mg/kg i.p.) and both DA and ACh release monitored at various time afterwards. Following reserpine administration both DA and ACh output underwent an initial increase (by 65 and 35% respectively) during the first two hours when animals were hyperactive, followed by a prolonged decrease (by 85 and 80% respectively, over at least 9 hours) when animals were sedated. Reserpine-induced increase in ACh output was prevented by SCh 23390 but was potentiated by (-) sulpiride. The results suggest that an increased output of endogenous DA causes an increased ACh release mediated by a preferential stimulation of D1 receptors.

116.6

DOPAMINERGIC REGULATION OF CORTICAL ACETYLCHOLINE RELEASE.

J. DAY* and H.C. FIBIGER. Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1Z3

Cortically projecting basal forebrain cholinergic neurons are thought to play a major role in cortical activation. The transsynaptic regulation of this cholinergic system is thus of great interest. We have previously reported that amphetamine-induced increases in cortical acetylcholine (ACh) release can be partially attenuated by dopamine (DA) receptor antagonists and that the non-selective DA receptor agonist apormorphine can increase ACh release. To further define the regulation of the basal forebrain cholinergic system by DA receptor mechanisms, *in vivo* microdialysis and HPLC-ECD was used in the present experiments to assess the effects of selective DA receptor agonists on cortical ACh release. The ability of selective DA receptor antagonists to block apomorphine-induced increases in ACh release was also studied.

The D_1 receptor agonist CY 208-243 (1.0 mg/kg) significantly increased cortical dialysate concentrations of ACh to 180% of baseline. The D_2 agonists quinpirole (0.2 and 0.5 mg/kg) and PHNO (0.05 mg/kg) did not significantly affect ACh release. In addition to having no effect on basal ACh release, quinpirole (0.2 mg/kg) did not potentiate the D_1-mediated increase. The increase in ACh release (220% of baseline) caused by apomorphine (1.0 mg/kg) was completely blocked by the D_1 receptor antagonist SCH 23390 (0.3 mg/kg). The D_2 receptor antagonist raclopride (1.0 mg/kg) did not significantly attenuate the apomorphine-induced increase. The above results confirm that the basal forebrain cholinergic system is regulated in an excitatory manner by DA and indicate that D_1 receptor mechanisms are preferentially involved.

116.7

DOPAMINE RELEASE AND TURNING BEHAVIOR EVOKED BY INTRANIGRAL CARBACHOL: A MICRODYALISIS STUDY. **S. Hernández, J.L. Góngora, D. Martínez-Fong*, M. Rosales and J. Aceves.**
Dept. of Physiology, CINVESTAV-IPN, Ap. Postal 14740, 07000 México, D.F., México.

A recent immunological study demonstrated that cholinergic terminals make synaptic contacts with dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) of the rat (Bolam et. al., Neurosci. 41:483, 1991). The aim here was to evaluate the striatal DA release and the turning behavior evoked by activation of acetylcholine receptors in the SNc. Male Wistar rats (280-320g) were anaesthetized and implanted with a guide cannula into right striatum. Five or seven days after surgery, DA was collected by a microdyalisis probe and measured by HPLC. Carbachol or vehicle were injected into right SNc. Carbachol (2 μg) administrated into caudal SNc increased the DA release (50%) and a concomitant contralateral turning was observed. In contrast, when carbachol was applied into rostral SNc, striatal DA decreased (46%) and ipsilateral turning was elicited. This results indicate the existence of at least two subpopulations of DA neurons in the SNc which differently respond to carbachol application.
(Supported by CONACYT No. P228CCOX891561)

116.8

MEASUREMENTS OF ACETYLCHOLINE (ACh) RELEASE FROM THE BARREL FIELD OF RAT SOMATOSENSORY CORTEX. M.E. Jiménez-Capdeville and R.W. Dykes*. Centre de recherche en sciences neurologiques Départements de physiologie Université de Montréal (Québec) CANADA.

ACh release is correlated with wakefulness and other factors related to levels of cortical activity. The technique of microdialysis in awake, unrestrained animals provides an opportunity to measure regional variations in ACh release that may be correlated with local neuronal activity. Three mm microdialysis probes (BAS) were obliquely inserted into the vibrissae representation of the primary somatosensory cortex. The collection of samples started 48 hours after the probe implantation and continued during a 24 hours period.The perfusion medium, a Ringer solution containing 5 μM neostigmine bromide, was pumped through the probe at a rate of 1.5 μl/min. One hundred and fifty μl aliquots were obtained each 100 min and immediatly injected into a reverse-phase HPLC column coupled to an electrochemical detector. The retention time for ACh in our system was 9 min and the limit of detection was 500 fmoles. After the Ach content of the sample was corrected for the probe efficiency,the results from 8 rats showed a release rate of 264 ± 22 fmol/min (x ± SEM, n=51). This value varied among animals ranging from x=189 fmol/min to x=525 fmol/min during the day and increasing up to twice these values in the evening. At the end of each experiment, the animal was sacrificed and the location of the probe in the brain was verified histologically.
Supported by the Medical Research Council of Canada MA 8700.

116.9

NICOTINIC AGENTS ENHANCE ACETYLCHOLINE RELEASE FROM RAT FRONTAL CORTEX. Kathleen L. Summers*, Ezio Giacobini and Elizabeth Williams. Dept. Pharmacology, Southern Illinois Univ. Sch. of Med., Springfield, IL 62794-9230

Nicotine has been shown to improve learning as well as short- and long-term memory. Patients with Alzheimer disease (AD) may benefit from treatment with nicotinic agents. We have studied the effects of nicotine and nicotine analogues on cortical acetylcholine (ACh) release in rat cortex in vivo with microdialysis and in vitro in slices. Nicotine (.59 mg/kg s.c.) produces a significant (40%) and long-lasting (3 hrs) increase in cortical ACh as determined by microdialysis without a cholinesterase inhibitor in the probe. We found that its presence decreases the duration of the nicotinic effect, probably by activating muscarinic autoinhibitory receptors. Nicotine (3.6 μM) also increases the potassium-evoked release of ^{3}H-ACh from cortical slices. Total ACh levels in cortex and striatum were found to remain constant or decrease slightly, supporting the hypothesis that nicotine increases release rather than synthesis of ACh. (Supported in part by R.J. Reynolds Tobacco Co.; NIA #P30 AG08014; S.I.U. Central Research Committee)

116.10

EFFECTS OF NICOTINIC ACETYLCHOLINE RECEPTORS ON MOTOR NERVE. R.J. Storella*, J. Hill and S.T. Begen. Dept. of Anesthesiology, Hahnemann U., Philadelphia, PA 19102

Previous studies on acetylcholine (ACh) overflow using anticholinesterases have not controlled for potential drug effects on muscarinic receptors and resting release. We measured ACh overflow from 6 mouse phrenic nerve diaphragms perfused with neostigmine (10 μM), atropine (20 μM) and d-tubocurarine (dTC). ACh was assayed by HPLC. Nerve was stimulated for 20 minutes with trains of 5 stimuli at 10 Hz, equilibrated first with 40 μM then 0.4 μM dTC. Perfusate was collected every 5 min. Resting release was greater with 0.4 μM than 40 μM dTC (0.66±.08[SE] vs 0.36±.09 pmole/min, paired T-test). ACh collected at steady-state release during stimulation (last 10 minutes) was greater for 0.4 than 40 μM dTC (2.04±.16 and 2.10±.22 vs. 1.77±.20 and 1.78±.17 pmole/min, repeated measures ANOVA). However there was no difference between 0.4 and 40 μM dTC (1.38±.11 and 1.44±.16 vs. 1.41±.14 and 1.42±0.11, repeated measures ANOVA) in evoked release (i.e. stimulated minus resting). Twitch tension was blocked completely at 40 μM, but only partially at 0.4 μM dTC (peak block = 59±4.7%, compared to drug free Krebs control). Thus, compared to 40 μM dTC, the 0.4 μM dTC allowed greater activation of nicotinic ACh receptors. We conclude that when muscarinic receptors are blocked, activation of nicotinic AChR may increase total ACh release but not evoked release.

116.11

NMDA RECEPTORS MODULATE THE SEPTO-HIPPOCAMPAL CHOLINERGIC PATHWAY THROUGH GABAERGIC INTERNEURONS.
M.G.Giovannini, L.Bianchi and G.Pepeu*. Dept.of Pharmacology, University of Florence, 50134 Florence, Italy.

The regulation of the septo-hippocampal cholinergic pathway was investigated by transversal microdialysis technique in freely moving male adult rats. ACh and GABA in the perfusate were quantified by HPLC methods. I.c.v. injections of the NMDA antagonists CPP, MK801 and AP-7 brought about an increase in ACh release while the non-NMDA receptor antagonist DNQX had no effect. The increase following CPP injection was dose-dependent, in the range of 1-50 nmol, and reached a maximum of about +500%. It was abolished by TTX perfused through the dialysis probe, and prevented by i.c.v. injection of muscimol. CPP perfusion through the septum resulted in a decrease in GABA release from the septum and a concomitant increase in ACh release from the hippocampus. Muscimol administration to the septum abolished the effect of CCP. These results demonstrate that in the septum NMDA receptors tonically activate GABAergic neurons which in turn inhibit the cholinergic septo-hippocampal neurons.
Supported by a grant from MURST, Italy.

116.12

EVIDENCE FOR FACILITATORY α_1 ADRENOCEPTORS ON NERVE TERMINALS OF THE RAT URINARY BLADDER G.T. Somogyi*, M. Tanowitz and W.C. de Groat, Department of Pharmacology, University of Pittsburgh, Pittsburgh PA, 15261.

Adrenergic facilitatory modulation of cholinergic transmission was studied in the urinary bladder (UB) of the rat. ^{3}H-ACh release and contractions evoked by electrical field stimulation were measured in UB strips prelabelled with ^{3}H-choline. Phenylephrine (PE 0.2-10 μM), an α_1 adrenoceptor agonist, increased 3H-ACh release and evoked contractions in a dose dependent manner. The α_1 adrenoceptor blocker terazosin (0.1-1 μM)(TRZ) or prazosin (1.0-5.0 μM) (PRZ) blocked both effects. TRZ was more potent than PRZ. The facilitation of the evoked contractions was also blocked by 0.1 μM WB 4101, a selective α_1 antagonist. Atropine (1 μM) reduced UB contractions, but did not block the facilitatory effect of PE. A reduction in the concentration of the Ca^{2+} in the perfusion fluid to 0.75-1 mM reduced ^{3}H-ACh release, but enhanced the facilitatory effect of PE. PE did not change the release of norepinephrine from UB strips. It is concluded that α_1 adrenoceptor-mediated facilitation occurs on cholinergic and non-cholinergic non-noradrenergic nerve terminals, but not on adrenergic terminals in the rat UB and that the magnitude of facilitation is inversely related to Ca_o^{2+}.
Supported by a grant from Abbott Labs.

116.13

ACETYLCHOLINE RELEASE IN THE MEDIAL PONTINE RETICULAR FORMATION (mPRF) IS INHIBITED BY SYSTEMIC MORPHINE. J.C.Keifer, H.A.Baghdoyan, R.Lydic (Spon:J.F.Biebuyck*) Dept. of Anesthesia, Penn State Univ., Col. Med., Hershey, PA 17033.

The mPRF has been well characterized as a site mediating the cholinergically induced REM sleep-like state and state-dependent respiratory depression. Recent studies in our laboratory showed that morphine sulfate microinjected into the mPRF decreased the REM sleep-like state and increased apneas (Keifer et al, *Neurosci. Absts. 17*:620, 1991). Since morphine suppresses acetylcholine (ACh) release in a variety of central nervous system structures, we are testing the hypothesis that morphine inhibits REM sleep by suppressing ACh release in the mPRF. The effect of systemic morphine on mPRF ACh release evoked by electrical stimulation of the pedunculopontine tegmental nucleus (PPT) in barbiturate anesthetized cats was measured. PPT stimulation caused a significant increase (241%) in mean ACh release (2.46 pmole) compared to mPRF dialysis with no PPT stimulation (0.72 pmole). Following the administration of intravenous morphine sulphate (283ug/kg) there was a significant reduction (41%) in PPT-evoked ACh release (1.45 pmole). These data support the hypothesis that morphine inhibits REM sleep by decreasing ACh release in the mPRF. *Support: Parker B. Francis Young Investigator Award (JCK), MH45361 (HAB), HL40881(RL).*

116.14

NIMODIPINE PREVENTS THE DECREASE IN HIPPOCAMPAL EXTRACELLULAR ACETYLCHOLINE RELEASE PRODUCED BY HYPOBARIC HYPOXIA. B. Shukitt-Hale*,[1] M.J. Stillman,[1] A. Levy,[2] D.I. Welch, J.A. Devine, and H.R. Lieberman, Military Performance and Neuroscience, U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760, [1]GEO-CENTERS, INC., Newton Centre, MA 02159, and [2]IIBR, Ness Ziona, Israel.

Hypoxia has been shown to decrease acetylcholine (ACh) synthesis and release *in vitro*, and to decrease ACh synthesis *in vivo*; however, it is not known whether it affects extracellular release of ACh *in vivo*. Since the calcium channel blocker nimodipine has been shown to increase extracellular ACh, it may provide protection from the effects of hypobaric hypoxia on the cholinergic system. The purpose of this study was to examine the effect of hypobaric hypoxia on extracellular ACh levels, as measured by microdialysis, and to test the effects of nimodipine under these conditions. Microdialysis guide cannulae were implanted into the hippocampal region of male Fischer rats so that probes would sample from the CA1 and DG regions. Animals were then exposed for eight hours to a simulated altitude of 5500 m (18,000 ft) or tested at sea level for an equivalent duration. High performance liquid chromatography with electrochemical detection was used for on-line analysis of the dialysates. At 5500 m extracellular ACh levels in the placebo-treated group were significantly lower than the sea level group values. This decrement was reversed by nimodipine administered i.p. immediately preceding altitude ascent (10 mg/kg) and 250 min post-altitude ascent (10 mg/kg). These data suggest that nimodipine may provide protection from the detrimental effects of hypoxia on hippocampal cholinergic functions.

ACETYLCHOLINE: MUSCARINIC RECEPTORS I

117.1

MUSCARINIC RECEPTOR SUBTYPES IN INTRAPARENCHYMAL MICROVESSELS FROM BOVINE AND HUMAN CEREBRAL CORTEX. D.G. Linville* and E. Hamel, Montreal Neurological Institute, McGill University, Montréal, Québec, Canada H3A 2B4

Acetylcholine is a recognized regulator of cortical cerebral blood flow, possibly via interaction with vascular muscarinic receptors. Since these receptors are highly heterogeneous, the subtypes present in bovine and human cerebral cortical microvessels (MV) were characterized pharmacologically by competition studies against [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) binding. Both bovine and human MV were highly enriched in marker enzymes alkaline phosphatase and γ-glutamyl transpeptidase. The [^{3}H]-NMS binding parameters were comparable in bovine and human MV (B_{max} = 29.8 $\pm$ 3.9 and 25.6 fmol /mg protein and K_D = 183 $\pm$ 57 and 178 pM, respectively). In competition studies, the antagonist vascular potencies (pK_D) were (bovine; human): HHSiD (7.15; 7.47); AF-DX 384 (7.33; 7.44); AQ-RA 741 (7.41; 6.91); methoctramine (7.41; 6.89) and DAU 6202 (8.16; 8.62). In bovine MV, pirenzepine delineated two sites, one of high (pK_{DH}=8.25) and one of low affinity (pK_{DL} = 6.88). In contrast, in human MV, pirenzepine was best fitted to a single site (pK_D = 7.87). Using correlation analysis with the antagonists published affinities at various muscarinic receptors, m_1 sites were identified in both bovine and human MV. The m_2 and m_5 subtypes were excluded in both species but the m_3 and m_4 remained as possible candidates for the low affinity site identified with pirenzepine in bovine MV. In conclusion, bovine MV appear to contain a heterogeneous population of muscarinic receptors while in human, a single population of m_1 sites was identified. These results suggest species differences in cholinergic regulation of local brain perfusion. Supported by MRC of Canada, FRSQ and Dr. Karl Thomae GmbH.

117.2

MOLECULAR CLONING OF BOVINE MUSCARINIC ACETYLCHOLINE m3 RECEPTOR. P.H.K. Lee*, P.K. Hodges, J. F. Glickman and K.-J. Chang, Division of Cell Biology, Burroughs Wellcome Co , Research Triangle Park, NC 27709.

With the use of the polymerase chain reaction (PCR), we have taken advantage of the sequence similarity at the seven transmembrane domains displayed by known G protein-coupled receptors to amplify and clone new gene members of this receptor family. Degenerate oligonucleotide primers were designed according to the conserved regions corresponding to transmembrane regions III and VI of cloned G protein-coupled receptors. cDNA obtained by reverse transcription of poly (A)+ RNA from bovine striatum was submitted to 30 cycles of PCR with the primers. When examined by 1% agarose gel, the PCR product revealed a major band at about 600 bp and another at 800 bp. The bands were excised and purified for subcloning in M13 and sequenced. Three clones had sequence characteristics similar to the G protein-coupled receptor gene family. One of these clones was used as a probe in screening of a bovine brain cDNA library by plaque hybridization method. Six clones were identified and one, S40A1, has 2538 bp and contains an open reading frame of 590 amino acids. The hydropathy profile analysis suggested that this protein comprised of seven putative transmembrane segments. Comparisons of S40A1 with those cloned G protein-coupled receptors show that the protein is highly homologous to porcine (98%), human (95%) and rat (92%) m3 receptors, therefore suggesting that S40A1 is encoding a bovine muscarinic acetylcholine m3 receptor.

117.3

DEVELOPMENT OF SITE-DIRECTED POLYCLONAL ANTIBODIES AGAINST m1, m2, and m4 MUSCARINIC CHOLINERGIC RECEPTORS. A. Hersi*, R. Quirion and P. Gaudreau, Douglas Hospital Research Ctr., Dept. Psychiatry, McGill University and Notre-Dame Hospital Research Ctr., Dept. Medicine, University of Montreal, Montreal, Canada.

Five subtypes (m1-m5) of muscarinic receptors have been cloned. However, their pharmacological and biochemical study is complicated in the brain because many regions express a mixed population of receptors. Consequently, we have developed site-directed polyclonal antibodies against muscarinic receptors. Peptides corresponding to subtype specific sequences of the carboxy-terminus of m1 and the third cytoplasmic loop of m2 and m4 rat muscarinic receptors were synthesized by solid-phase peptide chemistry. Rabbits were immunized with the purified peptides adsorbed onto methylated bovine serum albumin as carrier protein, in Freund's adjuvant. Both crude antisera and affinity purified IgGs are functioning in Western blot. In rat brain, a major band was labeled with each antisera while no signal was detected with pre-immune sera. This major band was erasable by pre-incubating the antisera with the corresponding but not hetero-logous peptides. Similar results were observed in human post-mortem brain tissues. The apparent molecular weight of these bands were 90, 88 and 60 Kd for m1, m2 and m4, respectively. These antibodies should prove useful in assessing the fate of muscarinic receptor subtypes in neurode generative processes such as Alzheimer's disease.

117.4

ONTOGENY OF m1-m5 MUSCARINIC ACETYLCHOLINE RECEPTOR GENE EXPRESSION IN THE RAT CNS. S. Vincent*, L. Tsiokas and M. Watson. Department of Pharmacology, UMDNJ-NJ Med. Sch., Newark, NJ 07103-2714.

The postnatal ontogeny of muscarinic receptor (mAChR) subtypes in rat brain (10um) sections at days 1,7,14,21, 28,35 and adult (3mo) was studied via quantitative ligand autoradiography and in situ hybridization histochemistry (ISHH). LiCl was used in cerebral cortical slices (350x350um) to study inositol phospholipid (IP) hydrolysis by prelabeling with myo[-2-3H]inositol (.5uM) in Krebs buffer (95%O_2,5%CO_2). [3H](-)quinuclidinyl-benzilate, a specific nonsubtype selective antagonist, [3H]cismethyldioxolane, a label for the super high affinity agonist state, [3H]pirenzepine, an M1 antagonist, [3H]AF-DX 384, an M2 antagonist and [3H]hemicholinium-3, a Na+-dependent high affinity choline uptake inhibitor, were each used as previously described to obtain quantitative autoradiograms. Quantitative ISHH data was done via 3'-end labeled oligonucleotide probes (^{35}S-dATP; >1,000 Ci/mmol) complementary to 4-48 or 4-51 base sequences of m1-m5 mRNA after verifying specificity via Northern blot analysis. Sections were incubated (25°C, 24h), washed (Tm=55°C;0.5xSSC), dried, apposed (~4wk; 0-4°C) to Hyperfilm-βmax and quantified via DUMAS densitometer. Differential progressive alterations in m1-m5 mRNA and mAChR levels occur during ontogeny. (MH-43024).

117.5

DENERVATION-INDUCED CHANGES IN HIPPOCAMPAL MUSCARINIC RECEPTOR SUBTYPES AND THEIR mRNA. Z. Zang*, G. Buzsaki, and Ian Creese. Center for Molecular & Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ 07102.

Alzheimer's disease is associated with cholinergic neuronal cell loss in the basal forebrain. Previous studies have shown that the major cholinergic projection to the hippocampus is derived from the medial septum and can be destroyed by lesions of the fimbria and fornix. The present study examined the expression of muscarinic receptor subtype mRNAs (m1 and m3) by solution hybridization/RNase protection assay and the level of pharmacologically defined M_1 and non-M_1 muscarinic receptors by autoradiography in rat hippocampus following fimbria/fornix aspiration lesion. The receptor autoradiography data demonstrated that 3 weeks following unilateral lesion there was an overall 20% increase in non-pirenzepine displaceable [^{3}H]QNB binding in the denervated hippocampus (CA3 +51%, CA4 +75% and dentate gyrus +16%). In bilaterally lesioned rats, the solution hybridization/RNase protection analyses indicated that hippocampal m3 receptor mRNA was significantly increased (1 week post lesion: +22.7%, 3 weeks: +54.2%). However, the level of m1 receptor mRNA was not significantly affected by the lesion. These results suggest that: (a) the non-M_1 receptors are coded by the m3 gene, (b) the localization of these receptors is probably post-synaptic to the septohippocampal cholinergic terminals, and (c) they are upregulated following denervation.

Supported by a grant from the Stanley Foundation.

117.6

PLASTICITY OF MUSCARINIC RECEPTORS FOLLOWING CHOLINERGIC DENERVATION OF THE RAT HIPPOCAMPUS. V. Ayyagari*, L.E. Harrell, A.D. Peagler and D.S. Parsons. Behavioral Neuroscience Program, Dept.s of Neurology and Psychology, VA Medical Center and U. of Alabama, Birmingham, AL 35294-0017.

Cholinergic denervation (CD) of the rat hippocampus following electrolytic lesions of the medial septum (MS) results in abnormal adrenergic innervation of the hippocampus [Hippocampal sympathetic ingrowth (HSI)] by sympathetic fibers that originate from the superior cervical ganglion (SCG) and are normally restricted to the cerebral blood vessels. We now report that CD and HSI alter binding parameters of the muscarinic antagonist, Quinuclidinyl benzilate, L-[benzilic-4,4'-^{3}H]- (QNB). Adult male Sprague-Dawley rats were divided into 3 surgical groups: Control; CD (MS lesion + Ganglionectomy: Gx); or HSI (MS lesions + sham ganglionectomy). Receptor binding studies were performed on hippocampal membranes 4 weeks post-surgery, using 1 μM Atropine to measure non-specific binding. Preliminary results indicate that compared to controls, K_D was increased in the CD group, while HSI appeared to "normalize" the K_D; and the B_{max} was increased in both the experimental groups. These results indicate that HSI, which normalizes both carbachol-stimulated PI hydrolysis, and protein kinase C (PKC) levels at this time point after surgery, may actually affect the entire cholinergic signal transduction pathway. This in turn may contribute to the observable behavioral deficits induced by HSI.

117.7

EFFECTS OF CHRONIC DFP ON MUSCARINIC AND NICOTINIC NEUROTRANSMISSION DURING RAT BRAIN DEVELOPMENT. M. Cimino, W. Balduini, F. Reno', P. Marini, A. Principalle, C. Tromba*, F. Clementi, F. Cattabeni. Inst. of Pharmacol. and Pharmacogn., Univ. of Urbino, Urbino; Inst. of Pharmacol. Sci. and CNR Center of Cytopharmacol. and Dept. of Medical Pharmacol. Univ. of Milano, Italy.

Organophosphates are widely used as pesticides and as experimental tools to study the regulation of cholinergic receptors due to their activity as acetylcholinesterase inhibitors. However, little is known about the effects elicited by the chronic exposure to these compounds during brain maturation. In this study, we have chosen two particular stages of brain development, postnatal day 10 (P10) and postnatal day 21 (P21), characterized by an active process of synaptic modeling. Rats at different ages were chronically treated with DFP 1 mg/kg the first day and 0.5 mg/kg the following days. Cholinesterase activity, binding of [^{3}H]-QNB and the accumulation of radiolabeled phosphoinositides ([^{3}H]-Ins Ps) after carbachol stimulation were measured in the cortex of P10, P21 and adult animals. The cholinesterase activity was reduced by 81%, 88% and 94% at P10, P21 and adult respectively. In P21 and adult animals, the treatment with DFP induced a statistically significant decrease in the Bmax of [^{3}H]-QNB binding and in the turnover of Ins Ps. In contrast, at P10 the treatment with DFP is still able to reduce the total number of cortical QNB binding sites but doesn't have any effect on the turnover of the Ins Ps. The Kd of the receptor for [^{3}H]-QNB was never affected by the treatment. This observation may suggest that at P10 there is an uncoupling of the muscarinic receptor with its transduction machinery, in contrast to what observed at P21 and in the adult where the receptor and the turnover of Ins Ps are both regulated by the treatment. We are currently evaluating the effects of DFP treatment on nicotinic parameters such as [^{3}H]-Nicotine, [^{125}I]-α bungarotoxin binding and the expression of some neuronal nicotinic receptor subunits using receptor autoradiography and in situ hybridization techniques.

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117.8

MUTATION OF ASP TO GLU IN TRANSMEMBRANE HELIX 3 OF THE MUSCARINIC RECEPTORS. K.M. Page, C.A.M. Curtis and E.C. Hulme. (SPON: Brain Research Association). Division of Physical Biochemistry, National Institute for Medical Research, Mill Hill, London, NW7 1AA, U.K.

The third transmembrane helix of the muscarinic acetylcholine receptors (mAChRs) contains a conserved aspartic acid residue (Asp105, m1 mAChR) found in all members of the cationic amine-binding subclass of G-protein-coupled receptors. It has been suggested that the carboxylate side-chain may form an ionic bond with the positively-charged headgroups of both agonists and antagonists. We have probed the function of this residue in detail by mutating it to glutamic acid in both m1 and m2 mAChRs, which differ in their primary G-protein selectivities. The binding properties of wild-type and mutant receptors were compared after expression in COS7 cells. In contrast to the large effect of homologous mutation on the β-adrenergic receptor, the affinity of the muscarinic antagonist N-methylscopolamine was reduced only 3-fold. ACh underwent a much larger reduction in potency, the corrected IC50 value increasing by 30-fold (m1) to 100-fold (m2). Analysis using a ternary complex model of binding suggested that induction of the agonist-receptor-G-protein complex was more strongly inhibited than formation of the binary agonist-receptor complex. Inhibition of ternary complex formation was also seen with several agonists structurally related to ACh. Interestingly, however, their overall potencies were less affected.

117.9

MUTAGENIC STUDIES OF A CONSERVED ARGININE IN THE RAT m1 RECEPTOR. P.G. JONES, C.A.M. CURTIS and E.C. HULME (SPON: Brain Research Association), Division of Physical Biochemistry, NIMR, LONDON. NW7 1AA, UK.

The muscarinic acetylcholine receptors are members of the "superfamily" of G-protein coupled receptors. These receptors form seven transmembrane domains and within the family, there are many conserved residues. These are likely to serve important roles in ligand binding, receptor conformation or G-protein interaction. One such residue is the arginine of the aspartate-arginine-tyrosine motif occurring at the cytoplasmic interface of the third transmembrane helix. The reversal of the arginine and tyrosine in rhodopsin, whilst not affecting the ground (non-G protein coupled) state, severely impairs its coupling to transducin.
Using oligonucleotide-directed mutagenesis, we have replaced this residue in the rat m1 receptor, by lysine and the mutant receptor was expressed in COS-7 cells at similar levels to the wild type. The mutation had little effect on the binding of the antagonist [^{3}H]-*N*-methylscopolamine, K_D for both wild type and mutant being approximately 0.1nM. This will allow detailed analysis to be performed and although ground state binding is unlikely to be affected, it is possible that this residue has an important role to play in receptor function.

117.10

REGIONAL DELETION OF THE THIRD INTRACELLULAR LOOP OF THE m1 MUSCARINIC ACETYLCHOLINE RECEPTOR IMPAIRS AGONIST-INDUCED DOWN-REGULUATION BUT NOT UNCOUPLING. Norman H. Lee* and Claire M. Fraser, Section on Molecular Neurobiology, Laboratory of Neurogenetics, NIAAA, ADAMHA, Rockville, MD 20852.

The role of the third intracellular (i3) loop in homologous and heterologous down-regulation of the m1 muscarinic acetylcholine receptor (m1 mAChR) was investigated upon expression in Chinese hamster ovary cells. Deletions were constructed where the majority or small sections of the i3 loop is missing, but leaving the membrane proximal portions intact. All mutants were able to fully stimulate phosphatidyl inositol (PI) turnover and were pharmacologically indentical to the wild-type receptor. In cell lines expressing wild-type m1 mAChR and β2 adrenergic receptors, homologous and heterologous down-regulation of the m1 mAChR occurs when cells are pretreated with the muscarinic agonist carbamylcholine (CBC) or adrenergic agonist isoproterenol (Iso), respectively. Deletions of amino acids 227-269 (del 227-269) at the amino terminal of i3 produced a mutant receptor with impaired ability to down-regulate when exposed to CBC for up to 12 hours or Iso for up to 24 hours. However, this same deletion did not impair the ability of CBC-pretreatment to desensitize receptor-mediated PI turnover. Del 275-348, where 74 amino acids from the carboxyl terminal side of i3 is deleted, was indistinguishable from wild-type m1 mAChR in terms of homologous and heterologous down-regulation. The deletion encompassing amino acids 227-341, in which the majority of i3 is missing, exhibited an impaired ability to down-regulate similar to del 227-269. In conclusion, a stretch of amino acids from 227-269 on the amino terminal side of i3 may play an important role in homologous and heterologous down-regulation of the m1 mAChR but is not necessary for CBC-mediated receptor uncoupling.

117.11

MUTATIONS OF ASPARTATE 103 IN HUMAN MUSCARINIC RECEPTOR SUBTYPE m2 EXPRESSED IN COS-7 CELLS: EFFECTS ON RECEPTOR BINDING AND SIGNAL TRANSDUCTION. C.J. Spencer*, R.D. Schwarz and L.L. Lauffer. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI, 48105

Based upon molecular biological characterization, five subtypes of the human muscarinic receptor have been cloned and expressed in mammalian cells in culture. Recent results using rat m1 receptors have shown that the Asp residue at position 105 was crucial for both agonist and antagonist muscarinic ligand binding. To gain a greater understanding of the involvement of this Asp in agonist/antagonist binding, Asp 103 in the Hm2 receptor (corresponding to Asp 105 in m1) was mutated to Asn, Ala, and Glu. Hm2 was chosen since both agonist (3H-CMD) and antagonist (3H-NMS) binding could be performed as well as measurement of alterations in signal transduction, e.g. adenylate cyclase activity. Mutagenesis was achieved by a modification of the Kunkle method and mutant and wild type receptors were expressed in COS-7 cells by transient transfection. Similar to the rat m1 receptor, changing Asp to Asn in Hm2 markedly inhibited 3H-NMS binding. Comparisons to this effect will be made for the Ala and Glu mutations. Thus, our results confirm the key role Asp 103 plays in binding of agonists and antagonists to the Hm2 receptor.

117.12

FUNCTIONAL ROLE OF PROLINE AND TRYPTOPHAN RESIDUES HIGHLY CONSERVED AMONG G PROTEIN-COUPLED RECEPTORS: A STUDY WITH m3 MUSCARINIC ACETYLCHOLINE RECEPTOR POINT MUTANTS. J. Wess*, S. Nanavati, Z. Vogel and R. Maggio. Natl. Inst. of Neurological Disorders and Stroke, Lab. of Molecular Biology, Bethesda, MD 20892.

A series of four proline and three tryptophan residues are well conserved among the entire superfamily of G protein-coupled receptors. All of these amino acids are located within the hydrophobic core of these receptors on transmembrane domains (TMD) IV, V, VI, and VII. To study their role in ligand binding and receptor function, the rat m3 muscarinic acetylcholine receptor was used as a model system. A series of mutant m3 muscarinic receptors in which the conserved proline and tryptophan residues were individually replaced by alanine and phenylalanine, respectively, were created and transiently expressed in COS-7 cells. As found in [3-H]NMS binding studies, Pro242->Ala (TMD V), Pro505->Ala (TMD VI), and Pro540->Ala (TMD VII) were expressed at >30 times lower levels than the wild-type receptor. Pro201->Ala (TMD IV) showed drastically reduced binding affinities (two to three orders of magnitude as compared with the wild-type receptor) for both muscarinic agonists and antagonists. In contrast to all other mutant receptors studied, Trp503->Phe (TMD VI), Trp530->Phe (TMD VII), and Pro540->Ala (TMD VII) proved to be severely impaired in their ability to mediate carbachol-induced stimulation of phosphoinositide hydrolysis (E-max ca. 20-40% of that of the wild-type receptor). These data suggest that the examined proline and tryptophan residues play important roles in ligand binding and receptor function. Since these residues are found in almost all G protein-coupled receptors, our findings may be of general importance for this whole class of receptors.

117.13

RECONSTITUTION OF FUNCTIONAL MUSCARINIC RECEPTORS BY COEXPRESSION OF N- AND C-TERMINAL RECEPTOR DOMAINS. R. Maggio, Z. Vogel, A. Zuddas*, S. Nanavati and J. Wess. Natl. Inst. of Neurol. Disorders and Stroke, Lab. of Mol. Biology, Bethesda, MD 20892 and *Institute of Pharmacology, University of Pisa, Italy

Truncated m2 and m3 muscarinic receptor genes were created and coexpressed in COS-7 cells with gene fragments encoding the C-terminal receptor portions. The truncated receptors (referred to as m2- and m3-*trunc*) contained transmembrane domains (TMD) I-V and the N-terminal portion of the third cytoplasmic loop (i3), whereas the C-terminal receptor fragments (referred to as m2- and m3-*tail*) contained TMD VI and VII and the adjacent intra- and extracellular sequences. Expression in COS-7 cells of any of these four polypeptides alone did not result in any detectable [^{3}H]NMS binding activity. Likewise, coexpression of m3-*trunc* with m2-*tail* did not give any [^{3}H]NMS binding. However, specific [^{3}H]NMS binding could be observed after coexpression of m2-*trunc* with m2- or m3-*tail*, and of m3-*trunc* with m3-*tail*. The "reconstituted" muscarinic receptors displayed agonist and antagonist binding properties similar to those of the wild-type m2 and m3 receptors. The "reconstituted m3-*trunc*/m3-*tail* receptor" was able to stimulate agonist-dependent phosphoinositide hydrolysis to the same maximal extent as did the wild-type m3 receptor. The data raise the intriguing possibility that muscarinic receptors could behave in a fashion analogous to multiple subunit receptors, with the i3 loop serving as a linker sequence between the two functional domains. Since the muscarinic receptors share high structural homology with all other G protein-coupled receptors, our findings may be of general importance for the entire superfamily of receptors.

117.14

DIFFERENTIAL DOWN-REGULATION OF STRIATAL m4 MUSCARINIC RECEPTORS DETECTED BY SUBTYPE-SPECIFIC RECEPTOR IMMUNOPRECIPITATION. K.A. Frey*, T. Desmond and A.I. Levey. *Neuroscience Laboratories, The University of Michigan, Ann Arbor, MI 48109* and *Department of Neurology, Emory University, Atlanta, GA 30322.*

Previous studies from our laboratories have identified muscarinic receptor down-regulation in the rat following one week of acetylcholinesterase (AChE) inhibition by repeated administration of diisopropylfluorophosphate (DFP). Subtype-selective muscarinic receptor autoradiographic assays indicate widespread changes in cerebral receptors of the M1 and M2, but not the M3 subtypes. Receptor decreases were greatest for non-M1 receptors within the striatum and olfactory tubercle. Due to enrichment in m4 mRNA in these regions, we hypothesized involvement of this subtype. In the present work we have specifically identified the receptors involved. Receptors were solubilized from cerebral cortices and striata of control and DFP-treated rats, radiolabeled with [^{3}H]methylscopolaine, and individual subtypes precipitated by addition of subtype-specific rabbit antisera followed by goat anti-rabbit secondary antibodies (Levey et al., *J. Neurosci.* 11:3218-3226, 1991). Precipitated receptors were then quantified by liquid scintillation spectroscopy. In control animals, receptors solubilized from frontal cortex consisted of relatively equal admixtures of the m1, m2 and m4 subtypes (25, 36, and 29% of total, respectively) while the m4 receptor predominated in the striatum (27, 12, and 48% attributable to m1, m2, and m4, respectively). Following DFP administration, total cortical binding was reduced by 25%, without differential effects on receptor subtypes. Striatal m4 receptors, however, were decreased by 50% compared to a reduction of only 20% in m1 receptors. These results provide evidence for specific effects on striatal m4 receptors during AChE inhibition. This may indicate distinct mechanisms of receptor regulation, or differential treatment effects on distinct cell populations expressing subsets of the muscarinic receptors.

117.15

USE OF CHIMERIC MUSCARINIC RECEPTORS TO INVESTIGATE EPITOPES INVOLVED IN ALLOSTERIC INTERACTIONS

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All five (m1-m5) muscarinic receptors are sensitive to allosteric regulation, but gallamine is considerably more potent at slowing the dissociation of [^{3}H]N-methylscopolamine (NMS) from the m2 subtype than from the m3 or m5 subtypes (Ellis *et al.*, Biochem. Pharmacol. 42:1927, 1991). In order to study the structural basis of gallamine's preference for the m2 subtype, we evaluated [^{3}H]NMS-gallamine interactions at chimeric receptors in which segments of the m5 receptor were systematically replaced with the corresponding m2 sequence. Substitutions that included the sixth transmembrane domain and third extracellular loop resulted in marked increases in gallamine's potency, but substitutions that did not include these regions were without effect. A similar substitution was investigated using m2/m3 chimeric receptors, in which a segment extending from the middle of the sixth transmembrane domain to the carboxyl terminal was exchanged. As with the m2/m5 constructs, substituting the m2 C-terminal segment into the m3 subtype significantly increased gallamine's potency. Furthermore, the converse substitution reduced the potency of gallamine dramatically, to approximately that seen for the m3 subtype itself. It appears that this portion of the receptor is very important for the binding of gallamine and/or the allosteric interactions between gallamine and [^{3}H]NMS. (Supported in part by R01 AG05214.)

118.1

THAPSIGARGIN (TGN), BUT NOT RYANODINE, INHIBITS NMDA-EVOKED INTRACELLULAR Ca^{2+} MOBILIZATION IN RAT CORTICAL NEURONS. Sizheng Z. Lei*, Dongxian Zhang, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Previously, we demonstrated that Ca^{2+} influx triggered by NMDA receptor activation can mobilize intracellular Ca^{2+} stores, and this effect can be inhibited by dantrolene or ionomycin (Lei et al., *Soc. Neurosci. Abstr.*, 1991;17:1291). Here, we further characterize the effect of NMDA on intracellular Ca^{2+} stores using digital calcium imaging with fura-2 and whole-cell recording techniques in cultures of rat primary cortical neurons. Thapsigargin (TGN; 10 μM), a tumor promoter known to block the Ca^{2+} pump of the endoplasmic reticulum, increases intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$. At a lower concentration, that does not by itself affect $[Ca^{2+}]_i$, TGN (1 μM) inhibited NMDA (10 μM)-evoked increases in $[Ca^{2+}]_i$ by 50%. In contrast, 1-2 μM TGN did not block kainic acid (50 μM)-evoked $[Ca^{2+}]_i$ responses. In whole-cell recordings, TGN (1 μM) did not decrease NMDA-activated currents. Also, application of 1-20 μM ryanodine, an alkaloid known to promote Ca^{2+} leak out of caffeine-sensitive intracellular stores in a use-dependent manner, failed to inhibit the $[Ca^{2+}]_i$ responses elicited by repetitive applications of NMDA. Taken together with our previous findings, these results suggest that NMDA-stimulated Ca^{2+} influx through ion channels leads to the mobilization of specific intracellular Ca^{2+} stores, and this increase in $[Ca^{2+}]_i$ can be inhibited ~50% with pharmacological manipulation of these stores.

118.2

NITRIC OXIDE (NO) GENERATION DOWN-REGULATES NMDA RECEPTOR ACTIVITY VIA A REDOX MODULATORY SITE. H.-S. Vincent Chen*, S.K. Aggarwal, S.Z. Lei, Z.-H. Pan, J. Hartman, N.J. Sucher, and Stuart A. Lipton. Department of Neurology, Children's Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

NMDA-evoked increases in $[Ca^{2+}]_i$ and whole-cell current of rat cortical neurons were monitored using fura-2 digital imaging and patch-clamp recording techniques. Extracellular application of nitroglycerin (NTG; 100 μM) or the NO-generating drug *S*-nitrosocysteine (SNOC; 100 μM) inhibited NMDA responses; most of this inhibitory effect persisted after extensive washing for several min. Partial prevention of NO formation from NTG (by prolonged pre-incubation with glutathione) attenuated the action of NTG, suggesting that the decrease in NMDA-evoked responses by this nitroso compound was related to its generation of NO. One possibility for these effects is that the thiol group(s) comprising the redox modulatory site of the NMDA receptor react in the presence of NO, analogous to the manner in which other oxidizing agents down-regulate NMDA receptor activity (Aizenman, Lipton, Loring, *Neuron* 1989;2:1257). Consistent with this notion, the reducing agent dithiothreitol (DTT; 2 mM for 2 min) reversed the effects of NTG or SNOC by ~90%. Moreover, alkylation of thiols with *N*-ethyl-maleimide (NEM; 1-5 mM for 2 min) completely prevented the inhibitory effects of NTG or SNOC on NMDA-evoked responses. NO-generating compounds also protected cortical cultures from NMDA receptor-mediated neurotoxicity, an effect blocked by complexing NO with hemoglobin. These results suggest a new NO regulatory pathway involving reactions with thiol groups on the NMDA receptor complex.

118.3

SIMILAR EFFECTS OF N^{ω}-MONOMETHYL-L-ARGININE (L-NMMA) AND SODIUM NITROPRUSSIDE (SNP) ON NMDA-INDUCED GLUTAMATE RELEASE AND ELEVATION OF INTRACELLULAR CALCIUM LEVELS ($[Ca^{++}]_i$), BUT OPPOSITE EFFECTS ON THE NMDA-INDUCED ELEVATION OF CYCLIC GMP IN NEURONAL CULTURE. S. Oh,, Z. Cai, R.W. Rockhold* and P. P. McCaslin, Dept. Pharmacol./Toxicol., Univ. Miss. Med. Ctr., Jackson, Ms., 39216

These studies were designed to examine the effects of a nitric oxide (NO) generator (SNP) and an inhibitor of NO synthase (L-NMMA) on glutamate neurotransmission. In primary cultures of cerebellar granule cells, the glutamate receptor agonist, N-methyl-D-aspartate (NMDA), resulted in the elevation of $[Ca^{++}]_i$ (measured by fura-2 fluorometry), the release of glutamate (measured by HPLC) and an increase in cyclic GMP levels (measured by RIA). Both L-NMMA and SNP partially prevented the NMDA-induced release of glutamate, but neither compound had effects on the basal release of glutamate. Elevation of $[Ca^{++}]_i$ induced by NMDA were very slightly elevated by L-NMMA and markedly antagonized by SNP when the compounds were added after the addition of NMDA. However, if cells were preincubated with L-NMMA or SNP 10 min before the addition of NMDA, the NMDA-induced elevation of $[Ca^{++}]_i$ was markedly reduced by L-NMMA and completely blocked by SNP. These compounds also had opposite effects on the NMDA-induced elevation of cyclic GMP; SNP greatly augmented cyclic GMP levels while L-NMMA completely blocked NMDA-induced increases in cyclic GMP. Both compounds had effects on basal levels of cyclic GMP in these cultures. These results show similar effects of compounds which are thought to increase (SNP) and decrease (L-NMMA) NO levels. It is unclear whether these effects result from a biphasic effect of NO or from partial antagonism of the NMDA receptor by L-NMMA.

118.4

INHIBITION OF N-METHYL-D-ASPARTATE-STIMULATED NEUROTRANSMITTER RELEASE BY SODIUM NITROPRUSSIDE NOT DUE TO NITRIC OXIDE. A.K. Stout* and J.J. Woodward. Dept. of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298.

Previous studies in our lab have suggested that nitric oxide (NO) may act as a feedback inhibitor of the N-methyl-D-aspartate receptor. In these experiments the NO generator sodium nitroprusside (SNP, 100 μM) inhibited NMDA-stimulated release of tritiated norepinephrine from hippocampal slices of adult male Sprague-Dawley rats by approximately 50% and also stimulated the production of cyclic guanosine monophosphate (cGMP) in primary neuronal cultures to over 400% of basal levels. However, our recent experiments suggest that the inhibition of transmitter release seen with SNP was not due to NO. Treatment of hippocampal slices with the photolysis degradation products of SNP, which presumably cannot generate NO, also inhibited NMDA-stimulated transmitter release (IC50 < 10 μM). In addition, potassium ferricyanide and potassium ferrocyanide complexes (100-300 μM) were also shown to inhibit release. Finally, the NO generator S-nitroso-N-acetyl-penicillamine (SNAP) stimulated cGMP formation to nearly 3000% of basal levels in primary neuronal cultures but failed to inhibit release in the hippocampal slices. Thus the role of nitric oxide in modulating NMDA-stimulated neurotransmitter release remains to be determined. Supported by NIAAA AA08089, DA 07027, and a grant from the Alcoholic Beverage Medical Research Foundation.

118.5

CARBACHOL POTENTIATES NMDA CURRENT VIA PROTEIN KINASE C IN RAT HIPPOCAMPAL SLICES. L. Aniksztejn, A. Kleschevnikov,Y. Ben-Ari and A.Represa*. INSERM U29, Hôpital de Port-Royal, 123 Bld de Port-Royal, 75014 Paris (France).

Activation of the protein kinase C (PKC) by a μ opioid agonist (Chen and Huang, Neuron 7, 319, 1991 and Nature, 356, 521, 1992) or Q_P agonists (Aniksztejn et al.,Eur.J.Neurosci. June 1992) enhances selectively the currents mediated by N-methyl-D-aspartate (NMDA). Markram and Segal (J. Physiol. 447, 513,1992) have described in CA1 hippocampal neurons a similar enhancement of NMDA current by stimulation of muscarinic receptors, however they suggested that this effect was not mediated by PKC since it was not blocked by intracellular injection of H-7, a broad spectrum unselective PKC blocker. We now report that in CA1 neurons, intracellular injection of the specific PKC (19-36) inhibitory peptide totally prevents the enhancement of NMDA currents induced by carbachol. Currents were recorded with the single electrode voltage clamp technique. Electrodes were filled with KCl (3M) . TEA (10mM), 4-AP (1mM), CsCl (3mM) and TTX (1μM) were added to the bath solution to block voltage dependent K^+ and Na^+ channels and improve space clamp. Bath application of NMDA (5-10μM; 90 sec) generated an inward current I_{NMDA} of 277 ± 75 pA (mean ± S.D. n=9) at mean holding potential of 59 ± 8mV. In presence of carbachol (10μM, 180-240 sec), NMDA peak current was potentiated (424 ± 115 pA). This effect fully reversed since 5 min after washing carbachol, I_{NMDA} was of 277 ± 80 pA. In contrast , in similar experiments performed with microelectrodes containing the PKC (19-36) inhibitory peptide (50μM), carbachol did not potentiate the NMDA current (228 ± 50 pA in control and 183 ± 50pA with carbachol (mean ± SD, n=9)). Therefore, in contrast to the data obtained by Markram and Segal, our results suggest that the enhancement of I_{NMDA} induced by carbachol is mediated by PKC.

118.6

EFFECTS OF PCP ON SINGLE NMDA CHANNELS IN EXCISED PATCHES FROM RAT HIPPOCAMPAL NEURONS R.C.Araneda*, R.S. Zukin, and M.V.L. Bennett. Dept. of Neuroscience, A. Einstein Coll. Med.,Bronx, NY 10461.

Phencyclidine (PCP) has been shown to produce a use- and voltage-dependent block of NMDA-activated currents. In whole cell recordings PCP block is relieved by depolarization during NMDA application. There is evidence that PCP enters closed channels, possibly from the intracellular side since it readily crosses cell membranes. To address this question we are studying the effects of PCP at the single-channel level using outside-out and inside-out patches from hippocampal neurons.

In inside-out experiments when the pipette contained NMDA (30 μM) and glycine (1 μM), PCP (1 μM) applied to the intracellular (bath) side blocked NMDA channels at negative potentials, but the block was relieved at positive potentials. In outside-out experiments when PCP (10 μM) was included in the pipette and the outside was perfused with NMDA (30 μM) and glycine (1 μM), the channel became blocked at -60 mV. Stepping to +40mV relieved the block and on repolarizing to -60 mV block developed again. PCP applied externally also blocked at -60 mV but not at +40 mV. In the outside-out configuration block by PCP from the inside seems unlikely to result from PCP crossing the membrane into the flowing solution and then acting on the NMDA channels from the extracellular side.

118.7

THE EFFECTS OF GLUTATHIONE ON N-METHYL-D-ASPARTATE MEDIATED CALCIUM UPTAKE IN DISSOCIATED NEURONS. R. D. Trent, P. K. Randall and S. W. Leslie*. Div. of Pharmacol. and Toxicol., and Inst. for Neurosci., Univ. of Texas, Austin, TX 78712.

Previous receptor binding studies in this laboratory have suggested a modulatory role for low micromolar concentrations of glutathione (GSH) at the agonist recognition site of the *N*-methyl-D-aspartate (NMDA) receptor complex. Functional studies using the intracellular calcium probe fura-2 in dissociated neurons isolated from newborn rat brain have demonstrated calcium uptake following GSH addition (EC_{50} = 914 μM) which is reversed by specific NMDA antagonists. The present experiments examined the effect of GSH (0.2 - 8000 μM) on NMDA-stimulated calcium uptake using fura-2 loaded dissociated brain cells from newborn rats. Dissociated cells were treated with or without GSH for 100 seconds prior to NMDA stimulation (4 - 500 μM NMDA). EC_{50}'s and minimum and maximum calcium uptake following NMDA addition were not significantly different from controls when cells were pretreated with GSH at concentrations from 0.2 to 200 μM. However, GSH concentrations within the range of 1000 to 8000 μM exhibited partial agonist characteristics at the NMDA receptor complex. Thus, GSH may interact with the NMDA recognition site to modify agonist responsiveness mediated by NMDA. High micromolar concentrations of GSH may be necessary for this interaction. (Supported by NIAAA grants AA05809 and AA08104)

118.8

PREGNENOLONE SULFATE ALLOSTERICALLY POTENTIATES THE NMDA CURRENT IN HIPPOCAMPAL NEURONS. M.R. Bowlby* and E.A. Kravitz. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Steroid hormones have profound influences on behavior. While many of the mechanisms of action of this class of substances appear to be at the level of transcriptional regulation, recent studies suggest that brain metabolites of steroids might exert important non-genomic modulatory effects on neuronal mechanisms (reviewed by Paul and Purdy, 1992, FASEB 6: 2311). In this regard, we examined the potentiation of the NMDA subtype of glutamate current by the endogenous neurosteroid pregnenolone sulfate in cultured hippocampal neurons using patch clamp techniques. The magnitude of the NMDA activated current was approximately doubled in the presence of 100 μM pregnenolone sulfate. The dose response curve predicts an EC_{50} of about 25 μM and a Hill coefficient of 1. Pregnenolone sulfate acts via an allosteric mechanism at a site distinct from the NMDA and glycine binding sites, as the dose response relationships for NMDA and glycine, even at saturating concentrations, are shifted upwards. The potentiation appears to be specific for the NMDA activated current; no modulation of non-NMDA currents are seen. The enhancement of the current probably is not due to activation of a second ionic conductance, as the reversal potential of the I-V curve does not shift in the presence of the steroid. The effect is specific for pregnenolone sulfate; corticosterone, tetrahydroprogesterone, and pregnenolone have no effect on the NMDA current. The potentiation by pregnenolone sulfate saturates in about 30 s, and reverses with a similar time course. (Supported by NINDS).

118.9

PREGNENOLONE SULFATE POTENTIATES NMDA-INDUCED RISES IN INTRACELLULAR Ca^{2+}: STUDIES IN CULTURED RAT HIPPOCAMPAL NEURONS WITH FURA-2 MICROSPECTROFLUORIMETRY S.M. Paul[1], R.P. Irwin[1], N.J. Maragakis[2], R.H. Purdy, D.H. Farb[3], M.A. Rogawski[4]*. [1]Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892, [2]University of Utah, Salt Lake City, UT 84132, [3]Boston University School of Medicine, Boston, MA 02118. [4]Epilepsy Research Branch, NINDS, Bethesda, MD 20892.

Recently it has been reported that pregnenolone sulfate, in addition to antagonizing GABA activated Cl^- currents, is also a potentiator of NMDA receptor responses in cultured fetal chick spinal cord neurons. We confirm and extend this observation by demonstrating that pregnenolone sulfate modulates NMDA receptor-mediated increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$). $[Ca^{2+}]_i$ was monitored in 7-14 day fetal rat hippocampal neurons using microspectrofluorimetry and the Ca^{2+} sensitive indicator fura-2. In these neurons NMDA (5 μM) produced a rapid and reversible rise in $[Ca^{2+}]_i$ of 145 ±28 nM. Coapplication of pregnenolone sulfate (5-250 μM) resulted in a concentration-dependent potentiation of the NMDA induced rise in $[Ca^{2+}]_i$ (E_{max} 789 %, EC_{50} 33 μM). The steroid failed to alter basal (unstimulated) $[Ca^{2+}]_i$ or modify the rise in $[Ca^{2+}]_i$ when hippocampal neurons were depolarized by K^+ (20 mM) in the presence of the NMDA receptor antagonist 3-[(±)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid (CPP). Structure activity studies on the pharmacophore for the steroid modulatory site on the NMDA receptor are in progress. Preliminary findings indicate a high degree of structural specificity for the modulatory actions of pregnene steroids. Our results suggest that pregnenolone sulfate or a structurally related steroid may be an endogenous modulator of NMDA receptor responses.

118.10

GLUTAMATE EXPOSURE RAPIDLY DECREASES INTRACELLULAR pH IN RAT HIPPOCAMPAL NEURONS IN CULTURE.

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The ability of glutamate to alter intracellular pH (pH_i) was studied in fetal rat hippocampal neurons (day 10-12 in culture) using the pH sensitive fluorescent indicator BCECF. Exposure (120 sec) of hippocampal neurons to glutamate (100 μM) reversibly reduced pH_i from 7.197±0.041 to 7.001±0.040 (n=49, $p \leq 0.001$) with recovery taking several minutes. In some neurons a reduction in pH_i of greater than 0.4 was induced by glutamate exposure (120 sec). The effect of glutamate in reducing the pH_i of hippocampal neurons was dependent on agonist concentration (0.01-1.0 mM) and time of exposure. NMDA (100 μM) also decreased pH_i and the latter effect was attenuated by the NMDA receptor antagonist 3-[(±)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid (CPP). Experiments in which extracellular Ca^{2+} was removed prior to glutamate exposure suggest that Ca^{2+} influx may be required for the decrease in pH_i induced by glutamate. This relatively rapid intracellular acidosis induced by glutamate exposure may be involved in the cascade of events leading to excitotoxic cell death.

118.11

EFFECTS OF ETHANOL ON NMDA RECEPTOR-CHANNELS IN SINGLE CHANNEL RECORDINGS. J.M. Wright* and F.F. Weight. Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Previous studies from this laboratory indicated that ethanol (EtOH) inhibits NMDA-activated currents in mouse hippocampal neurons in a concentration-dependent manner (1). We are currently extending the investigation on mechanism of action with single channel recordings from mouse cortical and hippocampal neurons.

Neurons from day 15-17 fetal mice were plated on collagen/polylysine-coated plates and cultured for 1-4 weeks in an enriched MEM medium prior to use. Single channel recordings were made from excised outside-out patches. Pipettes contained (in mM) 75 CsCl or NaCl, 75 CsAcetate, 10 HEPES (pH 7.2), 10 EGTA, 1 $CaCl_2$. The bath solution contained (in mM) 160 NaCl, 2.8 KCl, 1 $CaCl_2$, 10 HEPES (pH 7.2). Channel conductance was not changed by EtOH. EtOH effects were determined by comparing the steady state probability of opening (nPo) in 2-40 μM NMDA (1 to 12 min) followed by 0.25-100 mM ethanol (1 to 10 min) N=16. There was a 20% increase in nPo with application of 0.5 mM EtOH (N=3). 2-10mM EtOH had no effect on nPo (N=4). Inhibition of nPo was 47% in 100 mM EtOH, 38% in 50 mM and 19% in 20 mM. Changes in nPo at EtOH concentrations < 100 mM were primarily due to changes in frequency of opening with elementary open time changing < 10%. In 100mM EtOH mean open time decreased 30% and frequency of opening decreased 25%.

1) Lovinger, White, & Weight, Science **243**:1721-1724, 1989.

118.12

DIFFERENTIAL EFFECTS OF TRICYCLIC ANTIDEPRESSANTS AND BARBITURATES ON THE QUISQUALATE-, KAINATE- AND NMDA-INDUCED ELEVATION OF INTRACELLULAR CALCIUM LEVELS IN NEURONAL CULTURE. Z. Cai and P. P. McCaslin*, Dept. Pharmacol./Toxicol., Univ. Miss. Med. Ctr., Jackson, Ms., 39216

In these studies, we examined the ability of tricyclic antidepressants (TCAs) and barbiturates, in therapeutic concentrations, to block the elevation of intracellular calcium levels ($[Ca^{++}]i$) induced by excitatory amino acids (EAAs) in neuronal tissue culture. The glutamate receptor agonists, kainate and N-methyl-D-aspartate (NMDA), resulted in the elevation of $[Ca^{++}]i$ as measured with fura-2 fluorometry in primary cultures of cerebellar granule neurons. Several TCAs, amitriptyline and cyproheptadine, partially prevented this elevation induced by both of these EAAs, but not elevations of $[Ca^{++}]i$ induced by another EAA, quisqualate. The fast acting barbiturates, secobarbital and thiamylal were effective in reducing elevations induced by all three EAA agonists, while a slower acting barbiturate, pentobarbital, was only effective in reducing elevations of calcium induced by kainate but had weak or no effects on elevations of $[Ca^{++}]i$ induced by quisqualate or NMDA. Evidence suggests that this TCA and barbiturate interaction with EAAs may involve voltage-dependent calcium channels since the fast acting barbiturates and TCAs also partially blocked the elevation of $[Ca^{++}]i$ induced by membrane depolarization with 40 mM KCl. However, in the case of TCAs, the blockade is not reversed in high concentrations of extracellular calcium $[Ca^{++}]o$ as would be predicted by a direct interaction with calcium channels. These results show that both TCAs and barbiturates interact with EAA neurotransmission and suggest that this interaction may involve selective types of calcium channels differentially activated by EAAs.

118.13

CHRONIC BARBITURATE TREATMENT INCREASES NMDA RECEPTORS BUT DECREASES KAINIC ACID RECEPTORS IN MOUSE CORTEX. K. R. Short* and B. Tabakoff, Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

Several studies of sedative/hypnotic drugs have revealed that these drugs inhibit NMDA and kainate receptor function. Ethanol, pentobarbital, and phenobarbital all reduce the NMDA and kainate induced Ca^{2+} uptake in cells grown in culture. Furthermore, chronic treatment of animals with ethanol increases NMDA receptor number in hippocampus (but not cortex), and chronic barbital enhances cGMP response to kainate (but not NMDA) in cerebellum. These results suggest an adaptive change in glutamate receptors due to extended exposure to sedative/hypnotic drugs. We report the effects of chronic phenobarbital on NMDA and kainate receptors.

C57BL/6 mice had continuous access for 7 days to normal chow or chow containing 3.0 g phenobarbital (free acid) *per* kg food (increased to 3.5 g/kg after 5 days). Serum phenobarbital levels at the time of sacrifice were approximately 150 µg/ml. Equilibrium saturation [^{3}H]MK-801 binding (0.25 to 25 nM) to crude synaptosomal membranes was determined in the presence of 10 µM glutamate, 1 µM glycine, and 100 µM $MgSO_4$. Equilibrium saturation [^{3}H]kainate binding (1 nM) was determined in the presence of 0.5 to 64 nM kainate.

In cortex, the K_d values for kainate and MK-801 binding were unaffected by drug treatment ($ts < 1$, $p > 0.5$). The B_{max} for MK-801 increased 35% ($p < .05$) while the B_{max} for kainate decreased 37% ($p < .05$) in chronically barbiturate treated mice. In hippocampus, neither the K_d nor B_{max} for MK-801 binding was affected by drug treatment. B_{max} changes in cortex were uncorrelated with weight loss.

The decrease in kainate receptor number suggests a possible mechanism (in addition to their $GABA_A/Cl^-$ effects) by which barbiturates may exert anti-convulsant action following extended exposure. This may help explain the effectiveness of phenobarbital in the long-term treatment of epilepsy. (Supported by AA-09014 & AA-09005)

EXCITATORY AMINO ACIDS: RECEPTORS II

119.1

Evolutionary Homologies of Glutamate Receptors.

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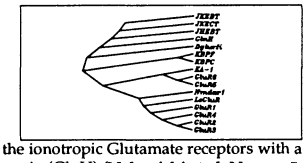

Tentative homologies were proposed in the literature for glutamate receptors, both iontropic (ie. Kainate, AMPA and NMDA) and metabotropic, based on pairwise sequence comparison:

1. Homology of a part of the N-terminal extracellular domain of the ionotropic Glutamate receptors with a procaryote amino acid binding protein (GlnH) **(Nakanishi et al: Neuron 5: 569, 1990).**
2. A domain homology of the N-terminal extracellular region of ionotropic and metabotropic Glutamate receptors. **(Masu et al: Nature 349: 760, 1991).**
3. Homology between the N-terminal extracellular domain of the metabotropic Glutamate receptors and a region similarly located in a family of receptor proteins having a guanylate cyclase domain located cytoplasmically **(Houamed et al: Science 252: 1318, 1991).**

Using multiple sequence alignments analysis, in case (2) above homology was ruled out, and the analysis supports the proposal that the ionotropic receptors constitute a single family. For the ionotropic receptors/procaryote amino acid binding proteins, molecular modelling indicated an invariant Arg 499 (GluR1 numbering) is essential in binding the α-amino acid carboxylate group, and steric restriction may be important in AMPA and NMDA selective recognition.

[1] Fellow of the Consejo de Investigaciones Cientificas y Técnicas (CONICET, INIBIBB), ARGENTINA, and partially supported by Fundación Antorchas (ARGENTINA) and The British Council.

119.2

CLONING OF A cDNA FOR A CGP 39653-BINDING PROTEIN FROM HIPPOCAMPAL cDNA LIBRARIES. **P. Johnson*, K. Kumar, M. Ahmad, D. Wong, K. Eggeman, J.-P. Wu, C. Bigge and E. Michaelis. Dept. of Pharmacol. and Toxicol., Univ. Kansas, Lawrence, KS 66045, and Neurosci. Chem., Parke-Davis, Ann Arbor, MI 48106.**

We previously reported on the isolation of a hetero-oligomeric complex of 4 proteins that has the ligand recognition sites and functional properties of an NMDA receptor. We have also reported the cloning of the largest subunit of this complex, an ~60 kDa glutamate-binding subunit (Nature 354, 70), and the isolation of a second subunit, the ~50 kDa competitive-antagonist-binding subunit (J. Biol. Chem. 265, 7768). We have recently raised polyclonal antibodies against the ~50 kDa CPP-binding protein and used these antibodies to screen a rat hippocampal cDNA library from which a 3.0 kb cDNA was isolated, cloned and sequenced. E. coli transfected with the vector pBluescript harboring the 3.0 kb insert express a fusion protein that is recognized by the anti-CPP-binding protein antibodies. The fusion protein was purified from IPTG-induced E. coli extracts using diaminophosphonopentanoic acid linked to a ReactiGel matrix. The fusion protein was eluted from the column by the introduction of 10 mM $MgSO_4$ and had high specific binding activity for [^{3}H]CGP 39653. This binding was completely inhibited by 2-AP5. We believe that this clone represents the cDNA for the antagonist-binding subunit of an NMDA receptor complex. Supported by grants from DAAL-03-88-K-0017 (ARO), AA04732 (NIAAA), DA05472 (NIDA) and an unrestricted grant from Parke-Davis.

119.3

ANTISENSE OLIGONUCLEOTIDES TO A 71 kDa GLUTAMATE-BINDING PROTEIN DECREASE EXPRESSION OF FUNCTIONAL NMDA RECEPTORS. **K.N. Kumar*, M.P. Mattson, H. Wang, B. Cheng and E.K. Michaelis. Depts. of Anatomy and Neurobiology, Univ. Kentucky, Lexington, KY 40536, and Dept. of Pharmacol. & Toxicol., Univ. Kansas, Lawrence KS 66045.**

NMDA receptors play important roles in neuronal development, plasticity and pathology in the mammalian CNS. Roles for specific NMDA receptor proteins in neuronal function have not yet been fully established. We recently isolated and cloned a 71 kDa NMDA receptor protein (NMDARP) from rat brain that comprises the glutamate binding subunit of a 4-protein NMDA receptor complex. Using antisense oligonucleotides directed against this NMDARP, we now demonstrate the requirement for this protein in calcium responses to NMDA and excitotoxicity in cultured hippocampal neurons. Western blot analysis and immunocytochemistry demonstrated that NMDA antisense oligonucleotides (50 uM) caused a selective suppression in NMDARP levels during 12-48 hr exposure periods. Elevations in intracellular Ca^{2+} levels normally caused by glutamate and NMDA were reduced in neurons exposed to NMDARP antisense oligonucleotides; Ca^{2+} responses to kainate were relatively unaffected. NMDARP antisense oligonucleotides protected the neurons completely against NMDA and partially towards kainate neurotoxicity. Thus, the 71 kDa NMDARP is a necessary component of the NMDA receptor-mediated Ca^{2+} responses and neurotoxicity in hippocampal neurons. [Supported by grants NS29001-01, AG05144-07, AA04732 and DAAL-03-88-K-0017]

119.4

MOLECULAR STRUCTURE AND PHARMACOLOGICAL CHARACTERIZATION OF humEAA2, A NOVEL HUMAN KAINATE RECEPTOR SUBUNIT. R.K. Kamboj#, D.D. Schoepp¶, S. Nutt#, L. Shekter#, B. Korczak# R.A. True¶, D.M. Zimmerman¶* and M.A. Wosnick#. #Allelix Biopharmaceuticals Inc., Mississauga, Ontario, Canada L4V 1P1, ¶Central Nervous System Research, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285, USA.

A cDNA encoding a novel human glutamate receptor subunit protein was isolated from a human hippocampal library. This cDNA termed human EAA2 (humEAA2) is most closely related to rat cDNAs for kainate receptor proteins, and when expressed in COS cells is associated with high-affinity kainate receptor binding. The relative potency of compounds in displacing ^{3}H-kainate binding was kainate > quisqualate > domoate > L-glutamate ≫ DNQX > dihydrokainate > CNQX > AMPA. Homomeric expression of humEAA2 does not appear to elicit ligand-gated channel activity. Nevertheless, the molecular structure and pharmacology of high-affinity kainate binding suggest the humEAA2 is a novel subunit protein of a human kainate receptor complex.

119.5

CLONING AND DISTRIBUTION OF MESSENGER RNA'S ENCODING FOR AVIAN GLUTAMATE RECEPTORS

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Glutamate receptors form part of a major excitatory neurotransmitter system. Three avian glutamate receptor subtypes, which have been designated pigeon GluR-62, GluR-26, and GluR-21, respectively have been cloned from a cerebellar library. Based on amino acid sequence homology the obtained sequences revealed that they encode for AMPA-sensitive glutamate receptors.

Northern blot analysis performed on total RNA from cerebellum and from optic tectum of the pigeon with radioactively labeled cDNA probes revealed hybridization to transcripts >5 kb. To investigate their patterns of expression in the pigeon brain, *in-situ* hybridization histochemistry on cryosections was performed using 35-S and Digoxigenin-labeled cRNA probes. Within the brain regions analyzed, the receptor subtypes showed distinct and regionally specific hybridization patterns. Particularly striking differences were seen for cell types within the cerebellar cortex: Purkinje cell perikarya showed very high messenger RNA expression for pigeon GluR-62 and -21, however no transcripts for pigeon GluR-26 were detected in this cell type. The non-neuronal Golgi epithelial/Bergmann glial cells were strongly positive for pigeon GluR-62 and -26 transcripts, and weakly positive for GluR-21. Granular cells showed a strong signal for GluR-62 only. The hybridization pattern is highly comparable to that found with glutamate receptor subtypes in rats, with high resemblance of GluR-62, -26 and -21 to the rat subtypes GluR-B, -D and -C respectively.

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119.6

AMPA RECEPTORS FROM THE CHICK BRAIN. M.E. Schneider, A.S. Niedzielski*, R.J. Wenthold. Lab of Molecular Biology, NIDCD, Natl. Inst. Health, Bethesda, MD 20892.

The chick auditory system is a useful comparative model for hearing science. Recent evidence suggests that the functionally mature cochlea can withstand enormous levels of noise exposure and suffer over 80 dB SPL threshold shifts followed by complete recovery. One mechanism for such recovery may be by replacing damaged hair cells of the reticular lamina. In order to study the role of the glutamate selective afferent receptors in the reinnervation of these nascent hair cells, suitable molecular probes must be developed.

An adult lambda gt10 chicken brain library was screened with various probes derived from rat cDNA sequences of the AMPA family. Using random primed probes containing the conserved transmembrane regions of each of the 4 members of the AMPA family, many partial clones representing all members of the AMPA family were identified. The majority of isolates cloned contained intronic material from incompletely spliced nuclear RNA's. The library was further screened using an oligonucleotide to the most 5' region of a chick GluR2 or with a degenerate pool of oligos based on the amino acid sequence from rat. Two clones were isolated using a specific oligonucleotide to GluR2. However, neither clone was correctly spliced. The presumptive correctly spliced gene was constructed from the two phage isolates and coexpressed in frog oocytes with rat cDNA's. Only one family member was cloned in its entirety (GluR2).

Based on both chick and rat sequences, PCR primers unique to each family member were synthesized and chick poly A RNA from total brain was reverse transcribed and the cDNA was PCR amplified, cloned and sequenced. AMPA family members 2 through 6 were detected in this manner.

119.7

CONANTOKIN PEPTIDES FROM MARINE SNAIL VENOM AFFINITY-LABELS SUBUNITS OF THE CONANTOKIN RECEPTOR. R. A. Myers*, J-P. Fernandez#, J. Rivier# and B. M. Olivera. Department of Biology, University of Utah, Salt Lake City, UT 84112, and #The Clayton Foundation Laboratories for Peptide Biology, Salk Institute, La Jolla, CA 92037.

The conantokins are small peptides (17-27 amino acids) which derive from the venom of *Conus* marine snails. The only known peptides which target the NMDA receptor, conantokins inhibit NMDA-induced currents, while eliciting no effect on either kainate or AMPA conductances. Conantokin analogs amenable to radioiodination were synthesized and radiolabeled. These probes were used to affinity-label crude rat brain membranes by reaction with divalent crosslinking agents. When detergent-extracted rat brain membranes were subjected to affinity chromatography, affinity crosslinking of the column retentate revealed enrichment of selectively (competed by excess cold ligand) and intensely labeled bands of high molecular weights, 90-160 kd. Components of this size are consistent with a glycosylated NMDA receptor polypeptide (10 putative glycosylation sites) sequenced by expression cloning by the Nakanishi group [*Nature* (1991) 354, 31-37]. Selectively labeled bands also observed at lower molecular weights presumably represent other subunits of the same receptor complex or different conantokin receptor subtypes. Size-exclusion HPLC indicates that these are subunits of a larger complex of several hundred kd. These receptors are presently being purified and characterized. (Supported by GM22737.)

119.8

TISSUE-SPECIFIC PROCESSING OF AMPA-TYPE GLUTAMATE RECEPTOR PRE-mRNA IN GOLDFISH VISUAL SYSTEM.

H. Ueda*# and D. Goldman. MHRI, Univ. of Michigan, Ann Arbor, MI 48109

Goldfish ganglion cells have the unique ability to regenerate their axons following optic nerve crush. Regeneration leads to synapse formation between retinal ganglion cell axons and their targets in the optic tectum, ultimately resulting in visual recovery. Glutamate is the major neurotransmitter used by ganglion cells to communicate with postsynaptic targets. In addition, glutamate receptors have been implicated to play a role in stabilization of appropriate retino-tectal synapses. In order to determine the number of different glutamate receptors expressed in the visual system and ultimately the role they may play in mediating retino-tectal synaptogenesis, we have begun to characterize glutamate receptor gene expression during optic nerve regeneration. Here we report the cloning of two different glutamate receptors expressed in retina and tectum. GFGR52 is most similar to rat GluR D-Flop with 91% amino acid identity. GFGR49 represents a partially processed RNA. It is most similar to GluR C-Flip with 92% amino acid identity. GFGR49 is not a full length clone and contains introns. The coding regions of this clone encode a Flip exon and transmembrane domain 4 with sequence extending 3'. Both these exons are flanked by intronic sequences. RNase protection experiments show genes corresponding to GFGR52 and 49 are expressed in retina and tectum. Using probes that correspond to intron and exon sequences of GFGR49 we find approximately 50% of the protected RNAs contain introns. Surprisingly the protected fragments differ between retina and tectum. These latter results suggest cell-specific splicing may contribute to the heterogeneity of glutamate receptors expressed in the visual system.

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119.9

CHARACTERIZATION OF A SPLICE VARIANT OF THE NMDAR1 RECEPTOR. J.W. Kusiak*, D.D. Norton and G.A. Higgins. Molecular Neurobiology Unit, NIA, NIH, Baltimore, MD 21224.

We have discovered a splice variant of the recently cloned receptor, NMDAR1. RT-PCR of rat brain RNA with primers near the carboxyl end gave two products, one of the expected size (~410bp) and one smaller (~300bp). Sequence of the smaller product was identical to the expected product but lacked 111 internal nucleotides. This sequence also contained an open reading frame identical to the expected product but lacked 37 internal amino acids. PCR of rat genomic DNA with these same primers gave an ~ 3.8 Kb product, which contained the deleted sequence bordered by consensus intron/exon junctions and adjacent upstream and downstream sequences suggesting that these two PCR products arise by alternative splicing of a single gene. A partial restriction map of the genomic fragment localized the coding region of the deleted sequence to an exon ~600 bp downstream of the common upstream exon. RT/PCR of RNA isolated from different brain regions showed differential expression of the two forms of the receptor with the deletion variant more abundantly expressed in the brain stem and cerebellum and the full length form more abundant in olfactory bulb, cortex, hippocampus, and striatum. Probing Southern blots of the RT/PCR reactions with splice junction, insert, and common probes confirmed the identity of the products. Preliminary *in situ* hybridization experiments show that both forms are widely distributed in the CNS. The results show that the NMDAR1 gene undergoes brain region specific alternative splicing to produce variants that differ in the carboxyl terminal portion of the receptor.

119.10

ALTERNATIVE mRNA SPLICING OF THE MOUSE GLUR2 GENE. J. A. Izzo, H. Endo*, J. W. Kusiak. Molecular Neurobiology, NIA, NIH, Baltimore, MD 21224

Molecular cloning of the cDNAs for the AMPA-selective glutamate receptors GLUR-1, -2, -3, -4 has shown that each gene produces several mRNA isoforms. The mutually exclusive splicing of two exons, termed "flip" and "flop", generates two protein isoforms which confer different electrophysiological properties to cells expressing these receptor subunits. The expression of flip and flop containing mRNAs is regulated during development with flop mRNAs appearing after the flip forms. Flip and flop mRNAs are also differentially expressed within specific regions of the adult brain. The molecular mechanisms responsible for the alternative splicing of the GLUR mRNAs are unknown.

We have cloned and characterized the genomic region surrounding the flip and flop exons. Sequence analysis revealed the presence of an unusually long polypyrimidine tract located between the two exons. Similar long polypyrimidine stretches have been shown to be important in the alternative splicing of muscle-specific genes. To investigate whether this unusual sequence is reponsible for the mutually exclusive splicing of the flip and flop exons, we constructed a minigene that contains the flip and flop genomic region, the upstream splice donor and downstream splice acceptor sites and flanking coding sequences. We have examined several cell lines for GLUR2 expression by RT-PCR. HeLa cells do not express either the flip or flop forms of GLUR2 mRNA. In contrast, PC12 cells induced to differentiate with nerve growth factor express both flip and flop-containing isoforms. Similarly, undifferentiated PC12 cells also express both flip and flop mRNAs.

119.11

CELL LINES STABLY EXPRESSING GLUA-D FLIP AND FLOP RECEPTOR SUBUNITS OR COMBINATIONS THEREOF. J. S. Rasmussen, L. S. Nielsen, A. Hansen, K. Hansen, E. Boel, K. M. Houamed, P. H. Andersen*. Depts. of Molecular Biology I and Molecular Pharmacology, Pharmaceutical Biotechnology, Novo Nordisk A/S, Novo Allé, DK-2880, Bagsvaerd, Denmark

Rat Glu A-D flip and flop subunits were cloned by PCR using rat cerebellar or hippocampal derived 1.strand cDNA's as template and sequence specific oligonucleotides as primers. Each cDNA was assembled from two or three PCR fragments ranging from 0.8 - 1.5 kb. The cDNA'S were transfected into BHK 570 cells under control of a constitutively active metallothionine promoter. Following addition of selection agent(s), subclones were isolated expressing high fmol - low pmol receptor/mg membrane protein. Using specific antibodies, the molecular weight of the various subunits was found to be in close agreement with previous reports investigating these proteins in rat brain tissue. Using binding of ^{3}H-AMPA, the pharmacological profile of the subunits or combinations thereof was investigated. These results indicated no difference between the flip and flop version of each subunit. Further, the profile of the A-D subunits was somewhat similar, showing high affinity for AMPA (10-30 nM) and quisqualic acid (2-20 nM), medium affinity for CNQX, DNQX and NBQX (60-600 nM), medium to low affinity for kainate (300-4000 nM) and very low affinity for ibotenic acid (>30 uM). Thus, the pharmacological profile of the glutamate receptors expressed in these cell lines corresponds to the profile of the high affinity AMPA binding site found in rat brain.

119.12

STABLE FUNCTIONAL EXPRESSION OF GLUTAMATE RECEPTOR SUBUNITS IN MAMMALIAN CELL LINES. K. M. Houamed*, J. S. Rasmussen, L. S. Nielsen, A. Hansen, K. Hansen, E. Boel, P. H. Andersen. Depts. of Molecular Pharmacology and Molecular Biology I, Pharmaceutical Biotechnology, Novo Nordisk A/S, Novo Allé, DK-2880 Bagsvaerd, Denmark.

Glutamate receptor subunits A - D, in flip and flop versions, cloned from rat brain (abstract by Rasmussen et al in this issue), have been stably expressed in the mammalian cell line BHK 570. Whole cell patch clamp recordings, at holding potential of -70 mV, from cells carrying single subunit or a binary combination, revealed sustained inward currents activated by the potent glutamate receptor agonist kainate. The currents became outward when the holding potential was made positive, indicating the activation of a mixed cation conductance.

The pharmacological profile of these cell lines, with respect to agonists, antagonists, and allosteric modulators, as well as the ionic mechanisms underlying the agonist induced conductances will be presented.

119.13

RAPID QUANTITATION OF NMDAR1 RECEPTOR mRNA BY SOLUTION HYBRIDIZATION (SH). S.O. Franklin, K. Elliott, Y-S. Zhu, C. Wahlestedt and C.E. Inturrisi*. Depts. of Pharmacology and Neuroscience, Cornell U. Med. College, New York, NY

To facilitate the study of the regulation of NMDA receptor gene expression, a rapid, sensitive and quantitative method for measuring the levels of the mRNA which codes for a protein sequence (NMDAR1) of a functional NMDA receptor was developed. The assay is based on ribonuclease protection of a 32P-labeled riboprobe (prepared from a 1413 bp fragment of the rat NMDAR1 cDNA (Moriyoshi et al., Nature 354, 1991). After hybridization for 4 hrs at 75°C, resistant riboprobe is precipitated with TCA and filtered using a cell harvester. Analysis was by scintillation counting and quantitation of mRNA was by use of a standard curve generated from a full length (4.2 kb) sense transcript of NMDAR1. The hybridization conditions produced a linear curve from 7.8 to 500 pg of sense transcript (i.e., NMDAR1 mRNA). NMDAR1 mRNA levels in RNA extracts from normal rat tissues were highest in cerebellum (26 pg/ug RNA), while olfactory bulb, frontal cortex, hippocampus, and striatum had similar values that ranged from 15 to 19 pg/ug RNA). Hypothalamic levels were (in pg/ug RNA) 12, spinal cord 7, liver <2 and E. coli tRNA undetectable. The distribution of NMDAR1 mRNA was similar in mouse CNS regions. These results demonstrate the sensitivity and utility of this method for the quantitation of NMDA receptor mRNA. (Supported by DA01457.)

119.14

ISOLATION OF NMDA RECEPTOR FROM ADULT RAT BRAIN. Y. Huang, M. Stanley* and K. Wu Div. of Neuroscience, New York State Psych. Inst., NY, NY 10032.

Abundant evidence suggests that NMDA (N-methyl-D-aspartate) receptor, a subclass of glutamate receptors, plays important roles in several neural processes in the central nervous system. The NMDA receptor appears to be associated with long-term potentiation, memory acquisition, learning as well as some neuronal disorder such as stroke and epilepsy. Remarkably, however, despite physiological importance, the molecular characteristics of NMDA receptor remain to be elucidated. Nearly all of the structural information about NMDA receptor has been deduced from cDNA sequence. To further characterize the NMDA receptor biochemically and functionally, we isolated the receptor from cerebral cortex of adult rat brain. Full characterization of the receptor will reveal whether the purified receptor exhibits properties characteristic of native receptor deduced from cDNA sequence and address the possibility of the post-translational modifications crucial to the physiological function of the receptors.

119.15

CULTURED CEREBELLAR GRANULE CELLS EXPRESS THE mGluR1 SUBTYPE OF METABOTROPIC GLUTAMATE RECEPTORS. G. Casabona[1], E. Aronica[2], P. Dell'Albani[2], A.A. Genazzani[1], V. Bruno[1], D.F. Condorelli[2], R. Bàlazs[2] and F.Nicoletti[1*]. Institutes of Pharmacology[1] and Biochemistry[2], University of Catania, Italy; and [2]Netherland Institute for Brain Research, Amsterdam, The Netherlands.

In cultured cerebellar granule cells, glutamate and quisqualate were more efficacious than 1$_s$,3$_R$-ACPD to stimulate polyphosphoinositide (PPI) hydrolysis, as observed in cells expressing the mGluR1 but not the mGluR5 receptor subtype (Masu M., personal communication). L-AP3 acted as a weak antagonist, and was devoid of intrinsic activity. Changes in agonist-stimulated PPI hydrolysis during cell maturation in vitro were paralleled by changes in the expression of the mRNA encoding the mGluR1 receptor protein. Metabotropic receptor agonists did not attenuate the stimulation of cAMP formation produced by forskolin, cholera toxin or norepinephrine, excluding the presence of receptor subtypes negatively coupled to adenylate cyclase. Rather, quisqualate stimulated cAMP formation in a concentration-dependent fashion, an effect that may be secondary to the mobilization of intracellular Ca^{2+} and activation of protein kinase C that follows the activation of mGluR1 receptors.

119.16

GROWTH CONDITIONS REGULATE THE EXPRESSION OF METABOTROPIC GLUTAMATE RECEPTORS IN CULTURED CEREBELLAR GRANULE CELLS. E. Aronica[1], F. Nicoletti[2], D.F. Condorelli[2], P. Dell'Albani[2], C. Amico[2], and R. Balàzs[1*]. Netherlands Institute for Brain Research, Amsterdam, The Netherlands[1]; and Institutes of Pharmacology[2] and Biochemistry[2], University of Catania, Italy. (Spon: ENA)

Cultured cerebellar granule cells grown in medium containing 25 mM K^+ (K25) express a metabotropic glutamate receptor coupled to polyphosphoinositide (PPI) hydrolysis. The functional expression of the receptor increased steeply during the first 4 DIV, and then progressively declined. This decline was prevented by conditions that deplete the glutamate present in the medium, suggesting that the latter down regulates metabotropic receptors. The decline in receptor function was also abolished when cultures were grown in medium containing 10 mM K^+ (K10) and NMDA. The action of NMDA was concentration-dependent, was antagonized by D-AP5, and was not mimicked by AMPA. Cultures at 7 DIV grown in K10 + NMDA also expressed higher levels of mGluR1 mRNA, as compared with cultures grown in K25. These results indicate that the expression of metabotropic receptors is regulated by growth conditions, which are known to exert a trophic influence on granule cells differentiating in culture.

119.17

EXCITATORY AMINO ACID (EAA) -MEDIATED SIGNAL TRANSDUCTION IN CULTURED NEURONS. Y.-H. Lee*, M. Yarom, D. L. Deupree and J.-Y. Wu.
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Two major G-protein-linked metabolic pathways, namely adenylate cyclase-triggered protein phosphorylation cascade and phospholipase C (PLC)-catalyzed phosphatidylinositol (PI) hydrolysis, are involved in transmitter-mediated signal transduction in mammalian CNS. In this communication, we described the effect of EAA on poly-PI turnover in the cultured neurons. Briefly, whole brains of 16-day-old rat embryos were used for the neuronal culture and cells at various time intervals, including early (4-6 days, referred to as DIV), middle (9-11 DIV), mature (16-18 DIV), and late (23-26 DIV) stages in culture were used. We used the ratio of inositol phosphates (IP_n) to PI as an index of poly-PI hydrolysis. For receptor activity, [^{3}H]-glutamate was used as a general ligand for all EAA receptor binding. It was found L-glutamate mediated stimulation of poly-PI turnover in the cultured neurons increased rapidly in the early stage, reached maximum in the mature stage, and decrease rather gradually in the late stage. A similar pattern was obtained for EAA receptor activity. Furthermore, AMPA and kainic acid-mediated poly-PI turnover appeared to be Ca^{2+}-dependent and expressed transiently, whereas those mediated by L-glutamate and trans-ACPD, a specific ligand for the metabotropic EAA receptor, were Ca^{2+}-independent and expressed over a longer time span. These results suggest that different EAA receptor subtypes are probably expressed and responsible for signal transduction at different stages of neuronal differentiation and maturation. [Supported in part by grants NS20978 (NIH) and BNS-8820581 (NSF).]

119.18

DEPOLARIZATION BY KCl MODULATES THE EXPRESSION OF METABOTROPIC GLUTAMATE RECEPTORS IN CULTURED CEREBELLAR NEURONS. M. Favaron*, J.M. Rimland, R.M. Manev, P. Candeo, D. Milani and H. Manev. FIDIA Research Laboratories, 35031 Abano Terme, Italy.

In primary cultures of rat cerebellar granule neurons the variations in the medium KCl content and KCl- mediated depolarizing growing conditions lead to a differential expression of the metabotropic glutamate receptor 1 (mGluR1) mRNA. The presence of 10-15 mM KCl in the growing medium allows a higher expression of mGluR1 mRNA as compared to the standard growing conditions of 25-30 mM KCl. The higher levels of mRNA correlated with the expression of a functional neuronal mGluR1 receptor as monitored by measuring the agonist-stimulated phosphoinositide formation and the agonist-induced rise in free intracellular Ca^{2+} levels. The exposure of established 8-12 days old cultures to a new environmental depolarizing condition lead to a novel pattern of mGluR1 expression. Hence, 15 mM KCl-grown neurons expressing high levels of mGluR1 can be shifted to low levels mGluR1 neurons after overnight exposure to an extra 5 mM KCl. Here we demonstrated that the mGluR1 expressing neurons quickly adapt to the new environmen- tal conditions by changing the level of receptors expressed even when maturation has been reached. The present model should help in understanding the mechanism of this plasticity.

GABA RECEPTORS: STRUCTURE

120.1

SPECIFIC EXPRESSION OF THE $GABA_A$-RECEPTOR α_3-, BUT NOT α_1-SUBUNIT, ON MONOAMINERGIC AND CHOLINERGIC NEURONS. B. Gao[1], J.P. Hornung*[2] and J.M. Fritschy[1] 1) Institute of Pharmacology, University of Zurich, 8006 Zurich, Switzerland; 2) Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.

The extraordinary structural heterogeneity of $GABA_A$-receptors is thought to reflect the existence of multiple subtypes with different sensitivities to GABA and pharmacological profiles. The allocation of receptor subtypes to identified neurons is a prerequisite for analyzing the functional relevance of $GABA_A$-receptor heterogeneity. Since monoaminergic (MA) and cholinergic neurons receive a prominent GABAergic input and can be influenced by benzodiazepines, the present study was undertaken to identify the type of $GABA_A$-receptor subunits expressed by these neurons. Rat and marmoset monkey (*Callithrix jacchus*) brain sections were processed for double-immunofluorescence staining using antibodies specific to the $GABA_A$-receptor α_1- or α_3-subunits and antibodies to markers for serotonergic, catecholaminergic (tyrosine hydroxylase) and cholinergic (choline acetyltransferase) neurons. In rat raphe nuclei, numerous neurons were immunoreactive for either the α_1- or the α_3-subunits. However, serotonergic neurons selectively expressed the α_3-subunit-immunoreactivity (IR) only. Catecholaminergic neurons of the A1-A9 cell groups were strongly labeled with the α_3-subunit antibody, as well. Throughout the rat brainstem, the α_1-subunit-IR decorated a population of neurons distinct from MA neurons. Similarly, cholinergic neurons in both rat and monkey basal forebrain were selectively labeled with the α_3-subunit-IR, whereas the α_1-subunit-IR decorated other cells in these nuclei. Thus, the results demonstrate that the $GABA_A$-receptor α_1- and α_3-subunits are expressed by different neuron populations in MA and cholinergic nuclei. The selective α_3-subunit expression by MA and cholinergic neurons suggest that these cells contain benzodiazepine type II receptors.

120.2

DEVELOPMENTAL EXPRESSION OF THE $GABA_A$ RECEPTOR α1 SUBUNIT POLYPEPTIDE IN RAT CEREBELLAR CELLS *IN VIVO* AND IN CULTURE. L. Nadler, K. Behringer, and R.E. Siegel*. Dept. of Pharmacology, Case Western Reserve Univ., Cleveland, OH 44106.

The $GABA_A$ receptor complex is a multisubunit ligand-gated ion channel that mediates the actions of GABA, the major inhibitory neurotransmitter in the CNS. The receptor is composed of multiple subunits, each of which exhibits distinct regional and temporal patterns of expression. Our previous studies demonstrated that α1 subunit mRNA levels in the rat cerebellar cortex are low in early postnatal development but subsequently increase 3- to 5-fold in the second week. To examine the relationship between the expression of the α1 subunit mRNA and the encoded polypeptide, we have generated an α1 subunit-specific antiserum. Use of this antiserum for Western blot analysis and immunohistochemistry demonstrated that α1 subunit polypeptide and mRNA expression change in parallel. While relatively weak signals are detected in early postnatal development, α1 subunit polypeptide levels rise several-fold in the second week. Subunit polypeptide expression peaks by postnatal day 21 (P21) after which it decreases to adult levels. In previous studies we demonstrated that the developmental changes in subunit mRNA expression appear to be mimicked in cerebellar neurons cultured from P10 rats. While low levels of the α1 subunit mRNA were initially found, 2.5-fold increases occurred between days 2 and 4 in culture. Immunohistochemistry indicates that subunit mRNA and polypeptide expression in the cultured cells also change in parallel. Together, these *in vivo* and *in vitro* studies suggest that the regulation of $GABA_A$ receptor α1 subunit expression occurs at the transcriptional level.

120.3

DEVELOPMENTAL CHANGES IN mRNA ENCODING HUMAN $GABA_A$ RECEPTOR SUBUNITS. A.R. Brooks-Kayal* and D.B. Pritchett. Children's Hosp. of Phila.; Children's Seashore House; Depts. of Peds., Pharm. and Neuro., Univ. of Penn, Phila., PA 19104.

The receptor for GABA, the major inhibitory neurotransmitter in the brain, is composed of multiple distinct subunits which confer pharmacological specificity for anticonvulsant drugs such as benzodiazepines. Recently $GABA_A$ receptor subunit mRNA levels have been shown to change in rat brain during development, with increases in α1 and decreases in α5 and β1 subunits. In this study we demonstrate similar changes in $GABA_A$ receptor subtype composition during human neurodevelopment. Samples of frontal cortex and cerebellum were collected at autopsy from patients without known neurological disease, aged 36 weeks post-conception to adult. $GABA_A$ receptor subunit mRNA levels were measured using the ribonuclease protection assay to hybridize radiolabeled antisense mRNA probes specific for α1 and α3 subunits to human brain mRNA. Levels of α1 were lowest at 36 weeks and highest in adult in both cortex (17-fold increase) and cerebellum (4-fold increase). By contrast, α3 mRNA levels in cortex were highest at 36 weeks and lowest in adult (3-fold decrease). At 36 weeks, α3 mRNA levels were 2-fold greater than α1 levels in cortex, but fell to only 4% of α1 levels by adulthood. Our findings suggest a marked change in $GABA_A$ receptor subunit composition during human development similar to that previously described in rats. Such a change could influence receptor activity, which might play a role in specific developmental patterns seen in some childhood seizure types as well as alter the efficacy of certain anticonvulsant drugs during development.

120.4

ISOLATION AND CHARACTERIZATION OF THE HUMAN $GABA_A$/BENZODIAZEPINE RECEPTOR α1-SUBUNIT GENE. M.D. Leach* and D.H. Farb. Dept. of Pharmacology & Experimental Therapeutics, Boston University School of Medicine, Boston, MA 02118.

The $GABA_A$ receptor is of great interest as it may have a large number of isoforms that mediate inhibitory synaptic transmission in the nervous system. There are α1-6, β1-4, γ1-3, δ1 and ρ1-2 subunit transcripts as well as alternatively spliced forms of β and γ subunits. *In situ* hybridization shows that each subunit is differentially distributed in the central nervous system, with the α1 subunit being the most abundant isoform. Our work has focused on identifying the gene structure and the regulatory elements that contribute to this subunit's pattern of expression.

Screening of a human chromosome 5 cosmid library using probes derived from human α1 cDNA sequences has identified a number of positive clones. Subsequent subcloning and sequencing of a 25 kb clone has yielded a 9 kb subclone which has sequence identity with the human α1 cDNA. This clone contains sequence for the last two introns and exons of the human α1 gene. We have previously reported the cloning and sequencing of the 5'-flanking region for the β1 gene of the $GABA_A$ receptor (S.J. Russek and D.H. Farb (1991) *Soc. Neurosci. Abst.* 17:526). We now report the isolation and sequencing of the 5' end of the human α1 gene. The 5' end of the human α1 gene was isolated using nesting and inverse polymerase chain reaction techniques. Primer extension analysis indicates the presence of a transcriptional initiation site. Various consensus sequences for regulatory elements have been identified and their potential roles in the control of gene expression are being evaluated.

120.5

CLONING AND CHARACTERIZATION OF 5' UPSTREAM REGULATORY REGION OF $GABA_A$ RECEPTOR ALPHA-1 SUBUNIT. I. Kang*, L.G. Miller. Dept. of Pharmacology and Experimental Therapeutics and Neuroscience Program, Tufts Univ. School of Medicine, Boston, MA, 02111.

From the published human $GABA_A$ receptor alpha-1 subunit cDNA sequence, a 200 bp stretch of DNA that encompasses the sequence upstream of the initiation codon was synthesized and has been used to screen human genomic library. Positive clones have been sequenced. Fragments of varying size have been fused to the upstream of lac Z gene and transfected into primary chick neurons in order to assess the presence of promoter activity and delineate the factors that regulate its function. Transcription start site is determined by S1 ribonuclease mapping.

120.6

CLONING AND SEQUENCING OF THE RAT α6 SUBUNIT GENE FOR THE $GABA_A$/BENZODIAZEPINE RECEPTOR. P.J. McLean*, S.J. Russek and D.H. Farb. Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA 02118.

The $GABA_A$ receptor is a member of the ligand-gated ion channel superfamily which includes the nicotinic acetylcholine receptor and the glycine receptor. *In situ* hybridization studies demonstrate that each subunit of the $GABA_A$ receptor has a differential distribution in the brain and spinal cord. We are identifying the regulatory elements of this gene family to study the differential regulation of $GABA_A$ receptor expression in the nervous system. Previously we reported the identification of the 5' flanking region of the human β1 gene and the position of several intron/exon boundaries with respect to the human and rat β1, β2 and β3 genes (S.J. Russek & D.H. Farb (1991) *Soc. Neurosci. Abst.* 17:526). We now report the cloning and sequencing of the rat α6 subunit gene for the $GABA_A$ receptor. The α6 gene is unique in that it is expressed in the cerebellum but not in other regions of the brain or spinal cord. The polymerase chain reaction (PCR) was used to amplify segments of rat genomic DNA using primers made to the rat α6 cDNA. Cloning and sequencing of the PCR products revealed sequence identity with the 5' end of the rat α6 cDNA, and several intron/exon boundaries have been mapped. Sequence comparison studies of alpha subunit genes suggest that the position of the introns at the 5' end of the α6 subunit gene are not conserved in the same way as was demonstrated for the beta subunits. Using inverse PCR, we have identified the 5' flanking region of the gene. This region may contain regulatory elements necessary for the control of the gene's expression in the central nervous system.

120.7

PCR-BASED HOMOLOGY PROBING REVEALS A FAMILY OF GABA RECEPTOR-LIKE GENES IN *DROSOPHILA*. Joseph E. Henderson, Patricia Marsella-Herrick, Douglas C. Knipple and David M. Soderlund.* Field of Environmental Toxicology and Dept. of Entomology, New York State Agric. Expt. Station, Cornell Univ., Geneva, NY 14456.

The GABA (γ-aminobutyric acid) receptor - chloride ionophore complex mediates synaptic inhibition in the central nervous system of both mammals and insects and is also a site of action for drugs and insecticides. The cloning and characterization of mammalian GABA receptors have identified genes encoding five groups of homologous subunits which, together with the gene encoding the strychnine-binding subunit of the glycine-gated chloride channel, comprise a family of ligand-gated chloride channel genes. We have taken advantage of a conserved octapeptide "signature motif" found in all members of this gene family to isolate genomic sequences encoding putative subunits of GABA receptors and other ligand-gated chloride channels in *Drosophila melanogaster* by employing a modification of the polymerase chain reaction (PCR) technique that depends on knowledge of only a single gene-specific amino acid sequence. The unique genomic sequence elements isolated and analyzed to date provide evidence for members of this gene family in *Drosophila* with varying degrees of homology to vertebrate GABA and glycine receptor subunit genes.

120.8

NATURAL MUTATION OF A CONSERVED TYR TO HIS IN THE M1 REGION OF THE MOUSE $GABA_A$ RECEPTOR DELTA SUBUNIT. G.L. Kamatchi, J.B. Wang, J. Yang and D.R. Burt*. Depts. of Pharmacology and Anesthesiology, Univ. of Maryland School of Medicine, Baltimore, MD 21201.

$GABA_A$ receptors are multisubunit inhibitory chloride channels which are opened by γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain. The δ subunit, originally detected by homology screening, has no known role in receptor function. In sequencing cDNAs for this subunit in audiogenic seizure prone (DBA/2J) and seizure resistant (C57BL/6J) inbred strains of mice, we detected in some clones from both strains a substitution of His for a conserved Tyr (#239) in the first putative transmembrane region (M1). This Tyr is conserved in other published $GABA_A$ and glycine receptor subunit sequences. As assessed by polymerase chain reaction amplification of tail genomic DNA, His was rare in both inbred strains. Its occurrence as an apparent allelic variant in one strain, let alone two, is extremely unusual, and it may not occur in homozygous form due to lethality. Further study of this mutation and its functional consequences, through breeding and expression studies, should help reveal the roles of δ subunits in $GABA_A$ receptor composition and function. (Supported by USPHS grants NS25525 and AA07559 to DRB)

120.9

MUTAGENESIS OF N-LINKED GLYCOSYLATION SITES OF THE $GABA_A$/BENZODIAZEPINE (BZ) RECEPTOR α1 SUBUNIT DECREASES THE LEVEL OF RO15-1788 BINDING IN TRANSFECTED 293 CELLS. G.A. Hastings, A.L. Buller, E.F. Kirkness and C.M. Fraser*. Section on Molecular Neurobiology, LNG, NIAAA, Rockville, MD 20852.

The addition of oligosaccharide chains to polypeptides by N-linked glycosylation is an important step in the maturation of membrane-bound proteins. The $GABA_A$/BZ receptor subunits have consensus sites for N-linked glycosylation which may affect GABA/BZ binding affinities and/or receptor complex formation. We have begun to assess the role of N-linked glycosylation on $GABA_A$/BZ receptor function by eliminating these sites on the α1 subunit by mutagenesis. There are two consensus N-linked glycosylation sites located on the extracellular portion of each α subunit. The rat α1 cDNA was mutagenized, changing Asn to Gln, at positions 10 (Gln^{10}) and 110 (Gln^{110}) or both ($Gln^{10/110}$). The wild-type and mutant α1, β1 and γ2 long-form cDNAs were transiently transfected into human kidney 293 cells. Saturation binding of the BZ antagonist [^{3}H]RO15-1788, with 25μM clonazepam as competitor, was performed with crude membranes prepared from transfected cells. There was no significant change in K_D between wild-type α1 (0.66 nM), Gln^{10} (0.54 nM) and Gln^{110} (0.50 nM). [^{3}H]RO15-1788 specific binding was undetectable in the $Gln^{10/110}$ mutant, however. There was a consistent decrease in B_{max} of 18-43% with the Gln^{10} and Gln^{110} mutants compared to wild-type. These results suggest that the N-linked oligosaccharide chains of α1 do not affect the affinity of the $GABA_A$/BZ receptor for BZs. However, α1 N-linked glycosylation is necessary for normal $GABA_A$/BZ receptor processing or stability.

120.10

CHARACTERIZATION OF STABLE CELL LINES EXPRESSING A CLONED $GABA_A$ BENZODIAZEPINE RECEPTOR Cl^- CHANNEL COMPLEX. B.J. Hamilton*, D.J. Lennon, D.D. McKinley, W.B. Im, H.K. Im, P.H. Seeburg, and D.B. Carter. CNS Research, The Upjohn Company, Kalamazoo, MI 49001 and Zentrum Fur Moleckulare Biologie, University of Heidelberg, Germany.

The $GABA_A$/Benzodiazepine receptor-Cl^- channel complex is composed of various combinations of 48-55kd subunits from at least three different classes of genes resulting in a highly diverse group of $GABA_A$ receptors. To elucidate the pharmacologies of various $GABA_A$ receptor subunit combinations, rat $GABA_A$ receptor cDNA's coding for α_1, α_3, α_5, α_6, β_1, β_2, and γ_2 were transfected into a human embryonic kidney cell line previously transfected with Adenovirus 5 (HEK-293). Stable cell lines expressing $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, $\alpha_6\beta_2\gamma_2$, and $\alpha_6\beta_1\gamma_2$ were obtained by selection in G418 and/or hygromycin containing media at a frequency of 0.008-0.04, as determined by Northern blot analysis. Subsequent Northern blot analysis of the four cell lines has shown stable expression of the three subunit messages over twenty-five passages. Membrane binding studies using the ligand flunitrazepam demonstrate saturable binding for the $GABA_A$ receptor subtype combinations $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, and $\alpha_6\beta_1\gamma_2$. While the $\alpha_6\beta_2\gamma_2$ combination shows no significant binding to flunitrazepam, saturable binding was demonstrated using the imidazodiazepine Ro15-4513. Electrophysiological studies of these cell lines reveal a typical dose response to GABA. The GABA response is potentiated by diazepam in the $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, and $\alpha_6\beta_1\gamma_2$ expressing cell lines, and by Ro 15-4513 in the $\alpha_6\beta_2\gamma_2$ expressing cell line. The expression of recombinant subunits in these stable cell lines produces functional $GABA_A$ receptor molecules which provides a tool for elucidating the features of the GABA mediated Cl^- channel and determining the intrinsic efficacy of drugs modulating the system.

120.11

IMMUNOHISTOCHEMICAL MAPPING OF $GABA_A$ RECEPTOR α SUBUNITS IN RAT BRAIN

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$GABA_A$ receptors are hetero-oligomeric membrane glycoproteins, in which five subunits are thought to assemble to form individual ion channel complexes. Five distinct classes, some containing several isoforms (α1-6, β1-3, γ1-3, δ and ρ1-2) encoded by separate genes have been identified by molecular cloning. To facilitate identification of putative native receptor subtypes, we have generated antibodies against the α1, α2, α3, α5 and α6 subunits, employing as antigens peptide sequences unique to the respective isoforms.

The affinity purified antibodies generated in rat brain sections characteristic patterns which could be blocked by preadsorption with their respective peptide antigens. α1, α2 and α3 subunit-like immunoreactivities were widespread, overlapped in some structures (e.g. neocortex and olfactory bulb) and were mutually exclusive in others (e.g. basal forebrain, substantia nigra). In contrast, α5- and α6 subunit-directed antibodies showed much more circumscribed staining patterns: α5 in CA3 cell fields in hippocampus, some cortical interneurones and thalamic reticular nucleus, and α6 principally in cerebellar granule cells. Double staining with antibodies to α1 and parvalbumin indicates that this subunit is very prominently expressed by GABAergic neurones.

The distributions are consistent with previous biochemical studies indicating that the majority of $GABA_A$ receptors contain only a single α subunit species, but do not exclude the possibility that in some brain areas different α subunits coexist within receptors.

120.12

CHARACTERIZATION OF STRUCTURAL AND FUNCTIONAL SUBTYPES OF GABA-A RECEPTORS WITH ANTIBODIES TO THE β3 SUBUNIT. **K.H. Huh, T.M. DeLorey, S. Endo, and R.W. Olsen, Dept. Pharmacology, UCLA, Los Angeles, CA 90024.**

β-Subunit subtype-specific antibodies were used to separate pharmacological subtypes of GABA-A receptors. GABA-A receptors are hetero-oligomers whose subunit composition is unknown. Antibodies were prepared against a synthetic peptide selective for the cow β3 subunit in the cytoplasmic loop. These antibodies immuno-precipitated 50% of [^{3}H]flunitrazepam binding sites from crude soluble extracts of bovine cortex, whereas antibodies which recognize all β subunits (Endo & Olsen, J. Neurochem. 1992) precipitated 100%. The GABA analog THIP differentially inhibits [^{3}H]muscimol photo-incorporation into different β polypeptides (Bureau & Olsen, Mol. Pharm. 37,497,1990), and binding in different brain regions (Olsen et al. Mol.Neuropharm.1992). Heterogeneity of THIP binding affinity is evident in membranes, soluble extracts, and affinity-purified receptors. [^{3}H]Muscimol binding sites in purified receptors immunoprecipitated with anti-β3 antibodies had higher affinity for THIP than the supernatant receptor subpopulations disenriched in β3 and enriched in other β subutypes. These results suggest that all receptors contain a β subunit but the nature of the β subunit varies and results in receptor subtypes showing differential pharmacological specificity at the GABA site. Supported by NIH grant NS28772.

120.13

IDENTIFICATION OF β_2 AND β_3 SUBUNITS OF THE $GABA_A$ RECEPTOR FROM RAT BRAIN WITH SUBUNIT SUBTYPE-SPECIFIC ANTIBODIES. Timothy M. DeLorey*, Shuichi Endo, Tina K. Machu, Michael D. Browning and Richard W. Olsen. Department of Pharmacology, UCLA, Los Angeles, CA 90024 and Colorado Health Sciences Center, Denver, CO 80262.

$GABA_A$ receptors are hetero-oligomeric complexes composed of a combination of polypeptides which are encoded by 15 different genes. These polypeptides have been subclassed into α, β, γ, δ, and ρ subunits. To date four different β subunit subtypes have been identified. Specific molecular weight assignments of the four β subtypes has been inferred by a variety of methods. Using subtype specific antibodies we were able to assign molecular weights to the β_2 and β_3 subunit subtypes. Purified $GABA_A$ receptors from bovine brain were separated by 7.5% acrylamide gel and transferred to nitrocellulose. Subunits were identified on Western blots using affinity purified β_2 and β_3 subtype-specific antibodies. Both antibodies were raised in rabbits against subtype specific peptides based on hydrophilic sequences in the cytoplasmic loop deduced from rat cDNA sequences. The β_2 subunit was identified as a protein band at 57 kDa and the β_3 was identified as a 54 kDa band. Supported by NIH Grant N528772.

120.14

LOCALIZATION OF THE ß3 SUBUNIT OF THE GABA-A RECEPTOR IN RAT FOREBRAIN. M. Esclapez, T.M. DeLorey, R.F. Tyndale, N.J.K. Tillakaratne, A.J. Tobin, R.W. Olsen and C.R. Houser*. Brain Research Institute, UCLA, and VA Medical Center, Los Angeles, CA 90024.

The ß3 subunit subtype of the GABA-A receptor was localized with *in situ* hybridization (ISH) and immunocytochemical (ICC) methods. ISH histochemistry using digoxigenin-labeled ß3 cRNA probes demonstrated strong neuronal labeling in pyramidal and granule cell layers of the hippocampal formation and pyriform cortex. Moderate levels of labeling were present in striatal neurons, and moderate to light labeling was observed in many cortical neurons. The antiserum used for ICC studies was produced in rabbits to a synthetic peptide sequence that was specific for the ß3 subunit and found within the cytoplasmic loop of the rat receptor sequence. Two types of labeling were observed: 1) a diffuse, fine punctate labeling that was present at high levels in dendritic layers of the hippocampal formation and at moderate levels in the striatum and cerebral cortex; and 2) a discrete outlining of some neuronal cell bodies and proximal dendrites that was most prominent around nonpyramidal neurons of the cerebral cortex. Double labeling studies demonstrated that a high percentage of the heavily outlined neurons contained GAD. While the results from the two methods are consistent with each other, they emphasize important differences between the cellular localization of the mRNA and the protein for this GABA-A receptor subunit. Supported by VA Medical Research Funds, NS29231, NS28772 and NS22256.

120.15

ANTIBODIES TO THE HUMAN γ_2 SUBUNIT OF THE $GABA_A$ RECEPTOR. L.P. Fernando, Z.U. Khan, P. Escribá, X. Busquets, C.P. Miralles and A.L. De Blas. Div. Molec. Biol. and Biochem., SBLS, UMKC, Kansas City, MO 64110

A γ_2 subunit (short form) of the $GABA_A$ receptor/benzodiazepine receptor ($GABA_AR$/BZDR) was cloned from an adult human cerebral cortex cDNA library in λgt11. The 261 base pair intracellular loop located between M3 and M4 was amplified using polymerase chain reaction (PCR) and inserted into the expression vectors λgt11 and pGEX-3X. Both ß-galactosidase (LacZ) and glutathione-S-transferase (GST) fusion proteins containing the γ_2 intracellular loop (IL) were purified and both rabbit antisera and monoclonal antibodies were made.

The rabbit antibody reacted with the γ_2IL of both LacZ and GST fusion proteins and immunoprecipitated the $GABA_AR$/BZDR from bovine and rat brain. The antibody reacted in immunoblots of the affinity-purified $GABA_AR$/BZDR from bovine cerebral cortex with a wide peptide band of 44,000-49,000 Mr. Immunoprecipitation studies with the anti-γ_2IL antibody suggest that in the cerebral cortex 87% of the $GABA_AR$ with high affinity for benzodiazepines and 70% of $GABA_AR$ with high affinity for muscimol contain at least a γ_2 subunit. Immunoprecipitations with various antibodies indicate that the most abundant combination (over 60%) of $GABA_AR$ subunits in the cerebral cortex involves α_1, γ_2 and β_2 and/or β_3. The monoclonal antibodies to γ_2IL are being characterized. This work was supported by grant NS17708 from NINDS.

120.16

IDENTIFICATION OF 5 DISTINCT $GABA_A$ RECEPTOR SUB-TYPES BY DOUBLE AND TRIPLE-IMMUNOFLUORESCENCE STAINING WITH SUB-UNIT SPECIFIC ANTIBODIES. J.- M. Fritschy, D. Benke, S. Mertens & H. Mohler. Inst. of Pharmacology, Univ. of Zurich, CH-8006 Zurich, Switzerland.

A family of at least fifteen sub-units provides the basis for an extraordinary structural heterogeneity of $GABA_A$ receptors in the CNS. To assess the functional relevance of $GABA_A$ receptor subtypes on defined neurones, physiological sub-unit combinations must be clarified at the cellular and sub-cellular levels. Co-localisation of prevalent $GABA_A$ receptor sub-units in rat brain neurones was assessed by confocal laser microscopy using double and triple immunofluorescence staining in restricted areas with polyclonal antisera recognising the α_1, α_3 and γ_2 sub-units and the monoclonal antibody bd17 ($\beta_{2/3}$ sub-units). Five distinct sub-unit combinations were identified. Four sub-units (α_1, α_3, $\beta_{2/3}$, γ_2) were co-localised in neurones of the basal forebrain, olfactory bulb and brainstem reticular formation. The triple combination, α_1, $\beta_{2/3}$, γ_2, again recognised neurones of the brainstem reticular formation plus the pallidum and substantia nigra reticulata. Olfactory bulb granule cells were immunofluorescent for α_3, $\beta_{2/3}$, and γ_2 sub-units whilst the combination of α_1, α_3, γ_2 sub-units was detected in brainstem motor nuclei neurones. Finally, a double combination, (α_3, γ_2) was observed in the inferior olive. Using single α_1 and α_3 sub-unit immunostaining, segregation of two different receptor sub-types within a single neurone was seen in Purkinje cells; whereas the α_3 sub-unit was found on dendrites and soma of these cells, the α_1 sub-unit was detected on the soma only. By demonstrating the existence of distinct $GABA_A$ receptor sub-types on identified neurone populations, the results provide the basis for a functional analysis of $GABA_A$ receptors *in situ* and may lead to novel therapeutic approaches based on sub-type-specific drugs.

120.17

AUTORADIOGRAPHIC LOCALIZATION OF $GABA_A$ AND $GABA_B$ BINDING SITES IN THE NUCLEUS TRACTUS SOLITARII OF THE CAT B.E. Maley*, Dept. Of Anatomy And Neurobiology, University Of Kentucky Medical Center, Lexington, KY 40536

Gamma-aminobutyric acid (GABA) is one of the major inhibitory neurotransmitters in the central nervous system. Previous studies from our laboratory have demonstrated a uniform distribution of GABA immunoreactive synaptic terminals throughout the neuropil of the various subdivisions of the cat nucleus tractus solitarii. In the present investigation we have studied $GABA_A$ and $GABA_B$ binding sites using *in vitro* autoradiography with [^{3}H] muscimol to label high affinity $GABA_A$ binding sites and [^{3}H]baclofen for $GABA_B$ binding sites. Each ligand was used at or below its calculated K_D. Autoradiograms of [^{3}H]muscimol and [^{3}H]baclofen labeled tissues demonstrated homogeneously moderate grain density over the entire nucleus tractus solitarii. There appeared to be no preferential localization of autoradiographic grains within specific subdivisions of the nucleus for either ligand used. The distribution of $GABA_A$ and $GABA_B$ binding sites reminiscent of the pattern for GABA immunoreactive terminals in the nucleus tractus solitarii, suggests a possible morphological correlate for GABAergic receptors in the cat nucleus tractus solitarii.

120.18

CHRONIC EXPOSURE OF CORTICAL NEURONS TO $GABA_A$ AGONISTS DOWN-REGULATES $GABA_A$/BENZODIAZEPINE RECEPTOR PEPTIDES ON THE CELL SURFACE. P.A. Calkin and E.M. Barnes, Jr.*, Dept. of Biochemistry, Baylor Col. of Med., Houston, TX 77030.

Chronic exposure of cortical neurons to GABA has been shown to reduce the density of [^{3}H]flunitrazepam binding sites and GABA-gated Cl^- currents by more than 60% [Hablitz et al. (1989) *Brain Res. 501*, 332-338]. In this study, we measured the levels of $GABA_A$ receptor (GaR) peptides on the surface of neurons which had been treated for 5 days with 100 μM GABA, isoguvacine or 4,5,6,7-tetrahydro-isoxazolo[5,4-c]pyridin-3-ol (THIP). GaR peptides on the surface of intact neurons were labeled with the cleavable, membrane-impermeant reagent 3,3'-dithiopropionyl 1-sulfosuccinimidyl glycyltyrosine[^{125}I]. Extracts from the [^{125}I]labeled cells were immunoprecipitated with polyclonal antibodies against affinity-purified chicken GaR. Analysis of the immunoprecipitates on nonreducing SDS-polyacrylamide gels showed incorporation of ^{125}I into 50- and 53-kD peptides. The label on these peptides was removed by treatment of intact neurons with 100 mM glutathione which cleaves the disulfide linkage. In cells chronically exposed to GABA or isoguvacine, the level of iodinated 50- and 53-kD peptides was greatly reduced, while a lesser effect was produced by the partial $GABA_A$ agonist, THIP. The effect of GABA on cell-surface GaR peptides was prevented by co-exposure of the neurons to 1 μM R 5135, a $GABA_A$ antagonist. Alone, R 5135 had no noticeable effect. The data suggest that agonist-dependent down-regulation reduces the levels of GaR subunit peptides on the neuronal surface.

Supported by DK17436, MH47715, and NS11535 from USPHS.

120.19

SULFHYDRYL MODIFICATION OF $GABA_A$ RECEPTORS HAS DIFFERENTIAL EFFECTS ON BENZODIAZEPINE BINDING. L.L. Duncalfe* and S.M.J. Dunn. Department of Pharmacology, University of Alberta, Edmonton, Canada T6G 2H7

The effect of sulfhydryl modification on benzodiazepine binding to the $GABA_A$ receptor has been studied in crude brain membranes prepared from bovine cerebral cortex. The bovine brain membranes were reduced with increasing concentrations of dithiothreitol (DTT). The binding of the partial inverse agonist [^{3}H]-Ro15-4513 (2.5 nM) was reduced to about 30% of control by 5 mM DTT, while the binding of the classical agonist [^{3}H]-flunitrazepam (2.5 nM) was unaffected by 30 mM DTT. Binding assays of [^{3}H]-Ro15-4513 in the presence of 10 mM DTT indicated that the K_D increased by more than 2-fold and the binding site density was reduced from 2.8 to 1.1 pmol/mg. DTT (10 mM) had no significant effect on either the K_D for [^{3}H]-flunitrazepam binding or the density of binding sites. DTT reduction of the membranes followed by alkylation of free sulfhydryl groups with iodoacetamide reduced the number of sites for both [^{3}H]-Ro15-4513 and [^{3}H]-flunitrazepam to about 60% of control, but had no effect on the equilibrium dissociation constants. This work was supported by the Medical Research Council of Canada, Alberta Heritage Foundation for Medical Research and Nordic Laboratories Inc.

120.20

IDENTIFICATION OF A [3H]FLUNITRAZEPAM PHOTO-AFFINITY SUBSTRATE IN BOVINE GABA-A RECEPTOR. **G.B. Smith and R.W. Olsen, Dept. of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024.**

We previously showed that the site of covalent modification by [3H]flunitrazepam ([3H]FLU) photoaffinity labeling of partially purified GABA-A receptor lies within the N-terminal 30kD of the α subunit. This would include the N-terminal extracellular domain and could extend up to the cytoplasmic end of the proposed first transmembrane spanning domain. Here, we have tentatively identified a hydrophobic tryptic peptide containing incorporated radioactivity in aromatic residues. The receptor was purified by benzodiazepine affinity chromatography from bovine cortex. After photoaffinity labeling with 10nM [3H]FLU, receptor polypeptides were separated by SDS-PAGE, sliced and counted for radioactivity, electroeluted and subsequently digested with TPCK trypsin. Digest products were separated by reverse phase HPLC and fractions containing label were manually collected. N-terminal sequencing showed a mixture of sequences one of which could be matched with 4/5 residues beginning at residue I223 of the published bovine α1 subunit. This sequence, following the tryptic cleavage site K222, begins at the extracellular surface of the first transmembrane domain and is conserved in all currently identified α subunit clones. Supported by NS 28772.

PEPTIDES: ANATOMICAL LOCALIZATION—NON-PRIMATES

121.1

LOCALIZATION OF PROLACTIN-IMMUNOREACTIVE NEURONS IN THE RABBIT CNS USING A MONOCLONAL ANTIBODY. R.J. Walsh*, M.F. Sherif, and J.G. Scammell Dept. of Anatomy, George Washington Univ., Washington, DC 20037, Dept. of Anatomy, Kuwait University, Kuwait, Dept. of Pharmacology, Univ. of South Alabama College of Medicine, Mobile, AL 36688

Evidence suggests that the CNS produces prolactin (PRL). Immunocytochemistry using 6F11, a monoclonal antibody which cross-reacts with PRL from several species including rabbit, was used to identify PRL-positive cells in the CNS and pituitary of female New Zealand white rabbits. Colchicine was injected into the lateral cerebral ventricle 20 h prior to perfusion with PBS and 4% paraformaldehyde. Cryostat sections were sequentially incubated in 3% H_2O_2, 0.2% Triton X-100, 10% goat serum and then for 2 days at 4° C with 6F11 diluted 1:1000 in PBS-1% BSA. Sections were exposed to avidin biotinylated goat anti-mouse IgG, washed, and incubated with DAB or AEC. Lactotrophs of the pituitary exhibited a prominent staining reaction. PRL-positive neurons occurred in the supraoptic, paraventricular, anterior hypothalamic, periventricular, lateral hypothalamic, mammillary, and perifornical hypothalamic nuclei. PRL-positive neurons also resided in the globus pallidus, zona incerta, entorhinal cortex, septal nuclei, and various thalamic nuclei. Preabsorption of 6F11 with PRL abolished the staining as did elimination of 6F11 from the incubation. The results indicate 1) 6F11 Mab recognizes PRL-positive neurons within the rabbit CNS, and 2) PRL-positive neurons have a broad distribution in the rabbit CNS.

121.2

IMMUNOCYTOCHEMICAL LOCALIZATION OF ACTH 4-10 IN RAT BRAIN. S.J. Lee,*C.J. Aoki, and F.L.Strand. Dept of Biology and the Center for Neural Science, New York University, New York, N.Y. 10003.

A naturally secreted neuropeptide, adrenocorticotropin (ACTH 1-39), contains all of its bioactivity within the peptide ACTH 1-24. While the corticotropic properties reside in the peptide 11-24, the neurotrophic properties appear to be in the peptides 1-10, 4-10, and 1-13 (α-MSH) (rev. Strand et al., 1991, Physio Rev 71: 1017). Although previous studies have shown immunoreactivity to anti-ACTH 1-39 and anti-ACTH 11-24 in the hypothalamus, thalamus, amygdala and periaqueductal gray area (Watson et al., 1978, Science 200:1180; Watson et al., 1978, Nature 275: 226), the immunoreactivity to anti-ACTH 4-10 has never been examined. Antisera against ACTH 4-10 were raised by immunizing male Sprague-Dawley rats. ACTH 4-10 was modified by cross linking to hemocyanin (30mg/ml) using glutaraldehyde (5%). The glutaraldehyde conjugated form of the peptide (4mg/ml) was mixed with complete Freund's adjuvant and injected subcutaneously into two rats. Sera were collected after the third immunization. Immunodot blot analysis indicated that amino acids 7-10 may be the major antigenic site. Antisera were not immunoreactive to ACTH 1-39 or ACTH 1-16. In brain sections, immunolabeling was seen within processes in the septal area and the perimeter of the third ventricle, including the median eminence. Furthermore, these processes, presumably axons, were more pronounced in young adult rats weighing 150-170gm than in adult rats weighing 250-270gm. However, perikaryal labeling was not observed in any of the brains. These results indicate that the neurotrophic fragment has a more restricted distribution than of its precursor ACTH 1-39. Supported by 2-S07-RR07062-26 to CA and Organon.

121.3

GASTRIN (CCK-8) IMMUNOHISTOCHEMICAL STAINING PATTERNS IN THE NORMAL AND NEONATALLY DEAFFERENTED TRIGEMINAL (V) BRAINSTEM COMPLEX. P.H. Young*, D.S. Zahm, M.C. Beinfeld & M.F. Jacquin. Depts. Anat. & Neurobiol. and Pharm. & Physiol. Sci., St. Louis Univ. School of Medicine, St. Louis, MO 63104.

Prior studies indicate that high levels of CCK in medullary dorsal horn (MDH) laminae I and II reflect a higher-order CCK-containing projection. However, it is unclear which neurons give rise to this projection because polyclonal CCK antibodies stain very few CNS neurons that project to the V complex. Elution of CCK immunoreactive peptides extracted from micro-punches of the superficial MDH indicated that a monoclonal antibody to Gastrin (courtesy of Dr. J. Walsh) detects mainly CCK8-like material in the MDH. In 16 adult rats, gastrin immunohistochemistry revealed a mean (+SD) of 8.6 ± 3.1 labeled cells per 50 μm transverse section through MDH laminae I and II. The largest #s were in lamina II and their processes formed a dense, continuous, circumferential and thin band that was prominent in lamina II. It is likely that these labeled neurons represent a significant source of CCK in the V complex. Dense terminal label was also noted in rostrally displaced pockets of substantia gelatinosa embedded in each of the V spinal tract and subnuclei interpolaris and oralis. Weak terminal staining was also seen in non-gelatinous regions of all 4 V subnuclei.

Prior studies also revealed elevated CCK levels in the MDH after infraorbital nerve section at birth. In 7 similarly lesioned rats, immunohistochemistry indicated a near total elimination of Gastrin + cells and more diffuse terminal staining in deafferented regions of the MDH. Thus, V primary afferent inputs may affect CCK expression, transport and/or release mechanisms. Support: NIH DE07662, DE07734, NS18667, NS23805.

121.4

SYNAPTIC CONNECTIONS IN THE PARACERVICAL GANGLION OF THE FEMALE RAT: CALCITONIN GENE-RELATED PEPTIDE-, GALANIN-, AND TACHYKININ (SUBSTANCE P AND NEUROKININ A)-IMMUNOREACTIVE NERVE TERMINALS. R.E. Papka* and D.L. McNeill. Dept. Anatomical Sciences, Univ. Oklahoma HSC, Oklahoma City, OK 73190

Many nerve fiber varicosities in the pelvic paracervical ganglia (PG) immunoreactive for calcitonin gene-related peptide (CGRP), galanin (GAL) and the tachykinins substance P (SP) and neurokinin A (NKA) are capsaicin-sensitive, originate in dorsal root ganglia and are considered primary afferents. Even though these varicosities are pericellular and closely adjacent to principal neurons, are they synaptically related? The present study addresses this question. In order to reveal the relationship between varicosities and neurons, PG of female rats were immunostained with antisera against synapsin I (SYN), a nerve terminal marker, and microtubule-associated protein-2 to define somata and dendrites. SYN endings were predominantly axosomatic with fewer being axodendritic. PG were processed for electronmicroscopic immunohistochemistry using standard immunogold and peroxidase-anti-peroxidase procedures. Unmyelinated fibers and varicosities immunoreactive for CGRP, GAL, SP and NKA were observed in the interstitium between neurons. Immunoreactive varicosities were within the satellite-cell sheath of the neuron somata and intimately associated with the membrane of the somata, somatic spines or with the proximal part of dendrites. Membrane specializations, indicative of synaptic contacts, between the fibers and the principal neurons were observed. It is suggested that these peptide immunoreactive sensory fibers and varicosities are involved in regulation of activity in the PG. (Supported by NIH NS 22526).

121.5

NEURONAL AND NON-NEURONAL ORIGINS OF CGRP IN THE GASTRIC WALL. G. Jakab,[1] K. Pacak[2], I. Salamon[3], L.Rosivall[3], H.deF.Webster[1]*. Lab. of Neuropathology[1], CNB[2], NIH, Bethesda, MD 20892 and Semmelweis Univ. Med. Sch. Budapest, Hungary[3]

The distribution of calcitonin gene-related peptide-immunoreactive (CGRP-IR) primary afferent fibers was studied in the rat gastric wall. The CGRP-IR fibers showed a characteristic arrangement in each layer of the gastric wall. The fine, varicose CGRP-IR fibers arborized in the basal region of the mucosa and followed either blood vessels or smooth muscle cells while running in the tunica propria toward the lumen of the stomach. The nerve fibers' branching pattern suggests that there may be columnar neurovascular regulatory units in the mucosa which are oriented perpendicular to the luminal epithelial surface and which do not overlap. When examined electron microscopically, boutons and axon terminals of these varicose nerve fibers contained large dense core vesicles. Also both immunohistochemistry and in situ hybridization histochemistry revealed the presence of a number of CGRP-producing cells located mainly in the lower mucosa. Some of them were inserted between the cells of gastric glands. Induction of gastric stress ulcers changed the distribution of CGRP mRNA and peptide. The results suggest that CGRP provided by these two systems may act as a neurotrophic factor. It may also regulate gastric microcirculation have a role in the pathogenesis of gastric ulcers.

121.6

GROWTH HORMONE RELEASING HORMONE (GHRH) IN THE GROWTH HORMONE (GH)-DEFICIENT DWARF MOUSE BRAIN. H. Dalcik[1]*, H. Endo[2], F. Talamantes[2] and C.J. Phelps[1], [1]Dept. Anatomy, Tulane Univ. Sch. Med., New Orleans, LA 70112, and [2]Dept. Biol, Univ. Calif., Santa Cruz, CA 95064.

Ames dwarf mice bear a genetic mutation which, in the homozygous condition (df/df), results in absence of GH and prolactin expression. These mice were used as a model to evaluate negative feedback regulation of hypothalamic GHRH in chronic GH deficiency. Hypophysectomy in adult rats has been reported to result in decreased GHRH (Merchenthaler and Arimura, Peptides, 1985, Leidy et al., Neuroendocrinology, 1990). Conversely, GHRH mRNA increases after hypophysectomy (Chomczynski et al., Mol. Endocinol., 1988) and in GH-deficient rats (Mizobuchi et al., Mol. Endocinol., 1990) and mice (Frohman et al., Mol. Endocrinol., 1989). Ames dwarf mice (df/df), which completely lack GH, also show increased hypothalamic GHRH mRNA (Hurley et al., Endocrine Soc. abs., 1991). Investigation of GHRH peptide expression in mice has been hampered by the absence of a homologous antiserum. This study employed an antiserum to a 25 aa c-terminal synthetic peptide specific to mouse-GHRH. In RIA, the antiserum did not detect several CNS peptides, including hGHRH, rGHRH, CRF and oxytocin. For ICC, some mice were treated with intracerebroventricular colchicine prior to sacrifice. Thirty μm coronal brain sections were incubated in anti-mGHRH antiserum (1:10,000), and processed using ABC method for ICC. In untreated mice, intensely stained GHRH terminals were apparent in both DF/? and df/df median eminence, but neuronal cell bodies were few, and limited to ventral hypothalamus and arcuate nuclei. In-colchicine treated normal (DF/?) and dwarf mice, immunoreactive cell bodies were noted in hypothalamic MPOA, and in ARC, VMH, DMH, PeN, SON and in PVN. GHRH-immunoreactive fibers were observed mainly in the vicinity of the cell bodies in both DF/? and df/df mice. The number of immunoreactive cell bodies, in colchicine-treated dwarf mice appeared to be increased in the PVN and in ARC. These results indicate that GHRH displays a unique distribution in the mouse, and that GHRH peptide and mRNA are similarly affected in the GH-deficient Ames dwarf brain. Supported by PHS grant NS25987 (CJP).

121.7

EXPRESSION OF A CHIMERIC GALANIN GENE IN TRANSGENIC MICE. Å. Rökaeus* and J. A. Waschek. Dept. of Biochemistry I, Molecular Endocrinology Unit, Karolinska Institute, S-104 01 Stockholm, Sweden and The Mental Retardation Research Center and Dept. of Psychiatry, Neurobiochemistry Research Group, University of California, Los Angeles, CA 90024-1759.

Galanin (GAL), a biologically active neuropeptide has been suggested to play a role in dementia of the Alzheimer's type and possibly also in diabetes. A linearized DNA fragment containing 5 kb of bovine GAL gene 5'flanking-sequence fused to the intronless cDNA encoding firefly luciferase have been introduced into fertilized eggs to raise transgenic mice, in order to establish lines of mice which could be used to identify upstream sequences mediating constitutive and inducible transcriptional activity in a tissue specific manner. Screening for transgenic founders (F0) and offspring (F1) was performed by slot-blot analysis and/or PCR.

Nine out of 25 developed mice became hybrydization positive; six of the latter mice mice appeared to express less then one copy of the gene, suggesting that these animals may be mosaic. When two low as well as one high expressing male were mated with non-transgenic females, only the latter male transmitted the gene-construct to the offspring (F1; 9 out of 21). When tissue extracts (2.5-10% of total) from two 12 week old PCR-positive female mice were analyzed for luciferase activity, detectable activity was found in the brain and spinal cord, uterus and heart, i.e. in organ with known GAL expresion, as well as in the thymus; no activity could be detected in the pituitary, adrenal glands, urinary bladder, jejunum or lung.

This set of limited data, indicate that 5 kb of bovine GAL gene 5'flanking-sequence appears to direct expression of luciferase activity to at least some of the organs with known vertebrate GAL expression.

121.8

THE IMMUNOHISTOCHEMICAL LOCALIZATION OF SUBSTANCE P IN THE SPINAL DORSAL HORN IN THE WOBBLER MOUSE. Y-H Du and J Xu*. Anatomy, Med Coll Jinan Univ, Guangzhou 510632, PR China; Neurology,* Med Univ SC, Charleston, SC 29425 USA.

The wobbler mouse mutant possesses a recessively inherited degeneration of motor neurons in spinal cord and has been considered a model for human hereditary motor neuron disease. It has been reported that Substance P (SP)-containing nerve fibers connect closely the motor-neurons in rat ventral horn plays a role in control and regulation of motor function.

Using PAP technique, the distribution of SP in the wobbler mouse spinal cord dorsal horn was studied. In the homozygous, distribution of SP immunoreactive (IR) fibers were widespread in the dorsal horn but more dense in laminae I and II than in other laminae. SP IR fibers concentrated in a cap-like area covering the margin of the dorsal horn. IR in the lateral area of the dorsal horn was usually higher than in the medial area. In laminae V and VI, SP IR nerve fibers formed large plexuses. The IR fibers passed from dorsal horn into the intermediolateral gray matter and the dorsal gray commissures and entered the ventral horn.

In the heterozygous, the distribution of SP IR in the dorsal horn were similar to that of the homozygous mouse. SP IR nerve fibers were concentrated mainly in the laminae I and II, and fewer in other laminae. No obvious difference was observed on SP IR in the dorsal horn between the homozygous and heterozygous mouse. The present results indicate that heredity only affect the motor neurons in ventral horn and do not involve dorsal horn even in heterozygous mouse.

121.9

NEUROKININ B PEPTIDE-2 NEURONS PROJECT FROM THE HYPOTHALAMUS TO THE SPINAL CORD OF THE RAT. Huang Zhuo and C.J. Helke*Dept. of Pharmacology, Uniformed Services Univ. of the Health Sciences, Bethesda, MD 20814.

Our studies of the spinal distribution of neurokinin B peptide-2 (NKB P2) (one fragment of the NKB precursor protein) showed that NKB P2-like immunoreactive (LI) fibers and terminals were mainly distributed in the superficial dorsal horn and central autonomic area. NKB P2-LI fibers and terminals were also found in the intermediate grey of the spinal cord. Neurons having NKB P2-LI were recently located in the hypothalamus, epithalamus, amygdaloid complex, and cerebral cortex (J. Comp. Neurol. 317:341, 1992). To explore the origins of spinal NKB P2, we combined the retrograde transport of Fluoro-Gold (FG) with NKB P2 immunohistochemistry (antibody from J. Krause, Washington Univ.) in the rat. Whereas neurons labeled with either NKB P2-LI or FG were found in many CNS regions, spinally projecting NKB P2-LI neurons were only found in the dorsal hypothalamic area. About 30% of the spinal projecting neurons in the dorsal hypothalamic area had NKB P2-LI. About 35% of the NKB P2-LI neurons in the dorsal hypothalamic area projected to the spinal cord. Most of these irregularly-shaped spinally projecting NKB P2-LI neurons have a cell size of 15 by 25μm. The results of the present study indicate that hypothalamic NKB-P2 neurons are the main CNS source of the spinal NKB P2-LI. (Supported by NIH grant NS20991)

121.10

ULTRASTRUCTURAL LOCALIZATION OF NEUROTENSIN (NT) IMMUNOREACTIVITY (IR) IN THE STELLATE GANGLION (SG) OF THE CAT. M.A. Morales, M. Bachoo, B. Collier, A. Beaudet, and C. Polosa*. Depts. of Pharmacology, Physiology and Neurology, McGill University, Montreal, Quebec, Canada H3G 1Y6.

The NT content of the SG is depleted by prolonged stimulation, and lost after chronic section, of the preganglionic input (Maher et al., 1991). Exogenous NT excites SG cells (Bachoo & Polosa, 1988). In anesthetized cats, the localization of NTIR in the SG was examined. Light microscopy showed NT varicosities in between ganglion cells throughout the ganglion, although the density around individual cells was variable. With electron microscopy, NTIR was localized exclusively to cholinergic-type synaptic boutons which mainly formed axo-dendritic synapses. The NT boutons had the same morphology as non-immunoreactive ones. Occasionally both types formed synapses on the same dendrite. Within the bouton, NTIR was most intense in large dense core vesicles (LDCV), but often cytoplasmic staining was also evident. Small, clear, synaptic vesicles were not labelled. Prolonged stimulation (20 min 40 Hz) of the preganglionic input to the ganglion reduced the number of LDCV per bouton (from 6.6 to 3.0) as well as the extent and intensity of NTIR within labelled boutons. The data suggest that NT is released in a stimulus-dependent manner from a subset of preganglionic axons in the cat SG. (With MRC support).

121.11

SEPARATE POPULATIONS OF CELLS IN THE NUCLEUS OF THE SOLITARY TRACT (NTS) PROJECT TO THE PARABRACHIAL NUCLEUS (PBN) AND THE SPINAL TRIGEMINAL NUCLEUS (Sp 5). R. D. Hofbauer, J. A. Yuan, C. Cozzari[1], B. K. Hartman and P. L. Faris*. Division of Neuroscience Research in Psychiatry, Univ. of MN 55455 and [1] Instituto Biologia Cellulare CNR, Roma, Italy.

Several neuropeptides, e.g., CCK and enkephalin, have been localized in NTS projections to both the PBN and the Sp5. This study was aimed at determining if these projections arise from separate populations of cells or if the same cells collateralize to innervate both areas. This was accomplished using two fluorescent retrograde tracers: Fluorogold (20 nl) and rhodamine-conjugated latex beads (200 nl) injected into the PBN and into the interpolar Sp5. Two distinct staining patterns resulted from the two injection sites. Cells projecting to the PBN were localized mainly to the medial subdivision of the NTS and the area postrema. Cells projecting to the Sp5 were localized to more lateral aspects of the NTS. Only an occasional cell (3-5 per brain) was found to be double-labeled. We are currently conducting additional experiments in which the Sp5 injections are centered in the caudal portion of this nucleus. In addition to the NTS labeling results described above, two other interesting findings arose from this study. First, injections of tracers into the PBN also resulted in extensive labeling in the Sp5. Since administration of the latex beads produces a small injection site, which was highly restricted to the dorsolateral PBN, it appears that the Sp5 and NTS both project to the same subdivision of the PBN. Thus, this confirms the role of the PBN in the integration of visceral and somatosensory information. Second, injections of a tracer into the Sp5 also resulted in cell labeling in the PBN. A large number of these retrogradely labeled cells lay within the PBN tracer injection site. Thus, it appears that the PBN-Sp5 projections are truly reciprocal, i.e. projecting to or from the same subdivision.

121.12

SOMATOSTATIN INTERNEURONS AND THE PATCH/MATRIX COMPARTMENT W.J.Rushlow*, C.C.G.Naus and B.A.Flumerfelt. Dept. of Anatomy, University of Western Ontario, London, Canada, N6A 5C1.

The patch/matrix compartments represent functionally distinct components of the caudate-putamen. It has been suggested that virtually no intermingling of the afferents and efferents to and from these compartments occurs but the GABAergic, cholinergic and somatostatinergic (SOM) interneurons may bridge the compartmental boundary. In particular, SOM interneurons have been chosen as likely candidates. Examination of SOM neurons and their corresponding fibre plexus using fluorescence microscopy reveals that SOM neurons may be found within the patch, within the matrix, and on or around the patch/matrix border. Dendrites of SOM matrix neurons may sometimes be traced over long distances (>50um) and occasionally approach and enter the patch compartment, suggesting an interaction. SOM interneurons found deep within the patch are also fairly common. In order to help clarify the possible role of SOM with regards to the patch/matrix a scanning laser confocal microscope was utilized. Using this technique it is possible to obtain three dimensional reconstructions of the area in and around the patch compartment. SOM interneurons found in and around the patch/matrix border sent dendrites into both compartments. Those dendrites which passed into the patch tended not to remain in the patch but exited to the matrix. Occasionally, a SOM interneuron sent an axon into the patch but it also exited to the matrix. SOM interneurons located deep within the patch were not common. The patch was almost devoid of SOM fibres which were prevalent in the matrix compartment. This suggests that SOM interneurons are involved in patch/matrix communication but the interaction is one-way. Though the SOM neurons may receive a synaptic input from both the patch and matrix, the target of the SOM neurons resides within the matrix. Supported by grants from MRC (B.A.F.) and NSERC (C.C.G.N.).

121.13

IDENTIFICATION OF KINDLING ACTIVATED NEURONS IN THE PARAVENTRICULAR NUCLEUS. D.T. Piekut*, S. Pretel and C. Applegate. Neuroendocrine Unit and Dept. of Neurology, University of Rochester, Rochester, NY 14642.

Neurons of the paraventricular nucleus of the hypothalamus are known to respond to stressful stimuli. We have used the immunocytochemical identification of the Fos protein to identify anatomically PVN neurons which are activated following kindling elicited epileptic seizures and subsequently combined this procedure with in situ hybridization to determine which peptides are synthesized in the activated, c-fos expressing neurons. Rats were kindled from sites in the a) amygdala, b) entorhinal cortex or c) exposed to a one time strong, electrical seizure evoking stimulation. The animals were perfused following survival times of 1-12 hrs. and vibratome sections were labeled for the immunocytochemical detection of the Fos protein using antisera provided by Drs. Slammon, Curran and from Oncogene. We observed a substantial increase in the immunocytochemical expression of Fos in specific and distinct CNS sites of the experimental animals. Numerous activated (i.e. Fos expressing) neurons were identified in specific parvocellular components of the paraventricular nucleus of rat hypothalamus, fewer in magnocellular components. Maximal immunolabeling was seen at 2-3 hrs. following the seizure stimulation, and a significant decrease of Fos immunolabeling was evident following a survival time of 4-6 hrs. ; few immunolabeled neurons remained at 12 hrs. following stimulation. Little Fos immunoreactivity was evident in control animals which received the same intensity of current as the kindled rats, but at a frequency not inductive of seizure behavior. Combined immunocytochemical and in situ hybridization procedures show that numerous PVN neurons activated in kindling express the CRF mRNA, while others express the ENK mRNA. Thus a classical population of stress associated PVN neurons appears to be activated during seizure. This indicates that seizure elicitation has an effect on the central control of the pituitary adrenal axis. NIH grant NS18626

121.14

COEXISTENCE OF FOS IMMUNOREACTIVITY AND SOMATOSTATIN mRNA IN THE HIPPOCAMPAL FORMATION. S. Pretel*, C. Applegate and D.T. Piekut. Neuroendocrine Unit and Dept. of Neurology, University of Rochester, Rochester, NY 14642.

The animal model of kindling is a well established electrophysiological paradigm for the investigation of neuronal mechanisms underlying neuroplasticity. The neuroanatomical basis for the occurrence of kindling is largely unknown. We have used the expression of c-fos to identify neurons which are activated during different stages of the kindling process and have begun to examine which peptides are synthesized in these neurons. Rats were stimulated from the entorhinal cortex and perfused following a) the elicitation of the first afterdischarge, b) the elicitation of stage 3 behavior and c) the elicitation of stage 5 behavior; control animals received the same intensity of current as the kindled rats, but at a frequency not inductive of seizure. Tissue sections were then processed for immunocytochemistry and in situ hybridization, using antibodies against the Fos protein and an oligonucleotide probe to label somatostatin mRNA (provided by Drs. Slammon, Curran and Young, resp.). The results of the single-labeled studies showed a progressive increase in the immunocytochemical expression of c-fos in animals perfused following the different stages of kindling in comparison to control animals. The results of the double-labeled studies showed that a majority (>60%) of the somatostatin-synthesizing neurons in the hippocampus and dentate gyrus were activated following seizure stimulation. It is apparent, however, that the somatostatin synthesizing neurons represent only one component of the kindling activated, i.e. c-fos expressing neuronal network. The data provide anatomical evidence for the hypothesis that the development of kindling involves the progressive recruitment of a neuronal network over time. The data also show that a substantial percentage of somatostatin synthesizing neurons in hippocampus and dentate gyrus are activated during kindled seizures. NIH grant NS18626

121.15

EXPRESSION OF mRNAs CODING FOR THE PROHORMONE CONVERTASES PC1 AND PC2 IN THE CENTRAL (CNS) AND PERIPHERAL (PNS) NERVOUS SYSTEMS IN ADULT AND FETAL MOUSE. M. Marcinkiewicz*, N.G. Seidah and M. Chrétien. Clinical Research Institute of Montreal, Montreal, Quebec, Canada, H2W 1R7.

Neural and endocrine tissues express the prohormone convertases PC1 and PC2 (PCs) which cleave precursor proteins into active products. Here, we studied the comparative tissue-specific expression of these convertases in the CNS and PNS of embryonic and adult mice, and during development. In the adult CNS both PCs are widely distributed from the olfactory bulb to the spinal cord including the cortex, hippocampal formation, amygdala and hypothalamus where a distinct distribution pattern and different (low to high) levels of hybridization are revealed. PC2 is very enriched in the caudate nucleus and thalamus. The localization of PC1 and PC2 overlaped with the sites of neuropeptides production including: AVP, OX, POMC peptides, enkephalins and LH-RH (hypothalamus) or SP (epithalamus). Furthermore, we noted that the nuclei which produce the biogenic amines (substantia nigra, raphe nuclei and locus coeruleus) also express PC1 and PC2. During ontogeny, PC1 and PC2 are first detected at embryonic day 11 (E11) in a small group of pontine neurons within the CNS. Later, the hybridization gradually increased and extended to many areas. Around the time of birth (E21), a high level of PC2 hybridization was detected in the caudate nucleus. In general, the CNS levels of PC1 and PC2 gradually increased until adulthood. In contrast, in the PNS, including the trigeminal and spinal ganglia, the levels of PC1 and PC2 mRNAs were much more elevated during embryonic development (E11-E21 with a peak between E14-E18) as compared to that in adult mice. These data demonstrate unique spatial and temporal expression patterns of PC1 and PC2 within the CNS and PNS.

121.16

DISTRIBUTION OF THE SUBTILISIN-LIKE PROCESSING ENZYME PC1 AND MET-ENKEPHALIN IN AtT-20 CELLS AND HYPOTHALAMUS. P.J. Hornby*, S.D. Rosenthal, O. Vindrola, J. P. Mathis, and I. Lindberg. Depts. of Pharmacology and Biochemistry, Louisiana State University Medical Center, New Orleans, LA 70112

The subtilisin-like prohormone convertase PC1, found in certain neuroendocrine organs and in brain, can accurately cleave the opioid peptide precursor proopiomelanocortin into bioactive peptide products. We have applied single and dual-staining immunocytochemical techniques to determine both the cellular localization of PC1 as well as its anatomical relationship to met-enkephalin in AtT-20 cells stably transfected with proenkephalin cDNA. Rat hypothalamic tissue was also studied. Antiserum to PC1 was generated in rabbit to a fragment of the mature protein corresponding to the presumed amino terminus. Using this antiserum, avidin-biotin immunocytochemistry revealed a dense immunoreaction product within AtT-20 cells immediately adjacent to the nucleus; staining was also apparent in the cell tips. This staining could be abolished by preadsorption with the immunizing peptide but not by a C-terminal fragment of the same protein. PC1-immunostained AtT-20 cells were then incubated in met-enkephalin antiserum and the glucose-antiglucose oxidase reaction product was used to stain met-enkephalin-containing areas blue. Met-enkephalin staining was present throughout the cell body and was especially prominent in the tips of the cells. The dense accumulation of PC1 immunoreaction products adjacent to the nucleus suggests that the Golgi apparatus may be a major site of PC1 in these cells. In vibratome-sectioned normal rat hypothalamus, diffuse light punctate staining of PC1 was noted in the supraoptic nucleus and the medial hypothalamus. Supported by an LSU Neuroscience Center incentive grant and PHS grants DK42714 (PH) and DA05084 (IL).

121.17

LHRH-IMMUNOREACTIVE TERMINAL NERVE PROJECTIONS IN THE NASAL CAVITY OF THE TIGER SALAMANDER. C.R. Wirsig-Wiechmann* Department of Neurobiology & Anatomy and Program in Neuroscience, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1010.

There is a paucity of information on the peripheral projections of the terminal nerve (TN) in most vertebrate species due to the weak immunoreactive labelling of this nerve in the nasal cavity. We have performed two experimental manipulations in the tiger salamander in an attempt to increase the luteinizing hormone-releasing hormone-immunoreactive (LHRH-ir) labelling of TN peripheral processes: 1) the TN was sectioned just caudal to the olfactory bulbs or 2) a 100 mg melatonin pellet was embedded subcutaneously for 3 days. The latter procedure decreases LHRH release thereby causing a buildup of LHRH in fibers. Following these procedures animals were sacrificed and tissue was processed for light microscopic analysis of the distribution of LHRH-ir processes.

LHRH-ir fibers were observed projecting 1) into the rostral olfactory epithelium, 2) to glands in the lamina propria of the rostral olfactory epithelium and to a collection of glands situated between the main nasal cavity and Jacobson's organ, 3) into the smooth muscle which controls nares movements, and 4) along trigeminal branches to reach the pterygopalatine ganglion (PPG). Some LHRH-ir fibers extended through the PPG and projected caudally along trigeminal branches to the trigeminal ganglion.

The lesion and hormone manipulations have allowed the detection of TN projections not seen previously with immunocytochemical procedures alone. The wide distribution of LHRH-ir TN processes in the nasal cavity and cranium suggests that this nerve may influence many different cranial structures during appropriate pheromonal or neuroendocrine events.

(Supported by NIH grant NS27586)

121.18

AFFERENT AND EFFERENT INNERVATION OF LEECH NEPHRIDIA OCCURS BY A SINGLE IDENTIFIED NEURON EXPRESSING FMRF-NH_2-LIKE IMMUNOREACTIVITY. A. Wenning*[1], U. Kaltenhäuser[1], U. Greisinger[1] and U. Hoeger[2]. [1]Fak. f. Biologie, Universität Konstanz, W-7750 Konstanz, FRG; [2]Inst. f. Zoologie, Universität Mainz, W-6500 Mainz, FRG.

Each of the 34 nephridia in the leech, *Hirudo medicinalis*, is individually innervated by a single peripheral neuron, the nephridial nerve cell (NNC). Resting potential and activity of the NNC depend on the extracellular Cl^- concentration (Wenning & Calabrese, J Comp Physiol , 168:53,1991). The NNC expresses FMRF-NH_2-like immunoreactivity (FLI), as shown here by injecting the NNC intracellularly with Lucifer yellow and subsequent processing for FLI using a polyclonal antibody. The numerous arborizations of the NNC in the nephridium are dotted with varicosities and, as seen in ultrathin sections processed for FLI, they directly innervate the urine-forming cells. Further, the NNC sends an axon branch to the sphincter of the urinary bladder.

The amount of FMRF-NH_2-like peptide present in one nephridium was determined using a competitive ELISA with an FMRF-NH_2- thyroglobulin conjugate in the solid phase, nephridial extracts, and the same polyclonal antibody as for immunocytochemistry. We found 1.3±0.75 pM peptide (±s.d., N=24) per nephridium. Application of FMRF-NH_2 at $1.7x10^{-10}$ M tripled urine flow in unstimulated, isolated nephridia (N = 9), suggesting that the NNC functions as a receptor *and* as a modulator. This is the first reported evidence that FMRF-NH_2-like peptides may modulate the activity of transporting epithelia.

The antibody was generously donated by R. Elde (U Minnesota). Supported by the Deutsche Forschungsgemeinschaft (We 745/4-1).

121.19

ANTHO-RF AMIDE-LIKE IMMUNOREACTIVITY IS PRESENT AT A TWO-WAY SYNAPSE IN THE SEA ANEMONE. J.A. Westfall*, C-Z Yu,[1] K. Sayvar,[1] and C.J.P. Grimmelikhuijzen[2] [1] Department of Anatomy and Physiology, Kansas State University, Manhattan, KS 66506, [2] Center for Molecular Neurobiology, University of Hamburg, 2000 Hamburg 20, Germany.

Ultrastructural evidence of synapses with clear, opaque, or dense-cored vesicles aligned at one or both sides of a pair of paramembranous densities has been demonstrated in several species of sea anemones. The localization of a specific neuropeptide in vesicles at one of these synapses has not been reported previously. To date, 13 novel neuropeptides, including Antho-RFamide (<Glu-Gly-Arg-Phe-NH_2), have been isolated from sea anemones. Our goal was to localize Antho-RFamide-like immunoreactivity at synaptic loci in tentacles of the sea anemone Anthopleura elegantissima. Tentacles were extirpated, fixed in 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1M phosphate buffer, pH 7.6, with 0.3N NaCl, for 2 hours at 4°C and osmicated for embedding in Epon/Araldite or nonosmicated and embedded in Lowicryl K4M. Immunogold staining of thin sections was done using rabbit anti-Antho-RFamide (#177IV or #177IVA at a dilution of 1:500) for 1-2 hours followed by 15-nm-gold-conjugated, goat antirabbit IgG diluted to 1:10 (Lowicryl) and 1:40 (Epon-Araldite). Opaque vesicles, ranging from 50-150 nm in diameter, were gold-labeled in nerve cells and on both sides of a two-way synapse. Vesicles associated with the paramembranous densities were smaller (~50 nm) than those not associated with synaptic loci. Clear vesicles and dense-cored vesicles with prominent halos were not labeled. Our findings suggest that a population of neurons containing 50-150 nm opaque vesicles is immunoreactive to Antho-RFamide antiserum and that these neurons are synaptically connected in the sea anemone Anthopleura elegantissima. (Supported by NSF grant no. IBN-9120161)

121.20

CHARACTERIZATION, LOCALIZATION AND EFFECT OF PACAP (PITUITARY ADENYLATE-CYCLASE ACTIVATING POLYPEPTIDE) IN FROG BRAIN AND PITUITARY. L. Yon[1], N. Chartrel[1], J.M. Conlon[2], M. Lamacz[1], F. Gracia-Navarro[3], L. Jeandel[1], A. Fournier[4], M. Feuilloley[1], A. Arimura[5], M.C. Tonon[1] and H. Vaudry[1]*. (1) Eur. Inst. Pept. Res., Lab. Mol. Endocr., CNRS URA 650, Univ. Rouen, France; (2) Regul. Peptide Center, Creighton Univ., Omaha, USA; (3) Dept. Cel. Biol., Univ. Cordoba, Spain; (4) INRS Santé, Québec Univ., Québec, Canada; (5) Biomed. Res. Lab., Tulane Univ., Belle Chasse, USA.

PACAP is a 38-amino-acid neuropeptide that was isolated from the ovine hypothalamus on the basis of its ability to stimulate adenylate-cyclase activity in anterior pituitary cells. The NH_2-terminal region of PACAP is structurally similar to VIP and thus identifies the peptide as a member of the secretin-glucagon family. In this study, we have purified PACAP from an extract of 1200 frog brains (Rana ridibunda) by gel chromatography and reverse phase HPLC and determined the primary structure of the peptide by automated Edman degradation. The sequence of frog PACAP shows only one amino acid substitution (Ile instead of Val at position 35) compared with the mammalian sequence. The distribution of PACAP-containing neurons was studied by immunofluorescence using an antiserum raised against PACAP(1-27). The main populations of immunoreactive perikarya were found in the medial and ventral diencephalon i.e. the ventral and ventrolateral areas of the thalamus, the preoptic and infundibular nuclei of the hypothalamus. In addition, beaded nerve fibers were found coursing from the ventral infundibulum to the external vascular layer of the median eminence. Frog PACAP and some analogs were synthesized and their biological activity tested. Frog PACAP(1-38) and PACAP(1-27) produced dose-dependent increases in cAMP concentrations in dispersed frog anterior pituitary cells and hypothalamic tissues whereas [Des-His^1] PACAP(1-38) was inactive. Frog PACAP(1-38) also stimulated cytosolic Ca^{2+} levels in all cell populations of frog pars distalis. Taken together these results indicate that, in amphibians, PACAP satisfies the criteria expected of a hypophysiotropic neurohormone.

122.1

A CANDIDATE SUBTILISIN-LIKE PROHORMONE PROCESSING ENZYME EXPRESSED BOTH IN THE NEUROENDOCRINE BAG CELLS AND IN THE EXOCRINE ATRIAL GLAND OF *APLYSIA CALIFORNICA*. W.R.A. van Heumen, S.L. Knock*, G.T. Nagle, and A. Kurosky. Department of Human Biological Chemistry and Genetics and Marine Biomedical Institute, Univ. of Texas Med. Branch, Galveston, TX 77555.

The neuroendocrine bag cells and the exocrine atrial gland of *Aplysia californica* synthesize multipeptidergic ELH-related precursors that upon enzymatic processing give rise to a number of well characterized (neuro)peptides. Using the polymerase chain reaction we have shown that the bag cells express a candidate prohormone processing enzyme that belongs to the subtilisin-related class of enzymes (see accompanying abstract, Nagle *et al.*). We have conducted *in situ* hybridization studies utilizing complementary oligonucleotide probes to establish the occurrence of the enzyme in the bag cells and atrial gland. Preliminary results indicate that in addition to synthesizing related peptide precursors, both the atrial gland and the bag cells use a similar enzyme for their processing. The occurrence of the subtilisin-like mRNA in the atrial gland is particularly interesting since it produces large amounts of ELH-related peptides and may be a relatively rich natural source for the subtilisin-related enzyme. Supported by NIH NS 29261 and the Robert A. Welch Foundation (H-1190).

122.2

A PUTATIVE SUBTILISIN-LIKE DIBASIC PROHORMONE PROCESSING ENZYME IN *APLYSIA* NEUROENDOCRINE BAG CELLS. G.T. Nagle*, W.R. van Heumen, S.L. Knock, A.T. Garcia and A. Kurosky. Marine Biomedical Institute and Department of Human Biological Chemistry and Genetics, Univ. of Texas Med. Branch, Galveston, TX 77555.

Strong evidence is accumulating that the endoproteases which process prohormones at dibasic residue cleavage sites are members of a subtilisin-related class of proteases. Using the polymerase chain reaction (PCR), we have isolated and characterized a cDNA product from *Aplysia californica* neuroendocrine bag cells that encodes a sequence which is highly homologous to the subtilisin-related class of processing proteases. The characterized cDNA PCR product showed the highest degree of residue identity with the furin-related group of proteins (human/mouse furin 71%; *Drosophila* furin 63%). These results establish that *Aplysia* contain a subtilisin-like gene and suggest that the expression of this gene may play a role in processing *Aplysia* precursor proteins in the bag cells and likely also in the endocrine atrial gland. Furthermore, these results, together with sequence results of other furin-like enzymes provide evidence that the furin-like enzymes may represent a separate subclass of the subtilisin-like processing enzymes. Supported by NIH NS 29261 and the Robert A. Welch Foundation (H-1190).

122.3

PROCESSING OF PRO-OPIOMELANOCORTIN (POMC) BY A YEAST ASPARTIC PROTEASE, YAP3: SIMILARITY TO THE MAMMALIAN POMC-CONVERTING ENZYME. A. Azaryan, M. Wong, F. Estivariz, Y.P. Loh*. Section on Cell. Neurobiology, LDN, NICHD, NIH, Bethesda, MD 20892.

The yeast aspartic protease gene, YAP3, is expressed in a mutant that lacks the KEX2 gene which encodes the processing enzyme for pro-α-mating factor (Egel-Mitani et al., 1990). In order to compare the YAP3 gene product with the aspartic protease, POMC converting enzyme (Loh et al., 1985) found in pituitary, that can cleave POMC at paired basic residues, we have overexpressed the YAP3 gene in yeast and characterized its activity. The YAP3 gene (a gift from Nova Nordisk, Denmark) was cloned into pEMBLyex plasmid and the YAP3 gene product was then overproduced in S. cerevisae strain BJ 3501. To purify YAP3 protease, a 10,000g supernatant of yeast cell lysate was applied on con A-agarose column followed by a pepstatin A-agarose chromatography. Protease activity was assayed with ^{125}I-B-lipotropin (B-LPH) as substrate. The enzyme was characterized as Mr~70,000 glycoprotein with a pH optimum of 4.0-4.5. It was activated by DTT and Ca^{++} and potently inhibited by pepstatin A. The YAP3 enzyme cleaved POMC at Lys-Arg pairs to yield Mr~21-23,000 ACTH and B-LPH. It also generates B-endorphin from B-LPH and Lys-γ-MSH and γ-MSH from bovine N-POMC$_{1-77}$ as identified by SDS-PAGE, RIA or HPLC. The YAP3 protease appears to share a number of properties (substrate specificity, inhibitor sensitivity, pH optimum) with the POMC-converting enzyme found in bovine pituitary secretory granules. YAP3 may be a homologue of this mammalian prohormone processing enzyme.

122.4

ROLE OF THE MAMMALIAN ENDOPROTEASES *PC1* AND *PC2* IN PROSOMATOSTATIN (PSS) PROCESSING. A.S. Galanopoulou, G. Kent, H.H. Zingg*, and Y.C. Patel. Fraser Laboratories, McGill University, Royal Victoria Hospital, Montreal, Quebec, Canada, H3A 1A1.

Mammalian PSS is processed at a dibasic Arg-Lys and a monobasic Arg site to produce SS-14 and SS-28 respectively. We have previously shown that PSS is capable of monobasic conversion to SS-28 in the constitutive secretory pathway of COS-7 cells by a *furin*-like enzyme (the only known endoprotease found in these cells) but that this conversion is relatively inefficient based on the amount of unprocessed PSS. We have also shown that efficient processing of PSS occurs in neuroendocrine cells and correlates with *PC1* and *PC2* expression. In this study we have determined directly the ability of *PC1* and *PC2* to influence PSS processing in COS-7 cells co-transfected with PKS5 (PSS expression vector) and either pmPC1 or pmPC2 (*PC1* and *PC2* expression vectors). Cell lysates and media were analysed for SS-14, SS-28 and unprocessed PSS by HPLC and RIA. *PC1* and *PC2* expression was confirmed by Northern analyses with cRNA probes.

	SS-14 (%)		SS-28 (%)		Unprocessed PSS (%)	
	cells	media	cells	media	cells	media
PKS5	3	7	35	5	62	88
PKS5+pmPC1	14	31	22	11	64	58
PKS5+pmPC2	4	14	31	2	65	84

CONCLUSIONS: (i) *PC1* is capable of dibasic cleavage of PSS to SS-14; such processing, however, is relatively inefficient. (ii) *PC1* is without effect on monobasic SS-28 conversion. (iii) *PC2* does not influence PSS processing. (iv) The relative inefficiency of *PC1*, and lack of activity of *PC2* on PSS processing in the constitutive pathway of COS-7 cells suggest that compartmentalization of proteolytic events in secretory vesicles or other, more specific endoproteases may be required.

122.5

THE DEVELOPMENTAL EXPRESSION OF FURIN, PC1, AND PC2 IN THE RAT. M. Zheng, R.E.M. Scott, T. Wood*, N.G. Seidah, and J.E. Pintar. Dept. Anat. and Cell Biol., Columbia P&S, NY, NY 10032 and Lab. Biochem. Neuroendocrinol., Clin. Res. Inst. Montreal, Montreal, Quebec H2W 1R7, Canada.

The genes for mammalian subtilisin-like endoproteases furin, PC1, and PC2 have recently been isolated and are implicated in endoproteolytic cleavage of precursor molecules, which is a key step in posttranslational maturation of polyproteins. We have begun to examine the expression of these genes during rat development by *in situ* hybridization and to compare their expression patterns to those of potential substrates. Furin mRNA thus far has been detected earlier than PC1 and PC2 mRNA and is present in the heart primordium as early as e9.5. At this age, proenkephalin mRNA is also present in the heart, but in a much lower proportion of cells. By midgestational ages (e12-e14), the expression domain of furin has expanded to encompass most tissues outside of the nervous system, with the highest level of expression detected in the liver. Furin, therefore, may contribute to endoproteolytic processing of precursor proteins in peripheral tissues during development. Unequivocal expression of both PC1 and PC2 thus far has been detected as early as e12.5. At this age, expression of PC2 is predominant in the peripheral cranial ganglia, DRG, and mesencephalon, while PC1 expression is detected in the ventral hypothalamus. By e13.5, PC1 is also detected in a cell population adjacent to the DRG. In the CNS, more widespread expression of both PC1 and PC2 occurs by e14. In general, the expression patterns thus far identified for PC1 and PC2 characterize regions throughout the nervous system containing differentiated neurons rather than neuronal precursors. PC1 expression is particularly evident in the ventral myelencephalon, hypothalamus, and ventral spinal cord. PC2 appears to be expressed at a relatively higher level than PC1; this difference is especially apparent in the mesencephalon, pons and hypothalamus. The different levels and partially overlapping expression patterns of PC1 and PC2 suggest that these endoproteases may dictate differential neuropeptide precursor processing in the developing nervous system. Supported by HD-18592 (JEP).

122.6

RECOMBINANT PROHORMONE CONVERTASE 1: CHARACTERIZATION AND PURIFICATION. Yi Zhou* and Iris Lindberg. Dept. of Biochemistry and Molecular Biology, LSU Medical Center, New Orleans, LA 70112.

Proteolytic cleavage of propeptides at dibasic residues is a very important processing event in neuropeptide biosynthesis. The mammalian subtilisin-like proteases may contribute to this processing. Prohormone convertase 1 (PC1; also known as PC3), one member of this family, has been identified by reverse transcription-PCR. While co-transfection experiments have provided evidence that PC1 is involved in propeptide processing, no work has been done thus far at the protein level. In order to characterize the biochemical properties and enzymatic activity of PC1, we established a stable recombinant Chinese hamster ovary cell line (CHO) which constitutively synthesizes and secretes PC1 protein, amplified by means of the dihydrofolate reductase-coupled method. Immunoblotting and pulse-chase labeling were performed to study PC1 protein biosynthesis in this recombinant cell line. The results demonstrated that PC1 protein was mainly synthesized and secreted in an 87 kDa form; 74 and 66 kDa forms were also found in both the cell extracts and cell medium. Neither the 74 nor the 66 kDa PC1 proteins were recognized by PC1 carboxyl-terminal antibody, suggesting that proteolytic cleavage at the carboxyl terminus occurred before secretion. The 87 kDa PC1 protein has been purified from conditioned medium to near homogeneity using ion exhange and hydrophobic interaction chromatography. Amino-terminal sequence analysis showed that the 87 kDa PC1 protein was generated by cleavage after the RSKR sequence at the amino terminus of the PC1 precursor. Weak calcium-dependent proteolytic activity was detected in conditioned medium with the fluorogenic substrate RSKR-AMC. This proteolytic activity, which was very labile, co-purified with PC1 immunoactivity upon hydrophobic interaction chromatography and could not be detected in the medium of control cells.

122.7

CHARACTERIZATION OF THE PROHORMONE CONVERTASE PC2 OVEREXPRESSED IN CHINESE HAMSTER OVARY CELLS. Fu-Sheng Shen and Iris Lindberg*. Dept. Biochem. and Mol. Biol. Louisiana State Univ. Med. Cen., New Orleans, LA 70112.

PC2 is a member of a family of recently discovered subtilisin-like prohormone converting enzymes, two of which (PC1 and PC2) are primarily distributed in neuroendocrine tissues where they are thought to play a role in the proteolytic conversion of neuropeptide precursors to active neuropeptides. Little information is available on the biochemical properties of these important cellular enzymes. In the present study, mouse PC2 was overexpressed in Chinese Hamster Ovary (CHO) cells using the dihydrofolate reductase-coupled genetic amplification method, purified from the conditioned medium, and characterized biochemically. Purification of recombinant protein from the medium was carried out using hydrophobic interaction and ion-exchange chromatography. The purified material shows one major Coomassie blue-stained band on SDS-PAGE gel with a molecular weight of 75 kDa. The results of cell labeling experiments showed that two proteins labeled with methionine could be immunoprecipitated from cell and medium samples using a carboxy-terminal specific antibody with molecular weights of about 75 and 72 kDa. Based on the work of others using oocytes, the 75 kDa protein may represent proenzyme and the 72 band might represent a processing intermediate. After a 5 h treatment of the cells with 5 ug/ml tunicamycin, the profile of labeled immunoprecipitated proteins was altered; the 75 and 72 kDa bands shifted down to 69 and 68 kDa, respectively. This result implies that CHO PC2 protein is glycosylated, and concanavalin A binding experiments support this idea. Further experiments are underway to identify each molecular weight form of the enzyme and to obtain an enzymatically active species.

122.8

BIOSYNTHESIS OF THE PROHORMONE CONVERTASE mPC1 IN AtT-20 CELLS. Osvaldo Vindrola* and Iris Lindberg. Depart. Biochem. and Mol. Biol., Louisiana St. Univ. Med. Cen., New Orleans, LA 70112.

A new family of mammalian subtilisin-like enzymes, probably involved in the processing of pro-proteins in regulated and constitutive cells at paired basic residues, has recently been discovered. Little information exists as yet concerning the biosynthesis of these endogenous subtilisin-like enzymes. In the present work we have studied the biosynthesis and release of the endogenous prohormone convertase PC1 in AtT-20 cells. As predicted from mRNA studies, AtT-20 cells contain high levels of PC1 protein. Through immunoblotting, 87 kDa and 66 kDa bands were detected with an amino terminally-directed antiserum; however, only the 87 kDa product was detected with carboxyl terminally-directed antiserum, indicating carboxyl-terminal truncation. Pulse-chase experiments, using [^{35}S]methionine/ cysteine, showed that after 20 min of pulse the main product in the cells was the 87 kDa protein. Cells chased for varying amounts of time exhibited a progressive increase in the intensity of a 66 kDa band, along with a corresponding decrease of the 87 kDa band. The 87 to 66 kDa conversion was nearly complete after 4 h of chase. This post-translational processing was inhibited by the ionophore monensin, a Golgi disruptor, with a corresponding accumulation of the 87 kDa protein within the cell. Both the 87 kDa and 66 kDa labeled proteins were detected as membrane-bound rather than soluble proteins. The 87 kDa protein was the main product secreted by non-stimulated AtT-20 cells, while the 66 kDa product was only released when the cells were stimulated with corticotropin releasing factor or $BaCl_2$ and Br-cAMP. In conclusion, our results show that PC1 in AtT-20 cells is initially synthesized as an 87 kDa membrane-bound protein which is then converted intracellularly to a 66 kDa species which can be released by the regulated pathway.

122.9

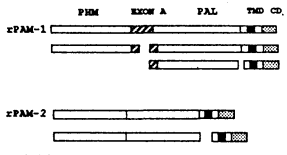
DIBASIC RESIDUE SPECIFIC ENDOPEPTIDASE IN ADRENAL MEDULLARY CHROMAFFIN GRANULES IS IMMUNOLOGICALLY RELATED TO PC1/PC2 ENZYMES. A.V. Azaryan* and V.Y.H. Hook. Dept. of Biochemistry, Uniformed Services University, Bethesda, MD. 20814.

Recently a new family of Kex2-like proteinases has emerged that is proposed to participate in the processing of hormone and neuropeptide precursors at paired basic sites; these include furin and prohormone convertases 1 and 2 (PCs) (Smeekens and Steiner, 1990; Seidah et al., 1990, 1991). We found that bovine adrenal medullary chromaffin granule membranes possess boc-Gln-Arg-Arg-MCA hydrolyzing proteolytic activity that is related to Kex2/furin/PC subtilisin-like superfamily of processing proteinases (Azaryan and Hook, BBRC, in press). This activity shows calcium sensitivity, pH optimum of 7.5-8.2 and belongs to the class of serine proteases based on inhibitor profile. Preference was demonstrated for cleavage at the carboxyl side of Arg-Arg and Lys-Arg, over Lys-Lys pairs. Specificity for Lys-Arg site was also indicated by potent inhibition by the active site-directed inhibitor [D-Tyr]-Glu-Phe-Lys-Arg-chloromethyl ketone. In addition, membrane solubilized boc-Gln-Arg-Arg-MCA cleaving activity was significantly immunoprecipitated by PC1 and PC2 antisera+. Our biochemical and immunological data indicate that this chromaffin granule dibasic residue specific endoproteolytic activity is related to PC1/PC2 enzymes. +(We thank Dr. Y. Peng Loh (NICHD, NIH for the generous gift of PC1 and PC2 antisera)

122.10

IDENTIFICATION OF MAJOR PAM PROTEINS IN HYPOTHALAMIC AND HIPPOCAMPAL SYNAPTOSOMES. A.M. OYARCE AND B.A. EIPPER*. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Peptidylglycine α-amidating monooxygenase (PAM) (EC1.14.17.3), a bifunctional enzyme, catalyzes the COOH-terminal amidation of many neuropeptides. The reaction requires the sequential action of peptidylglycine α-hydroxylating monooxygenase (PHM) and peptidyl-α-hydroxyglycine α-amidating lyase (PAL). Alternative splicing of the single copy gene for rat PAM generates the two major forms of PAM mRNA (rPAM-1 and -2) found in the central nervous system. Both proteins are integral membrane proteins (TMD=putative transmembrane domain) with an NH_2-terminal signal sequence



that is removed and differ by the presence of a non-catalytic domain, exon A, between the PHM and PAL domains only in rPAM-1. Subcellular fractionation of adult rat hypothalamus and hippocampus demonstrated the localization of PHM and PAL activity to synaptosomal fractions. Following separation of synaptosomes into soluble and particulate fractions, 70% of the PHM and PAL activity was recovered in the particulate fraction. The major PAM proteins identified by Western blot analysis using antisera specific to PHM, PAL, Exon A and the COOH-terminal domain (CD) are illustrated. Major products include soluble monofunctional PHM and PAL proteins along with intact PAM-1 and PAM-2 and a monofunctional integral membrane PAL protein. A significant proportion of the PHM and PAL proteins lacking the transmembrane domain remain membrane associated following removal of peripheral proteins by treatment with carbonate. Following exocytosis, the membrane associated PAM proteins may go to the cell surface where they may remain or from which they may be internalized. Supported by DA-00266 and DA-00098.

122.11

DYNORPHIN PROCESSING ENZYMES IN BRL3A CELLS, A LIVER-DERIVED CELL LINE; IMPLICATIONS FOR NEUROPEPTIDE BIOSYNTHESIS. Lakshmi Devi, Julie Zikherman, Suzanne Petanceska, and Lloyd D. Fricker*. Dept. of Pharm., New York Univ. Medical Center, NY, NY 10016, and *Dept. of Molec. Pharm., Albert Einstein College of Medicine, Bronx, NY 10461

A key question regarding neuropeptide biosynthesis concerns the specificity of the peptide processing enzymes. Dynorphin is produced from prodynorphin by a series of endoproteolytic cleavages at dibasic and monobasic sites, followed by a carboxypeptidase which removes the C-terminal basic residues. BRL3A cells secrete insulin-like growth factor II (IGF-II), which also requires dibasic and monobasic endopeptidase, and carboxypeptidase activities.

To examine whether the IGF-II and dynorphin processing enzymes are similar, we transfected BRL3A cells with rat prodynorphin; transfected cells express low levels of immunoreactive dynorphin B, indicating that the appropriate monobasic endoprotease and carboxypeptidase activities are present in these cells. Medium from BRL3A cells was directly examined for enzyme activities. A monobasic 'dynorphin converting enzyme' (DCE) and carboxypeptidase E (CPE) were detected; these activities showed similar inhibitor sensitivities and pH optima as DCE and CPE previously characterized from brain and pituitary.

The properties of these enzymes were further studied by partial purification. The DCE activity eluted from ion exchange FPLC under identical conditions as the rat brain DCE. The CPE activity bound to a substrate affinity column under selective condition, and the molecular weight of the purified CPE was similar to that of CPE purified from brain and pituitary (approximately 50 kDa). Also, CPE mRNA was detected on Northern blots of BRL3A RNA. Both DCE and CPE are secreted from the BRL3A cells via a regulated secretory pathway. Secretion of both enzyme activities is stimulated several-fold by secretagogues such as 8-bromo cAMP, KCl, and phorbol esters. These results suggest that DCE and CPE are general peptide processing enzymes that function in the biosynthesis of peptide growth factors, as well as peptide hormones and neurotransmitters. This is consistent with the similarities between the cleavage sites of most secreted bioactive peptides.

122.12

DIFFERENTIAL ENDOPROTEOLYTIC CLEAVAGE OF MONKEY PROOPIOMELANOCORTIN (mPOMC) AT TWO LYS-LYS PROCESSING SITES: POSSIBLE ROLE OF SURROUNDING SEQUENCES. H. Lin, N. Day, N. Seidah, and H. Akil.* Mental Health Research Institute, University of Michigan, Ann Arbor MI 48109.

POMC processing at dibasic cleavage sites within a given tissue is a function of several factors, including the complement of expressed endoproteases, the specific sequence of the dibasis signal (e.g. Lys-Arg vs Lys-Lys), as well as other factors yet to be determined. For example, Lys-Arg cleavage within POMC is mediated via the action of at least two different enzymes, termed PC1 and PC2, which exhibit selectivity toward the various Lys-Arg signals. This suggests that sequences surrounding the dibasic cleavage site may direct enzymatic specificity. We have recently demonstrated a similar phenomenon for Lys-Lys cleavage within POMC. We compared Lys-Lys processing at one site at the amino-terminus of β-MSH and at another site in the carboxy-terminus of β-Endorphin (βE). mPOMC cDNA was transfected into two endocrine cell lines: AtT-20 and Rin m5F. Our results have demonstrated both tissue-specific and site specific factors controlling Lys-Lys cleavage. AtT-20 cells do not to process at either site. On the other hand, Rin m5F cells fail to process at the site in the carboxy-terminus of βE, but do process, to a significant extent the site N-terminal to β-MSH. To determine whether this differential processing was due to a conformational hindrance of the βE sites, we mutated both sites to Lys-Arg. Rin m5F cells fully processed at both locations, suggesting that the sites were both accessible to endoproteases. These results suggest that the differential processing of the two Lys-Lys sites may be due to sequences beyond the dibasic residues, which may determine likelihood of cleavage. Mutants altering the residues surrounding the Lys-Lys sites have been constructed and the effect of these mutations on processing will be described.

122.13

IDENTIFICATION OF TWO MEMBRANE-ASSOCIATED NEUROPEPTIDE-DEGRADING ENZYMES FROM OVINE MEDIAN EMINENCE ENDOTHELIAL CELLS. R.A. Lew, K.E. Sheppard* and A.I. Smith. Baker Medical Research Institute, Prahran, Victoria, Australia 3181.

The capillary endothelial cells (EC) of the median eminence (ME) represent a potential site of regulation for both circulating and hypothalamic neuropeptides passing through the hypophysial portal blood system toward the pituitary. The present study examines in vitro the metabolism of gonadotropin releasing hormone (GnRH) and other peptides by cultured EC from sheep ME. Non-vascular tissue was dissected away prior to enzymatic dispersion of the capillary bed of ovine ME, and the cells obtained were cultured using standard techniques. After 2-13 passages, confluent EC (positively identified by staining for von Willebrand factor) were removed from culture plates, frozen and thawed three times to lyse the cells, and centrifuged at 100,000g. The pellet was washed, re-centrifuged, resuspended in buffer, aliquoted and frozen at -70°C. Aliquots (0.2 mg protein) were incubated with synthetic GnRH (10-50 μg) in the presence or absence of a variety of protease inhibitors. The GnRH fragments generated were separated by HPLC and identified by amino acid analysis as GnRH1-5 and GnRH1-3. The degradation profile produced by EC differed from that produced by bovine aortic EC and AtT-20 cell membranes. Generation of GnRH1-3 was diminished in the presence of thiol protease inhibitors such as leupeptin and iodoacetamide, while formation of GnRH1-5 was inhibited by PMSF, a serine protease inhibitor. EDTA, phosphoramidon and angiotensin converting enzyme (ACE) inhibitors were ineffective, indicating that neither endopeptidase 24.11 nor ACE is involved. These novel membrane-bound peptidases may also be involved in the regulated degradation of other peptides such as angiotensin, bradykinin and neuropeptide Y by these cell membranes. Thus the capillary endothelium of the ME expresses peptidases capable of cleaving hypothalamic and cardiovascular peptides en route to the pituitary; their role in the fine regulation of both pituitary function and local blood flow is currently under investigation.

122.14

PURIFICATION, CHARACTERIZATION, AND IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF A CATHEPSIN D-LIKE PROTEASE FROM BOVINE CHROMAFFIN GRANULES. T.J. KRIEGER, Y.H. Kang, and V.Y.H. Hook*. Dept. of Biochem., Uniformed Services University, Bethesda, MD. 20814, and Naval Medical Research Institute, Bethesda, MD.

A chromaffin granule aspartyl protease (CGAP), preferring as substrate the model tachykinin precursor (^{35}S-(Met)-ß-preprotachykinin) over the enkephalin precursor (^{35}S-(Met)-preproenkephalin), was purified by ConA-Sepharose, Sephacryl S200, and chromatofocusing. CGAP was composed of 47, 30, and 16.5 kDa polypeptides that migrated as a single band in a non-denaturing gel system, and coeluted with an apparent M_r of 45-55 kDa by gel filtration. These results suggest that two forms exist: a single 47 kDa polypeptide and a complex of 30+16.5 kDa associated subunits. Microsequencing and amino acid composition studies showed that the three CGAP polypeptides were similar, but not identical, to bovine cathepsin D. CGAP cleaved at a dibasic Lys-Arg site and at hydrophobic residues (Leu-Tyr, Phe-Val). Processing of proendothelin in chromaffin granules requiring cleavage at both hydrophobic and dibasic residues would be compatible with CGAP's cleavage specificity. Cleavage at hydrophobic residues, like cathepsin D, also suggests that CGAP may be involved in degrading precursor segments that do not become part of active peptide sequences. Immunoelectron microscopy with immunogold confirmed colocalization of CGAP with (Met)enkephalin within chromaffin granules. These results show that secretory vesicles of adrenal medulla possess a cathepsin D-like protease that may be involved in peptide precursor processing.

122.15

CARBOXYPEPTIDASE M IN BRAIN AND PERIPHERAL NERVES A. Nagae, P.A. Deddish, R.P. Becker, C.H. Anderson, M. Abe, F. Tan, H.L. Jackman*, R.A. Skidgel, E.G. Erdös Lab. of Peptide Res., Depts. of Pharmacol., Anesthesiol., and Cell Biol., U. of Ill., Coll. of Med., Chicago, IL 60612.

Carboxypeptidase M (CPM), a plasma membrane-bound enzyme with a neutral pH optimum, cleaves C-terminal Arg or Lys of peptides such as kinins and Arg^6- or Lys^6- enkephalins. It is present in several organs and cultured cells, and was purified, cloned and sequenced from human placenta. We investigated CPM activity in human, baboon, and dog brain and dog peripheral nerves. Brain areas were dissected, homogenized, fractionated by centrifugation and assayed with Dansyl-Ala-Arg. The corpus callosum, pons and the pyramidal and optic tracts had high activity, while the basal ganglia, cerebellum, amygdala and cortex were low in CPM. This carboxypeptidase activity was due to CPM, as shown by membrane attachment, substrate hydrolysis, inhibition pattern and immunoprecipitation of activity with anti-human CPM antiserum (85% of human and baboon, 66% of dog brain CPM). CPM co-purified with myelin-rich fractions from the brain where it was membrane-bound via a phosphatidylinositol glycan anchor. The mRNA of CPM was detected in Northern analysis of the baboon brain. Dog sciatic and vagus nerves also had high activity and in immunohistochemical studies, the outer aspect of myelin sheaths and Schwann cells stained. In conclusion, CPM is present in the CNS and peripheral nerves, and in some areas it is closely associated with myelin and/or myelin forming cells. Supported by NIH, HL36473, 36082 and DK41431.

122.16

CHARACTERISTICS OF N-ACETYL-ASPARTATE AS AN ACETYL DONOR IN THE RAT BRAIN. V. Mehta and M.A.A. Namboodiri*, Lab. Mol. Neurobiol, Dept. Biol., Georgetown Univ., Washington, DC 20057.

Recent findings have suggested that the incorporation of acetyl groups from N-acetyl-aspartate (NAA) into lipids may involve a mechanism independent of free acetate. In the present study, we have investigated this question by analyzing the products formed when a brain slice preparation (300-400 microns) incubated with (C-14)-NAA (250 uM) in an artificial cerebrospinal medium in presence of 95% O2/5% CO2. Parallel incubations were performed using (C-14)-acetate (250 uM). The tissue was extracted with chloroform/methanol (2:1) and the chloroform phase was analyzed using TLC. The aqueous phase was analyzed using an anion exchange HPLC. Significant amounts of radiolabeled lipids were detected on TLC analysis in both cases, with (C-14)-acetate sample showing 2-3 fold higher incorporation. Analysis of the aqueous phase from the NAA samples showed that a significant amount of free acetate was formed during the incubation. In addition, an early eluting peak which is distinct from free acetate and NAA was observed. Radiolabel incorporation into this peak was two to three fold higher in the case of the NAA samples. These preliminary studies showing a differential radiolabel incorporation pattern between NAA and acetate suggest that NAA may be metabolized with and without the formation of free acetate.

122.17

POST-MORTEM STABILITY STUDY OF METHIONINE ENKEPHALIN, SUBSTANCE P,AND BETA-ENDORPHIN IN RAT PITUITARY. X.Zhu* and D.M.Desiderio. Stout Neurosci. Mass Spec. Lab., Univ. of Tenn., Memphis, TN 38163.

The post-mortem stability of three neuropeptides, methionine enkephalin(ME), substance P(SP), and beta-endorphin(BE) was studied in the rat pituitary to test the hypothesis that significant changes occur post-mortem in the concentration of those neuropeptides. Those neuropeptides represent the three gene products proenkephalin A(ME), tachykinins(SP), and proopiomelanocortin(BE). Post-mortem stability data of neuropeptides from the rat pituitary provide important reference data for our qualitative and quantitative studies of neuropeptides in human post-mortem pituitary tissues and of the genesis of human pituitary tumors. The Spokes-Koch model, which simulates human autopsy conditions, was used to monitor and to control the time-vs.-temperature cooling curve of the rat pituitary. Decapitated heads were incubated for various time before pituitaries were dissected and neuropeptides extracted. Radioimmunoassay (RIA) was used to measure HPLC fractions for the three peptides, and the confirmation of the molecular weights of those peptides is proveded by using mass spectrometry(MS) to detect the corresponding protonated molecule ions, MH+. The data demonstrate the importance of: 1) measuring HPLC-purified neuropeptide-like immunoreactivity rather than only the total peptide-immunoreactivity; and 2) accurately defining a time-course series of measurements-especially in the critical portion of the cooling curve when rapid temperature changes occur, rather than measuring peptid immunoreactivity at only one, generally later, time. Statistically increases and decreases are demonstrated within five hours for peptide-like immunoreactivities from the proenkephalin A and the proopiomelanocortin neuropeptidergic systems.

123.1

SUBSTANCE P ELEVATES $[Ca^{2+}]_i$ IN NEONATAL DORSAL HORN CELLS WITH NEURONAL AND GLIAL PROPERTIES. M.J.S. Heath and A.B. MacDermott*. Departments of Physiology and Anesthesiology, and Center for Neurobiology and Behavior, Columbia University, New York, NY 10032.

We previously defined two different populations of spinal cord dorsal horn cells that respond to substance P (SP) by elevation of intracellular Ca^{2+} ($[Ca^{2+}]_i$). In one population (type E cells), SP promotes Ca^{2+} entry while in the other population (type R cells), SP evokes elevation of $[Ca^{2+}]_i$ in the absence of extracellular Ca, indicating that Ca is released from internal stores. Both populations were believed to be neurons (Womack et al, Neurosci Abs. 15:184, 1989).

In the present study, we have used additional criteria to further define these two populations of cells. Cells were acutely dissociated from P0-P4 rat spinal cords and $[Ca^{2+}]_i$ was monitored using the Ca^{2+} indicator, indo-1. SP sensitive cells were first classified as type E or R by removal of bath Ca^{2+} and addition of La^{3+}. Type E cells were large and smooth, with few processes. Type R cells were smaller than type E cells and surrounded by multiple fine filamentous processes. Type R were morphologically indistinguishable from 04-immunoreactive oligodendrocytes, and often expressed GD3, a marker of immature neuroectodermal cells and oligodendrocyte precursors. The type R response exhibited longer latency and faster rise time than the type E response. Both type E and type R cells responded to glutamate, but only type E cells responded to NMDA. Thus, while type E cells exhibit the morphological and pharmacological characteristics of mature neurons, type R cells may represent a population of immature neuronal or glial precursors. (Supported by NIH GM32009 and the FAER with a grant from Abbott Laboratories)

123.2

BLOCKADE OF NK1 RECEPTORS BY CP-96345 ENHANCES THE DOPAMINE EFFECTS OF METHAMPHETAMINE IN RATS. S.P. Gygi, J.W. Gibb, and G.R. Hanson*, Department of Pharmacology and Toxicology, University of Utah. S alt Lake City, UT, 84112.

The major extrapyramidal dopaminergic pathway to the striatum is a nigrostriatal projection. Striatonigral substance P (SP) neurons are proposed to have an excitatory effect on this dopamine (DA) system. However, the lack of a specific SP antagonist has made it impossible to determine the precise physiological role of this SP pathway. Recently, a nonpeptide, NK1 receptor antagonist, CP-96345 (CP), was developed by Pfizer Inc. which is centrally active when administered systemically. We combined CP (15 mg/kg:PNAS [1991] 88:10042) with the DA-releasing stimulant, methamphetamine (METH) (10 mg/kg) for 5 doses, 6 hours apart. The animals were sacrificed 18 hours after the last dose, and neuropeptide content (i.e., SP, neurotensin [NT], and dynorphin) was determined by RIA in order to assess the response of DA activity to METH treatment. CP did not alter any peptide content when given alone; however, when given in combination, CP usually substantially enhanced METH-induced increases in both striatal and nigral neuropeptide-like immunoreactivity. For example, NT-like immunoreactivity in the striatum increased 209% of control after METH treatment alone, while in combination with CP the increase was 367% of control. CP had similar effects on METH-induced striatal and nigral changes in SP and dynorphin content. Surprisingly, our findings suggest that the physiological role of the SP pathway is to antagonize the dopaminergic extrapyramidal systems. (We thank Pfizer Inc. for their generous gift of CP-96345. Research supported by DA00869 and DA04222.)

123.3

Inhibitory Effects of an NK-1 (Substance P, SP) Receptor Antagonist, (±)CP96345 (CP), on Visceral Nociceptive Activity and Non-nociceptive Bladder (BL) Activity in Rats. Iyengar, S.*, Muhlhauser, M.A., Howbert, J.J. and Thor, K.B. Lilly Research Laboratories, A Division of Eli Lilly and Co., Indianapolis, IN.

SP is contained in BL primary afferent neurons, sacral spinal interneurons, and bulbospinal neurons. SP binding sites are preferentially distributed in spinal autonomic and somatic nuclei that regulate BL function. The present study examined the effects of a potent and selective NK-1 antagonist, (±)CP96345 (CP), in a model of visceral nociception (see Thor, K.B., this meeting). BL activity (cystometry) and anal and urethral sphincter activity (EMG) were recorded in urethane-anesthetized rats. In 6 rats, the BL was infused with 0.5% acetic acid (a.a.), which consistently produced increases in BL and anal activity. Within 1-4 minutes, CP produced dose-dependent (0.1 - 30 mg/kg, i.v.) decreases in a.a.-induced anal activity (to 10% of control) and BL activity. (The inhibitory effects of CP (0.3 mg/kg) partially recovered after 45 min). In rats with saline-infused BL (n=3), CP also produced decreases in bladder activity and increases in bladder capacity (up to 4 fold) beginning at 1 mg/kg and abolished BL contractions at doses of 10 or 30 mg/kg. In contrast, functional activity of the urethral sphincter was maintained even at high doses of CP, as indicated by significant elevations in intravesical pressure before overflow incontinence ensued. Thus, these studies are suggestive of NK-1 receptor involvement in a.a.-induced vesicoanal nociceptive activity and BL activity, as well as distension-induced bladder activity whereas urethral sphincter activity appears to be less dependent upon NK-1-mediated SP transmission

123.4

INTERACTIONS OF THE SUBSTANCE P NH_2-TERMINAL METABOLITE SP(1-7) WITH THE SIGMA RECEPTOR IN VIVO (BEHAVIOR) AND IN VITRO (BINDING). D.D. Mousseau, X. Sun and A.A. Larson*, 295 AnSci/VetMed Bldg, Univ. on Minnesota Dept. of Veterinary PathoBiology, St. Paul, MN, 55108.

Sensitization of caudally-directed biting and scratching (CBS) in mice induced by repeated intrathecal kainic acid injections is used to investigate effects of compounds on spinal excitatory amino acid systems. Interactions between the substance P (SP) NH_2-terminal metabolite SP(1-7) and the sigma receptor were investigated given their similar distributions in the mammalian CNS. Both the sigma agonist 1,3-di-*o*-tolylguanidine (DTG; 5 nmol 30 min) and SP(1-7) (22.5 pmol 30 min) potentiated kainic acid-induced CBS activity. In contrast, the sigma antagonist haloperidol (HAL; 1 nmol 5 min) attenuated the initial response without affecting sensitization, whereas the SP(1-7) antagonist D-SP(1-7) (1 nmol 30 min) blocked behavioral sensitization but did not affect the initial response to kainic acid. Radioligand binding revealed that DTG and HAL were almost as potent at competing for the [^{3}H]DTG-labeled binding site but that SP(1-7) and D-SP(1-7) were much less potent at competing for this site, although they were equipotent in their respective effects on [^{3}H]DTG binding. The relevance of the effect of SP(1-7) was demonstrated when SP itself, but not its COOH-terminal metabolite SP(5-11), inhibited [^{3}H]DTG binding in a similar fashion. While the mechanism of action of SP(1-7) with sigma sites is unclear, the ability of SP(1-7) to mimick the effect of DTG on behavior, while their respective antagonists D-SP(1-7) and HAL antagonize these actions clearly indicates a similar mode of action of these two classes of compounds. (Supported by NIDA 04090, 04190, 00124).

123.5

EVIDENCE THAT THE N- BUT NOT THE C-TERMINUS OF SUBSTANCE P (SP) POTENTIATES NON-NMDA ACTIVITY BY AN INTERACTION WITH PCP RECEPTORS. X. Sun*, K. Kovács and A. A. Larson. Department of Veterinary Pathobiology, University of Minnesota, Saint Paul, MN 55108, U.S.A.

Behavioral sensitization to kainic acid (KA) administered intrathecally in mice appears to be mediated by the N-terminus of substance P (SP) released from primary afferent C-fibers. To determine whether KA sensitization, like pain, is sensitive to PCP ligands, we compared effects of MK801, PCP, d-APV and CPP on sensitization. At doses that inhibited NMDA-induced behavior, d-APV and CPP had no effect whereas MK801 and PCP inhibited sensitization to KA. Pretreatment with 10 nmoles of SP1-7, but not SP5-11, 24 hr prior to testing appeared to downregulate SP1-7-sensitive sites and attenuate KA sensitization. MK801, but not d-APV or haloperidol, blocked downregulation of SP1-7 sites, preventing the effect of SP1-7-pretreatment on KA sensitization. These data suggest a direct interaction of SP1-7 with PCP sites. Binding of SP and its N- and C-terminal fragments, SP1-7 and SP5-11, respectively, to PCP sites was studied using tissue homogenates of whole mouse brain. Whereas high concentrations of SP and SP1-7 displaced [^{3}H]TCP, the C-terminal fragment, SP5-11, did not compete for this binding site. When MK-801 was added to the reaction mixture, displacement of [^{3}H]TCP by SP1-7 was increased in an additive manner. These results provide evidence that the N-terminus of SP modulates non-NMDA mediated activity by an interaction with PCP receptors. (Supported by NIDA 04190, 04090, 00124)

123.6

THE NK-1 RECEPTOR ANTAGONIST (±)-CP-96,345 PRODUCES BIPHASIC CHANGES IN RAT STRIATAL DOPAMINE RELEASE WHEN ADMINISTERED VIA A MICRODIALYSIS PROBE. L. A. Phebus*, J. J. Howbert and J. A. Nixon. The Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46285.

Substance P, contained in striatal neurons which project to the substantia nigra, may modulate striatal dopamine release by a direct action on striatal dopamine-containing terminals, or by an action on dopaminergic cell bodies and dendrites in the substantia nigra. We used microdialysis to examine the effects of striatal NK-1 blockade on striatal dopamine release.

Male Wistar rats were anesthetized with isoflurane and placed in a stereotaxic apparatus where a plastic loop-style dialysis probe was implanted into the striatum and cemented in place. The next day, after recovery from anesthesia, the dialysis probe was perfused with artificial CSF (pH=7.4), at a rate of 1.3 microliters / minute. Striatal dialysate dopamine, DOPAC, HVA and 5-HIAA were assayed every 15 minutes using "on line" HPLC. After baseline samples were measured, 15 microliters containing 3 micrograms of (±)-CP-96,345 in artificial CSF (pH=7.4) were perfused through the probe.

Shortly after striatal exposure to (±)-CP-96,345, striatal dialysate dopamine levels increased briefly and then decreased, reaching a level of about 50% baseline. Dopamine levels remained significantly decreased for several hours. Striatal dialysate DOPAC and HVA levels were significantly decreased. The biphasic and prolonged effects of brief NK-1 antagonism on dopamine release indicate that this interaction may have a complex mechanism.

123.7

EFFECTS OF SUBSTANCE P (SP) FRAGMENTS ON EVOKED RESPONSES OF RAT SPINAL NOCICEPTIVE NEURONS.
D. Budai,* G. L. Wilcox and A. A. Larson. Departments of Pharmacology and Veterinary Pathobiology, University of Minnesota, Minneapolis, MN 55455, U.S.A.

The effects of N- and C-terminal fragments of substance P, SP1-7 and SP5-11, respectively, were tested on nociceptive neurons in the dorsal horn of the anesthetized rat. Neuronal activity was evoked by iontophoretic application of excitatory agents (NMDA, AMPA, kainic acid) or by electrical stimulation of the receptive field and recorded extracellularly. While SP(1-7) had no effects when applied iontophoretically by itself, it was a potent and long-lasting modulator of spinal nociception. NMDA responses were transiently decreased (by an average of 36% of control at minimum) by SP(1-7) followed by a more sustained increase (by 76% at time of maximum effect). In contrast, AMPA and kainate responses were only increased by SP(1-7) (by 81 and 105 % at time of maximum effect, respectively). SP(1-7) reduced both the initial responses and the frequency dependent potentiation (wind-up) of the neurons to repeated C-fiber stimulation. Inhibitions by SP(1-7) of chemical- as well as peripheral stimulation-evoked responses proved to be naloxone sensitive. On the other hand, the C-terminal fragment, SP(5-11), exerted naloxone insensitive increase in the responses of nociceptive neurons. These results show that the C- and N-terminal fragments of SP may exert their effects through different mechanisms. The inhibitory component of the SP(1-7) effects is related to opioid receptors. *(Supported by NIDA Grants RO1-04090 and KO2-00124 to A.A.L. and RO1-04274, RO1-01933 and KO2-000145 to G.L.W.)*

123.8

EVIDENCE FOR POSSIBLE INTERACTIONS BETWEEN MORPHINE AND CGRP IN THE DEVELOPMENT OF TOLERANCE TO OPIATES IN THE SPINAL CORD.
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Spinal administration of morphine induces an antinociceptive effect through the activation of receptors localized in the substantia gelatinosa. Rapid tolerance develops to this effect. The mechanism involved in this phenomenon mostly remains to be established but clearly does not involve a down regulation of opioid receptors. A possible alternative relates to an action via nociceptive sensory inputs containing neuropeptides such as Calcitonin Gene-Related Peptide (CGRP). The present investigation was undertaken to determine if any relationship existed between tolerance induced by morphine and CGRP in the dorsal horn of the cord. Morphine sulfate (7.5μg/h/μl) was continuously administered at lumbar level L4, for a 3 day or 5 day time period, using Alzet mini-osmotic pumps. The analgesic effect monitored by tail-flick test from the 3-day treatment was similar to the analgesia produced by a single intrathecal injection of morphine sulfate (7.5μg). In contrast, tolerance to nociceptive action of morphine was evident at day 5 of treatment. Following behavioral evaluation, animals were sacrificed and L4 segments processed for either CGRP immunohistochemistry or quantitative *in vitro* [^{125}I]hCGRP receptor autoradiography. While no changes were detectable for either parameters by day 3, CGRP-like immunoreactivity was apparently increased while [^{125}I]CGRP binding decreased (25-30%) in the dorsal horn at day 5 of morphine treatment. This may suggest the possible involvement of CGRP and its receptors in the phenomenon of tolerance to the antinociceptive effect of morphine. Supported by MRCC.

123.9

CAPSAICIN PRETREATMENT REDUCES PHOSGENE LUNG INJURY
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Phosgene (CG) is an irritant gas which elicits lung inflammation and noncardiogenic edema when inhaled. We examined the role of airway neuropeptides in CG-induced lung injury. Anesthetized adult guinea pigs were injected with vehicle, or capsaicin (CAP, 5 mg/kg or 50 mg/kg, sc) to deplete neuropeptides from airway sensory nerves. One week later, guinea pigs were exposed to air or CG (200 mg/m^3) for ten minutes. Four hours after exposure, lung wet weight to dry weight ratios were obtained as indices of pulmonary edema. Substance P- (SP) and neurokinin A- (NKA) like immunoreactivity (LI) in trachea, bronchi and lungs were measured by radioimmunoassay. CG-exposed guinea pigs had higher lung weight ratios than air-exposed guinea pigs. Edema indices of CG-exposed guinea pigs treated with 5 mg/kg CAP were lower than those of 50 mg/kg CAP- or vehicle-treated guinea pigs. SP-LI at all levels of the respiratory tract was ≥75% lower in CAP-treated guinea pigs. NKA-LI was reduced only in 50 mg/kg CAP-treated guinea pigs. Vehicle-treated, CG-exposed guinea pigs had 60% lower lung SP-LI and bronchial NKA-LI than those exposed to air. We conclude that 1) CG inhalation evokes lung SP and bronchial NKA release; 2) SP release in the lung may contribute to CG-induced pulmonary edema. Precise mechanisms of 5 mg/kg CAP effect are to be determined.

123.10

INTRIGUING EFFECTS OF NEUROTENSIN ON RETINAL DOPAMINE METABOLISM
Henni H., Drumheller A. and Jolicoeur F.B * Depts. of Psychiatry and Pharmacology, Faculty of medicine. University of Sherbrooke, Sherbrooke Qc. Canada J1H 5N4.

Neurotensin has been found in a subpopulation of amacrine cells in the retina of several vertebrates. Also, we have reported previously that this neuropeptide may be co-localized within dopaminergic retinal cells in mammalian retina (Invest. Ophthalmol. Visual Sci. 28: 351, 1987).The purpose of this study was to investigate possible neurochemical effects of neurotensin on dopamine systems in the retina. Various doses of neurotensin were injected in the vitreous of anesthesized rats. Retinal concentrations of dopamine and its main metabolites, DOPAC and HVA were determined using HPLC with electrochemical detection 15 min following injections. Serotonin and its metabolite 5-HIAA were quantified simultaneously to verify the specificty of effects. Experiments were conducted in both light and dark adapted animals. The results indicate that, while concentrations of dopamine remained unaffected, the turnover of dopamine, as evaluated by metabolite concentrations as well as by the metabolites to dopamine ratios, was significantly reduced in a dose related fashion by the peptide. Threshold doses for producing these effects under light and dark conditions were 0.78 μg and 12.5 μg respectively demonstrating a markedly greater influence of the peptide when retinal dopamine release is increased by photopic stimulation. Retinal concentrations of serotonin or its metabolite were unaffected by the treatment, indicating a selective action of the peptide on dopaminergic transmission. The observed decrease in retinal dopamine metabolism is contrary to the well known enhancing effect of the peptide found in other areas of the CNS. At the present time the reasons for these opposite effects cannot be adequately explained, but they surely reveal the complexity of the dopamine-neurotensin interaction in the CNS. Supported by MRC Canada, Grant No P6-287

123.11

NEUROTENSIN EXCITES DOPAMINERGIC NEURONS IN RAT VENTRAL TEGMENTAL AREA (VTA) BY REDUCING POTASSIUM AND INCREASING SODIUM CONDUCTANCES.
Z.G. Jiang*, M. Pessia and R.A. North, Vollum Institute, Oregon Health Sciences Univ., Portland, OR 97201.

Whole cell recording techniques were used to study the actions of neurotensin (NT) on VTA neurons in rat midbrain slices. NT dose-dependently (EC_{50} = 37 nM) caused an oscillatory inward current (up to 260 pA at holding potential -60 mV) in 81% of principal (presumed dopaminergic) neurons (n=106), but 30% of secondary cells (n=47). TTX (1 μM) or glutamate-antagonists reduced the NT-induced current in 20% of cells; thus, either one or both were added. NT-induced current was associated with a small reduction in instantaneous conductance at -60 mV. NT-current was not blocked by TEA (10 mM), Ba (1 mM) or Cs (3 mM). In most cells, the NT-current became smaller with depolarization and larger with hyperpolarization; but in some cells the current became smaller with hyperpolarization and even reversed polarity at about -100 mV. With internal and external solutions to suppress potassium and calcium currents, the NT-induced current had a reversal potential between -10 and 30 mV, and was inhibited 70 - 90% by low Na^+(20 mM). The results suggest that both potassium codncutance decrease and cation condutance increase contribute to the depolarization caused by NT.

123.12

ALTERATIONS IN THE ACTIVITY OF CENTRAL CATECHOLAMINERGIC NEURONS FOLLOWING SYSTEMIC ADMINISTRATION OF A SUBSTITUTED NEUROTENSIN ANALOG
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The effects of systemic administration of a substituted neurotensin analog (Sub-NT_{8-13}) were examined on the activity of central catecholaminergic neurons in the male rat. The activity of mesolimbic (ML), tuberoinfundibular (TI) and incertohypothalamic (IH) dopaminergic (DA) neurons was estimated by measuring the concentrations of the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in regions of the brain containing terminals of these neurons (i.e. nucleus accumbens [NA], median eminence [ME] and dorsomedial nucleus [DMN], respectively). Sub-NT_{8-13} (sc) produced dose- and time-dependent increases in DOPAC concentrations in the NA, ME and DMN. Sub-NT_{8-13} also increased dopamine concentrations in the DMN, but had no effect in the NA or ME. To determine if concurrent increases in DOPAC and dopamine reflect an increase in the activity of noradrenergic neurons the effects of Sub-NT_{8-13} were examined on concentrations of the norepinephrine metabolite 3-methoxy-4-hydroxyphenylethylene glycol (MHPG). Sub-NT_{8-13} (1.0 mg/kg; sc) increased MHPG concentrations in the DMN, and this was accompanied by a decrease in concentrations of norepinephrine. Furthermore, neurochemical-induced lesions of the ventral noradrenergic bundle blocked the ability of Sub-NT_{8-13} to increase concentrations of dopamine, but not DOPAC, in the DMN. Taken together, these results indicate that systemic administration of Sub-NT_{8-13} increases the activity of DA neurons comprising the ML, TI and IH systems, and noradrenergic neurons terminating in the DMN. (Supported by NIH Grant NS15911).

123.13

EVIDENCE FOR PROTEIN KINASE C MEDIATION OF THE NEUROTENSIN INDUCED ACTIVATION OF TUBERO-INFUNDIBULAR DOPAMINE (TIDA) NEURONS. G.A. Gudelsky* and S.A. Berry, Depts. of Psychiatry and Neuroscience, Case Western Reserve University, Cleveland, OH 44106

The purpose of the present study was to determine whether neurotensin acts within the arcuate nucleus/median eminence to activate tyrosine hydroxylase (TH) within TIDA neurons and to assess the role of protein kinase C in this activation. The activity of TH within TIDA neurons was assessed by quantification of the formation DOPA in the arcuate nucleus/median eminence under in vitro conditions after inhibition of DOPA decarboxylase. Neurotensin (0.1-10 nM) increased the activity of TH within TIDA neurons under in vitro conditions by approximately 80%. The activity of TH also was increased approximately 55% by the phorbol ester, TPA (1-1000 nM), which activates protein kinase C. Sphingosine (10 μM), an inhibitor of protein kinase C, attenuated the activation of TH within TIDA neurons that was induced by TPA and neurotensin. Sphingosine alone did not alter the activity of TH nor did it alter the dibutyryl-cAMP-induced activation of TH. It is concluded that neurotensin acts directly within the arcuate nucleus/median eminence to activate TIDA neurons. Furthermore, it is suggested that the activity of TH within these neurons is enhanced following the activation of protein kinase C and that protein kinase C may mediate the neurotensin-induced activation of TIDA neurons.

123.14

EFFECT OF SYSTEMIC AND CENTRAL ADMINISTRATION OF NATURAL AND NOVEL NEUROTENSIN ANALOGUES ON BEHAVIOR AND NEUROTRANSMITER TURNOVER IN THE RODENT BRAIN. L.W. Cooke*, D.J. Wustrow, T.A. Pugsley, H. Akunne, T.G. Heffner, W. Cody and M.D. Davis. Neuroscience Section, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105.

Neurotensin (NT) is a tridecapeptide with a broad range of activity and is distributed along with its receptor in discrete peripheral and brain regions. This study evaluated the central effects of both native and novel NT fragments.

Neither NT1-13 nor NT8-13 had any measurable effects on locomotor or biochemical activity in rodents when given systemically (0.1-10mg/kg S.C.). In contrast, a nanomolar-affinity analogue of NT8-13 with selective amino-acid substitutions produced a profound and prolonged suppression of locomotor activity (ED50 <0.2 mg/kg S.C.) in rats and mice. This fragment also evoked elevations in dopamine and acetylcholine turnover in rats, being more pronounced in the nucleus accumbens than in the striatum. In addition, NT1-13, NT8-13 and other modified fragments all produced similar effects when administered ICV. These results suggest that certain modified NT fragments can be made resistant to peptidase degradation and are able to cross the blood-brain barrier.

123.15

NITRIC OXIDE PARTICIPATES IN THE INHIBITION OF UTERINE CONTRACTION BY CALCITONIN GENE-RELATED PEPTIDE. R.L. Shew*, J.A. Yee, R.E. Papka and D.L McNeill. Department of Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190 and Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE 68178

We have demonstrated that calcitonin gene-related peptide (CGRP) inhibits substance P- (SP) stimulated uterine contraction in vitro. In an attempt to elucidate the mechanism of action of CGRP, the present study was undertaken to examine the role nitric oxide might have in the inhibitory action of CGRP on uterine contraction. The effect of CGRP on SP- and neurokinin A- (NKA) stimulated uterine contraction was examined in vitro on uterine horns from diethylstilbestrol-treated rats (50 µg/rat,i.p. 14-16 hrs prior to the study). These experiments were done in the absence or presence of two arginine analogs which inhibit nitric oxide formation (N^G-monomethyl-L-arginine [CH_3-ARG] and N^G-nitro-L-arginine [NO_2-ARG]). CGRP (10^{-7} M) significantly reduced SP- (10^{-5} or 10^{-6} M) and NKA- (10^{-6} M) stimulated uterine contraction. The inhibitory action of CGRP on SP- and NKA-stimulated uterine contraction was blocked by 10^{-3} M CH_3-ARG. In contrast, 10^{-3} M NO_2-ARG had no effect on the inhibitory action of CGRP on NKA-stimulated uterine contraction. Neither CH_3-ARG or NO_2-ARG alone had any effect on SP- or NKA-stimulated uterine contraction. These data demonstrate that CH_3-ARG, a blocker of induced nitric oxide formation, suppresses the relaxant effect of CGRP on myometrial activity and that NO_2-ARG, a blocker of constitutive nitric oxide formation, has no effect on CGRP inhibition of SP- and NKA-stimulated uterine contraction. These data suggest that the inhibitory action of CGRP may be dependent on induced nitric oxide formation.

123.16

CALCITONIN GENE-RELATED PEPTIDE AND MUSCLE ACETYLCHOLINESTERASE REGULATION. C.A. Hodges-Savola* and H.L. Fernandez. Neuroscience Res. Lab., US Dept. Veterans Affairs Med. Ctr., Kansas City, MO 64128

Calcitonin gene-related peptide (CGRP) in mammalian skeletal muscles is predominantly localized within motor nerve terminals and it has been shown that this peptide modulates the metabolism/function of nicotinic acetylcholine receptors (nAChRs). Here we describe data suggesting that CGRP also influences muscle acetylcholinesterase (AChE), particularly the globular tetrameric (G_4) molecular form. Young adult (3 month-old) male Sprague-Dawley rats received two subcutaneous injections of rat CGRP [40-120 ug in 0.3 ml phosphate buffered saline (PBS)], one at time 0 and the second 24 hr later. Immediately after the first injection, the anterior gracilis muscle in one leg was denervated. Controls included other similarly denervated animals that were either injected with PBS or that received no injections. In all cases, muscle samples enriched in motor endplates (+EPs) were removed 48 hr after the initial injection/denervation and processed for AChE isoform analysis. We observed the expected AChE isoform changes in non-treated, as well as PBS-treated denervated muscle +EPs; i.e., a significant increase in G_4 AChE, and marked declines in the G_1, G_2, and A_{12} molecular forms. In turn, CGRP did not dramatically alter the denervation-induced changes in G_1, G_2, and A_{12} AChE, whereas the peptide (at doses of 80 and 120 ug) completely eliminated the tetramer's response to denervation; i.e., CGRP had an "innervation-like" effect that was specific for G_4 AChE. These observations may be explained by assuming that the exogenous CGRP served to replenish that peptide which is known to decrease following denervation. The foregoing results, together with other data from our laboratory, suggest that CGRP may not only serve as a trophic agent for nAChRs, but may also participate in the regulation of G_4 AChE.

Supported in part by the Marion Merrell Dow Scientific Education Partnership and the Veterans Administration Medical Research Service.

CATECHOLAMINES: RECEPTORS I

124.1

IMMUNOHISTOCHEMICAL LOCALIZATION OF THE D1 DOPAMINE RECEPTOR IN THE RAT BRAIN. Q. Huang[1*], D. Zhou[1], K. Chase[2], J.F. Gusella[3], N. Aronin[2] and M. DiFiglia[1]. Lab. of Cellular Neurobiology*, Lab. of Molecular Neurogenetics[3], Massachusetts General Hospital, Boston, MA 02114 and Dept. of Medicine and Cell Biology[2], Univ. of Massachusetts Med. Ctr., Worcester, MA 01655.

Localization of the D1 receptor was examined using a purified polyclonal anti-peptide antibody, which immunoprecipitated a protein of approx. 45 kDa on a silver-stained 8% SDS-PAGE. The regional distribution of D1 immunoreactivity overlapped with all dopamine-containing pathways and with patterns of D1 receptor binding and mRNA localization previously reported. D1 was heavily concentrated in cortical and subcortical neurons and neuropil of the limbic forebrain. Regions included the prefrontal, cingulate, parietal, piriform and entorhinal regions, the hippocampal formation, the amygdala, septal area, substantia inominata, thalamus (eg., medial dorsal complex), hypothalamus (eg., mammillary complex) and the neurohypophysis. Axonal transport of receptor protein was indicated by the prominent staining found in fiber bundles which connect limbic areas and in axons traversing the hypothalamus and other subcortical regions. Intense immunoreactivity was also seen in neurons and fibers of the thalamic reticular nucleus, a region known to integrate ascending basal forebrain inputs with thalamocortical and corticothalamic pathways, and throughout the basal ganglia (see also D. Zhou et al., this meeting). Results provide the first direct immunohistochemical evidence for the regional distribution of D1 receptors in the brain. The high density of D1 receptors in the limbic system and thalamic reticular nucleus supports an important role for this receptor in mediating functions involved with learning, memory, and cognitive processing. (Supported by NIH NS 16367 to MD and JFG and NSF BNS8819989 to NA).

124.2

ULTRASTRUCTURAL LOCALIZATION OF DOPAMINE D1 AND D2 RECEPTOR PROTEINS IN RAT STRIATUM. S. M. Hersch*, S. M. Edmunds, B. J. Ciliax, and A. Levey. Dept. of Neurology, Emory University School of Medicine, Atlanta, GA 30322

A family of at least five dopamine receptors (D1-5) mediate motor, cognitive, and endocrine effects of dopamine neurotransmission. D1 and D2 receptor mRNAs are most abundant in striatum and are differentially expressed in medium spiny neurons projecting to substantia nigra and globus pallidus, respectively. To better understand the synaptic organization of neurons expressing D1 and D2, we developed subtype-specific antibodies and used them for electron microscopic immunocytochemistry. Antibodies were raised to fusion proteins derived from divergent regions of the i3 loop and C-termini, affinity-purified, and used to localize the native D1 and D2 receptors (Kitt et al., 1991). The antibodies are specific for the respective fusion proteins, cloned receptors, and native striatal receptors on immunoblots. These antibodies were applied to 40µm thick vibratome sections from rat striatum and visualized using the avidin-biotin method. At the ultrastructural level, D1 immunoreactivity was localized to selected somata with the typical characteristics of medium spiny cells; to medium sized spiny dendrites, and to the postsynaptic elements of axospinous synapses in the neuropil. No axonal labeling was noted. D2 immunoreactivity was similar with the additional finding that some axon terminals were also labeled. In each, label was visible within the cytoplasm of these neural elements and also at postsynaptic densities. D1 and D2 immunoreactivity was present in subpopulation of spiny somata and dendrites.

124.3

IMMUNOCYTOCHEMICAL LOCALIZATION OF DOPAMINE D5 RECEPTOR IN RAT BRAIN. B.J.Ciliax*, N. Nash, C.Heilman and A. Levey. Dept. of Neurology, Emory University School of Medicine, Atlanta, GA 30322 and Nova Pharm. Corp., Baltimore, MD 21224

A human D5 dopamine receptor gene, closely related to D1, was recently cloned. However, the encoded D5 protein has yet to be identified in tissues because selective ligands are unavailable. A recombinant protein derived from D5 (<8% identity to D1) was prepared by PCR amplification from human genomic DNA, subcloned into pGEX2T, and expressed in *E. coli*. Rabbit antibodies to the purified protein were produced, affinity-purified, and shown to have no cross-reactivity with D1 or D2 fusion proteins on immunoblots. D5-like immunoreactivity (D5LI) was localized in rat brain by immunocytochemistry. In general, D5LI was highly consistent with the distribution of D5 mRNA, and distinct from D1 and D2. D5LI was most dense in the olfactory tubercle, including the Islands of Calleja, and in the outer molecular layer of piriform cortex. Less dense D5LI was present in the neuropil in superficial layers of other cortical regions, and in pyramidal neurons in layers 2/3 and 5. In hippocampus, D5LI was present in pyramidal neurons and stratum radiatum, especially in CA3. Diffuse, weak D5LI was present throughout dorsal and ventral striatum. Scattered, large striatal neurons expressed more intense D5LI. Small neurons in the substantia nigra, ventral tegmental area, hypothalamus, raphe and several other brainstem regions were also labeled. Preadsorption of antibodies with D5 protein eliminated specific labeling. These results suggest D5 may be relevant in a variety of dopaminergic pathways. The antibodies should be useful for further studies of the D5 receptor, including its regulation and subcellular distribution.

124.4

LOCALIZATION OF THE D5 DOPAMINE RECEPTOR IN THE CENTRAL NERVOUS SYSTEM AND PERIPHERAL TISSUE OF THE RAT. D.K. Grandy*[1,2], J. Meador-Woodruff[3], C. Saez[1], Y. Zhang[2], R.A. Johnson[1], A. Mansour[3], S.J. Watson, Jr[3], and O. Civelli[1,2]. [1]Vollum Institute for Advanced Biomedical Research and [2]Dept.Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR 97201; [3] Department of Psychiatry and the Mental Health Research Insitute, University of Michigan, Ann Arbor, MI 48019.

Dopamine affects a number of physiological processes in both the central nervous system and select peripheral tissues. In the brain, acting as a neurotransmitter, dopamine stimulates at least five different receptor subtypes: D1, D2, D3, D4 and D5, which are coupled via G proteins to the formation of cAMP, potassium channel conductance, and the mobilization of intracellular Ca^{+2}. In the periphery dopamine influences several processes including the absorption of Na^{+} by renal tubules (natriuresis) and the hemodynamics of the renal and mesenteric vasculature. These effects are thought to be mediated by two different subtypes of G protein-coupled receptors designated DA1 and DA2 to distinguish them from their central nervous system counterparts. However, whether or not these two peripheral dopamine receptor subtypes are molecularly distinct from those expressed in the central nervous system remains to be determined. We have begun to address this question by asking whether the recently described rat D5 dopamine receptor is expressed in tissues of both the central nervous system and periphery. Using *in situ* hybridization and the polymerase chain reaction (PCR) we have obtained evidence for the expression of the rat D5 dopamine receptor in both rat brain and kidney. In the brain the D5 mRNA is expressed at very low levels in the hippocampus and the parafascicular nucleus of the thalamus. In the kidney we find that the D5 receptor mRNA is also expressed at low levels in the proximal convoluted tubules.

124.5

D_1, D_2 and D_3 DOPAMINE RECEPTOR DEVELOPMENT IN THE INFANT AND JUVENILE HUMAN BASAL GANGLIA. C.E. Adams* and S.J. Boyson. Depts. of Neurology and Pharmacology, Univ. Colo. Hlth. Sci. Cntr., Denver, CO 80262.

D_1, D_2 and D_3 dopamine receptor densities within the caudate (Cd), putamen (Put), external (GPe) and internal globus pallidus (GPi) of 3 infant, 2 juvenile and 4 adult human brains were examined using quantitative autoradiography. D_1 receptors were labeled with a single concentration of ^{3}H-SCH-23390. D_2 receptors were labeled with ^{3}H-spiperone in adjacent tissue sets under two conditions; the first with the D_3-selective antagonist 7-OH-DPAT (DPAT/D_2) and the second without 7-OH-DPAT (non-DPAT/D_2). All buffers also contained 300 nM ketanserin. Nonspecific buffers also contained 2 μM (+)-butaclamol. The specific density of D_3 receptors was calculated by subtracting the density of total D_2 receptors in a DPAT/D_2 section from the density of total D_2 receptors in the adjacent non-DPAT/D_2 section. In general, the density of D_1 receptors was found to decrease with increasing age in all structures, most markedly within the GPi. D_2 receptor density also decreased with increasing age within the Put and GPe, but did not change within the Cd or GPi. D_3 receptors were present at low, highly variable levels in all structures examined but the density of D_3 receptors did not change systematically with maturation. These results suggest that the D_1 receptor, which is elevated throughout the basal ganglia in early life may play a role in development, as may the D_2 receptor in the Put and GPe. D_3 receptor levels are very low, as might be predicted from mRNA studies in rat. Supported by NIH NS019199.

124.6

POSTNATAL DEVELOPMENT OF DOPAMINE D_1, D_2, and D_3 RECEPTOR mRNAs IN RAT BRAIN. L.K. Srivastava* and R.K. Mishra. Depts. of Psychiatry and Biomedical Sciences, McMaster University, Hamilton, Ont., Canada, L8N 3Z5.

The postnatal development of the mRNAs for brain dopamine D_1, D_2, and D_3 receptors was investigated in Sprague-Dawley rats by Northern blotting and polymerase chain reaction. The levels of expression of striatal dopamine D_1 receptor mRNA was appreciable at birth, increased to a maximum at day 14, and remained at that level until day 28. There was a significant decline of D_1 mRNA level in six-month and one-year old rats. The expression of D_2 mRNA followed a similar pattern of development except that maximal level mRNA was observed at day 28. In contrast, very low level of D_3 receptor mRNA was observed at birth. The D_3 mRNA expression increased to a maximum at day 14 and then showed a gradual decline until age one-year. The development of the D_1 and D_2 receptors as measured by [^{3}H]SCH 23390 and [^{3}H]spiroperidol followed the pattern of their respective mRNAs except that there was a lack of correlation between ligand binding and message level especially during early developmental periods. For example, although the message levels of both receptors at birth were 70-75% of the maximal level seen at day 14-28, the respective ligand bindings were only 15-20%. PCR analysis of alternatively spliced forms of D_2 receptor mRNA showed that the developmental expression of the two isoforms proceeds in parallel. *In situ* hybridization revealed a differential developmental profile of D_2 mRNA in major dopaminergic regions of brain such as caudate-putamen, nucleus accumbens, olfactory tubercle, and substantia nigra.

124.7

PROMOTER ANALYSIS OF THE D1A DOPAMINE RECEPTOR GENE. M. T. Minowa, T. Minowa, Y-S. Lau* and M. M. Mouradian. Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

The D1A dopamine receptor plays an integral role in propagating neural information in the basal ganglia and other brain structures. We recently reported the primary structure of the promoter of the human D1A gene and found it to be TATA-less with multiple transcription start sites between -1061 and -1040 relative to the adenosine in the first ATG codon (Minowa et al., PNAS, 89:3045-3049, 1992).

In the present report, we carried out detailed promoter analysis by making deletion mutants of the -1343 to -1103 region of the gene which showed strong transcriptional activity in the D1A expressing NS20Y cells. Results indicated that the -1197 to -1120 region retains high promoter activity with specificity to these cells. Gel shift and footprinting suggested that recombinant human AP2 binds to two of its consensus sites in the D1A promoter between -1197 and -1070 but no Sp1 binding could be detected. Gel retardation with NS20Y nuclear extract suggested that additional factor(s) are involved in the transcriptional regulation of this gene.

We conclude that the D1A dopamine receptor gene is subject to complex regulatory control even though its promoter has features of "housekeeping" genes.

124.8

ORGANIZATION OF THE 5'-REGION OF THE RAT D_1 AND HUMAN AND RAT D_2 DOPAMINE RECEPTOR GENES. K.J. Buck[1,2*], Q.-Y. Zhou[1], C. Li[1], D.K. Grandy[1], and O. Civelli[1,3]. Vollum Institute[1], Department of Medical Psychology[2], and Department of Cell Biology & Anatomy[3], Oregon Health Sciences University, Portland, OR 97201.

Anomolous function and/or expression of dopamine receptors has been implicated in several neurological disorders including schizophrenia and Parkinson's disease. Genomic and cDNA clones encoding the rat D_1, rat D_2 and human D_2 dopamine receptors were isolated and sequenced. The rat D_1 receptor gene (rDRD1) is organized into two exons separated by a small intron in the 5'-UT region. The transcription start site is located 864 bp upstream from the translational initiation site. The 5'-flanking sequences of rDRD1 do not contain TATA and CAAT canonical sequences, but have a high GC content, potential CRE and GRE sequences and binding sites for transcription factors, e.g. Sp1, Ap1 and Ap2. Transfection studies using CAT gene fusion constructs demonstrated that 735 bp of the 5'-flanking region of DRD1 is sufficient to confer cell-specific expression. The rDRD1 promoter responds to cAMP induction, suggesting the possibility of an autoregulation mechanism by which stimulation of the D_1 receptor exerts a positive feedback on its own gene expression. This effect was enhanced by PMA and dexamethasone, although they alone had no effect.

Recent studies indicate that the human DRD2 gene extends over 270 kb and includes an intron of approximately 250 kb separating the 5'-region of DRD2 from the 7 exons encoding the receptor protein. We have identified an exon, putatively exon 2, in the 5'-UT region of the DRD2 gene. Exon 2 is 80% identical between human and rat, and is 331 bp and 333 bp in length, respectively. Primer extension analyses indicate that 200 bp of human and rat D_2 dopamine receptor mRNAs are encoded by an upstream exon(s). (Supported by MH45614, AA07468 and S.C.S.R.)

124.9

MOLECULAR CLONING AND CHARACTERIZATION OF THE 5'-FLANKING REGION AND PROMOTER OF THE HUMAN DOPAMINE D5-RECEPTOR GENE. T.V. Beischlag*, A.E. Tirpak, R.K. Sunahara, H.H.M. Van Tol, P. Seeman, and H.B. Niznik. Depts. of Pharamacology and Psychiatry, University of Toronto, Toronto, Canada, M5S 1A8, and The Laboratory of Molecular Neurobiology, The Clarke Institute of Psychiatry, Toronto, Canada, M5T 1R8.

The human dopamine D5-gene encodes for a protein that is a member of the guanine nucleotide-binding protein-linked superfamily of receptors. It has a higher affinity for dopamine than either the D1 or D2 receptors. Furthermore, the D5 gene has 2 closely related pseudogenes that are expressed but do not produce functional proteins. In order to study the transcriptional regulation of this gene a 3.6 Kb genomic clone 5' of the first ATG codon was isolated using a Sac 2.4 Kb fragment that overlapped the coding and 5' non-coding region of the D5- receptor gene. Amplification of human putamen and temporal cortex cDNA libraries by PCR using specific oligonucleotides to different regions of this clone yielded partial cDNA colones that suggest transcription of this gene initiates 1.4-1.9 Kb away from the first ATG codon. Sequence analysis reveals that this area and the region immediately 5' to it lacks conventional TATA and CAAT boxes, similiar to the D1 receptor gene promoter. It does however, contain several transcription factor binding domains and two antisense *Alu* motifs which contain transcription initiation sites for DNA polymerase III. To test for transcriptional activity, various regions of genomic DNA from the 5'-flanking region of the D5 gene have been fused in front of the firefly luciferase reporter gene. Data concerning the transient expression of these mutants in human and non-human immortal cell lines will be presented and reverse transcription of RNA from human putamen by PCR will be performed in order to delineate the exact site of transcription initiation.

124.10

ALU REPEAT INVOLVED IN THE DUPLICATION OF THE HUMAN D5-DOPAMINE RECEPTOR GENE A. Marchese, T. Beischlag, T. Nguyen, H. Niznik, L. Grupp*, P. Seeman, and B.F. O'Dowd. Dept. of Pharm., Univ. of Toronto, and Addiction Research Foundation, Toronto, Canada M5S 1A8.

We have previously reported the cloning of the functional D5-dopamine receptor DR and the two related pseudogenes (PGs) (Gene, 109: 211-218, 1991). These three genes are the most recent duplications in the large family of G-protein linked receptor genes, and they represent an opportunity to study the mechanism by which genes in this family have evolved. We have sequenced both the 5' and 3' regions flanking the coding region of the D5 DR and the equivalent regions in the two PGs. So far these three genes share close homology spanning a total region of at least 5 kb. In the 5'-untranslated region of these genes following 1.9 kb of homologous sequence, a transition to non-homologous sequence occurs in the D5 DR gene. The transition site to non-homologous sequence occurs at an Alu sequence that is present in both of the PGs, but is not found in the D5 DR gene. It is possible, given the position of the Alu repeat in the PGs, that this element was involved in the duplication of D5 DR which produced the first PG. The Alu sequences in both PGs are of the monomeric type (162 bp) and are composed of the right side of the Alu repeat with the characteristic poly-A tract terminating the repeat element and direct repeats flanking the entire structure. The orientation of the Alu is opposite to that of the PGs. Each of the PGs continue to be homologous beyond the Alu repeats suggesting that they were not involved in the duplication event forming the second PG. We have detected transcription of at least one PG in several brain areas and this mRNA transcript could produce a truncated receptor of 154 amino acids. We have determined that genomes of related primates, such as gorilla, also contain two PGs, however transcription of these genes would produce a truncated protein of only 95 amino acids.

124.11

EXPRESSION AND REGULATION OF THE D_{1A} DOPAMINE RECEPTOR IN STABLY TRANSFECTED CHO CELLS. E.M. Smyk-Randall*, L.-J. Zhang, F.J. Monsma, Jr. & D.R. Sibley. Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

To investigate the functional and regulatory properties of the D_{1A} dopamine receptor, we have stably expressed a cDNA encoding the rat D_{1A} receptor in Chinese Hamster Ovary (CHO) cells. Following transfection and selection in G418, several clonal lines were isolated which exhibited a wide range of D_1 receptor binding activities as determined using the D_1-selective antagonist [^{3}H]-SCH-23390. One cell line, expressing about 1.8 pmol/mg protein, was selected for further characterization. Saturation analysis revealed a K_d value of about 0.3 nM for [^{3}H]-SCH-23390 in good agreement with radioligand binding assays using rat striatal membranes. Dopamine produced a dose-dependent and pharmacologically-specific stimulation of adenylyl cyclase activity in both intact cell and membrane preparations. Dopamine's EC_{50} for stimulating cAMP accumulation was about 1 μM with a maximal response at 100 μM which represented a 20-fold stimulation over basal levels. Pretreatment of the cells with dopamine was found to produce a profound desensitization of the dopamine-stimulated cAMP response and a loss of [^{3}H]-SCH-23390 binding. The dopamine-induced desensitization/down regulation was time-dependent reaching maximal levels after 20 hr with a $t_{1/2} \geq 5$ hr. The dopamine dose-response for promoting these regulatory effects shows an EC_{50} of about 10 nM with an EC_{max} of 10 μM. After maximal desensitization, the EC_{50} of dopamine for stimulating cAMP accumulation is unchanged whereas the maximum response is decreased by about 75-90%. Similarly, there is no change in the affinity (K_d) of [^{3}H]-SCH-23390 but a >50% decrease in maximum binding capacity is observed. The agonist-induced desensitization is pharmacologically-specific being mimicked by 6,7-ADTN, fenoldopam, and SKF38393. The biochemical mechanisms responsible for these regulatory effects are currently under investigation.

124.12

CHARACTERISTICS OF DOPAMINE-STIMULATED PHOSPHOINOSITIDE METABOLISM IN RAT BRAIN. A. S. Undie and E. Friedman*. Departments of Psychiatry and Pharmacology, Medical College of Pennsylvania, 3200 Henry Avenue, Philadelphia, PA 19129.

Previous investigations have demonstrated that dopamine enhances inositol phosphate formation in rat brain slices, and that this action is mediated through a D_1-like dopamine receptor. The present studies were directed at elucidating the mechanism of this dopaminergic response in rat striatal slices incubated in the presence of LiCl. Neomycin, a polyphosphoinositide phospholipase C inhibitor, dose-dependently and completely inhibited SKF38393-stimulated formation of inositol phosphates. Moreover, time course studies in slices simultaneously treated with [^{3}H]inositol and SKF38393 showed that [^{3}H]inositol trisphosphate was the first inositol phosphate to accumulate following agonist stimulation. These observations suggest that phosphatidylinositol-4,5-bisphosphate is the preferential or primary substrate for dopamine-stimulated phospholipase C.

Using a number of benzazepine and other dopamine D_1 receptor agonists at concentrations from 10^{-8} to 10^{-3} M, we studied the relative efficacies of these drugs in stimulating phosphoinositide metabolism. Whereas dopamine and several benzazepines including SKF38393 and SKF82526 were highly potent or efficacious in stimulating inositol phosphate accumulation, several otherwise potent D_1 receptor agonists were without effect. This differential pattern of agonist effects suggests that the phosphoinositide-linked dopamine receptor is different from the classical D_1 receptor. SUPPORTED BY USPHS GRANT #NS-29514 AND BY THE TOURETTE SYNDROME ASSOCIATION.

124.13

BINDING OF A FLUORESCENT D_1 DOPAMINE RECEPTOR ANTAGONIST TO SINGLE LIVE CULTURED NEURONS. K.R. Hoyt* and I.J. Reynolds. Department of Pharmacology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Fluorescent D_1 and D_2 dopamine receptor antagonists have recently become available (Monsma *et al.*, J. Neurochem. (1989) **52**, 1641-1644). Using these ligands, we are attempting to identify dopamine receptors on live cultured striatal neurons. We are using quantitative fluorescence microscopy (ACAS 470, Meridian Instruments, Okemos, MI) to characterize the binding of a fluorescent D_1 dopamine receptor antagonist (bodipy-SCH 23390; Molecular Probes, Eugene, OR) to live cultured striatal neurons from fetal rats (E17). We have been able to demonstrate specific binding as assessed by competition of the fluorescent ligand with an unlabelled competitor, (+)butaclamol. Total fluorescent binding of bodipy-SCH 23390 to individual striatal neurons has been quantified and the median total binding per neuron was 20189 arbitrary fluorescence units (AFU) (n = 256 cells) and median total binding in the presence of excess unlabelled (+)butaclamol was 2329 AFU (n = 252 cells). In addition, in kinetic experiments, bodipy-SCH 23390 binding to individual neurons can be reversed with unlabelled (+)butaclamol. This specific labelling of live neurons that possess D_1 dopamine receptors should allow the study of functional responses (e.g. changes in $[Ca^{2+}]_i$) in single cells that specifically express certain dopamine receptor subtypes, rather than in a heterogeneous population of neurons or in a random fashion.
Supported by NIMH (MH18273).

124.14

POTENCY OF D_1-RECEPTOR AFFINITY LIGANDS WITH ALKYLATING MOIETIES. M Hartmann*, RJ Baldessarini, N Kula, D Huston-Lyons, N Baindur, & JL Neumeyer, Department of Psychiatry & Neuroscience Program, Harvard Medical School, Mailman Research Center, McLean Hospital, Belmont, MA 02178 & Research Biochemicals, Inc., Natick, MA 01760.

Derivatives of D_1-selective dopamine receptor antagonists were prepared and tested for affinity and apparent alkylating potency in rat striatum with radioreceptor assays using tritiated SCH-23390 (D_1) or YM-09151-2 (D_2) as radioligands. The *p-bis*-chloroethylamino (*b*-CEA) derivative of SKF-83566 (7-Br-8-OH-3-methyl-1-(4'-*bis*-chloroethylaminophenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine), had 132x higher D_1 affinity (K_i = 0.8 ± 0.1 pM) and 1176x greater D_1/D_2 selectivity (1.7 x 10^6) than the parent compound SKF-83566 with striatal homogenates. It also was more potent and D_1-selective than previously reported congeners containing other alkylating groups, in order of D_1 affinity (K_i = 0.5–17 μM): chloroethylamino > chloracetamido ≥ bromoacetamido ≥ isothiocyanato > ethylfumaramido > maleimidoacetamido. For *in vitro* tests of D_1 alkylation, striatal minces were preincubated with test agents (37° C, 40 min), extensively washed, then homogenized and assayed with *ca.* Kd concentrations of the radioligands. Again, the *b*-CEA phenylbenzazepine showed very high potency and D_1/D_2 selectivity (D_1 K_i = 180 ± 30 nM; D_1/D_2 selectivity = 580x). Congeners had K_i values of 1–3.4 μM, and selectivity ratios of 8–500x. Scatchard analysis of inhibition of ^{3}H-SCH-23390 binding after preincubation of striatal tissue with the *b*-CEA derivative showed concentration-dependent decreases in B_{max} without changes in ligand affinity; these were prevented by coincubation with nonalkylating D_1, but not D_2, agents. These results are consistent with potent and selective alkylation of striatal D_1 receptors *in vitro*. [Supported by NIMH awards 14275, 31154, 36004, 45692 & 47370 & Deutsche Forschungsgemeinschaft.]

124.15

DIHYDREXIDINE BINDS DIFFERENTLY TO HUMAN CLONAL AND RAT STRIATAL D_1 DOPAMINE RECEPTORS. Val J. Watts[1,*], David M. Mottola[1], Olivier Civelli[2], Robert A. Johnson[2], David E. Nichols[3] and Richard B. Mailman[1]. University of North Carolina[1]., Chapel Hill, NC 27599, Oregon Health Sciences University[2]., Portland OR, and Purdue University[3]., W. Lafayette IN.

Dihydrexidine (trans-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine) (DHX) is a full efficacy D_1 agonist in rat striatum and has been shown to bind D_1 receptors with an IC50 of 10 nM. SKF82958 (6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzapine) has also been characterized as a full efficacy agonist at D_1 dopamine receptors with an IC50 value of 8 nM. Although both agonists display high potency in striatal homogenates, their receptor recognition characteristics differ. Several studies have reported that SKF82958 binds only to the high affinity state of the D_1 receptor, whereas DHX displays a typical agonist binding profile, binding to both high and low affinity states of the receptor. This study was undertaken to examine and characterize the interactions of D_1 agonists in a homogeneous population of D_{1a} receptors. The cloning and stable expression of D_{1a} receptors in Ltk- cells provides an ideal system in which to examine the guanine nucleotide-dependent binding properties of DHX and other full efficacy agonists. The results from these studies showed that SKF82958 binds to D_{1a} receptors with a potency similar to that observed in striatal homogenates (IC50 $\approx$ 4.0 nM). In contrast, DHX binds to D_{1a} receptors in this cell line with more than five-fold lower potency (IC50 $\approx$ 60 nM) compared to binding in striatal homogenates (IC50 $\approx$ 10 nM); both ligands had Hill slopes significantly < 1.0. In this cell line, the addition of GTP shifted competition curves for both drugs such that they fit a one site model (n_H $\approx$ 1.0) of a low affinity state. There are at least three possible mechanisms that may explain these data. First, there may be a quantitatively important D_1-like receptor in the striatum that is as yet uncloned. Second, the expression of D_1 receptors in clonal cell lines may result in processing that yields a receptor that is subtly different from the one in mammalian brain. Third, the human D_{1a} receptor may be significantly different from the rat striatal D_1 receptor in its recognition of these members of two distinct drug classes. (Supported by MH40537 and MH42705)

124.16

Substitutions on the Pendent Phenyl Ring of Dihydrexidine Alter D_1:D_2 Selectivity. D.H. Mooney[1,4*], D.M. Mottola[2], S.B. Southerland[4], T.A. Knoerzer[5], D.E. Nichols[5], and R.B. Mailman[1,2,3,4]. Curriculum in Toxicology[1], Departments of Pharmacology[2] and Psychiatry[3], and Brain and Development Research Center[4], University of North Carolina, Chapel Hill, NC, 27599 and School of Pharmacy Purdue University[5], West Lafayette, IN, 47907.

We have demonstrated previously that dihydrexidine (DHX) is a high potency, full efficacy D_1 agonist with significant D_2 potency (e.g., Brewster et al., J. Med. Chem. 33:1756, 1991). Our molecular modeling studies (Mottola et al., SN Abstr. 16:79, 1990) have predicted that substitutions at the 2- and 3- positions of DHX (the pendent phenyl ring) might affect dopamine receptor affinity and/or selectivity. Thus, the 2-methyl, 3-methyl, and 2-phenyl analogs of DHX where synthesized and tested for their ability to compete for D_1 and D_2 binding sites (labeled with ^{3}H-SCH23390 and ^{3}H-spiperone, respectively) in rat brain striatal homogenates. Similar to earlier studies, the affinity (IC50) of DHX for D_1 binding sites was ca. 8 nM whereas the D_2 affinity was ca. 100 nM (D_1:D_2 selectivity of ca. 13). Addition of a methyl group to the 2-position of dihydrexidine (2-MeDHX) had slight effects on D_1 potency (IC50=14 nM), whereas D_2 affinity is decreased more (to ca. 650 nM), thus increasing the D_1:D_2 selectivity to ca. 46. Conversely, addition of a methyl group to the 3-position did not significantly affect the D_1 potency (IC50 = 7 nM), whereas the D_2 affinity of 3-MeDHX was doubled (45 nM) compared to DHX. Substitution of a bulkier phenyl ring at the 2-position of DHX drastically reduces D_1 affinity (IC50=290 nM), but has less effect on D_2 affinity (IC50=185nM). The functional activity of these analogs was also tested using adenylate cyclase assays in rat striatal homogenates. Preliminary studies indicate that 2-MeDHX and 3-MeDHX were, like DHX, full efficacy agonists. The functional potency of 2-MeDHX relative to DHX, however, appears to be affected more than its competition for ^{3}H-SCH23390. While studies of phenyltetrahydrobenzazepines suggest great tolerance for steric bulk on the pendent phenyl ring, these data indicate that this region may be an important site regulating interaction of hexahydrobenzophenanthridines with dopamine receptors. (Supported, in part, by PHS Grants ES07126, MH40537 and MH42705).

124.17

AGONIST-INDUCED CHANGES IN DENSITY OF D1, D2 AND CHIMERIC D1/D2 RECEPTORS. L.B. Kozell*, S. Starr, C.A. Machida, R.L. Neve and K.A. Neve. VA Medical Center and Oregon Health Sciences Univ., Portland, OR; Primate Research Center, Beaverton, OR; and McLean Hospital, Belmont, MA.

Treatment of C_6 glioma cells expressing recombinant D1 dopamine receptors with the agonists dopamine or NPA decreased the density of receptors by 51% and 39%, respectively. In contrast, the density of D2 receptors on C_6 glioma cells transfected with $D2_{444}$ cDNA was increased by 41%, 130% and 59%, respectively, after treatment with dopamine, NPA or quinpirole. NPA-induced up-regulation of receptor density (26%) was also seen in LZR1 cells, a fibroblast cell line expressing recombinant $D2_{415}$. Agonist-induced increases in receptor density do not involve G-proteins or adenylyl cyclase inhibition. Thus, neither treatment with pertussis toxin nor mutation of Asp-80, which prevents interaction with G proteins, blocked NPA-induced D2 receptor proliferation. The density of D1/D2 chimeras containing D1 sequence from the amino terminus to the amino-terminal end of transmembrane domain (TM) 6 or 7 was decreased by treatment with NPA or dopamine, whereas agonist treatment of a D1/D2 chimera with D1 sequence only up to the amino-terminal end of TM 5 increased the density of the receptors. These results suggest that the 3rd cytoplasmic loop or TM 5 may confer agonist-induced changes in receptor density. (MH 45372, VA Merit Review Program)

124.18

BIOLOGICAL ACTIVITIES OF DOPAMINE RECEPTORS IN VARIOUS CELL ENVIRONMENTS. C. Bouvier*, J.A. Salon‡, R. Johnson, J.R. Bunzow, H.H.M. VanTol† and O. Civelli. Vollum Institute, Oregon Health Science University, Portland, OR 97201, ‡Synaptic Pharmaceuticals, Paramus, NJ 07652,†Department of Pharmacology, University of Toronto, Toronto, Canada M5S1A8

The dopaminergic system implicated in several human disorders including schizophrenia, Parkinson's disease and prolactinomas, exerts its effects through several dopamine receptors coupled to G proteins. Upon stimulation, the D_1 and D_2 dopamine receptor respectively activates and inhibits the adenylate cyclase. Recently, these receptors have been found to couple to several other second messenger pathways, and moreover these couplings were found to be dependent on the cellular environment. In order to understand the metabolic response of dopamine receptors upon stimulation with dopaminergic agents, we used a silicon-based microphysiometer which allows real-time measurement of metabolic activity of living cells. The use of several cell lines transfected with the cDNA of the D_1 and D_2 receptors confirm the importance of the cellular environment not only for the coupling of these receptors but also for their metabolic response to a stimuli. The possibility to determine the coupling of these receptors by studying their metabolic response will be examined. Other subtypes of dopamine receptors have recently been cloned, the functions of which are still under investigation. The coupling and metabolic responses of these receptors will be discussed.

124.19

D1/D2 RECEPTOR INTERACTIONS: STIMULATING BOTH RECEPTORS INDUCES AN EFFECT OPPOSITE TO THAT OF STIMULATING D1 ALONE IN THE SUBSTANTIA NIGRA PARS RETICULATA AFTER RESERPINE TREATMENT. K.-X. Huang* and J.R. Walters. NINDS, Bethesda, MD 20892.

Synergistic interactions between D1 and D2 receptor processes have been described in normal and dopamine (DA)-depleted rats; mechanisms remain undefined. Present studies show D1/D2 synergy in responses of globus pallidus (GP) neurons to DA agonists after subchronic reserpine treatment (1 mg/kg/day for 6 days, s.c., then a 5-day wash-out). This treatment altered the response of GP cells to the D1 agonist SKF 38393 (10 mg/kg, i.v.): 14/17 neurons had firing rates increased by 50%. Combining SKF 38393 with quinpirole (0.16 mg/kg, i.v.) caused greater increases in firing rates while quinpirole alone had only a limited effect. These results are similar to those in rats with 6-hydroxydopamine (6-OHDA)-induced nigrostriatal lesions. Interestingly, another type of D1/D2 interaction was found in the substantia nigra pars reticulata (SNpr) of reserpine-treated rats. SKF 38393 increased the firing of SNpr neurons by 91% (n = 18). Quinpirole itself had no effect but, given with the D1 agonist, it reversed the excitatory effect of SKF 38393 and induced a profound inhibition (80%, n = 11). In contrast, in lesioned rats, SKF 38393 alone has been shown to inhibit SNpr activity, an effect potentiated by quinpirole. Thus, consequences of D1/D2 interaction differ in the GP and SNpr and after reserpine and 6-OHDA treatments; an effect of one of these treatments, other than DA depletion, appears to alter DA processes. The possibility is raised that D1-mediated effects in reserpinized rats reflect a basic ability of D1 receptors, when acting independently of D2 stimulation, to mediate inhibition of striatal output, while concomitant D2 stimulation leads to striatonigral excitation and enhanced striatopallidal inhibition.

124.20

INTRASTRIATAL RESERPINE ADMINISTRATION DECREASES DOPAMINE RECEPTOR mRNA LEVELS IN RAT STRIATUM. T. Mukherjee, P. Seeman* and S.R. George, Depts of Medicine and Pharmacology, University of Toronto, Toronto, Ont, CANADA M5S 1A8

Peripheral reserpine treatment has been shown to increase and then decrease D1 (DRD1) and D2 (DRD2) receptor density in rat striatum in a time dependent manner. To determine the effect of localized dopamine depletion on the synthesis of DRD1 and DRD2,reserpine was administered stereotactically via unilateral implantation of cannulas into the rat striatum using coordinates (from bregma): 0.2mm anterior, 3mm lateral, 5.5mm ventral (atlas by Paxinos and Watson). Positioning of the cannula was verified by microscopic section analysis. Alzet minipumps were attached to the cannula and implanted subcutaneously. Reserpine was infused at 10ug/day for a period of 2 weeks. Northern blot analysis was used to measure D1 and D2 receptor mRNA using a 500bp cDNA probe for D1 and D2 was hybridized using a 48bp synthetic oligonucleotide corresponding to bases 782-826 of the D2 receptor gene. A decrease in both D1 and D2 receptor mRNA was observed in the striatum of the treated rats compared to control. Furthermore, within the treated group, a decrease in D1 and D2 receptor mRNA was observed in the striatum ipsilateral to the injection site compared to contralateral. To correlate with RNA changes, D1 binding analysis was performed on striatal tissue homogenate using the D1 antagonist 3H-SCH23390. Results showed a 53% decrease in D1 receptor density on the ipsilateral side of the injection compared to contralateral. No changes were observed in Kd. Thus, the decrease in D1 binding is consistent with the decrease in D1 mRNA on the ipsilateral side of the treated animals. Our results suggest that localized administration of reserpine may cause decreased synthesis of dopamine receptors.

125.1

ONTOGENY OF NIGROSTRIATAL DOPAMINE NEURON AUTORECEPTORS: IONTOPHORETIC STUDIES. L. Wang* and D.K. Pitts, Dept. of Pharm. Sci., Coll. of Pharmacy & A.H.P., Wayne State Univ., Detroit, MI 48202.

Antidromically (AD) identified nigrostriatal dopamine (DA)-containing (NSDA) neurons were studied during postnatal development using standard extracellular iontophoretic techniques under chloral hydrate anesthesia. Stimulation of somatodendritic (SD) D2 receptors on adult NSDA neurons results in membrane hyperpolarization and inhibition of spontaneous activity. Pitts et al. (Synapse 6:309, 1990) examined NSDA neuron SD DA autoreceptor sensitivity to cumulative i.v. doses of either apomorphine or quinpirole. NSDA neurons from 2- and 4-week-old rats were found to have lower sensitivity to apomorphine (D2/D1 agonist) than NSDA neurons from adults. However, the sensitivity of NSDA neurons to quinpirole (D2 agonist) was not found to be age dependent. To resolve this discrepancy between test agonists given by the i.v. route both quinpirole and apomorphine were applied iontophoretically. 2-week-old, 4-week-old and adult rats were found to be equally sensitive to both apomorphine and quinpirole. These results suggest that adult-like SD DA autoreceptor sensitivity is exhibited as early as the second postnatal week of development. (Supported by MH-47857 [DKP])

125.2

NEONATAL NIGROSTRIATAL DOPAMINE NEURONS ARE LESS RESPONSIVE THAN ADULT NEURONS TO THE INHIBITORY EFFECTS OF AMPHETAMINE G. Zhang* and D.K. Pitts. Dept. of Pharm. Sci., Coll. of Pharmacy & A.H.P., Wayne State Univ., Detroit, MI 48202.

The inhibition of DA neuronal impulse flow has been attributed to an enhancement of both the stimulation of somatodendritic (SD) dopamine (DA) autoreceptors by dendritically released DA and the activity of inhibitory feedback pathways which are activated by DA released from neuronal terminals in forebrain regions. In the present study standard extracellular electrophysiological techniques were used to examine the responses of antidromically identified nigrostriatal DA-containing (NSDA) neurons to amphetamine. NSDA neurons from both urethane and chloral hydrate anesthetized 2-week-old (postnatal day 14 or 15) rats were less sensitive to the inhibitory effects of cumulative i.v. amphetamine doses than similarly anethetized adults. These results confirm those of Trent et al. (Eur. J. Pharmacol. 204:265, 1991) using the i.p. route of administration. In the present study excitatory responses to i.v. amphetamine were also observed in 2-week-old rats but not in adults. Since SD DA autoreceptor sensitivity in 2-week-olds appears to be similar to that of adults (see Wang and Pitts, Soc. Neurosci. Abstr., 1992) these results suggest that the functional expression of feedback pathways and/or the dopamine transporter are not adult-like at this postnatal age. (Supported by MH-47857 [DKP]).

125.3

GABAERGIC MODULATION OF NIGROSTRIATAL DOPAMINE NEURON ACTIVITY IN NEONATAL RATS. D.K. Pitts* and L. Wang. Dept. of Pharm. Sci., Coll. of Pharmacy & A.H.P., Wayne State Univ., Detroit, MI 48202.

Nigrostriatal dopamine (DA)-containing (NSDA) neurons were antidromically identified in chloral hydrate anesthetized rats using standard extracellular recording techniques. Iontophoretically applied GABA inhibited NSDA neurons from 2-week-old and adult rats in a similar fashion indicating the presence of functional GABA receptors early in postnatal development. Likewise functional GABA receptors could be demonstrated on zona reticulata (ZR) neurons using iontophoretic techniques. DA neurons in the zona compacta (ZC) are thought to receive inhibitory afferents from ZR neurons. Grace and Bunney (Eur. J. Pharmacol. 59: 211, 1979) have shown that in adult rats: (i) GABA has more potent inhibitory effects on ZR neurons than ZC DA neurons, and (ii) the GABA-A agonist, muscimol, when given i.v., excites ZC DA neurons by a mechanism most likely involving selective inhibition of ZR neurons and disinhibtion of ZC DA neurons. In the present study i.v. muscimol (0.1 to 1.6 mg/kg) was found to excite NSDA neurons from both adults and 2-week-old rats. This suggests that a tonic endogenous inhibitory input to NSDA neurons is present at the postnatal age of 2-weeks. Preliminary results also suggest that muscimol is more likely to induce or increase burst discharge activity in adults than in 2-week-old pups. (Supported by MH-47857 [DKP]).

125.4

EFFECTS OF DOPAMINE ANTAGONIST ADMINISTRATION ON THE ACTIVITY OF MIDBRAIN DOPAMINE NEURONS RECORDED *IN VITRO*. M. L. Pucak* and A. A. Grace. Depts. of Behavioral Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Stimulation of somatodendritic autoreceptors by dendritically released dopamine (DA) has been proposed to be an important mechanism regulating the activity of DA neurons in the substantia nigra. We have assessed 1) whether dendritically released DA modulates the activity of DA neurons in the *in vitro* slice preparation and 2) the mechanism of this regulation by examining the effect of blocking somatodendritic autoreceptors with DA antagonists. Intracellular recordings from DA neurons were performed in rat midbrain slices containing the substantia nigra. Administration of 10 µM haloperidol caused the input resistance to decrease by approximately 30% in most DA neurons tested. Haloperidol also depolarized the membrane potential by approximately 5 mV; this was associated with an increase in spontaneous firing rate and a 10-100% increase in the slope of the slow depolarization which precedes spontaneously generated action potentials. The amplitude of the afterhyperpolarization was decreased by 20-60% in most of the neurons. In addition, there was a small (10%) decrease in the amplitude of the action potentials. Thus, dendritically released DA does appear to regulate the activity of at least some DA neurons *in vitro*. The haloperidol-induced depolarization and associated membrane changes are consistent with the previously observed DA-induced hyperpolarization of DA neuron membrane potential; furthermore, the effects of haloperidol suggest that the actions of endogenous DA on DA neuron activity may be mediated by more than a single mechanism. Supported by MH42217, MH45156, NS19608, and MH09873 (to MLP)

125.5

NUMERICAL SOLUTION TO THE DIFFUSION EQUATION WITH NON-LINEAR MICHAELIS-MENTEN UPTAKE FOR DOPAMINE IONTOPHORESIS IN THE STRIATUM. C. Nicholson* and M. E. Rice. Dept. Physiology and Biophysics, New York University Medical Center, 550 First Avenue, New York, NY 10016.

The migration of dopamine released into the extracellular space (ECS) of the striatum is governed by diffusion and by an avid uptake system that obeys non-linear Michaelis-Menten (MM) kinetics (Wightman et al. Neurosci. 25: 513, 1988). We previously showed the validity of analytical solutions to the diffusion equation with linear uptake for the movement of tetramethylammonium through the ECS of the striatum (Rice and Nicholson, J. Neurophysiol. 65: 264, 1991). The addition of MM kinetics, however, necessitates a numerical solution. Here we describe a solution representing a typical experimental paradigm.

The spherically symmetric partial differential diffusion equation with MM uptake was converted to an integral equation. Using Simpson's rule a set of simultaneous equations was constructed which also incorporated the boundary conditions representing iontophoresis from a micro-electrode. These equations were solved by the Newton-Raphson method using LU decomposition. This solution was programmed in Turbo Pascal and required 2-6 hours of run time on a 33 Mhz 486 PC.

Preliminary experiments using fast cyclic voltammetry in slices of the rat striatum confirmed that typical literature values (Wightman et al., ibid) of the MM parameters, V_{max} and K_m, are appropriate. Further comparisons of numerical solutions with experiment are expected to refine the values of these parameter.

This study was supported by NIH Grant NS 28642.

125.6

ELECTROPHYSIOLOGICAL RESPONSES OF MESENCEPHALIC DOPAMINE NEURONS TO DORSAL RAPHE STIMULATION. C. Rouillard* and J. Gervais. Lab. of Neurobiology and Dept. of Pharmacology, Laval University, Québec, Canada G1J 1Z4.

Neuroanatomical data indicate that mesencephalic dopamine (DA) neurons receive a serotonergic (5-HT) input from the dorsal raphe (DR) nucleus and electron microscopic evidence have shown that a fair proportion of this 5-HT input is aimed at dendrites of DA neurons. Recent electrophysiological studies have suggest that the 5-HT input from the DR may act in a phasic rather than tonic manner to regulate presynaptic functions of DA neurons without affecting neuronal excitability as a whole. The present study was designed to examine the effects of dorsal raphe stimulation on the spontaneous activity of substantia nigra pars compacta (SNpc) and ventral tegmental area (VTA) DA neurons. Extracellular single unit recordings were performed *in vivo* on male Sprague Dawley rats, anesthetized with chloral hydrate. DA neurons in the SNpc and VTA were recorded using single barrel micropipettes and were identified by their location, waveform, firing rate and pattern. DR stimuli consisted of monophasic square wave pulses of 800 µA intensity and 350 µsec duration delivered at a rate of 0.5 Hz. Peristimulus time histograms were generated on line by cumulating 250 consecutive stimuli. Two different types of response were found both in SNpc and VTA. Some DA cells exhibited an inhibition-excitation sequence while in others DA neurons, the initial response was an excitation followed by a long lasting inhibition. In SNpc, 53,3% of the DA cells recorded were initially inhibited (latency: 23±8 msec; duration: 109±22 msec) and 33,3% of the DA neurons were initially excited (latency: 20±5 msec; duration: 80±31 msec). However, in VTA 56% of the DA cells recorded were initially excited (latency: 31±6 msec; duration: 79±11 msec) and 37% were initially inhibited (latency: 8±2 msec; duration: 75±11 msec). In some DA cells, DR stimulation also induced profound changes in the firing rate and/or firing pattern. These data strongly suggest that the 5-HT input from the DR nucleus can modulate in a complex manner the spontaneous activity of the majority of mesencephalic DA neurons. (Supported by FRSQ.)

125.7

EFFECTS OF CRUS CEREBRI LESIONS AND LONG-TERM AMPHETAMINE TREATMENT ON PATTERNS OF SPONTANEOUS UNIT ACTIVITY OF NIGRAL DOPAMINERGIC NEURONS. B.A. Heidenreich* and G.V. Rebec. Prog. Neural Science, Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

The spontaneous activity of dopamine- (DA) containing neurons in the substantia nigra compacta (SNC) *in vivo* consists of irregular spikes and bursts of 2-10 spikes fired rapidly (Grace and Bunney, J. Neurosci., 4: 2877, 1984). Because disruption of feedback from forebrain target areas (Bunney and Aghajanian, Science, 192: 391, 1976) and chronic treatment with DA agonists (Kamata and Rebec, Neuropharmacology, 22: 1377, 1983) have marked effects on DA neurons, we examined the firing patterns of DA units in adult male rats after electrolytic lesions of the crus cerebri (CC) and repeated d-amphetamine (AMPH) administration. One week after surgery, rats received either 5.0 mg/kg AMPH or saline twice daily for 6 days. On the following day, single-unit activity of DA neurons was recorded in the SNC under urethane anesthesia. Firing patterns were analyzed off-line with a PC-based program (courtesy of Dr. L.A. Chiodo). Mean spontaneous firing rate was reduced by AMPH treatment ($p<.05$), but not CC lesions. With burst categorization added as a third factor, bursting cells had markedly faster firing rates ($p<.01$), but the effect of AMPH treatment was reduced to a non-significant trend ($p=.13$). Units from CC-lesioned animals showed a higher number of bursts during the period analyzed ($p<.05$). The number of bursts and the mean number of spikes/burst were significantly correlated with firing rate in all treatment groups except AMPH-treated shams. Taken together, these results demonstrate the complex influences on the rate and pattern of DA cell activity.

Supported by USPHS grant DA 02451.

125.8

QUINAZOLINONE CCK-B ANTAGONISTS DECREASE THE NUMBER OF SPONTANEOUSLY ACTIVE DOPAMINE NEURONS. M. J. Yu*, J. F. Czachura, M. E. Stockton, J. R. McCowan and K. Rasmussen. *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.*

Cholecystokinin (CCK) has been shown to exist in a subpopulation of dopaminergic neurons in the ventral tegmental area (A10). These CCK-containing dopaminergic efferents project to limbic regions, in particular, the caudodorsomedial part of the nucleus accumbens. Although the functional relationship between CCK and dopamine is complex, these pathways may be associated with a number of neuropsychiatric disorders. Since CCK has potent excitatory effects on dopamine neurons and can potentiate dopamine mediated behaviors, CCK receptor antagonists may reduce midbrain dopaminergic hyperactivity associated with schizophrenia and thereby act as an antipsychotic agent. This study evaluated the effects of two structurally related and novel CCK-B antagonists, 2-[2-(1H-indol-3-yl)ethyl]-3-phenyl-4(3H)-quinazolinone (**1**) and a 5-chloroindole derivative (**2**), on the number of spontaneously active midbrain dopamine neurons using extracellular, single-unit recordings in anesthetized rats. Acute intraperitoneal administration of either **1** or **2** decreased the number of spontaneously active A10 dopamine cells. Thus, in contrast to currently prescribed antipsychotic drugs, these compounds as CCK-B receptor antagonists may exhibit antipsychotic effects without a delayed onset of action.

125.9

ELECTROPHYSIOLOGICAL EFFECTS OF DIPHENYLPYRAZOLIDINONE CCK-B AND CCK-A ANTAGONISTS ON A9 AND A10 DOPAMINE CELLS. *J. F. Czachura, J. J. Howbert*, and K. Rasmussen. Lilly Research Labs, Eli Lilly & Company, Indianapolis, IN 46285.*

Chronic administration of antipsychotic drugs has been shown to decrease the number of spontaneously active dopamine (DA) cells in the ventrotegmental area (A10) and the substantia nigra (A9). The selective CCK-B antagonist LY262691 (a diphenylpyrazolidinone) has recently been demonstrated to decrease the number of spontaneously active A9 and A10 DA cells following acute administration. The present study evaluated the effects of structural analogs of LY262691 on the number of spontaneously active A9 and A10 DA cells using extracellular, single-unit recordings in the anesthetized rat. Three diphenylpyrazolidinone CCK-B antagonists (LY262684, LY242040, and LY191009) decreased the number of spontaneously active A9 and A10 DA cells. However, both an inactive analog (LY206890) and a CCK-A selective analog (LY219057) did not affect the number of spontaneously active A9 and A10 DA cells. In addition, L-365,260, a benzodiazepine CCK-B antagonist, also decreased the number of spontaneously active A10 cells. Unlike typical antipsychotics (e.g., haloperidol), which produce pronounced catalepsy, these compounds showed no cataleptogenic effects. These results indicate that the firing of A9 and A10 DA neurons is suppressed specifically by antagonism of CCK-B, but not CCK-A, receptors. CCK-B antagonists may therefore represent a novel class of antipsychotic drugs. Further, since CCK-B antagonists have no cataleptogenic effects, they may also have a reduced propensity for producing extra-pyramidal side effects. In addition, these actions on midbrain DA neurons may contribute to the known anxiolytic activity of CCK-B antagonists.

125.10

LOCALIZATION OF RECEPTORS MEDIATING EFFECTS OF THE SELECTIVE CCK-B ANTAGONIST LY262691 ON A9 AND A10 DOPAMINE CELLS: LESION AND MICROINJECTION STUDIES. *M. E. Stockton, J. J. Howbert, and K. Rasmussen* , Lilly Research Labs, Eli Lilly & Co., Indianapolis, IN 46285.*

Previous studies have shown that peripherally administered LY262691 (LY), a selective CCK-B antagonist, decreases the number of spontaneously active dopamine (DA) neurons in the substantia nigra (A9) and ventral tegmental area (A10) of rats. This study examined the localization of the receptors mediating the effects of LY on A9 and A10 cells. The number of spontaneously active A9 or A10 DA cells was determined using extracellular, single-unit recordings in anesthetized rats following either radio-frequency lesions or intracerebral microinjection of LY in: n. accumbens (NA), striatum (ST), or medial prefrontal cortex (PC). Lesions of the NA decreased by 75% ($p<0.01$) the inhibitory effects of LY (10mg/kg, i.p.) on A10, but not A9, DA cells. ST lesions decreased by 34% ($p<0.05$) the inhibitory effects of LY on A9, but not A10, DA cells. PC lesions completely blocked the effects of LY on A10 DA cells and decreased by 25% ($p<0.05$) the inhibitory effects of LY on A9 DA cells. Microinjections of LY (1µg in 1µl) into the NA and PC decreased the number of spontaneously active A10 [by 54% ($p<0.01$) and 52% ($p<0.01$), respectively], but not A9, DA cells. Microinjections of LY into ST decreased the number of A9 (by 62%, $p<0.01$), but not A10, DA cells. Vehicle control injections had no effect on the number of spontaneously active DA cells. These data indicate that, consistent with known feedback pathways between forebrain structures and midbrain DA neurons, the effects of LY262691 on A9 and A10 DA cells are mediated, at least in part, by actions on CCK-B receptors located in the ST and PC/NA , respectively.

125.11

COMPARISON OF THE EFFECTS OF THE CCK-B RECEPTOR ANTAGONIST, PD 134308, AND THE CCK-A RECEPTOR ANTAGONIST, L-364,718, ON A9 AND A10 DOPAMINE NEURONAL ACTIVITY. C.L. Christoffersen*, K.A. Serpa and L.T. Meltzer. Dept. of Pharmacology, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., 2800 Plymouth Rd., Ann Arbor, MI 48105.

Extracellular single-unit recording techniques were used to study the effects of selective CCK-A and CCK-B antagonists on the basal firing activity and apomorphine (APO)-induced inhibition of DA neurons in chloral hydrate anesthetized rats. Neither L-364,718 (CCK-A; 0.1-1.6 mg/kg i.v.) nor PD 134308 (CCK-B; 0.1-6.4 mg/kg) altered the basal firing rate of A9 or A10 DA neurons. Pretreatment with L-364,718 (0.6 or 6.4 mg/kg i.v.), produced dose-related, significant shifts to the right of the APO dose-response curve. However, these effects were small in comparison to the haloperidol (0.1 mg/kg i.p.)-induced shift of the APO curve. These data suggest that in the substantia nigra, there may be a tonic release of CCK, that, through actions on CCK-B receptors, may modulate DA agonist-induced inhibition of DA neuronal activity.

125.12

CHOLECYSTOKININ (CCK) B-TYPE RECEPTOR ANTAGONIST ATTENUATES STRESS-INDUCED INCREASES IN DOPAMINE (DA) METABOLISM IN RAT FRONTAL CORTEX IN VIVO. M. Marien*, S. Tacconi, L. Romanelli, G. Gaviraghi and E. Ratti. Glaxo Res. Labs., Verona, Italy.

Activation of the mesocortical DA pathway has been suggested to be a neurochemical correlate of stress and anxiety, since stressors (eg. immobilization) or anxiogenic agents (eg. the benzodiazepine receptor inverse agonist FG7142) preferentially augment DA release and metabolism (DOPAC levels) in the rat prefrontal cortex (PFC) and amygdala (AMYG) in vivo. We have used this biochemical index to evaluate the effect of PD134308, a selective CCK-B receptor antagonist which has non-sedating anxiolytic effects in animal models of anxiety. FG7142 (30 mg/kg i.p.) increased levels of DOPAC in microdialysates from the PFC of conscious rats by up to 75%; pretreatment with PD134308 (1 mg/kg i.p.) 2h prior to FG7142 attenuated this effect to 39%. Physical restraint of rats for 20 min increased ex vivo tissue DOPAC:DA ratios in the PFC and AMYG by 75% and 67%, respectively; PD134308 reduced these changes to 23% and 15%. Results confirm a stress-induced activation of DA metabolism in the rat PFC and AMYG, demonstrate a suppression of this effect by a CCK-B antagonist, and suggest that some anxiolytic properties of these agents might involve modulation of the mesocortical DA system.

125.13

ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF MIDBRAIN DOPAMINE NEURONS IN CULTURE CORRESPOND TO THEIR PROPERTIES REPORTED *IN VIVO*. D.L. Cardozo*
Department of Neurobiology, and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Midbrain neurons have been dissected from neonatal rat pups and maintained in dissociated cell culture for several months. The dopaminergic (DA) phenotype was confirmed by catecholamine fluorescence and by tyrosine hydroxylase immunocytochemistry. Recordings have been made from over 70 DA neurons identified for electrohysiology by 5,7-DHT fluorescence. The neurons had the following properties: (1) Resting potential: -52 ± 5 mV; (2) Action potential amplitude: 66 ± 12 mV; (3) Action potential duration: 2.9 ± 1.3 ms; (4) 50% of neurons displayed pacemaker-like activity with a spontaneous firing frequency of 2.3 ± 1.3 Hz; (6) 65% of neurons displayed anomalous rectification; (7) 38% of neurons displayed bursting activity, typically having 2-6 action potentials per burst; (8) 90% of neurons expressed the D2 receptor; (9) 100% of neurons had glutamate and GABA receptors. Application of the neuroleptic haloperidol significantly increased the duration of the action potential in DA neurons. The pharmacology and physiological mechanism of the action of haloperidol is currently under investigation. (Supported by funds from the MA Mental Health Ctr;DA 04582; Mahoney Foundation and Scottish Rite.)

125.14

IN VITRO ELECTROPHYSIOLOGICAL PROPERTIES OF SOMA-TO-TERMINAL DOPAMINE NEURONS IN EXPLANT CORES OF RAT NIGROSTRIATAL AND MESOLIMBIC PROJECTIONS. M.D. Davis* and Melinda Hyde.
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Electrophysiological measurements of dopamine neurons have been studied in vitro for some time now. However, past studies have been limited for the most part to recordings made from cell bodies in tissue slices of the substantia nigra pars compacta (A9) and ventral tegmental nucleus (A10) regions where all their axons have been severed. In this investigation, we report on measurements obtained from whole dopamine cells using a novel explant technique.

Male, 14-21 day old rats were decapitated, their brains removed and the intact nigrostriatal or mesolimbic projection "cored" out using a sharpened cannula. The resulting cylinder of tissue was perifused with media in vitro and standard extracellular electrophysiological tests applied. In successful explants, putative A9/A10 dopamine neurons were antidromically activated by stimulating electrodes placed in the terminal regions (striatum or accumbens). The dopamine D2 agonist LY171555 depressed, while neurotensin elevated the firing rate of these neurons.

125.15

COOPERATIVE D1:D2 RECEPTOR INVOLVEMENT IN DOPAMINERGIC MODULATION OF GLUTAMATE'S EXCITATORY EFFECT ON STRIATAL NEURONS IN VIVO. X.-T. Hu* and F.J. White. Dept. Psychiatry, Wayne St. Univ. Sch. of Med., Detroit, MI 48207

In addition to the often noted inhibitory effect of iontophoretic dopamine (DA) on striatal neurons, this transmitter can also potentiate the excitatory effect of glutamate. The specific DA receptor subtypes involved in this modulatory effect have not been established. In the present study, extracellular single cell recording and microiontophoresis were used to investigate this issue. At low ejection currents (1-8 nA), DA facilitated glutamate-evoked activity of quiescent striatal neurons and also enhanced the firing of spontaneously discharging cells. This effect of DA was mimicked by both the selective D1 agonist SKF 38393 and the selective D2 agonist quinpirole as well as by the (Na^+-K^+)ATPase inhibitor ouabain. In contrast, high currents (≥ 16 nA) of these compounds suppressed the firing of striatal neurons. The DA receptor antagonist haloperidol blocked either the excitatory or inhibitory effects of DA, but not of ouabain. Although acute depletion of DA produced by combined treatment with reserpine and the DA synthesis inhibitor α-methyl-p-tyrosine failed to alter the excitatory effects of DA or ouabain, it completely abolished similar effects of both SKF 38393 and quinpirole. Coadministration of SKF 38393 and quinpirole restored the excitatory response in acutely depleted rats. These findings indicate that the ability of DA to modulate the excitatory response of striatal neurons to glutamate requires both D1 and D2 receptor stimulation. Moreover, the similarity of this effect to that produced by ouabain suggests the possibility that inhibition of (Na^+-K^+)ATPase activity may be involved in the effect (Bertorello et al., Nature 347:386, 1990). Supported by USPHS Grants MH 40832 and DA 04093 to FJW.

125.16

EFFECTS OF PCP, MK-801 AND DTG ON RAT MIDBRAIN NON-DOPAMINERGIC NEURONS. A.S. Freeman* and J. Zhang. Cellular and Clinical Neurobiology Program, Department of Psychiatry, Wayne State University School of Medicine, Detroit, MI.

Low i.v. doses of phencyclidine (PCP) stimulate the firing rate of midbrain DA neurons. This response has been attributed to occupation of the "PCP receptor" which results in the noncompetitive blockade of the NMDA subtype of glutamate receptor. The selective noncompetitive NMDA antagonist, MK-801, also excites DA neurons. Blockade of excitatory glutamatergic inputs to DA neurons would, however, be expected to decrease not increase DA cell firing. The current experiments begin to test the hypothesis that the excitatory effects of noncompetitive NMDA antagonists on DA neuronal firing rate are due to disinhibition resulting from blockade of NMDA receptors on DA cell afferents.

As reported previously, substantia nigra pars reticulata (SNPR) neurons in chloral hydrate-anesthetized rats were either excited {P(+)} or not excited {P(-)} by tail-pinch. P(+) cells are putative GABAergic interneurons that inhibit DA neurons. PCP (1-8 mg/kg, i.v.) and MK-801 (0.1-3.2 mg/kg, i.v.) inhibited the spontaneous activity of P(+) SNPR neurons but excited or had no effect on P(-) SNPR neurons. Lower doses of these drugs were inactive. The $sigma_1/sigma_2$ ligand, DTG (0.125-2 mg/kg, i.v.) did not significantly alter the firing rate of either type of SNPR neuron. The doses of MK-801, in particular, that inhibited P(+) SNPR neurons correlate well with doses known to stimulate the firing rate of nigrostriatal DA neurons. These results suggest that the excitatory effects of noncompetitive NMDA antagonists on DA cell firing rate are at least partly due to disinhibition as the result of inhibition of non-DA interneurons. (Supported by DA07844.)

125.17

EFFECTS OF LIGANDS FOR PCP AND SIGMA RECEPTORS ON NMDA-EXCITATION OF A9 DA NEURONS. J. Zhang* and A. S. Freeman. Cellular and Clinical Neurobiology Program, Department of Psychiatry, Wayne State University School of Medicine, Detroit, MI.

Electrophysiological studies in chloral hydrate-anesthetized rats assessed the effects of six *sigma* and/or PCP receptor ligands on microiontophoretic N-methyl-D-aspartate (NMDA)-induced activation of substantia nigra pars compacta (A9) dopaminergic (DA) neurons. I.V. administration of the selective *sigma* ligands DTG (cumulative doses of 0.02-2.22 mg/kg) and (+)-pentazocine (0.04-12.44 mg/kg) did not alter the stimulatory effects of NMDA. The nonselective *sigma* ligand BMY 14802 (0.04-4.44 mg/kg) increased A9 DA neuronal firing rate as previously reported, but did not affect NMDA-induced stimulation. Co-iontophoresis of BMY 14802 with NMDA also did not alter the response to NMDA. In contrast, the selective PCP receptor ligand, (+)-MK-801 (0.02-2.2 mg/kg), and the mixed PCP receptor/*sigma* receptor ligands, PCP (0.05-4.55 mg/kg) and (+)-SKF 10047 (0.05-12.55 mg/kg), dose-dependently blocked the NMDA-activation with an order of potency ((+)-MK-801 > PCP > (+)-SKF 10047) consistent with the affinities of these drugs for the PCP receptor. Co-iontophoresis of (+)-MK-801 or PCP with NMDA also blocked the response to NMDA.

In conclusion, *sigma* receptor occupation does not appear to modulate the NMDA-induced activation of A9 DA neurons. The antagonism of NMDA produced by PCP and (+)-SKF 10047 is likely due to the noncompetitive NMDA antagonist properties of these drugs that are due to PCP receptor occupation. (Supported by DA07844.)

125.18

EFFECTS OF ETHANOL ON EXCITATORY AMINO ACID-EVOKED ELECTROPHYSIOLOGICAL ACTIVITY OF NUCLEUS ACCUMBENS NEURONS. R. Shen*, A.S. Freeman and L.A. Chiodo. Cellular and Clinical Neurobiology Program, Department of Psychiatry, Wayne State University, School of Medicine, Detroit, MI.

Recent evidence indicates that acute and chronic ethanol administration may alter neuronal activities by modifying the functions of the *N*-methyl-*D*-aspartate (NMDA) receptor. In the present study, we utilized *in vivo* extracellular single-unit recording techniques to investigate the effects of acute ethanol administration on the electrophysiological activity evoked by agonists of the NMDA receptor and the other two major excitatory amino acid (EEA) receptors, the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor and the kainate receptor. The agonists were administered microiontophoretically to nucleus accumbens neurons in chloral hydrate- or urethane-anesthetized rats. Ethanol (40% w/v) was administered intravenously in cumulative doses of 0.125 g - 2.0 g/kg. Ethanol exhibited a dose-dependent inhibitory effect on the neuronal discharge evoked by all three EEA receptor agonists. At lower doses (0.125-0.5 g/kg) a transient excitation was regularly observed following the initial inhibition. Ethanol also showed a greater potency in inhibiting the electrophysiological activity evoked by AMPA than the activity evoked by kainate and NMDA. Experiments to study the interactions between selective EEA antagonists and ethanol are currently in progress.

These findings indicate that acute ethanol administration produces a general inhibitory effect on the EEA receptor-stimulated electrophysiological activity of nucleus accumbens neurons. [MH41557, DA07844]

126.1

IN VIVO EFFECTS OF INTRAPALLIDAL MORPHINE MICROINJECTIONS AS STUDIED BY BRAIN MICRODIALYSIS AND LOCOMOTOR ACTIVITY ANALYSIS.

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Injections of morphine into different parts of the globus pallidus (GP), are associated with increases in locomotion, as we showed in earlier studies. The present study was designed to determine if the observed changes in locomotion are (a) mediated by opiate receptors, and (b) correlated with DA and its metabolites concentrations in the nucleus accumbens and striatum. In the first series of experiments, male Sprague-Dawley rats were treated with subcutaneous injections of 1 mg/kg naloxone or vehicle 10 min before the intrapallidal (dorsal, medial, ventral) administration of morphine (7.5 µg/0.5µl/site) and tested for locomotion. Blockade of opiate receptors with naloxone resulted in an inhibition of the effect of morphine on locomotion.
In the second series of experiments microdialysis probes (2.3 or 4.0 mm) were implanted unilaterally in the nucleus accumbens (NAC) , or antero-medial striatum, or antero-lateral striatum of male Sprague-Dawley rats. Microinjections of morphine were made through microdialysis probes which were implanted in ventral pallidum (VP) (10 µl of 0, 2.6, 4.0, 13, 26 mM) or dorsal GP (10 µl of 26 mM). Samples were collected every 45 min (flow rate: 0.75 µl/min) over 3 and 6 hrs before and after challenging rats with morphine respectively. Dopamine (DA) and metabolites (DOPAC, HVA) were determined in NAC and striatal dialysates using HPLC-EC coupled. We have found that (a) Morphine microinjections in VP are associated with dose dependent increases in the concentration of DOPAC and HVA in NAC, (b) Morphine microinjections in GP increased both DA and its metabolites concentrations in NAC, and DA concentrations in antero-medial striatum.No changes of either DA or metabolites were seen in anterolateral striatum. These findings provide evidence that the increases in locomotor activity observed following intrapallidal morphine microinjections are mediated through opiate receptors and associated mainly with an enhancement of DA overflow in NAC rather than in the striatum.

126.2

CHOLECYSTOKININ POTENTIATES MORPHINE ANTICONVULSANT ACTION THROUGH BOTH BRAIN CCK-A AND CCK-B RECEPTORS. A. Legido, M. W. Adler , C. Karkanias, E. B. Geller *, J. I. Greenstein and W. D. Grover. Temple Univ. Sch. of Med., Dept. of Pediatrics, Pharmacology and Neurology & Merck Res. Lab., Philadelphia, PA 19140

The role of cholecystokinin (CCK) in epileptogenesis is unclear. Exogenously applied to the hippocampus CCK evokes a marked increase in neuronal excitability, but an anticonvulsant effect has also been suggested after subcutaneous (sc) or intracerebroventricular (icv) CCK administration. The anticonvulsant action of opioids (e.g., morphine) has been well documented in the past. CCK interacts with morphine (M) in the regulation of some physiologic functions; e.g., CCK blocks opiate analgesia. However, the possible interaction of CCK and M in epileptogenesis is unknown.We studied the effect of sc M and icv administered CCK octapeptide sulphate ester (CCK-8-SE), and CCK-A (MK 329) and CCK-B (L 365260) receptor antagonists (Ant) (Merck Lab.) on seizures induced by maximal electroshock (MES). Male Sprague-Dawley rats weighing 250-300 gm were used in all experiments (N=7-34). CCK-8-SE (100 ng), CCK-A Ant (9 µg) and CCK-B Ant (9 µg) were injected through chronically implanted icv PE-10 cannulae. MES were induced, 30 min after M administration and 10 min after CCK-8-SE, CCK-A and CCK-B infusion, through electrode-gel coated ear clip electrodes by a high voltage, high internal resistance constant current generator (50 mA for 200 msec). The % change (%C) of the tonic component (TC) from the mean of the TC from controls (C) was (mean±SE): -3.6±5.7 for CCK (NS =not significant from C), 21.7±2.1 for M ($p<0.001$ vs. C), 45.2±7.7 for M+CCK ($p<0.01$ vs. M), 20.8±3.4 for M+CCK+CCK-A Ant (NS vs. M), and 14.0±1.8 for M+CCK+CCK-B Ant (NS vs. M). The %C of the TC after icv administration of CCK-A and CCK-B Ant alone was not significant: -5.4± 5.5 and -7.5±3.5, respectively. We conclude that CCK potentiates the antinconvulsant action of M through both brain CCK A and B receptors. *(Funded by Laboratorios AUSONIA (Spain), SCHC Academic Development Fund and Grant DA 00376 from NIDA.)*

126.3

DIFFERENTIAL BEHAVIORAL INTERACTIONS BETWEEN DOPAMINE D1/D2 AGONISTS AND N-METHYL-D-ASPARTATE ANTAGONISTS IN MONOAMINE-DEPLETED MICE. M.L. Carlsson*, A. Svensson and A. Carlsson. Department of Pharmacology, University of Göteborg, Medicinaregatan 7, S-413 90 Göteborg, Sweden.

Previous work in our laboratory has shown that the noncompetitive N-methyl-D-aspartate (NMDA) antagonist MK-801 (dizocilpine) interacts synergistically with the mixed dopamine (DA) receptor agonist apomorphine to promote locomotion in an animal model of Parkinson's disease (monoamine-depleted mice). The purpose of the present study was to investigate the relative importance of DA D1 receptors compared to DA D2 receptors in this interaction. To that end, MK-801 was given in combination with either the DA D1 receptor agonist SKF 38393, or the DA D2 receptor agonist quinpirole or the preferential DA D2 agonist bromocriptine. Locomotor stimulation was generally more pronounced if MK-801 was combined with a DA D1 receptor agonist than with a DA D2 receptor agonist, although baseline dopaminergic activity seems to be an important factor that determines whether MK-801 will have a weakening or a potentiating effect. Experiments with local applications point to an important role of the nucleus accumbens in generating the locomotor response.

Moreover, the effects on rotation following a unilateral injection of the competitive NMDA antagonist AP-5 into the nucleus accumbens in situations with varying dopaminergic tone were studied. AP-5 was found to induce contralateral rotation in monoamine-depleted mice when given alone or in combination with a systemic injection of SKF-38393. In contrast in intact mice, as well as in monoamine-depleted mice receiving quinpirole systemically, AP-5 induced predominantly ipsilateral rotation.

The results will be discussed in relation to 1) what is known about the cellular distribution of DA D1 and DA D2 receptors in the (ventral) striatum 2) the anatomy and function of the so-called direct and indirect pathways projecting from the striatum to the thalamus.

126.4

DETERMINATION OF MONOAMINES IN HUMAN CEREBRAL SPINAL FLUID (C.S.F.) AFTER FLUNITRAZEPAM (FLU) AND HYDROXYZINE (HYD) ADMINISTRATION.

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Recently, the study of various actions of benzodiazepines (BZDs) highlighted the role of noradrenergic and serotoninergic system. To elucidate this role in human we have determined levels of monoamines in C.S.F. after administration of drugs mostly used in pretreatment of rachianesthesia : benzodiazepine (FLU 0.01mg.kg^{-1} i.v.) or HYD (1.5mg.kg^{-1} Per Os), a sedative antihistaminic agent. FLU was administered 10 min. and HYD 60 min. before lumbar puncture (L3-L4) for rachianesthesia.

(ng.ml^{-1})	Control (n=15)	BZD (n=11)	Hydroxyzine (n=6)
MHPG	60.78±4.93	21.47±4.10***	51.25±8.81 NS
5-HIAA	18.12±1.56	7.62±1.41 ***	20.48±2.34 NS
HVA	29.66±3.52	3.15±1.92 **	45.27± 7.28 *

(Unpaired Stutent t test -$p<0.05$ * - $p<0.01$** - $p<0.001$***).
These results, in accordance with animal pharmacological data, demonstrate that Flunitrazepam interferes with metabolism of serotonin, dopamine and norepinephrine, while hydroxyzine only significantly increases homovanillic acid.

126.5

DIRECT CATECHOLAMINE INHIBITION OF ENKEPHALINASE. F.C. Westall* and M.S. Graige. Institute for Disease Research, P.O. Box 890193, Temecula, CA 92589-0193 and Dept. Chem., Calif. State Poly. University, Pomona, CA.

Root-Bernstein and Westall (Brain Res. Bull. 12, 425, 1984; ibid. 17, 519, 1986; ibid. 18, 509, 1987; ibid. 25, 827, 1990, ibid. 25, 817, 1990; Endogenous Sleep Factors, Inoue and Krueger, eds. p289, 1990) have shown the possibility that many drugs, hormones, neurotransmitters, and their metabolities can interact directly. In this study we examine the effect of dopamine and norepinephrine binding to met-enkephalin on the hydrolysis of the peptide by enkephalinase. Using paper chromatographic techniques, we find that as the concentration of dopamine or norepinephrine increases the ability of enkephalinase to digest met-enkephalin drops. Furthermore, chromatography easily shows that the catecholamines bind met-enkephalin. As was also shown previously using spectral techniques, this binding is quite specific. Therefore, we have found that not only catecholamines will bind met-enkephalin, but that this will occur in the presence of an enzyme which in vivo utilizes enkephalin. The actions of enkephalinase and several peptidases are considered the prime processes for inactivating the enkephalins. Our results suggest that binding of enkephalin to the catecholamines acts as a second mechanism in enkephalin inactivation.

126.6

ATIPAMEZOLE, A SELECTIVE ALPHA$_2$-ANTAGONIST, INCREASES NORADRENALINE AND HISTAMINE RELEASE IN THE RAT HYPOTHALAMUS. K. Laitinen*, L. Tuomisto, E. MacDonald. Dept Pharmacol and Toxicol, Univ Kuopio, 70211-Kuopio, Finland.

Recent studies suggest that histamine (HA) release in the hypothalamus is modulated by α_2-adrenoceptors located on histaminergic neurons. Noradrenaline (NA) release is primarily regulated by presynaptic α_2-autoreceptors. We used in vivo microdialysis to examine the effects of the selective α_2-adrenergic antagonist, atipamezole (ATI) on HA and NA release in the hypothalamus. Rats were anesthetized and microdialysis probe was implanted into the medial hypothalamus. ATI was given either via the probe (50 µM) or s.c. (1 mg/kg). NA and HA in the dialysate were analyzed by HPLC methods. Basal levels of NA and HA were 83±7 and 53±7 fmol/30 min, respectively. ATI, through the microdialysis probe and s.c. injection, increased NA release up to about 250 % of the control values, reaching max effect at 90 min after drug administration. ATI injection (s.c.) had no effect on basal HA release, but ATI infusion (1 h) via probe into the hypothalamus increased HA release and returned to the basal level at 120 min. The results suggest that there is a potential interaction between histaminergic and noradrenergic neurones in the hypothalamus.

126.7

EFFECTS OF PHENCYCLIDINE AND (+)MK801 ON CEREBRAL SUBSTANCE P CONTENT IN THE RAT. Y.Shirayama, H.Mitsushio, T.Nishikawa and K.Takahashi*. Div. Mental Disorder Res., Natl. Inst. Neurosci., NCNP, Tokyo, Japan.

The effects of a schizophrenomimetic drug, phencyclidine (PCP), on substance P contents have been investigated in the discrete brain areas of the rat. To this end, a simple and sensitive enzyme-immunoassay for substance P has been developed. An acute administration of PCP (10 mg /kg, intraperitoneally (i.p.)), which is a non-competitive antagonist of N-methyl-D-aspartate (NMDA) typed excitatory amino acid (EAA) receptor, reduced the concentration of the peptide in the prefrontal cortex, limbic forebrain (nucleus accumbens, septum, olfactory tubercule, etc.), striatum and substantia nigra, but not in the ventral tegmental area, 60 min thereafter. PCP no longer diminished substance P content in these areas at 120 min post-injection. A selective non-competitive NMDA antagonist, (+)MK801 (1 mg/kg, i.p.) also caused a decrease in substance P content in the prefrontal cortex and limbic forebrain, but failed to alter the contents in the striatum, substantia nigra and ventral tegmental area, 30 min later. There was no change in the peptide concentration 60 min following i.p. injection of (+)MK801. The present results suggest that EAAergic transmission might be involved in the regulation of substance Pergic systems in the prefrontal cortex and limbic forebrain.

126.8

COMPARISON OF EXCITATORY-AMINO-ACID RECEPTOR AGONIST RESPONSES IN CULTURED CEREBELLAR PURKINJE NEURONS. E.A. Fox* and D.L. Gruol. The Scripps Res. Inst., La Jolla, CA, 92037.

We previously reported that corticotropin-releasing-factor (CRF) modulates Quisqualate (Quis) responses in rat cerebellar Purkinje neurons (PNs; Neurosci. Abst. 16:521, 1990). As a first step toward determining whether CRF interacts with the ionotropic or the metabotropic excitatory amino acid receptor subtypes activated by Quis, we are characterizing the responses of PNs to selective agonists using extracellular recordings. AMPA (10μM) and (1S,3R)-1-aminocyclopentane-1,3,-dicarboxylic acid (ACPD; 100μM) were used to activate the ionotropic and metabotropic receptor subtypes, respectively, and Quis (1μM) was used to activate both.

Micropressure application (50-200 ms) of Quis, AMPA, or an AMPA+ACPD mixture produced characteristic and repeatable biphasic responses (excitatory phase followed by inhibitory phase). In contrast, 300-900 ms applications were required to elicit biphasic ACPD responses and repeated applications often produced variable responses. Some characteristics of the biphasic response were agonist-dependent. These included: (1) The duration of the excitatory phase was 2.5-fold greater for Quis than for AMPA or AMPA+ACPD. (2) The duration of the inhibitory phase for AMPA+ACPD was 20% shorter than for Quis or AMPA. (3) Quis produced a higher firing rate during the inhibitory phase than did AMPA, ACPD or AMPA+ACPD. (4) Both Quis and AMPA produced more bursting during the excitatory phase than AMPA. Co-application of ACPD with AMPA slightly increased this bursting relative to AMPA alone but it was still much less than that produced by Quis or ACPD. The interaction of CRF with these responses is under investigation. Supported by NS21777 and AA0756.

126.9

SEROTONIN MANIPULATIONS ALTER LEVELS OF mRNA CODING FOR THYROTROPIN-RELEASING HORMONE IN MEDULLARY RAPHE NEURONS L.A. Riley* Dept. Biol. Sci., Rutgers Univ., Newark, NJ 07102.

Thyrotropin-releasing hormone (TRH) and substance P (SP) are colocalized with serotonin (5-HT) in neurons of the medullary raphe. To begin to understand the role and regulation of multiple neurotransmitters housed within individual neurons, we have sought to determine whether changes in serotonin levels alter the biosynthesis of the colocalized neuropeptides. Previously we showed that pharmacological manipulations of serotonin alter levels of mRNA coding for the prohormone precursor of SP (PPT; Walker et al., Mol. Brain Res., 1990; Riley et al., Mol. Cell. Neurosci., 1991).

In general, pharmacological manipulations of serotonin altered levels of TRH mRNA like that of PPT mRNA. Decreases in serotonin following a one week constant infusion of the serotonin synthesis inhibitor pCPA led to a 117% ($p<0.001$) *increase* in TRH mRNA. Increases in local serotonin induced by a 5 day treatment with the serotonin selective reuptake blocker zimelidine *decreased* TRH mRNA 69% ($p<0.001$).

However, in several respects PPT and TRH responded differently to changes in serotonin neurotransmission. After 14 days of zimelidine treatment, levels of TRH mRNA were still 66% ($p<0.001$) below control, while in the same animals, PPT mRNA levels recovered. PPT mRNA levels were decreased 38% ($p<0.02$) 1 day after treatment with 0.2 mg/kg of 8-OH-DPAT, but TRH mRNA levels were unchanged. Finally, levels of TRH but not PPT mRNA were increased 50% after a 5 day treatment with 2 mg/kg/day 8-OH-DPAT. While TRH and PPT react similarly to changes in serotonergic neurotransmission these differences suggest differential mechanisms may be involved. Supported by the Tourette Syndrome Assoc.

126.10

GLYCINE MODULATES ENHANCEMENT OF NMDA RESPONSE BY SOMATOSTATIN IN VAGAL MOTONEURONS D. Bieger*, M. Zhang, Y.T. Wang and R.S. Neuman. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3V6.

NMDA receptors contribute to the fast rising phase of EPSPs in neurons of the nucleus ambiguus compact formation (AMB_c), synaptically driven spike production in these neurons being dependent on NMDA receptor activity (1). Recently we have shown that exogenous SST augments NMDA-induced depolarization and endogenous somatostatin (SST) is required for the expression of NMDA receptor activity during the EPSP (submitted). Here we report that glycine inhibits the permissive action of SST.

Intracellular recordings of EPSPs and responses to pressure-ejected or bath-applied test substances were obtained from sagittal slices of rat medulla containing the AMB_c and the solitario-ambigual pathway (SAP). Bath-applied glycine (100μM) had little effect on AMB_c depolarizing responses to NMDA (0.5-1.0pmol) pulses delivered by pressure-ejection; however, in the presence of glycine, SST (0.1pmol) failed to augment the NMDA response. SAP-driven EPSPs in AMB_c neurons were inhibited by glycine both in the presence and absence of strychnine (1-50μM). This effect was abolished by depletion of SST with cysteamine (100-200μM).

In conclusion, our data provide evidence for modulation of the NMDA channel by a novel mechanism involving an interaction between glycine and SST. Supported by MRC (Canada).

[1] Wang, Y.T., Bieger, D. and Neuman, R.S., 1991 Brain Res. 567:260-266.

126.11

PURINERGIC MODULATION OF GLUTAMATE LEVELS IN THE NUCLEUS TRACTUS SOLITARII. C.L. Beck*, M. Appalsamy, D. Robertson and R. Mosqueda-Garcia. Dept. of Pharmacology, Vanderbilt University, Nashville, TN 37232.

Adenosine (Ado) generally has been considered an inhibitory neuromodulator. Recent work from our lab, however, has challenged this concept. We showed that Ado has excitatory cardiovascular effects in the nucleus tractus solitarii (NTS) which are associated with selective increases in glutamate (Glu) levels. In this study we further characterize the effect of Ado on Glu levels. Male New Zealand white rabbits (3-4kg) were anesthetized with sodium thiopental and urethane. A carotid artery and jugular vein were cannulated for recording of blood pressure and heart rate. A tracheostomy tube was placed for airway patency. The rabbits were placed in a stereotaxic frame and following a partial occipital craniotomy, a microdialysis probe (CMA-11, BAS) was implanted into the right NTS. Basal levels of Glu were obtained over a 2 hour resting period (saline perfusion; 5 μl/min.) followed by perfusion of increasing concentrations of Ado (10^{-6} to 10^{-2}M) or the A_2 selective agonist CGS 21680 ($4.5x10^{-3}$M). At the end of the study, the brains were removed and fixed for histological confirmation of probe location. Samples were analyzed for free amino acids by HPLC.

μM	Basal	Ado 10^{-4}M	Ado 10^{-3}M	Ado 10^{-2}M	CGS $4.5x10^{-3}$M
Glu	1.5±0.1	4.5±2.6	13.9±3.4	63.6±37.2	2.3±0.4
n	3	3	3	3	8

Purinergic substances in the NTS increase Glu levels in a dose-dependent manner. This effect, however, could involve more than A_2 receptor activation.

126.12

THE EFFECTS OF DYNORPHIN A(1-13) ON GLUTAMATE AND SYNAPTIC RESPONSES OF RAT SPINAL DORSAL HORN NEURONS. M. Kolaj, R. Cerne, M.C. Jiang, T.H. Lanthorn* and M. Randic. Dept. Vet. Physiol. Pharmacol. Iowa State University, Ames, IA 50011 and Neurological Diseases Research, G.D. Searle & Co., Skokie, IL 60077.

The effects of dynorphin A(1-13) on inward currents induced by excitatory amino acids (EAA) and the evoked excitatory synaptic potentials (EPSPs) were studied in dorsal horn (DH) neurons of young rats using whole-cell voltage-clamp and intracellular recording techniques. When the freshly isolated DH cells were clamped to -60mV, application of 1-10 nM dynorphin A(1-13) reversibly enhanced the peak amplitude of the initial transient component of the glutamate-induced current in about 60% of the examined cells. With 0.1-10μM dynorphin A(1-13), the reduction of the glutamate response was seen. In addition, 1-50 nM dynorphin A(1-13) potentiated the initial peak N-methyl-D-aspartate (NMDA) current by 176.4 ± 17.2% in 8 of 10 tested cells and reduced it when 0.5-10μM of the peptide was used. The enhancing effect was absent in 2 cells pre-treated with norbinaltorphimine, a kappa receptor antagonist. Both, a small increase (128.2 ± 13.7%, n=6) and a decrease (90.7 ± 1.6%; n=4) in the transient component of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-induced current was seen with dynorphin A(1-13). The enhancing effect was frequently preceded by a transient depression of the responses to glutamate, NMDA and AMPA. In a transverse slice preparation, electrical stimulation of a dorsal root evoked EPSPs in the substantia gelatinosa neurons that were sensitive to NBQX. We found that 1-10 μM dynorphin A(1-13) reduced or completely abolished the fast EPSPs in 4/4 tested cells. These results are consistent with the possibility that the activation of kappa opioid receptors may contribute to the regulation of the strength of EAA-mediated primary afferents neurotransmission, including nociception (Supported by BNS 8418042 and Searle CNS Research).

127.1

TRANSIENT TRANSLOCATION OF PKC BY HIPPOCAMPAL SLICE PREPARATION.

Helen Valsamis, Maria Naik, and Todd C. Sacktor*
(SUNY-HSC at Brooklyn, NY, 11203)

In order to characterize the effect of hippocampal slice preparation on protein kinase C, a time course was determined for the subcellular distribution of PKC following dissection and placement in a recording chamber. Each of the 8 known PKC isozymes was identified by Western blot using affinity-purified carboxy-terminal antibodies, which recognized the proteins at their reported molecular weights. With the PKCζ antibody, an additional band was noted at 51 kDa, which may be the isozyme's catalytic fragment. All isozymes translocated to membrane, corresponding to activation, 15-30 min after hippocampal slice preparation. The membrane-associated level of each isozyme then declined to a stable baseline, with the exception of PKCζ51, which in some experiments continued to rise. The concerted activation of PKC isozymes may be due to the trauma of slice preparation with concomitant release of neurotransmitters. Slice preparation may activate a sequence of molecular steps similar to that which occurs following synaptic activation by tetanus. In addition, PKCζ51 may play a special role in long-term synaptic modulation (Osten and Sacktor, this volume).

127.2

EVIDENCE FOR A NEW ISOFORM OF PKC SPECIFIC TO RAT BRAIN.

Elizabeth Sublette*, Maria Naik, Xiaolan Jiang, and Todd C. Sacktor (SUNY-HSC at Brooklyn, NY)

Protein kinase C is a gene family of isozymes important in signal transduction and which, in brain, participates in the regulation of synaptic strength. The PKC gene family is divided into two groups: "conventional" cPKC's, regulated by both Ca^{2+} and lipid second messengers, and "novel", activated only by the latter. All known isozymes are present in the rat hippocampus. In addition, antibodies toward the carboxy-terminus of the relatively lung-specific isoform nPKCη (generous gift from Dr. Shin-ichi Osada, Yokohama, Japan) detect on Western blot a protein of molecular weight higher than that of the known PKC's, including nPKCη from lung. This hippocampal protein does not cross-react with antisera made toward the amino-terminus of nPKCη. Nonetheless, the protein translocates to membrane following applications of phorbol esters to hippocampal slices. The protein is detectable by Western blot in most areas of the brain but not in other tissues of the rat. Since this protein contains a phorbol-ester binding site and cross-reacts with an antibody made largely to the conserved catalytic domain of nPKCη, we believe that it may be either alternatively spliced nPKCη or a new isozyme.

127.3

CHARACTERIZATION OF HIPPOCAMPAL PROTEIN KINASE C ISOZYMES IN DBA/2 AND C57BL/6 MICE.

B.J. Bowers*, C.L. Parham, J.M. Sikela and J.M. Wehner. Instit. for Behavioral Genetics, Univer. of Colorado, Boulder, CO 80309.

We have previously shown that DBA/2 and C57BL/6 strains of mice differ in performance on the Morris water task. A genetic analysis of 11 B x D recombinant inbred strains indicates that performance of the spatial task is correlated with hippocampal protein kinase C (PKC) activity. The PKC enzyme is composed of a family of isozymes. Differences in PKC activity between C57BL and DBA mice may originate from variations in the isozymes at the molecular level. In order to examine possible molecular differences we have begun a characterization of three PKC isozymes, α-, β-, and γ-PKC in DBA and C57BL hippocampus. Gene expression was evaluated by Northern analysis. Both strains of mice expressed the three isozymes, indicating that the difference in PKC activity is not due to a missing isozyme(s). Western blots measuring α-, β-, and γ-PKC protein expression in hippocampus confirmed that the proteins are also expressed in both strains of mice. In order to compare primary gene and amino acid sequences, we have isolated clones for the α-, β_{II}-, and γ-isozymes from C57BL and DBA brain cDNA libraries, provided by David Burt (Dept. Pharm., Univ. of Maryland, Sch. Med.). A comparison of a full length C57BL cDNA clone and partial length (90%) DBA cDNA clone of the γ-PKC isozyme indicates no differences in amino acid sequences between the strains. Future sequence comparisons of the remaining isozymes as well as quantitative characterizations of PKC isozymic gene and protein expression may reveal differences that are responsible for the observed differences in PKC activity.

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127.4

Purification and Characterization of Two Forms of Protein Kinase C ε From Rat Brain.

F. L. Huang*, K. Osada, and H. Nakabayashi, NICHD, NIH, Bethesda, MD 20892

Protein kinase C (PKC) ε was purified to near homogeneity from rat brain by column chromatography on DEAE-cellulose, phenyl-Sepharose, Sephacryl S-200, polylysine-agarose, Mono Q and hydroxyapatite. In the initial three steps PKC ε was copurified with other PKCs (α, β and γ) and was separated from them on polylysine-agarose. Subsequently on Mono Q, PKC ε was separated into two activity peaks, eluted at 0.2 and 0.4M KCl (designated ϵ_1 and ϵ_2, respectively) and both exhibited immunoreactivities toward ε-specific antibodies at 94-96 kDa. ϵ_2 is pure at this step, and ϵ_1 was further purified on hydroxyapatite. Using myelin basic protein or peptide (MBP4-14) as substrate, ϵ_1 displayed absolute requirement of PS and DG but not Ca^{2+} in the absence of NP40, however, Ca^{2+} was also required in the presence of detergent. In contrast, ϵ_2, while showing no proteolysis, was active in the absence of any activator with or without NP40, and PS and DG were only slightly stimulatory. ϵ_1 could be phosphorylated when incubated with PS/DG, ATP/Mg, and Ca^{2+}, as evidenced by autoradiography after SDS/PAGE and mobility change in immunoblot analysis. This incubation also gradually converted ϵ_1 from activator-dependent into -independent enzyme similar to that of ϵ_2 without detectable proteolysis. Furthermore, this conversion of ϵ_1 to ϵ_2-like activity also occurred in the absence of ATP, suggesting that phosphorylation of the enzyme is not important for such conversion. However, this process absolutely required Ca^{2+} in addition to PS/DG, even though autophosphorylation occurred in the absence of Ca^{2+}. It appears that PKC ε may exist as an inactive form of enzyme like ϵ_1, which is only active in the presence of activators, and an active form like ϵ_2, which is spontaneously active even in the absence of activator. It is possible such a conversion and activation of PKC ε occurs physiologically during agonist-mediated signal transduction.

127.5

PROTEIN KINASE C β IS ABSENT IN HUMAN GLIOMA CELL LINES.

N.P. Dooley*, J-G. Villemure and V.W. Yong. Neuroimmunology Unit, Montreal Neurological Institute, Montreal, Quebec, CANADA, H3A 2B4.

Investigations in our laboratory have shown that the total protein kinase C (PKC) enzyme activity in both malignant and non-malignant human glia positively correlates with their rate of proliferation. As well, the PKC enzyme activity in glioma cell lines was found to be significantly greater than that in both non-transformed fetal and adult astrocytes.

To date, nine isoforms of PKC have been identified of which seven are highly enriched in the central nervous system. In order to determine whether both malignant and non-malignant human glia express the same isoforms of PKC, oligonucleotide primers specific for the group one isoforms of PKC (α, β_1, β_2 and γ) were employed in a reverse transcriptase - polymerase chain reaction (RT-PCR). Total RNA was isolated from cultured cells using a modified guanidinium thiocyanate-phenol-chloroform protocol. Adult human astrocytes, oligodendrocytes, and microglia as well as fetal human astrocytes were found to express PKC α but not PKC γ. Glioma lines (A172, U251, U373, U178) with growth rates ranging from fast to slow, exhibited this same pattern of PKC expression. However, unlike non-transformed glia, the glioma cell lines did not express RNA for either PKC β_1 or PKC β_2. This finding is of particular interest since astrocytes, which do express PKC β, are believed to be the antecedents of glioma cells. Given that recent studies in colon cancer cell lines have indicated a role for PKC β_1 in tumor suppression (Choi et al., Mol Cell Biol 10, 1990), we believe that the absence of PKC β in glioma cell lines may underlie their abnormally high rate of proliferation.

127.6

EFFECT OF CARBACHOL ON PROTEIN PHOSPHORYLATION PRODUCED BY PKC IN HIPPOCAMPAL NEURONS.

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Neuronal fractions from cultures were immediately prepared in presence of 10 mM $ZnCl_2$ and 1.0 mM PMSF. Protein samples of 70ug from each fraction were placed in 3MM Whatman Discs dried and treated by Mans and Novelli's method. Quadruplicate samples of 300,150 and 100 Ug of protein from cell homogenates, synaptosomal or nuclear fractions in the presence of Krebs-Hepes buffer pH 7.4 and of 60nM of (^{3}H)-PDBu in a final volume of 600 Ul were incubated at 37ºC for 40 min. Samples filtered in GF/B discs pretreated with 0.4% polyethylenimine. Thus, carbachol in hippocampal plasma membrane fractions from neuronal cultures, in a dosis response manner produced first a 40% increase of PKC density and then returned to basal values. A dosis-response curve in the P_2 fraction, carbachol produced a maximal increase of about 300%. In the nuclear fraction, carbachol produced a significant decrease of 35%. A decrease of 35% is induced in hippocampal cells nuclei, from rats injected with 0.085mg/kg of carbachol. Carbachol produced significant increase of protein phosphorylation in both the synaptosomal (20%) and cytoplasmic fraction (12%), but an important decrease in the nuclear fraction (-60%). In nuclear subfractions carbachol produced a decrease in the chromatine subfraction of 25%, a smaller decrease in the nuclear membrane (11%) and an increase in the nucleoplasm (12%). These PKC changes were significantly reverted for synaptosomal, cytoplasmic and the nuclear fraction by 20 mM polymyxin B.

127.7

ETHANOL ACTIVATES PROTEIN KINASE C (PKC) IN CULTURED BOVINE ADRENAL MEDULLARY (BAM) CHROMAFFIN CELLS. R.K.Tuominen*, S.Lustig, P.T.Männistö and M.Viluksela. Dept. of Pharmacology and Toxicology, Univ. of Helsinki, SF-00170 Helsinki, Finland.

Effect of ethanol on PKC-activity in primary cultured BAM cells was studied. The cells were dissociated by using a retrograde collagenase digestion and purified by a renografin gradient. The cells were used after growing for 2 days in fetal calf serum (10%) supplemented and for one day in serum free DME/F12 medium. Phosphorylation of calf thymus histone in vitro was used to measure PKC activities of soluble and particulate protein exstracts. Continuous incubation of BAM cells with ethanol increased particulate PKC activity at 30 min and at 24 hrs. The effect of ethanol was concentration dependent (0 - 200 mM). Time-course study showed a biphasic effect of ethanol (300 mM) on BAM cell PKC activity; a significant activation at 1 and 3 min, no effect at 10 min and a significant activation at 30 min. These results suggest a role for PKC in the signal transduction of the effects of ethanol in BAM cells. The biphasic effect may indicate different mechanisms of activation of PKC during the short- and long-term exposures to ethanol.

127.8

PHORBOL 12-MYRISTATE-13-ACETATE ATTENUATES GABA-MEDIATED INHIBITION IN THE HIPPOCAMPUS. R. Mora and S. Springfield*. Dept. of Biology, City College of New York, NY 10031.

This investigation examined the effect of a protein kinase C (PKC) activator, phorbol 12-myristate-13-acetate (PMA), on GABA-mediated inhibition in the hippocampal slice. PMA is able to activate PKC by mimicking the action of the second messenger, diacylglycerol (DAG). Previous studies in this laboratory have shown that serotonin attenuates GABA-mediated inhibition. Ultimately, we wish to determine if PKC plays a role in the neuromodulatory action of serotonin.

Hippocampal slices (350-400 μm) were obtained from adult male Sprague-Dawley rats weighing 150-300 g. Slices were constantly superfused with a bicarbonate-buffered balanced salt solution and aerated with 95%O_2/5%CO_2. After a 1 hr equilibration period, twin stimuli (0.2-3.0 msec; 1-10 V; 1/60 Hz) of interstimulus intervals of 5 to 15 msec were applied to the stratum radiatum at the border of the CA1-CA2 region. Extracellular recordings were taken from the CA1 pyramidal cell layer. Local inhibition occurred when the amplitude of the test (second) population spike was smaller than the conditioning (first) spike. This reduction in the amplitude of the test spike is attributed to the action of a GABA-mediated inhibition superimposed upon orthodromic excitation.

Application of a hydrophobic phorbol ester, PMA (10^{-4}-10^{-7} M) resulted in a significant decrease in GABA-mediated inhibition (n=33). This result may indicate an increase in the excitability of hippocampal pyramidal cells. Experiments are now in progress using structurally analogous synthetic compounds of DAG and a hydrophilic phorbol ester to further explore a possible neuromodulatory mechanism of serotonin involving PKC. (Supported by NSF 860641 and NIH/NIGMS GM 08168.)

127.9

NEUROTRANSMITTERS AND CALCIUM STIMULATE EXOGENOUS PHOSPHOINOSITIDE HYDROLYSIS BY PHOSPHOLIPASE C IN HUMAN BRAIN MEMBRANES. F.T. Crews, P. Kurian and G. Freund*, Departments of Pharmacology and Medicine*, University of Florida College of Medicine, Gainesville, FL 32610-0267.

Agonist stimulated phosphoinositide (PPI) hydrolysis is a major signal transduction pathway in brain. Previous studies have shown that muscarinic and serotonergic receptors activate PLC in membranes incubated with a mixture of [3H]PtdIns (87 %), [3H]PtdIns(4)P (10.4 %) and [3H]PtdIns(4,5)P_2 (2.6%) which is comparable to that found in intact tissue. To determine if postmortem delay and other factors associated with obtaining human brains would disrupt receptor - PLC coupling, rat brain membranes were prepared from fresh, brains incubated for 4 hours at 24°C and then frozen and brains incubated at 24°C for 4 hours and then at 4°C for 24 hours. All three preparations showed similar receptor stimulation of PLC suggesting that human postmortem tissue may be reliably studied for differences in receptor - PLC coupling. The time course for GTPγS and GTPγS - carbachol stimulated PPI hydrolysis by human brain membranes (frontal cortex) was linear for at least 20 min. GTPγS stimulated [3H]InsP formation was enhanced by carbachol (232 %) and 5-HT (147%). There was no effect of carbachol in the absence of GTPγS and atropine prevented carbachol enhancement of PPI hydrolysis. The EC50 for carbachol was approximately 8 μM. SAX-HPLC separation of [3H]inositol polyphosphates indicated that the major isomer of $InsP_3$ was Ins(1,4,5)P_3, the expected product of PtdIns(4,5)P_2 hydrolysis. No Ins(1,3,4,5)P4 was formed in this assay. Two isomers of $InsP_1$ were formed, Ins(1)P_1 and Ins(4)P_1. These isomers could be formed by direct hydrolysis of [3H]PtdIns substrate and/or phosphatase activity within our membrane preparation [Ins(1)P_1 from PtdIns and Ins(4)P_1 from PtdIns(4)P and PtdIns(4,5)P_2]. Ca^{++} increased PPI hydrolysis progressively from 100 nM through 50 μM. Calcium synergistically enhanced carbachol - GTPγS stimulation up to 1 μM.. ACPD and angiotensin II did not stimulate [3H]PPI hydrolysis in the presence of GTPγS. This PLC assay will be useful for studying agonist stimulated PPI hydrolysis in human brain. (Supported by a grant from NIA).

127.10

MUSCARINIC RECEPTOR-MEDIATED INCREASE IN ZETA-PKC EXPRESSION. J. Baumgold* and K.D. Dyer. Dept. of Radiology, George Washington Univ., Washington, DC 20037.

The PKC family of enzymes include at least 8 isotypes, each with a discrete structure, tissue distributions, calcium and lipid sensitivity. ζ-PKC is insensitive to calcium and lacks one of the two zinc fingers necessary for phorbol ester and DAG activation, thus raising questions regarding its physiological function. To address this, we used antipeptide antibodies to each PKC isotype to study receptor-mediated activation of PKC isozymes in SK-N-SH human neuroblastoma cells. Western blot analysis of these cells revealed that they express only the α-PKC and ζ-PKC isozymes. As expected, stimulation of these cells with PMA (1 μM, 10 min) caused the α-PKC but not ζ-PKC to translocate from a cytosolic to a particulate fraction. Stimulation of these cells with 0.5 mM carbachol or with 10 μM bradykinin elicited PIP_2 hydrolysis and a small, transient translocation of α-PKC. Suprisingly, stimulation of these cells with either carbachol or bradykinin also induced a several-fold increase in immunoreactive ζ-PKC, most of which remained in the cytosolic fraction. The amount of ζ-PKC increased over time and was maximal after a 60 min carbachol stimulation, and gradually decreased thereafter. This response was blocked by atropine. In order to determine whether this increase was the result of increased protein synthesis, we pre-treated SK-N-SH cells with 1 μM cycloheximide and found that this pre-treatment prevented the carbachol-mediated increase in ζ-PKC. These findings demonstrate that stimulation of muscarinic and of bradykinin receptors induce increased synthesis of ζ-PKC by a mechanism that is calcium and DAG-independent.

127.11

SUBCELLULAR DISTRIBUTION AND GLUTAMATE-STIMULATED PHOSPHORYLATION OF MARCKS AND A NOVEL 48 KDA PROTEIN IN DEVELOPING CULTURED HIPPOCAMPAL PYRAMIDAL NEURONS. W. K. Scholz* and H. C. Palfrey. University of Chicago, Dept Pharmacol./Physiol. Sci., Chicago, IL 60637

Glutamate stimulation of cutured rat hippocampal pyramidal neurons results in the rapid transient phosphorylation of 3 PKC substrates (Scholz and Palfrey, 1991; J. Neurosci. 11, 2422). The 3 phosphoproteins are very acidic (pI 4-5), of 48, 87 and 120 kDa. The 87 kDa protein has been indentified as myristoylated alanine-rich C kinase substrate (MARCKS). The 48 kDa protein is related to MARCKS by virtue of its very similar isoelectric point, elution from a mono-Q column, phosphopeptide fingerprint, and time course of phosphorylation/dephosphorylation following glutamate stimulation, but could be distinguished from MARCKS by its failure to react with a polyclonal anti-MARCKS antibody and lack of expression in the adult brain. The 48 kDa phosphoprotein is not GAP-43. To assess the subcellular distribution of these proteins, cells were prelabeled with 32P, stimulated with glutamate and neuronal proteins were separated into cytosolic, membrane bound and cytoskeletal bound fractions by sequential extraction of cultures with digitonin, triton-X-100 and SDS-sample buffer. Following glutamate stimulation there is an immediate increase in 32P-labeled 87 and 48 kDa in cytosolic fractions. The subcellular distribution of MARCKS and the 48 kDa protein was also examined during development of cultured neurons. In neurons cultured for 2 days, at a stage when growth cones are most prevalent, these phosphoproteins are found in membrane bound and cytosolic fractions. At 2 wks in culture, after most process outgrowth has been completed, MARCKS and the 48 kDa phosphoprotein are found predominantly in membrane and cytoskeletal bound fractions with very little in cytosolic fractions. Immunostaining of pyramidal neurons with antibodies recognizing MARCKS shows intense staining of growth cones in neurons cultured for 2-3 days, and cell soma, processes and some growth cones of older neurons. These results suggest that the subcellular distribution of both MARCKS and the 48 kDa protein shifts from primarily cytosolic to membrane/cytoskeletal association during development.

127.12

Protein Phosphorylation by Protein kinase C in Rat Hippocampus: Effect of Cis-fatty Acid and Diacylglycerol. S.G. Chen and K. Murakami*, Department of Biochemical Pharmacology, SUNY-Buffalo, Buffalo, NY 14260

Protein phosphorylation has been indicated to play an important role in the regulation of synaptic plasticity. It has been shown that cis-fatty acid (CFA) activates protein kinase C (PKC) either independently of or in concert with diacylglycerol (DAG). We have recently shown that rat brain PKC, particularly type III (α) PKC, can be synergistically activated by CFA and DAG in the presence of phosphatidylserine (Biochem. J. 282:33-39,1992). The synergism can also be supported by phosphatidylcholine, a neutral lipid, but inducing a different activation state of PKC (Biochem. J. 284:221-226,1992). These indicate that both CFA and DAG can act as second messengers for PKC activation and that the different activation state may lead to selective protein phosphorylation. In order to examine whether synergistic PKC activation mode is of physiological relevance, we studied the effect of CFA and DAG on protein phosphorylation in rat hippocampus. We have found that at least three hippocampal proteins are responsive to the synergistic activation of type III PKC by CFA and DAG *in vitro*. Further studies using intact hippocampal slices showed that phosphorylation state of the two of those proteins can be altered by the treatment with CFA. These results suggest that synergistic mode of PKC activation is operative in hippocampus.

127.13

SPHINGOSINE AND PSYCHOSINE ALTER PHOSPHOINOSITIDE HYDROLYSIS IN CULTURED ASTROCYTES. T. Ritchie*, A. Rosenberg and E.P. Noble. Neuropsychiatric Institute, Univ. California, Los Angeles, CA 90024.

The effects of sphingosine and psychosine on phosphoinositide (PI) hydrolysis in primary cultured astrocytes were investigated. Astrocytes prelabeled for 24 hrs with ^{3}H-inositol were incubated with sphingosine for 1 hr. PI hydrolysis was measured subsequently by incubating the intact cells with 10 mM Li^+ for 30 min at 37°C and isolating the generated inositol phosphates. Exposure to sphingosine produced a dose-dependent stimulation of PI hydrolysis requiring the presence of external Ca^{2+} for optimal activity. The addition of 10 µM norepinephrine (NE) resulted in a stimulation additional to that with sphingosine. The α_1-antagonist prazosin completely inhibited NE-induced PI hydrolysis but had no effect on that produced by sphingosine. Psychosine (50 µg/ml), when co-incubated with sphingosine, produced complete inhibition of sphingosine-induced PI hydrolysis at all doses of sphingosine tested (10-200 µg/ml). Likewise, psychosine totally inhibited NE-induced PI hydrolysis. The protein kinase C inhibitor staurosporine (1 µM) had no effect on sphingosine-induced PI hydrolysis. Taken together, these findings suggest that lysosphingolipids such as sphingosine and psychosine may play an important role in the regulation of PI turnover in astrocytes by a mechanism dependent on extracellular Ca^{2+} and independent of the α_1-adrenergic receptor and protein kinase C. (Supported by grant AA 07653.)

127.14

PHORBOL ESTER-INDUCED REGULATION OF THE MYRISTOYLATED ALANINE RICH C KINASE SUBSTRATE (MARCKS) IN IMMORTALIZED HIPPOCAMPAL CELLS IN CULTURE. D.G. Watson[1], B.H. Wainer[2] and R.H. Lenox[1]*. Molecular Neuropharmacol, Dept Psychiatry, Univ Vermont Coll Med[1], Burlington, VT 05405 & Dept Pathol & Pharm/Physiol, Univ Chicago[2], Chicago, IL 60637.

We have previously reported that chronic lithium administration results in a marked reduction in a major protein kinase C (PKC) substrate (MARCKS) in rat hippocampus (Lenox *et al*, Brain Res 570:1991). In an effort to better understand the mechanism of action of lithium we have initiated studies to examine the regulation of PKC and MARCKS in an immortalized line of mouse hippocampal cells (HN33) characterized for neuronal properties (Lee *et al*, J Neurosci 10:1990). These cells were shown to possess muscarinic receptors using [^{3}H]NMS binding and could be further differentiated in the presence of 10µM retinoic acid. Western Blot analysis with MARCKS antibody (J. Patel, ICI) was used to identify the major (80kDa) phosphoprotein substrate of PKC in this cell line. Exposure of the HN33 cells to the phorbol ester, PDBu (1µM), for 12 to 24 hrs. revealed a progressive reduction of MARCKS in the soluble fraction. These findings are similar to recent data demonstrating a phorbol ester induced down regulation of MARCKS in non-neuronal cell populations (Brooks *et al*, EMBO J 10:1991; Otsuka and Yang BBRC 178:1991). Ongoing studies are examining the action of lithium on MARCKS in these hippocampal cells and the possible role that PKC plays in its mechanism of action in the brain. (Supported in part by MH41571 and NS25787)

127.15

PURIFIED 14-3-3 PROTEIN FROM BOVINE BRAIN ACTIVATES BOTH TYROSINE HYDROXYLASE AND PROTEIN KINASE C. R. Horwitz, M. Tanji, and J. C. Waymire*. The University of Texas Medical School, Houston, TX, 77030

14-3-3 protein, a soluble acidic protein present in high concentrations in the brain, is believed to function in monoamine biosynthesis through its capacity to activate tyrosine hydroxylase and tryptophan hydroxylase in the presence of phosphorylation by Ca^{2+}/calmodulin-dependent protein kinase II (Ichimura, T., et al.FEBS Letters 219, 79-82 (1987)). Moreover, Toker, A., et al. Eur. J. Biochem. 191, 421-429 (1990) suggest that 14-3-3 protein also inhibits protein kinase C.

In the present study, protein bovine 14-3-3 was purified from forebrain by assessing the presence of 14-3-3 with an antibody that cross-reacts with 14-3-3 protein (gift of Dr. G.C. Rosenfeld, Department of Pharmacology, U. Texas Med School, Houston, TX). The purified protein was estimated to be homogeneous as judged by native gradient-gel electrophoresis with a M_r of 50,000. The subunit composition on SDS-PAGE revealed a pattern, M_r 32,000, 30,000, and 29,000, similar to that previously reported for 14-3-3. Although the purified bovine 14-3-3 activates purified bovine adrenal tyrosine hydroxylase in the presence of Ca^{2+}/calmodulin-dependent phosphorylation, it activates, rather than inhibits, protein kinase C. Moreover, the concentration dependence of the activity of 14-3-3 was the same for the activation of tyrosine hydroxylase and protein kinase C, and is the same as that previously reported to inhibit protein kinase C. Supported by USPHS NS 11061.

127.16

IMMUNOLOCALIZATION OF PROTEIN KINASE C-delta IN PARASAGITTAL BANDS OF THE CEREBELLAR CORTEX. S. Chen* R. Bing, T.T. Aung, and D.E. Hillman, Department of Physiology and Biophysics, New York University Medical Center, New York, NY 10016.

Protein kinase C (PKC) is a key phosphorylation enzyme in signal transduction. So far, eight isozymes have been isolated (α, βI, βII, γ, δ, ε, ζ & η) and are encoded by different genes. Using commercially available antibodies (GIBCO-BRL, Gaithersburg, MD), we found that α,γ, & ζ labeled Purkinje cells uniformly while the δ isozyme labeled groups of Purkinje cells as parasagittal bands. Beta and ε did not label Purkinje cells at all. Variable-width bands of Purkinje cell somas and their dendrites were interspersed with negative zones in the caudal vermis of coronal sections. The hemispheres had lighter bands while the floccular and paraflocular lobules were quite uniformly intense. In addition, interneurons in the molecular layer exhibited regional variations which were reciprocal to that of Purkinje cells. Strong labelling of Purkinje cell somas lacked basket axonal plexuses, in contrast to the negative Purkinje somas, which were cupped by intense basket staining. Parasagittal compartments in the cerebellar cortex have been found, previously, by AchE, B1, motilin, cytochrome oxidase, zebrin, 5' nucleotidase, GAD and taurine, and have been attributed to grouping of terminal fields for climbing or mossy fibers. Additionally, compartmentalization of PKC δ isozyme could be due to: 1) function-related signal-transduction, or 2) genetic coded proteins of neurons and glia. Supported by NIH-NINCDS NS-13742 & NIH- NIA AG-09480

127.17

CHARACTERIZATION OF HIPPOCAMPAL PHORBOL ESTER BINDING DIFFERENCES BETWEEN C57BL/6 AND DBA/2 MICE. R. Paylor* J.R. Pauly, and J.M. Wehner, Institute for Behavioral Genetics, University of Colorado, Boulder, 80309.

The present study was designed to characterize differences in C57BL/6 and DBA/2 brain protein kinase C (PKC) activity which have previously been shown to be important for spatial learning performance. Using quantitative autoradiography, we observed that C57BL/6 mice had higher levels of [3H]PDBu binding compared to DBA/2 mice in CA regions of the hippocampus and the substantia nigra. Filtration receptor binding experiments on hippocampal homogenates indicated that C57BL/6 mice have higher levels of [3H]PDBu binding in particulate, but not cytosolic fractions. To determine if the strain difference in hippocampal [3H]PDBu binding is simply quantitative, or if there are also qualitative differences in the binding protein, heat denatuarion experiments at 43° C using Bmax concentrations of PDBu showed that over time both C57BL/6 and DBA/2 mice [3H]PDBu binding decreased. Although there appeared to be no overall difference in the rate of denaturation between the two strains, C57BL/6 tissue appeared to lose more binding sites in the first minute of exposure to heat than DBA/2 tissue. C57BL/6 mice showed a significant decrease in both the Bmax and Kd values when saturation analyses were performed at 33.5° C compared to at 22 ° C. Bmax and Kd values were not different in DBA/2 tissue at the two temperatures. Finally, we examined the development the hippocampal [3H]PDBu binding. The difference in the amount of [3H]PDBu binding sites emerged around 24 days of age. These results suggest that hippocampal PKC activity differences between C57BL/6 and DBA/2 mice may be due to decreased amount of the PKC protein as measured by [3H]PDBu binding that appear before adulthood. This strain may result from a difference in both the quantitative as well as the qualitative nature of the PKC protein. Supported by NSF BNS 8820076.

127.18

EFFECT OF PROTEIN KINASE C(PKC) ON CYCLIC AMP ACCUMULATION IN RAT BRAIN SLICES AND PC12 CELLS. N. W. DeLapp*, C. A. Luttman and R. D. Saunders. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind 46285.

Phorbol ester activation of PKC augments or inhibits receptor-mediated increases in cAMP. A recent report showed that PKC activation reduced (controls-PKCα) or augmented(PKCγtransfected) cAMP levels in NIH 3T3 cells(Gusofsky et. al., Mol Pharm 39: 124, 1991). This study explored the suggestion that PKCγ may augment cAMP increases in brain slices and PC12 cells.

cAMP was determined in 3H-adenine prelabeled brain slices and in PC12 cells using an ELISA. Phorbol dibutyrate(PDBu) augmented 2-chloroadenosine(2CA)-stimulated cAMP accumulation equally in adult and neonatal rat cortex slices although neonatal levels of PKCγare low(Yoshida et. al., JBC 263: 7868, 1988). PDBu reduced 2CA and forskolin-stimulated cAMP increases in PC12 cells in contrast to the findings of Gusofsky et. al. Immunoblots of PC12 extracts showed theα and γ isoforms. These results indicate that PKCγ augmentation of cAMP responses may depend upon the cell type in which the γ isoform is expressed.

127.19

TUBULIN MODIFIES NEURONAL SIGNAL TRANSDUCTION THROUGH THE ASSOCIATION WITH G PROTEINS IN RAT CEREBRAL CORTEX AND STRIATUM. S. HATTA*, N. AMEMIYA, H. OHSHIKA, T. SAITO, AND H. OZAWA. Depts. of Pharmacology and Neuropsychiatry, Sapporo Med. Coll., Sapporo 060, Japan.

Tubulin dimers, when polymerized with GppNHp (tubulin-GppNHp) or azidoanilido GTP (AAGTP) (tubulin-AAGTP), have been shown to form a complex with and transfer guanine nucleotide to Giα or Gsα thereby regulate adenylate cyclase (AC). The present study examines effects of tubulin on the regulation of receptor affinity for its agonist via nucleotide transfer from tubulin to Giα or Gsα. Addition of the tubulin-[^{32}P]AAGTP to rat cerebral cortex membranes resulted in incorporation of [^{32}P]AAGTP into Giα, indicating transfer of AAGTP from tubulin to Giα. Similar experiments with striatal membranes showed the transfer of [^{32}P]AAGTP from tubulin to Giα and Gsα. To ascertain whether tubulin-GppNHp would affect agonist binding affinity, we examined the isoproterenol competition of [^{3}H]CGP12177 binding in cerebral cortex membranes and the dopamine competition of [^{3}H]spiperone binding in striatal membranes. Tubulin-GppNHp decreased agonist binding affinities for the β-adrenergic receptor and the dopamine D_2-receptor (i.e., right shifted the agonist competition curves) with potency similar to that of GppNHp. These results suggest that tubulin participates in the regulation of receptor affinity by transferring guanine nucleotide to Gi and Gs.

127.20

A NOVEL IN VITRO ASSAY FOR PHOSPHOLIPASE D ACTIVITY. J. Horwitz* and L. L.Davis. Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Neurotransmitters activate a phospholipase D that is thought to specifically hydrolyze phosphatidylcholine. This enzyme has a unique property called transphosphatidylation; in the presence of an appropriate nucleophilic receptor such as alcohol, phospholipase D will catalyze the production of phosphatidylalcohol. We have studied phospholipase D using an in vitro assay that uses high specific activity [^{3}H]butanol (15 Ci/mmol) as an acceptor. In the presence of [^{3}H]butanol and phosphatidylcholine, a microsomal membrane fraction from rat brain catalyzed the production of phosphatidyl[^{3}H]butanol. Phospholipase D activity was dependent upon a detergent. At a phosphatidylcholine concentration of 2.5 mM the optimal sodium oleate concentration was between 4 and 6 mM. The Rf of the phosphatidyl[^{3}H]butanol on thin-layer chromatography was identical to the Rf of the phosphatidylbutanol formed when [^{3}H]phosphatidylcholine was incubated with 100 mM butanol. These data confirm the identity of phosphatidyl[^{3}H]butanol. This assay has two important advantages. It is not necessary to add high concentrations of alcohol that may affect the regulation of the enzyme. The other advantage is that the substrate does not need to be labeled. Microsomal membranes were found to catalyze the production of phosphatidyl[^{3}H]butanol from all four phospholipid classes. These data suggest that under appropriate conditions brain phospholipase D is not specific for phosphatidylcholine. This assay should be useful in furthering our understanding of this important enzyme. (Supported by the Alcoholic Beverage Medical Research Foundation and NIA-AG00532)

PAIN PATHWAYS: HYPERALGESIA

128.1

CUTANEOUS INJECTION OF THE CAPSAICIN ANALOG, NE-21610, PRODUCES ANALGESIA TO HEAT BUT NOT MECHANICAL STIMULI IN MAN. K.D. Davis*, R.A. Meyer, J.L. Turnquist, M. Pappagallo, T.G. Filloon[1], and J.N. Campbell, Johns Hopkins Univ., Baltimore, MD 21205 and [1]Procter & Gamble Co., Cincinnati, OH 45329.

The present study examined the effects of the capsaicin analogue, NE-21610, on heat and mechanical sensation in human volunteers. In the first series of experiments, subjects received intradermal (ID) injections (30 µl) of the drug (0, 0.3, 3.0, 10 µg) in ascending order into different sites on the volar forearm. Subjects were randomly assigned to one of three protocols to examine drug-evoked pain (n=8), or drug-induced alterations in sensitivity to mechanical (n=8) or heat (n=8) stimuli. In a subsequent study, subjects (n=8) rated sensations evoked by mechanical and heat stimuli before and after subcutaneous (SC) injections (300 µl) of the drug (0, 100 µg). The peak pain (rated by means of a visual-analog scale) occurred at the time of injection, was of short duration, and was similar for vehicle and the highest drug dose. A dose-related algesic effect of NE-21610 was detected by summing the pain ratings for the 10 minutes after injection. Von Frey thresholds for detection, sharpness, and pain at the injection site (measured 24 hr after injection) were not significantly altered by either ID or SC drug administration. However, pain to stepped heat stimuli (ranging from 40 to 48°C, 1 s) was reduced in a dose-dependent fashion for both types of injection. At the highest doses (10 µg ID and 100 µg SC), analgesia to heat stimuli was still present one week after injection. This dissociated loss of heat but not mechanical pain-sensibility may be due to: 1) a selective action of the drug on heat transducers in nociceptors responsive to both heat and mechanical stimuli, or 2) a selective action on a subset of nociceptors. (Supported by Procter and Gamble.)

128.2

INTRADERMAL INJECTION OF THE CAPSAICIN ANALOG, NE-21610, PREVENTS THE DEVELOPMENT OF HYPERALGESIA FOLLOWING A BURN. R.A. Meyer*, K.D. Davis, J.L. Turnquist, T.G. Filloon[1], M. Pappagallo and J.N. Campbell, Johns Hopkins Univ., Baltimore, MD 21205 and [1]Procter & Gamble Co., Cincinnati, OH 45329.

Intradermal and subcutaneous injection of the capsaicin analogue, NE-21610 (Procter & Gamble), produces analgesia to heat but not mechanical stimuli in man. The present study examined whether pretreatment of the skin with NE-21610 prevents the development of hyperalgesia following a thermal injury. On day one, eight volunteers received an intradermal injection (30 µl) of vehicle to one volar forearm and drug (10 µg) to the other volar forearm. On day two, the subjects used a visual-analog scale to rate the pain to mechanical and heat stimuli before and after a burn (48°C, 120 s) to each injection site. At the vehicle site, the pain evoked by the burn was rated as moderate to strong. In addition, primary hyperalgesia to heat stimuli, secondary hyperalgesia to mechanical stimuli, and flare were observed after the burn. In contrast, the pain evoked by the burn at the drug treated site was rated as weak, and primary hyperalgesia to heat stimuli did not develop. In addition, the zones of secondary hyperalgesia and flare appeared smaller than those observed at the vehicle site. These data suggest that pretreatment with the capsaicin analog, NE-21610, may be helpful in ameliorating the pain associated with injury. (Supported by Procter & Gamble.)

128.3

MECHANISMS OF NEUROPATHIC PAIN FOLLOWING CHROMIC GUT LIGATION OF THE SCIATIC NERVE IN THE RAT. T.J. Maves,[2]* P.S. Pechman,[2] G.F. Gebhart,[1] and S.T. Meller.[1] Departments of Pharmacology[1] and Anesthesia,[2] University of Iowa, Iowa City IA, 52242, USA.

The aim of this study was to evaluate the mechanisms involved in the development and maintenance of the behavioral changes produced by loose chromic gut ligation of the sciatic nerve in a model of neuropathic pain.

Four loose ligatures of either silk (4-0), plain gut (4-0), or chromic gut (4-0, 3-0, or 2-0) were placed around the left sciatic nerve of male Sprague-Dawley rats. An additional group of rats had 8 pieces (0.5 cm pieces each) of 4-0 chromic gut laid in adjacent to the left sciatic nerve. The right sciatic nerve was exposed in all rats as a sham surgery. Rats were tested prior to surgery (day 0) and on post-operative days 3, 5, 10, 20, and in some groups on day 30.

Chromic gut sutures (4-0, 3-0, and 2-0) tied loosely around the left sciatic nerve produced a 'dose-dependent' decrease in thermal withdrawal latency that was maximal on postoperative day 3 (28 $\pm$ 4%, 38 $\pm$ 5%, and 46 $\pm$ 6%, respectively) which returned to baseline by day 20 (4-0) or day 30 (3-0 and 2-0). 4-0 chromic gut laid in adjacent to the sciatic nerve produced a decrease in thermal withdrawal latency of 21 $\pm$ 5% that was maximal on day 5 and returned to baseline by day pp20. In contrast, neither 4-0 silk nor 4-0 plain gut produced any evidence of thermal hyperalgesia on any day tested. There was no evidence of mechanical hyperalgesia on any day tested in rats from any of the 6 groups. After chromic gut, but not plain gut or silk ligatures had been placed around or laid next to the sciatic nerve, rats altered their posture and gait, suggestive of both spontaneous pain and allodynia. Sections of the sciatic nerve through the ligation site revealed no change in content of large or small diameter myelinated fibers compared to the right (sham) sciatic nerve in silk, plain gut or 4-0 chromic gut groups. There was a variable change in the content of large and small diameter myelinated fibers in 2-0 or 3-0 chromic gut treated rats. These data support the hypothesis that a chemical component of chromic gut sutures interacts with sciatic/sympathetic nerves, and that a mechanical insult is not a requirement for the development of the behavioral symptoms in this model of neuropathic pain.

128.4

NITRIC OXIDE (NO) MEDIATES THE THERMAL HYPERALGESIA PRODUCED IN A MODEL OF NEUROPATHIC PAIN. P.S. Pechman,[2]* G.F. Gebhart,[1] T.J. Maves,[2] and S.T. Meller.[1] Departments of Pharmacology[1] and Anesthesia,[2] University of Iowa, Iowa City IA, 52242, USA.

Loose ligation of the sciatic nerve with chromic gut sutures (4-0) produces behavioral evidence of neuropathic pain in rats, including a marked thermal hyperalgesia. It has been suggested that activation of NMDA receptors in the dorsal horn are critical to the development of thermal hyperalgesia. In other regions of the CNS, such as the cerebellum, NMDA receptor activation has been shown to result in a Ca^{2+}-dependent increase in cGMP through the production of NO, or an NO-containing moiety from free L-arginine. Therefore, the aim of the present study was to determine whether the thermal hyperalgesia produced in a model of neuropathic pain is mediated through the production of endogenous NO and subsequent activation of soluble guanylate cyclase (GC).

Four loose chromic gut ligatures (4-0) were tied around the left sciatic nerve. The right sciatic nerve was exposed as a sham surgery. Thermal withdrawal latencies of the left and right hindpaws were measured prior to surgery (day 0), and on the day of maximal thermal hyperalgesia (day 3 post-surgery). On day 0 there was no left hindpaw/right hindpaw difference in withdrawal latencies, but on day 3 all rats showed a significant thermal hyperalgesia of the left hindpaw compared to the right. One hour after determining the magnitude of the thermal hyperalgesia, the same rats were given an intrathecal (i.t.) dose of either saline, an alternate substrate for NO synthase, N_W-nitro-L-arginine methyl ester (L-NAME, 20 nmol) or the GC inhibitor, methylene blue (MB, 20 nmol). Testing began 10 min later and continued every hour until thermal hyperalgesia was reestablished. I.t. administration of L-NAME and MB, but not saline, blocked the thermal hyperalgesia for 2 and 4 hours, respectively. L-NAME (20 nmol, i.t.) or MB (20 nmol, i.t.) produced no change in thermal nociceptive thresholds in sham rats.

These results suggest that production of endogenous NO and activation of GC within the dorsal horn of the spinal cord are necessary for the expression of the thermal hyperalgesia produced in a model of neuropathic pain.

128.5

PERSISTENT HINDLIMB FLEXION IS ASSOCIATED WITH AN EXPERIMENTAL PERIPHERAL MONONEUROPATHY. S.Hanning, D.J.Mokler, & B.J. Winterson* Depts. Physiol.& Pharmacol., Univ. New Eng.Coll.Ost.Med., Biddeford, ME 04005.

Loose ligation of the common sciatic nerve produces an experimental mononeuropathy (EMN), a syndrome of hyperalgesia, trophic changes and "guarding" of the hindlimb. We have been studying the persistent hindlimb flexion induced by prolonged percutaneous electrical stimulation. We examined rats with EMN to determine whether they also developed persistent hindlimb flexion.

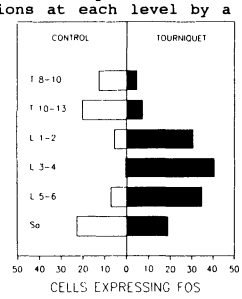
EMN was produced in Long-Evans rats by the method of Bennett and Xie (Pain, 1988) (LIGATE). On days 3,7,10, and 14 rats were anesthetized with pentobarbital and persistent flexion was measured by applying known weights until leg lengths were equal. LIGATEd rats routinely showed flexion on the ligated side (3d: X=6.2g; 7d: X=4.8g; 10d: X=6.4g; 14d: X=7.1g). Sham surgical controls showed little flexion (Overall X= 1.1g). To test whether the flexion was due to the hypersensitivity of the foot, the foot was anesthetized with lidocaine. There was no reduction in flexion (X= 7.1 g). Rats were spinalized at T7 and the flexion remained (X= 6.0 g). The results suggest that the persistent flexion of EMN is a segmentally maintained process that does not depend upon sensory input from the foot. These features are similar to persistent hindlimb flexion induced by electrical stimulation. (supported by AOA)

128.6

C-FOS MAPPING OF CENTRAL NOCICEPTIVE PROJECTIONS IN ACUTE COMPRESSION-RELATED NEUROGENIC PAIN. JC Crews*, CS Sehlhorst, TM Reed, MM Behbehani. Dept. of Anesthesia, Univ. of Cincinnati, Cincinnati, OH 45267-0531.

To determine the nociceptive pathways for acute compression-related pain, c-fos immunohistochemical labeling techniques were used to identify activation of spinal cord and central nociceptive neurons in anesthetized rats. A pneumatic tourniquet was applied to the animals' right lower extremity and inflated to 300 mmHg for 120 mins. The animals were perfused, and the spinal cord and brain were sectioned and prepared for c-fos immunohistochemical labeling using the ABC technique. Brain and spinal cord sections were labeled for R and L sides and cells staining for c-fos expression were counted in 5-10 spinal cord sections at each level by a blinded observer.

Fos expression was greatest in the ipsilateral lumbar spinal cord levels, consistent with c-fos expression following formalin injection of the hind paw. However, bilateral nociceptive activation of dorsal horn neurons in the thoracic levels suggests that novel peripheral nociceptive pathways may be associated with acute compression-related pain. Fos expression was detected bilaterally in LRN, NRM, LC, and PAG, but no expression was detected in the hypothalamus.



128.7

SUTURE MATERIAL AFFECTS HINDPAW POSITION AFTER CHRONIC CONSTRICTION INJURY (CCI). C.H. Pollock, J.D. Coltz, G.L. Wilcox, and K.C. Kajander*. Depts of Psychology, Pharmacology, Oral Science & Prgm in Neuroscience, Schools of Med and Dent, U of Minnesota, Mpls, MN 55455.

A model of neuropathic pain in the rat (CCI) is produced by ligating the sciatic nerve with chromic gut (Bennett and Xie, 1988). Attal et al. (1990) report that after the CCI ipsilateral hindpaw position is abnormal. In this study, we investigated whether ligating with other suture materials produces this effect on the hindpaw position.

Rats stood with hindpaws flat on the glass testing surface before surgery. We ligated the left sciatic nerve of each rat with chromic gut (n=6), gut (5), or vicryl (6). Control rats (6) received a sham surgery. After surgery all rats were evaluated for two months (13 sessions, 180 s each). A "blind" observer recorded the total time each rat held the left hindpaw in one of eight specified positions during each session.

Significant differences in hindpaw position existed between treatments (Kruskal-Wallis, $p < .05$). In sessions 1-13, rats in the sham surgery group spent 100% of the time with the left hindpaw flat on the glass surface. In sessions 1-5, rats in the vicryl group spent 67% of the time in one of two positions: paw ventroflexed or paw resting on its the medial surface. In sessions 6-13, these rats spent 100% of the time in the hindpaw flat position. In sessions 1-8, rats in the chromic gut and gut groups spent nearly 100% of the time with the hindpaw in the paw ventroflexed, elevated, and medial surface positions. In sessions 9-13, these rats still spent 50% of the time with the hindpaw off the glass surface.

The effect of chromic gut or gut ligatures on hindpaw position differed from the effect of vicryl ligatures or sham surgery. It is possible that suture material also alters other behavioral sequelae.

128.8

REFLEX SYMPATHETIC DYSTROPHY (RSD): PERIPHERAL NERVE STIMULATION AND ANIMAL MODEL INVESTIGATION. S. J. Hassenbusch*, N. A. Mekhail, D. N. Doss, M. S. Stanton-Hicks, J. G. Walsh. Dept. Neurosurgery and Pain Management Center. Cleveland Clinic, Cleveland, OH 44195.

Peripheral nerve stimulation was utilized in 23 patients with Stage III RSD and failure to improve significantly with conservative measures and blocks. RSD symptoms/findings were located entirely or mostly in the distribution of one major peripheral nerve (median, ulnar, common peroneal, posterior tibial). All patients underwent placement of a peripheral nerve stimulator electrode with an implanted generator. The patients were followed for one year with frequent generator reprogramming, thermography, McGill pain questionnaire and verbal pain scale ratings. Long-term, all patients experienced mild-marked relief of changes. Best results were seen in marked reduction or elimination of allodynia and cutaneous flow changes. Deep, stimulation-independent pain was improved moderately and motor weakness was least improved. Chronic peripheral nerve stimulation is a valuable treatment for high-stage RSD patients but more information concerning stimulation voltages, on/off periods, and detailed selection criteria is required.

In order to examine physiologic and biochemical effects of peripheral nerve stimulation for RSD, an animal model for upper limb involvement is being investigated. This model is produced by application of 4 ligatures around the median nerve similar to the sciatic nerve compression model of Bennett & Xie (1988). Apparent vasomotor changes and exaggerated thermal stimulation responses developed in affected forelimbs. Thermography, 7 days after operation, of 8 Sprague-Dawley rats revealed significant ($p<0.01$) decreases (1.065 ± 0.27°, mean ± s.e.m.) in average skin temperature in operated forelimbs as compared to contralateral sham forelimbs. Effects of this model upon stellate sympathetic neurons and granule-containing cells (electron microscopy) and distal arterial wall catecholamine stores (fluorescent microscopy) are being quantitated for comparison to effects of peripheral nerve stimulation in the same model.

128.9

RESPONSES OF VLO NEURONS TO NOXIOUS COLD PRESSOR IN RATS WITH AND WITHOUT SCIATIC NERVE LIGATION. B. Wang, M. Backonja and V. Miletic*. Dept. Comp. Biosci. and Neurol., Univ. of Wisconsin, Madison, WI 53706.

We compared the responses of ventrolateral orbital cortex (VLO) neurons to noxious cold pressor in two groups of animals. In 13 rats loose ligatures were tied around the sciatic nerve subsequent to its exposure. In 12 rats the sciatic nerve was similarly exposed but no ligatures were tied. Extracellular recordings were performed 14-28 days after surgery by an experimenter who was unaware as to which animal underwent sciatic ligation. At the time of recording none of the ligated or sham-operated animals exhibited hind limb elevation (i.e., neuropathic pain). To date responses of 33 VLO neurons to noxious cold pressor (immersion of a hind-limb in ice-cold water for 3 min) have been adequately characterized. Statistical analysis (*t-test* with Bonferroni corrections) was used to differentiate responding from nonresponding neurons. In ligated rats a greater proportion of units responded to the cold pressor stimulus than in sham-operated animals (12/15 vs. 11/18). In addition, the average afterdischarge to the cold pressor was longer in ligated than in sham-operated animals (4.69±6.30 vs. 1.93±2.60 min, $p<0.05$). There was no difference in the average background firing rate (2.24±1.80 vs. 1.94±1.45 spikes/sec), or the magnitude of response to the cold pressor (189±206 vs. 189±193% increase) between VLO neurons in ligated and sham-operated animals. Sciatic ligation apparently increases the number of VLO neurons responding to noxious cold pressor. [NIH NS21278].

128.10

MORPHINE EFFECTS HYPERALGESIA AND ALLODYNIA DIFFERENTIALLY IN EXPERIMENTAL PERIPHERAL NEUROPATHY. J.S. Kroin*, E.N. Tanck, R.J. McCarthy, R.D. Penn. Departments of Neurosurgery and Anesthesiology, Rush Medical College, Chicago, IL 60612

Sciatic nerve ligation produces a syndrome in rats exhibiting both hyperalgesia and allodynia. The effect of analgesic drugs on the various aspects of the model has not been fully examined as yet. Fourteen young adult (250-300 g) rats underwent ligation of the left sciatic nerve (Bennett & Xie, Pain, 33:87, 1988). A sham operation was performed on the right side. After 2 weeks, rats developed allodynic responses to cool water (withdrawal) and mechanical stimulation (vocalization) and a hyperalgesic response to hot water (faster withdrawal) on the lesioned side. Rats were tested 3 weeks after surgery with systemic injections of morphine sulfate (0,0.5,1,2, and 5 mg/kg i.p.). In tests for allodynia, morphine produced a dose-dependent increase in both the withdrawal latency to cool water and the number of non-vocalizations to mechanical stimulation on the lesioned side. In comparison, withdrawal to hot water was not significantly increased at morphine doses up to 2 mg/kg. At 5 mg/kg, there was a large increase in withdrawal latency on the lesioned side but it was not significantly greater than the increase on the sham side. Therefore, in this model, morphine can alleviate allodynia at moderate doses, but high doses are required to have a significant effect on hyperalgesia.

129.1

SYMPATHECTOMY INDUCES NOVEL ADRENERGIC EXCITATION OF CUTANEOUS NOCICEPTORS D.F. Bossut* and E.R.Perl, Physiology, Univ. N. Carolina, Chapel Hill 27599-7545

Partial injury of a peripheral nerve induces some remaining C-fiber cutaneous polymodal nociceptors (CPMs) to develop an excitatory response to nor-epinephrine (NE) and sympathetic stimulation (Sato and Perl, Science 251, 1991). CPMs from normal animals are excited neither by NE nor by sympathetic stimulation. The role of sympathetic denervation in the induction of adrenergic responsiveness was tested by unilateral excision of the superior cervical ganglion in deeply-anesthesized domestic rabbits. After survival for 4 to 55 days, recordings were made under areflexic anesthesia from discharges of single C-fibers with established receptive characteristics of CPM units in filaments of the ipsilateral great auricular nerve. In sympathectomized animals, close arterial injection of 200ng NE evoked responses unrelated to vasoconstriction from about 50% of CPM units. The evoked activity varied from a few to many impulses at latencies of 1 to 94 sec. A number of CPM units developed ongoing discharge after NE trials, sometimes of a crescendo quantity. This effect of sympathectomy implicates loss of sympathetic innervation in development of adrenergic responsiveness of cutaneous nociceptors and in the etiology of sympathetically-aggravated pain states such as causalgia. (Supported by grant NS-10321 from the NINDS and NRSA DE05585 to DFB.)

129.2

RECEPTIVE FIELDS OF HYPERALGESIA CONFINE TO DISTRICTS OF INJURED NERVES; FIELDS "EXPAND" IN "RSD" WITHOUT NERVE INJURY.

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Mechanical hyperalgesia (MH) (Allodynia) is a symptom of causalgia/RSD.

Animal studies have reported striking expansion of receptive fields of Wide Dynamic Range neurons (WDRN's) secondary to nerve injury, enabling mechanoreceptor input from broad cutaneous fields to activate WDRN's[a]. Under the premises that a) WDRN's may signal pain and, b) that patients with chronic "neuropathic" pains following (even mild) tissue injury often express broad areas of MH, it has been hypothesized that post-injury MH that trespasses nerve districts is due to 2[n] changes in WDRN's. Since animal studies forbid optimal mapping of fields of MH it becomes imperative to test, in humans expressing MH, the clinical hypothesis that generated by extrapolation from animal nerve injury.

Thirty six patients expressing post traumatic MH underwent rigorous neurological evaluation plus Quantitative Sensory, Sympathetic reflex, Telethermography, and electrophysiological testings. Eighteen (10 F, 8 M) had histologically proven nerve injury and another 18 (11 F, 7 M) had evidence of absence of organic nerve dysfunction. Magnitude of dynamic MH, defined as an unpleasant subjective response to brushing the skin[b], was rated, and the fields were mapped and photographed. The nerve to symptomatic skin was then anesthetized. In the 18 patients with partial neuromas, the district of lidocaine-induced hypoesthesia (LIH) matched the field of dynamic MH. In contrast, in "RSD" patients with evidence of absence of nerve injury, cutaneous hyperalgesia overlapped multiple nerve districts in 10, and covered an extremity in 8. In these patients the districts of LIH were confined to the anatomical nerve territory.

Conclusions: a) The expansion of physiological receptive fields following animal nerve injury is usually not matched by expansion of psychophysical fields of MH after human nerve injury. b) It remains to be proven that physiological changes in dorsal horns explain "neuropathic" MH in "RSD" patients without nerve injury.

Refs: a) Dubner R., Proc.VI World Congress of Pain, p.269, 1991.
b) Ochoa et al., Soc. Neurosc. Abst., Vol 15 p. 472, 1989.

129.3

HYPOESTHESIA ERASED BY SOMATIC NERVE BLOCK, OR PLACEBO: A PSEUDONEUROPATHIC, PSYCHOGENIC SIGN, IN CAUSALGIA/RSD.

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Diagnostic anesthetic nerve blocks provide clues on neuropathic pain mechanisms. While abolition of hyperalgesia by somatic block is a stereotype, the variable behavior of spontaneous pain predicts peripheral versus central mechanisms. Many patients with neuropathic pains also express cutaneous hypoesthesia (CH); however, its outcome in response to somatic blocks has not been duly investigated.

Abolition of sensory deficit during placebo-controlled somatic block was tested in 19 adult patients with chronic regional causalgiform syndromes who estimated CH as 40 to 95% (average 72%) loss. The area of CH (pinprick and/or light touch) was finely mapped and magnitudes of spontaneous pain (SP), dynamic and static mechanical hyperalgesias (MH) and Tinel sign (TS) were rated. Saline was injected close to the site of pain. After thirty min., CH, SP, MH, and TS were again rated. A nerve serving the area of pain was then blocked. Thirty min. later, the area of lidocaine-induced CH was mapped, and SP, MH, and TS were again rated. Pre-existing CH was finely remapped and rated. After saline there was abolition of CH in 58% of patients and significant improvement in another 21%. SP diminished by 50% or more in 93% of patients whose CH improved with placebo. After lidocaine the residual CH, beyond the area of lidocaine-induced hypoesthesia, improved in all 19 patients, 95% of whom volunteered abolition of CH (active placebo effect).

That this phenomenon is psychogenic is supported by:

a) In 95% of the patients there was evidence of absence of nerve lesion behind the pseudoneuropathic symptoms, by electrophysiological, quantitative sensory, thermographic and sympathetic function criteria. b) In 89.5% the area of hypoesthesia was non-anatomical c) In 95% of patients there was explicit evidence of psychogenic origin for sensory-motor complaints, such as presence of hysterical anesthesia, or weakness due to cortical effort *interruptus*, even in asymptomatic limbs. (Supported by NIH NS 24766 and 28747)

129.4

BEHAVIORAL RESPONSES FOLLOWING AN EXPERIMENTAL NEUROPATHY IN PRIMATES. H.A. Lekan*, S.H. Kim, J.M. Chung and S.M. Carlton. Marine Biomedical Institute, UTMB, Galveston, TX 77555-0843.

An experimental peripheral neuropathy was induced by tight unilateral ligation of spinal nerve L_7 in one monkey (Macaca fascicularis). Behavioral responses to mechanical and thermal stimuli were determined before and for 14 days after surgery. The stimuli employed consisted of a graded series of von Frey filaments (0.068 - 15.0g), brushing with a camel hair brush, application of 68 and 128 HZ tuning forks, 5 s heat pulses (39° - 55°C), 15 s cooling pulses (28° - 8°C) and application of acetone to the plantar and dorsal surface of the feet. Thermographic temperature readings of the ventral feet were also obtained.

Prior to surgery, withdrawal responses were not obtained with the mechanical and cold stimuli. The threshold for response to the heat stimuli was 55°C. There was no temperature difference between sides according to thermographic temperature readings. Following the induction of neuropathy, robust bilateral responses to all von Frey filaments and to brushing developed within 24 hours and were still present 14 days following surgery. During this time period, the threshold for a withdrawal response to heat stimuli decreased to 49°C on the neuropathic side only. Responses to the acetone stimuli were seen bilaterally on day 5 and 6. No responses developed to the cold pulses or vibratory stimuli. Thermographic temperature readings indicated the neuropathic foot was consistently warmer than the contralateral foot by at least 1°C.

The behavioral responses are compatible with electrophysiological findings in this model (see Palecek, adjacent poster). The behavioral manifestations of this model, which parallel the human condition, are indicative of mechanical allodynia, cold allodynia and thermal hyperalgesia and will be very useful for investigations of the mechanisms underlying neuropathic pain. Supported by NS11255, NS27910, NS21266, and Bristol-Myers Squibb Corp.

129.5

RESPONSES OF SPINOTHALAMIC TRACT NEURONS TO MECHANICAL AND THERMAL STIMULI ARE INCREASED IN AN EXPERIMENTAL MODEL OF PERIPHERAL NEUROPATHY IN PRIMATES. J. Palecek*, P.M. Dougherty, S.H. Kim, V. Paleckova, H. Lekan, J.M. Chung, S.M. Carlton, and W.D. Willis. Marine Biomedical Institute, UTMB, Galveston, TX-77555 - 0843.

An experimental peripheral neuropathy was induced in three monkeys (Macaca fascicularis) by tight ligation of spinal nerve L_7. Behavioral responses to innocuous mechanical stimuli were tested before and after the surgery. Responses of spinothalamic tract (STT) neurons to stimuli applied to the skin were recorded under general anesthesia 14 days after the ligation: graded mechanical stimuli (BRUSH, PRESS, PINCH and SQUEEZE), von Frey filaments (0.077 - 19.05g), 5 s heat stimuli (39° - 53°C) and 15 s cooling stimuli (32° - 8°C).

Innocuous stimulation of the foot did not evoke hindlimb withdrawal in the animals before surgery. Within 24 - 48 hours after nerve ligation, the animals showed hindlimb withdrawal to the same innocuous stimuli. Responses of 51 STT neurons on the side of the ligation showed several statistically significant differences when compared to responses of 33 STT cells on the sham operated side and STT neurons in unoperated animals. These differences included: 1) increased spontaneous activity, 2) higher responses to BRUSH stimulus (mean evoked frequency 35 Hz compared to 15 Hz), 3) lower thresholds and higher discharge frequencies with von Frey filament stimulation, 4) decreased threshold temperatures and increased responses to suprathreshold heat stimuli, 5) responses to cooling stimuli with a threshold around 28°C.

Our results strongly suggest that tight ligation of spinal nerve L_7 in monkey results in generation of behavioral and electrophysiological changes that parallel symptoms found in humans with chronic neuropathic pain - mechanical allodynia, thermal hyperalgesia, cold allodynia and possibly spontaneous pain. NS 11255, NS 27910, NS 09743, NS 21266, NS 08660 and a grant from Bristol-Myers Squibb Corp.

129.6

ANATOMICAL CHANGES IN A PRIMATE MODEL OF EXPERIMENTAL PERIPHERAL NEUROPATHY (EPN) S.Carlton*, S.Kim, H.Lekan, J.Palecek, V.Paleckova, P.Dougherty, J.Chung and W.Willis. Marine Biomedical Institute, UTMB, Galveston, TX 77555-0843.

In the present study, we adapted a rat model of EPN (Kim and Chung,'91) to the primate. In 3 monkeys (M. fascicularis), the L_7 spinal nerve was tightly ligated. Compared to presurgery levels, all showed increased behavioral responses bilaterally to application of innocuous mechanical stimuli on the ventral surface of the feet; responses were more dramatic on the operated side. Following 14 days, the animals were perfused and the L6, L7 and S1 spinal cord segments removed and immunostained. Compared to the contralateral side, the dorsal horn ipsilateral to the surgery demonstrated a decrease in the staining density for SP and CGRP. In contrast, there was an increase in galanin and in glial (GFAP) staining. These changes occurred in L7 and L6 but were more dramatic in L7, the segment which sustained the greatest peripheral deafferentation. Minimal changes were observed in staining densities in S1. The increased glial staining was most likely due to glial hypertrophy, which can occur in response to excess glutamate. Electrophysiological studies in the same animals demonstrated that STT cells in the L7 segment were generally non-responsive to peripheral stimulation, however, STT cells in L6 showed maximal changes in response to mechanical and thermal stimulation. These findings indicate that changes in dorsal horn peptide levels may be related to the allodynia and hyperalgesia demonstrated in the behavioral and electrophysiological studies (see Palecek et al and Lekan et al adjacent posters). Elucidating the neuroplasticity occurring in this model of peripheral neuropathy may clarify underlying mechanisms which might be clinically exploited. Supported by NS11255, NS27910, Bristol-Myers Squib.

129.7

CORRELATION OF AXONAL NUMBERS AND BEHAVIORAL SYMPTOMS IN A RAT EXPERIMENTAL NEUROPATHY MODEL.
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Chronic partial constriction of rat sciatic nerve produces hyperalgesia and allodynia, symptoms also found in human neuropathies (Bennett & Xie 1988, 1989). A proposed mechanism is loss of central inhibitory controls due to the preferential interruption of large diameter afferents (Gautron et al., 1989; Basbaum et al., 1991), but severe losses of unmyelinated and myelinated fibres of all sizes are also found in this injury (Basbaum et al.,1991; Carlton et al., 1991). The problem with all these studies, however, is that they were all done at one time point and the neuropathic symptoms are not static. We have tested 19 rats for paw withdrawal latency (PWL) and sustained paw elevation time (PET) before nerve constriction, at weekly intervals and then immediately before sacrifice at 3, 5, 14, 28 and 56 days post-op. Myelinated and unmyelinated axons were counted in E.M. montages of the sciatic nerve segments proximal and distal to the site of injury. Results show that behavioral symptoms are maximal at 3 days and virtually gone by 28 days (PWL) or 14-21 days (PET), whereas axon numbers are most diminished distally at 14 days, are recovering by 28 days and approximately equal to proximal numbers at 56 days. We suggest that behavioral changes initially correspond with axon loss, particularly large myelinated axon loss, but at later stages the behavioral symptoms and axonal changes do not coincide. (Supported by NS10161, NS11255, NS27910 and Bristol-Myers Squibb).

129.8

THE TIME COURSE OF HISTOCHEMICAL CHANGES AND THE TRANSYNAPTIC INDUCTION OF A 29KD LECTIN IN THE DORSAL HORN IN A RAT MODEL OF NEUROPATHY.
A.A.Cameron*, P.M. Dougherty, C.J. Garrison, W.D. Willis and S.M. Carlton. Marine Biomedical Institute, UTMB, Galveston, TX, 77555-0843.

In a rat model of peripheral neuropathy (Bennett and Xie, 1988), behavioural hyperalgesia is observed. We have investigated the time course of effect this peripheral nerve lesion has on the histochemistry of the dorsal horn. Four loose ligatures were tied around the sciatic nerve in anaesthetised rats. All animals demonstrated behavioural hyperalgesia. At various time points (3, 5, 14, 28 days and 10 weeks) the animals were perfused and sections of the L4 and L5 spinal cord were stained with soybean agglutinin (SBA) and immunostained for the lectins RL-14.5 and RL-29, the growth associated protein GAP-43 and the neuropeptides substance P and calcitonin gene-related peptide (CGRP). In the superficial dorsal horn, the densities of reactivity to SBA and RL-29 were unchanged at 3 days and increased at 5, 14, 28 days and 10 weeks. The density of GAP-43 was unchanged at 3 and 5 days, and increased at 14 days, 28 days and 10 weeks. The density of RL-14.5 was unchanged at 3 days, increased at 5, 14 and 28 days, and decreased at 10 weeks. Reactivity to SP was unchanged at 3 and 5 days and decreased at other time points. Reactivity to CGRP remained unchanged at all time points. In addition, RL-29 reactivity was induced in a subpopulation of neurons in lamina V in the dorsal horn ipsilateral to the injury.

The results suggest that following sciatic nerve damage, complex degenerative and regenerative changes occur in sensory neurons and that these are reflected in the L4 and L5 dorsal horn within five days of injury. It appears that the temporal sequence of changes in the numbers of axons in the sciatic nerve in animals with the neuropathic lesion is coincident with the changes in dorsal horn histochemistry. (Supported by NS11255, NS09743, NS27910, NS08660 and Bristol Myers-Squibb).

129.9

QUANTITATIVE ANALYSIS OF SUBSTANCE P AND CALCITONIN GENE-RELATED PEPTIDE IMMUNOHISTOCHEMICAL STAINING IN THE DORSAL HORN OF NEUROPATHIC RATS TREATED WITH MK-801.
C.J. Garrison*, P.M. Dougherty, S.M. Carlton. Marine Biomedical Institute, UTMB, Galveston, TX 77555-0843.

Neuropathic pain remains a major complication following various forms of peripheral nerve injury in humans. An animal model of peripheral neuropathy resulting in a unilateral thermal hyperalgesia has recently been developed. It is the purpose of this study to quantitate the immunohistochemical changes in Substance P (SP) and Calcitonin Gene-Related Peptide (CGRP) within the spinal cord of neuropathic animals and examine these changes under experimental conditions which limit excitatory primary afferent input into the dorsal horn. The model is induced by placement of 4 loose ligatures around the sciatic nerve (Bennett & Xie, 1988) of 24 rats. On post-surgical days 7 and 14, immunohistochemical staining of SP and CGRP within the lumbar spinal cord was conducted on non-treated animals (n=8) and animals treated for 7 days with MK-801 (0.5mg/kg or 1mg/kg, n=16). Non-treated animals demonstrated thermal hyperalgesia and an ipsilateral decrease in SP staining density. MK-801 treatment resulted in a dose dependent attenuation of the hyperalgesia. In addition, the lower dose of MK-801 eliminated the decrease in SP and the higher dose resulted in an ipsilateral increase of SP. Although MK-801 treatment increased both peptides in non-neuropathic rats, no side to side change was demonstrated in CGRP staining in neuropathic rats. It can be concluded that blocking excitatory amino acid transmission can eliminate or reverse the changes in spinal cord SP induced by a nerve constriction, and that the changes in SP are not accompanied by changes in CGRP staining in this model. Supported by NS1255, NS27910, and Bristol Myers-Squibb.

129.10

SENSORY RECEPTORS RESPONSIBLE FOR MECHANICAL ALLODYNIA IN A RAT NEUROPATHIC PAIN MODEL.
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Mechanical allodynia in causalgia is thought to be signalled by activity of low threshold mechanoreceptors. The aim of this study is to determine the exact type of mechanoreceptors mediating allodynia using an animal model for neuropathic pain.

Neuropathic animals were produced by ligating the L5 and L6 spinal nerves of one side in rats. These animals showed behavioral signs of mechanical allodynia. One week after neuropathic surgery, single fiber recordings were made from fine filaments dissected from the L4 dorsal root under urethane anesthesia.

We found an unusually large number of intermediate mechanoreceptors, which are characterized by weak sustained discharges during maintained mechanical stimulation. Furthermore, they changed to purely rapidly adapting receptors after an intravenous injection of phentolamine (0.5mg/kg). The drug had no effect on other types of mechanoreceptors.

The data suggest that the rapidly adapting mechanoreceptors change their responsiveness in causalgia and are responsible for allodynia. (Supported by NIH grants NS21266 and NS11255 and a grant from Bristol-Myers Squibb Co.)

129.11

NEURAL ACTIVITY OF MEDIAL AND LATERAL THALAMUS IN A DEAFFERENTATION MODEL.
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Neuronal hyperactivity ("bursting") has been identified in the somatosensory system following neurological insults in animal and human studies, and recently in nociceptive pain patients. This study characterizes neuronal activity in medial as well as lateral thalamic nuclei in a deafferentation model. Male rats (n=26) underwent unilateral dorsal rhizotomy. Following signs of active autotomy, thalamic (VPL/VPM, MD, CM, CL) extracellular recordings were obtained in sedated rats. Control data were obtained from thalamic recordings in naive rats. Our results show that 80% of the deafferented animals exhibited autotomy 2 to 59 days after rhizotomy (mean 20.8). A total of 208 thalamic neurons were studied in 10 deafferented and 9 control rats. In the deafferented group, 65% (96/148) of the neurons showed spontaneous bursting, whereas only 21% (12/56) of the cells from controls burst. In the medial thalamus, 19% of the neurons in deafferented and 5% in control rats were responsive to noxious stimuli. In the lateral thalamus, 24% of the cells in deafferented and 17% in control rats responded to pain. Computerized interval histograms and burst analyses were completed for 23 cells in deafferented and 10 cells in control rats. Bursting cells in deafferented rats showed longer bursts (4-10 spikes) than controls (2-7 spikes). In control rats, the average duration for successive intervals between spikes in a burst was progressively longer. Bursting cells in deafferented rats showed intervals that decreased in duration from the first to the third one and increased thereafter. We conclude that increases in noxious-responsive cells and bursting activity of neurons occur early following deafferentation in both medial and lateral thalamus. Deafferentation produces longer bursts by increasing both the number of spikes per burst and the duration of the intervals between spikes. Our data indicate an important role for medial as well as lateral thalamic hyperactivity in deafferentation.

129.12

ACTIVITY-DEPENDENT INTERACTIONS AMONG DORSAL ROOT GANGLION NEURONS BEFORE AND AFTER PERIPHERAL NERVE INJURY
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A variety of afferent excitability changes are known to occur after peripheral nerve injury; most of the experimental work to date has focused on either the initial site of injury or within the CNS to determine the mechanisms of these alterations in sensation. Single fiber recordings from peripheral nerve have shown, however, that it is likely that cross-excitation does occur among the cell bodies of dorsal root ganglion (DRG) neurons (Devor and Wall, J. Neurophys., 64:1733-1746, 1990). To verify this possibility a combination of intracellular recordings and extracellular potassium measurements were obtained from whole excised DRGs from uninjured rats and potassium measurements were obtained from the DRGs 20 days after ligation and transection of the sciatic nerve. In the control DRGs the dorsal root was split into two branches allowing for separate stimulation of either branch; the S1 branch could then be identified as containing the axon of the impaled neuron and the S2 branch would contain only the axons of neighboring neurons. Tetanic stimulation of the S2 branch (10 sec., 50 Hz) resulted in a frequency- and amplitude-dependent depolarization of the neuron whose axon projected to the S1 branch, clearly indicating that the depolarization in this recorded neuron was due to activity in neighboring neurons, and not from stimulation of its own axon. A rise in extracellular potassium with a timecourse similar to the depolarization was also observed in the control (1.42 $\pm$0.86 mM) and ligated (1.85 $\pm$0.57 mM) DRGs. The potassium increase observed in the control DRGs was significantly greater than that seen in the dorsal roots (0.66 $\pm$ 0.25 mM). An activity-dependent accumulation of extracellular potassium within the DRG could account for some of the pathologic sensory disturbances seen after peripheral nerve injury.

129.13

COMPARISONS BETWEEN REGIONAL CHANGES IN SPINAL CORD NEURAL ACTIVITY AND MEMBRANE-BOUND PROTEIN KINASE C IN A RAT MODEL OF PAINFUL PERIPHERAL MONONEUROPATHY

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Spatial distributions of spinal cord neural activity and membrane-bound protein kinase C (PKC) were compared in a rat model of painful peripheral mononeuropathy (Bennett & Xie, Pain, 1988, 33:87) following chronic constrictive sciatic nerve injury (CCI) by employing the [^{14}C]-2-deoxyglucose ([^{14}C]-2-DG) metabolic mapping and the [^{3}H]-phorbol-12,13-dibutyrate ([^{3}H]-PDBu) binding assay, respectively. CCI rats exhibited demonstrable thermal hyperalgesia and spontaneous pain behaviors 10 days after nerve ligation when [^{14}C]-2-DG and [^{3}H]-PDBu experiments were carried out. Reliable increases in both metabolic activity and PKC membrane binding occurred bilaterally in the lumbar dorsal horn (laminae I-VI of L_2-L_5) of CCI rats as compared to sham-operated rats. In both cases, activity was higher in the ipsilateral than in the contralateral spinal cord grey matter of CCI rats. In addition, regional increases in both activities occurred over an extensive rostro-caudal area from L_2-L_5. However, major differences between the two patterns of increased activity are that increases in PKC binding peaked in laminae I-II and did not occur in the ventral horn, whereas neural activity (2-DG) peaked in laminae V-VI and increased considerably within the ventral horn (lamina VII). While the spatial overlap between PKC binding and neural activity in CCI rats indicates a possible causal relationship between the two, much of the increased neural activity within the deep dorsal horn and ventral horn may occur as a consequence of excitation from more superficial dorsal horn neurons that undergo PKC-mediated neuronal plastic changes.

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129.14

PAIN-RELATED INCREASES IN SPINAL CORD MEMBRANE-BOUND PROTEIN KINASE C AFTER PERIPHERAL NERVE INJURY

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Mechanisms underlying post-injury neuropathic pain are thought to involve excitatory amino acid-mediated central nervous system neuronal plastic changes. Using a rat model of painful peripheral mononeuropathy (Bennett & Xie, *Pain, 1988, 33:87*), we examined changes in spinal cord membrane-bound protein kinase C (PKC) resulting from PKC translocation, a Ca^{++}-dependent process known to mediate central nervous system neuronal plasticity, by employing the [^{3}H]-phorbol-12,13-dibutyrate ([^{3}H]PDBu) autoradiographic assay. Consistent with demonstrable thermal hyperalgesia and spontaneous pain behaviors in sciatic nerve-ligated (chronic constrictive injury, CCI) rats 3 and 10 days after nerve ligation, increases in [^{3}H]PDBu binding also occurred in these CCI rats in the fourth lumbar spinal cord segment (laminae I-II, III-IV, and V-VI) both ipsilateral and contralateral to the ligated side as compared to corresponding regions of sham-operated rats. The [^{3}H]PDBu binding was reliably higher on the side ipsilateral as compared to contralateral to nerve ligation in CCI rats. In addition, three daily intrathecal treatments with 80 nmol GM1 ganglioside (a glycolipid shown to prevent PKC translocation/activation) beginning 1 hr after nerve ligation reliably reduced both increased levels of [^{3}H]PDBu binding and nociceptive behaviors in CCI rats when examined 24 hrs (day 3 after nerve ligation) but not 7 days (day 10 after nerve ligation) after the last GM1 treatment, suggesting a possible causal relationship between increases in membrane-bound PKC and pain-related behaviors. Thus, central mechanisms underlying persistent neuropathic pain may be mediated by the initiation of central nervous system neuronal plastic changes resulting from the increase in membrane-bound PKC. Supported by Fidia Pharmaceuticals.

129.15

ELECTRON MICROSCOPIC ANALYSIS OF AXON FIBER SPECTRUM FOLLOWING MONONEUROPATHY HYPERPATHIA PRODUCED BY FIXED DIAMETER NERVE CONSTRICTION.

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A constriction mononeuropathy model was developed in which the degree of compression exerted on the nerve could be standardized. Thick wall polypropylene tubing ranging in diameter from 0.010"-0.030" was cut either in short (<0.5 mm) rings or in longer (3 mm) tubes. The walls of 4-6 rings were slit, and the rings were applied to either sural or sciatic nerves at intervals of <1 mm over a distance of 3-6 mm. Tubes were slit longitudinally and applied to sciatic nerves. The walls of the rings and tubes were resilient enough to appose the edges of the slit and lightly constrict the nerve. Clear tubing allowed for verification of continued blood flow through epineurial vasculature. Constriction produced behavioral signs of hyperpathia and allodynia in a majority of animals similar to that described by Bennett and Xie ('88) using multiple suture constriction. Electron microscopic determination of fiber spectrum in sections of nerves distal to the constriction showed degeneration in a large proportion of heavily myelinated axons and in a smaller proportion of lightly myelinated axons. Unmyelinated axons were distinctly more resistant to constriction. The ability to regulate the extent of compression exerted in this model can be useful for future studies of the alterations produced in sensory ganglia. The cutaneous sural nerve provides a model for analysis of sensory fiber spectrum relating to pain pathology.

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129.16

EXPLOSIVE AUTOTOMY (AT) INDUCED BY SIMULTANEOUS DORSAL COLUMN (DC) LESION AND LIMB DENERVATION : A POSSIBLE MODEL FOR ACUTE DEAFFERENTATION PAIN.

N.E. Saade'*, M.Z.M. Ibrahim, S.F. Atweh and S.J. Jabbur. Faculty of Medicine, American University of Beirut, Beirut, Lebanon.

In rodents, AT appears 2-3 weeks after limb denervation and lasts for 2-3 weeks progressing from distal to proximal parts of the leg; it has served as an experimental model for chronic deafferentation pain. We examine here the neural basis of a new form of AT (explosive AT) characterized by rapid onset (1-2 days), short duration (1-2 days) and unpredictable progression. It occurs when rats are subjected to simultaneous DC lesion and leg denervation but fails to occur when these procedures are immediately preceded by lumbar spinal anesthesia. Regular, rather than explosive, AT occurs with DC lesion carried out one week before or after leg denervation, with simultaneous lesion of other spinal tracts and leg denervation, and with simultaneous DC lesion and leg denervation immediately preceded by lumbar spinal anestheseia. These results support the concept that explosive AT could be due to simultaneous injury to peripheral and central ends of primary afferent neurons involved in nociceptive transmission and modulation, combined with injury to other afferent fibers involved in nociceptive transmission. (Supported by grants from the Lebanese National Research Council and the D.T. Sabbagh Foundation)

129.17

SPONTANEOUS PAIN AND COLD HYPERALGESIA IN A RAT NEUROPATHIC MODEL.

Y. Choi*, H.S. Na, S.H. Kim and J.M. Chung. Marine Biomed. Inst., Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX, 77555.

We previously developed an animal model for causalgia by tightly ligating the L5 and L6 spinal nerves unilaterally in rats. These rats showed a long-lasting heat hyperalgesia and mechanical allodynia. The aim of this study is to extend the model by demonstrating the existence of two additional important characteristics of causalgia: spontaneous pain and cold hyperalgesia.

Each rat was placed on a brass plate at a neutral (30°C) or a cold (4°C) temperature. The total duration of hind paw elevation during a 5 min observation period was used as a pain rating for spontaneous pain or cold hyperalgesia, respectively.

After neuropathic injury, pain ratings of both spontaneous pain and cold hyperalgesia were significantly increased on the affected hind paw as compared to either the control side or the preoperative state. This occurred as early as 2 days after the operation and continued for the 2 week test period. These rats also showed heat hyperalgesia and mechanical allodynia, confirming the previous data.

These results suggest that two additional features of causalgia, spontaneous pain and cold hyperalgesia, are also present in our rat model for causalgia. (Supported by NIH grants NS21266 and NS11255 and a grant from Bristol-Myers Squibb Co.)

129.18

THE SITE OF SYMPATHECTOMY ALLEVIATING MECHANICAL ALLODYNIA IN A RAT NEUROPATHIC PAIN MODEL.

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Sympathectomy is known to be the most effective treatment of causalgia. The aim of this study is to localize the critical site of sympathetic-afferent interactions for mechanical allodynia in causalgic symptoms.

Neuropathic injury was produced by tightly ligating the L6 spinal nerve of one side in 20 rats. These animals showed behavioral signs of mechanical allodynia. Two weeks later, a differential sympathectomy was performed and the result was verified by examining noradrenergic axons with the glyoxylic acid reaction at various sites. One group received a sympathectomy at the injured site, and the other group in the intact segment.

Signs of mechanical allodynia remained in animals which received a sympathectomy at the injured segment. On the other hand, a sympathectomy at the intact (uninjured) segment alleviated the mechanical allodynia almost completely.

The data suggest that sympathetic nerve fibers innervating the intact sensory receptors play a critical role in mediating the mechanical allodynia of causalgia. (Supported by NIH grants NS21266 and NS11255 and a grant from Bristol-Myers Squibb Co.)

129.19

THE ROLE OF INJURED FIBERS FOR NEUROPATHIC PAIN IN AN EXPERIMENTAL ANIMAL MODEL. J.M. Chung* and K. Sheen. Marine Biomed. Inst., Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX, 77555.

The aim of this study was to determine the role of injured fibers for the initiation and maintenance of neuropathic pain in an experimental animal model.

Neuropathic pain was produced by injuring spinal nerves in rats and signals entering the spinal cord from the injured fibers were blocked by dorsal rhizotomy at different times. Behavioral tests were performed to determine the presence of mechanical allodynia and heat hyperalgesia for several weeks after neuropathic injury.

Resection of the L5 and L6 spinal nerves produced neuropathic pain, as did tight ligation of the nerves. However, dorsal rhizotomies (at the L5 and L6 segments) which were preceded by the same spinal nerve injury prevented the development of neuropathic pain. Furthermore, dorsal rhizotomies (at the injury segments) which were performed 3 weeks after neuropathic injury abolished the signs of neuropathic pain.

The results suggest that signals entering the spinal cord from injured fibers are important for both initiation and maintenance of neuropathic pain in an experimental animal model. (Supported by NIH grants NS21266 and NS11255 and a grant from Bristol-Myers Squibb Co.)

129.20

INTRANEURAL MICROSTIMULATION OF LOW THRESHOLD MECHANORECEPTORS IN PATIENTS WITH CAUSALGIA/RSD/SMP R. Dotson*, J. Ochoa, M. Cline, P. Marchettini, D. Yarnitsky.
Depts. of Neurology, Good Samaritan Med. Ctr. and Oregon Health Sci. Univ., Portland, OR, and Medical College of Wisconsin, Milwaukee, WI.

We directly tested hypothetical models of neural mechanisms for spontaneous pain and hyperalgesia in causalgia/RSD/SMP which propose that low threshold mechanoreceptors (LTMs), or alternatively nociceptors, activate sensitized W.D.R. dorsal horn neurons that transmits signals subjectively decoded as pain. The microneurographic technique was used to record identified mechanoreceptor units from peripheral nerves supplying the painful/hyperalgesic area of skin in 9 patients with clinical diagnoses of causalgia/RSD/SMP conventionally endorsed by temporary relief of pain by peripheral sympathetic blockade (SMP). Thirty LTMs (12 RA, 5 PC, 9 SAI, and 4 SAII) all but one of each type innervating glabrous skin, were isolated and identified by receptive field (RF) and stimulus-response characteristics. Intraneural microstimulation up to threshold intensity for first sensation, with trains of square wave pulses at 2-30 Hz was performed for each unit. Patients reported subjective quality of the initial projected sensation as stimulus intensity reached threshold, usually by 0.2-0.3 V. Receptor responses were re-evaluated after stimulation to confirm that the unit retained proximity to the electrode throughout the stimulus period. Each of the identified units had normal RF and stimulus-response characteristics, and the RF and the projected field of sensation matched in space. This test consistently evokes intermittent tapping or vibration in control subjects(Ochoa and Torebjork,1983).

In patients, threshold stimulation of RA, PC, and SAI units also resulted in painless sensations. As in controls, stimulation of SAII units resulted in no perceived sensation in patients.

We conclude that stimulation of single LTMs at liminal intensity in patients with causalgia/RSD/SMP does not evoke pain. Therefore, in these patients LTMs are either not responsible for peripheral encoding of signals for pain or spatial/temporal summation is required for this to occur.

PAIN MODULATION: DORSAL HORN

130.1

EFFECTS OF NMDA AND SUBSTANCE P ON EVOKED DISCHARGE OF SINGLE DORSAL HORN NEURONES.
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The excitatory amino acid (EAA) glutamate and the peptide substance P (SP) are important in nociceptive transmission in the dorsal horn of the spinal cord. The NMDA receptor subtype is involved in the phenomenon of wind-up, and both the NMDA and the SP receptors have been suggested to play a role in long-term changes in nociceptive modulation.

The experiments were carried out in Halothane/N_2O-anaesthetized rats. Deep dorsal horn neurones were recorded extracellularly using tungsten electrodes. All cells responded to both noxious and non-noxious stimulation of the ipsilateral hind paw. The receptive field was stimulated electrically (16 pulses, 2 ms wide, 0.5 Hz, 0.3–4.5 mA). Drugs were applied directly onto the cord, akin to intrathecal application, dissolved in 50 µl saline.

With stimulation at 1.5 × C-fibre threshold, the effect of NMDA was to increase the C-fibre response at low doses and reduce it at high doses, while the response to electrical stimulation at 3 × C-fibre threshold (mA) was not altered by intrathecal NMDA. Conversely, SP alone had little effect, but coadministration of SP inhibited the increase in response induced by NMDA.

These experiments confirm the facilitatory role of NMDA-receptor stimulation during nociceptive transmission in the dorsal horn. The lack of effect when strong stimulation was used may be due to high synaptic EAA release with already maximal NMDA receptor stimulation, and therefore no additional effect after application of NMDA. The reduction of facilitation when SP and NMDA were coadministered may be due to the induction of inhibitory systems.

130.2

RESPONSE BEHAVIOR OF RAT NOCICEPTIVE MEDULLARY DORSAL HORN NEURONS TO DEFINED CO_2 PULSES APPLIED TO THE NASAL MUCOSA.
P. Peppel and F. Anton (Spon: European Neuroscience Association). Dept. of Physiology I, University of Erlangen-Nürnberg, F.R.G.

The response behavior of chemo-nociceptive medullary dorsal horn neurons was determined by extracellular single unit recordings in halothane anesthetized rats.

For noxious stimulation we applied 2s lasting CO_2 pulses of different concentrations (25-100%) to the nasal mucosa. The observed neuronal discharges revealed that WDR and NS neurons encoded the stimulus intensities in a linear fashion. Although NS cells generally displayed weaker responses, the discriminatory capacity of both categories of neurons was comparable. We compared stimulus response functions (SRFs) obtained with different interstimulus intervals (ISIs: 30s and 120s) and consistently found the neuronal responses to be depressed under the short ISI condition. Stable responses to identical stimuli were obtained with 120s ISIs, proving that the chemical stimuli themselves do not per se alter the response characteristics.

In a second set of experiments we examined whether the SRFs were altered by subsequent heterotopic conditioning. Once stable baseline SRFs had been recorded, a mixture of inflammatory agents was applied to the ipsilateral cornea or a radiant heat pulse (55ºC; 100s) was applied to the ipsilateral upper lip. The subsequent SRFs of some neurons became steeper following heterotopic conditioning, reflecting central sensitization. In other neurons we observed a flattening of the SRFs, possibly due to dominating effects of descending inhibition. (supported by DFG)

130.3

NOCICEPTIVE AND PHYSIOLOGICAL BEHAVIOR IN THE FLUOROCARBON EXCHANGED-TRANSFUSED RAT. S.A. Green*, C.L. Cleland and G.F. Gebhart, Department of Pharmacology, University of Iowa, Iowa City, IA 52242

The relation between sensory stimulation and the perception of pain is subject to modulation by psychological, neural, local and humoral factors. The investigation of humoral factors, however, has been complicated by the complexity of blood constituents. Consequently, we have developed a new model system for investigating the role of circulating factors in the modulation of nociception - the fluorocarbon, exchanged-transfused whole rat preparation.

Awake male Sprague-Dawley rats, chronically instrumented with femoral venous and arterial catheters and EMG electrodes, were continuously infused with artificial blood through the femoral vein at 1ml/minute for 70 ml. Simultaneously, blood was withdrawn manually from the arterial line via a 1ml syringe at 1ml/minute. Responses to nociceptive thermal (tail immersion in hot water), mechanical (von Frey stimulation of the tail) and visceral (colorectal distension, CRD) stimuli were measured before, during and following transfusion.

We found that the exchange-transfused rat can exhibit normal responses to thermal, mechanical and visceral nociceptive stimuli following transfusion. The sensitivity to nociceptive stimuli, however, varied during the course of the transfusion. Overall, transfusion decreased the sensitivity to all three nociceptive stimuli. Individually, different nociceptive responses exhibited different trends: sensitivity to mechanical stimuli decreased rapidly, sensitivity to heat decreased slowly, and the visceromotor response to CRD initially decreased but later returned toward control levels. These results provide a baseline against which the effects of blood-borne factors can be experimentally tested by manipulating the contents of the blood in conditions such as inflammation or nerve injury where circulating factors are likely to influence nociceptive transmission. Funded by NS19912.

130.4

CELLULAR AND SYNAPTIC MODULATION OF NOCICEPTIVE TRANSMISSION IN THE SPINAL CORD OF THE FLUOROCARBON PERFUSED RAT. C. L. Cleland*, M. C. Jiang and G. F. Gebhart, Department of Pharmacology, University of Iowa, Iowa City, IA 52242

Nociceptive transmission in the spinal cord can be modulated by synaptic and cellular processes. Repetitive synaptic activity can produce wind-up, slow post-synaptic potentials (sEPSPs), central sensitization and hyperalgesia. Cellular properties such as plateau potentials can influence neuronal responses to synaptic input for prolonged periods of time. Our goal is to evaluate the relative contributions of synaptic and cellular properties to the modulation of nociceptive processing in the spinal cord of the rat.

Intracellular recordings from 42 spinal cord neurons in the L4-L5 spinal segments of the adult rat were obtained using the fluorocarbon perfused, whole rat preparation. The perfused preparation allows stable intracellular recordings in excess of 2 hours in intact rats at normal physiological temperature. Synaptic input was tested by natural stimulation of receptive fields and electrical stimulation of the sciatic nerve. Cellular properties were tested by intracellular current passage.

We have identified several processes that could underlie long-term modulation of nociceptive processing. Wind-up ($n=13$) or wind-down ($n=2$) was observed in most neurons tested ($n=15$) and three neurons exhibited long-lasting sEPSPs following repetitive sciatic nerve stimulation. Four neurons displayed long-lasting plateau potentials following depolarization evoked by intracellular current passage and one neuron exhibited reproducible oscillations in membrane potential. We are currently investigating the characteristics, mechanisms and significance of these synaptic and cellular processes. Funded by NS 19912.

130.5

HABITUATION OF THE RESPONSES OF TRIGEMINAL BRAIN-STEM NEURONES TO TOOTH-PULP STIMULATION IN CHRONICALLY PREPARED, ANAESTHETIZED CATS.
D. Banks, M. Kuriakose and B. Matthews*. Department of Physiology, University of Bristol, BS8 1TD, England.

The jaw opening reflex (JOR) evoked by electrical stimulation of tooth-pulp (TP) in chronically prepared cats habituates at frequencies >0.5Hz to 20-25% of its initial value after 5-10 minutes. In the present experiments we have examined the responses of trigeminal brain-stem neurones (main sensory and spinal pars oralis) under similar conditions to determine whether their responses also habituate. Three male cats were prepared for chronic recording of the JOR and from brain-stem neurones as described previously (*J. Neurosci. Meth.* **38**, 35-40, 1991; *J. Physiol.* **446**, 78P, 1992). Following complete recovery the cats were anaesthetized and recordings made from 12 neurones responding to TP stimulation (thresholds 20-100μA, 0.1ms, 0.3Hz). Changes in the number of spikes and their latency, and in the integral of the digastric EMG were recorded during a period of TP stimulation (0.1ms, 40-1000μA, 10Hz, 30s). In all cases the JOR habituated but the responses of the neurones fell into two distinct patterns. Of the total, 10 were short latency (3-6ms, SL) neurones and showed habituation whereas the remaining 2 were long latency neurones (15-17ms) and did not habituate. Changes in the responses of most SL neurones parallelled those of the EMG. The habituation of both the JOR and SL neurones was tooth specific, i.e. after habituation to upper tooth stimulation, the response from the corresponding lower tooth was unchanged from its initial value. We suggest that the SL neurones may be interneurones in the JOR pathway.

130.6

NOCICEPTIVE SPINAL DORSAL HORN NEURONS RECEIVE ABUNDANT SYNAPTIC CONTACTS FROM SENSORY FIBRES CO-LOCALIZING SUBSTANCE P AND CGRP IMMUNOREACTIVITIES. AN ULTRASTRUCTURAL, TRIPLE-LABELLING STUDY IN THE CAT.
A.Ribeiro-da-Silva*, W. Ma, Y. De Koninck, A.C.Cuello and J.L.Henry. Depts. of Pharmacology & Therapeutics, Psychiatry, Physiology & Anaesthesia Research, McGill University, Montréal, Québec, Canada H3G 1Y6

In a previous study (De Koninck et al., PNAS, in press), we demonstrated at ultrastructural level that nociceptive neurons receive an abundant substance P (SP)-immunoreactive (IR) input, whereas non-nociceptive neurons have minimal SP-IR input. However, SP-IR fibres originate from sensory afferents, intrinsic spinal neurons and brain stem neurons. As calcitonin gene-related peptide (CGRP) is a useful marker for peptidergic primary sensory fibres, we studied the ultrastructural distribution of SP and CGRP immunoreactivities in association with physiologically classified dorsal horn neurons which had been labelled by intracellular iontophoresis of HRP. SP immunostaining was demonstrated by means of a bi-specific anti-SP/anti-HRP monoclonal antibody, using a DAB based pre-embedding protocol. CGRP immunoreactivity was revelaed by means of a post-embedding immunogold protocol. CGRP and SP immunoreactivities were co-localized in many varicosities contacting wide-dynamic range and nociceptive-specific cells, although other boutons were immunoreactive exclusively for SP or CGRP. Immunoreactivities for these peptides were infrequent in boutons apposed to non-nociceptive neurons. These results confirm the primary sensory origin of many of the SP-immunoreactive boutons which are presynaptic to nociceptive neurons in the cat spinal dorsal horn. (supported by NIH)

130.7

RESPONSES OF SPONTANEOUS AND NOXIOUS EVOKED DORSAL HORN CELL ACTIVITY IN CAT TO PROLONGED TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION (TENS) AND CHANGE IN TENS FREQUENCY.
D.W. Garrison* and R.D. Foreman. Depts. of Physical Therapy and Physiol. and Biophys, Univ. of Okla. Hlth. Sci. Ctr, Okla. City, OK. 73190.

We have shown that TENS applied to somatic receptive fields for short periods of time (20-30 s), decreases spontaneous and noxious evoked activity of dorsal horn neurons. This study examined the effects of cell activity during prolonged TENS application and changes in stimulation frequency (Hz). In 9 alpha-chloralose anesthetized cats, spontaneously firing dorsal horn neurons were recorded from the lumbar spinal cord. Continuous TENS was applied for 5 minutes to dermatomes corresponding to cord level. Of 17 spontaneously firing cells, 88% had a mean decrease from control activity of 17 ± 2.4 imp/s to 7 ± 1 imp/s. Two cells did not respond. For 86% of 7 noxiously evoked cells, mean control activity decreased from 34 ± 8 imp/s to 10 ± 1 imp/s (Medtronic TENS unit:mode = norm; pulse duration = 100 us; 5-125 Hz; 5-60 mA). Spontaneous and evoked activities were reduced consistently for 5 min. When the TENS unit was turned off activity immediately increased for all cells but at a level which was less than control for the majority of cells. Responses to changing frequency (5-125 Hz) during TENS were recorded for 10 spontaneously active cells and were found to be frequency dependent for each. The results indicate that 1) TENS can maintain decreased cell activity for a prolonged period of time and 2) variation in the frequency (Hz) has a graded response on dorsal horn cell activity. (Supported by OCAST Proj. HR1-063, Contr. 4089, Presbyt. Hlth Fdn. and NIH HL22732)

130.8

DO WDR NEURONS PARTICIPATE IN THE HIND LIMB NOXIOUS HEAT EVOKED FLEXION WITHDRAWAL REFLEX?
K. Nishioka, Y. Harada, L.M. Kitahata* and J.G. Collins. Dept. of Anesthesiology, Yale Univ. Sch. of Med., New Haven CT 06510 (USA)

The role of WDR neurons in a withdrawal reflex(WR) evoked by noxious heat applied to the hind limb is not well understood. This IACUC approved study examined the relationship between WDR neuronal activity and WR evoked by the same heat stimulus in the same animals. Male rats were initially anesthetized with an injection of pentobarbital I.P. for tracheostomy, a jugular vein and a carotid artery cannulation. Anesthesia was maintained with I.V. pentobarbital. Animals were artificially ventilated, and $PaCO_2$, arterial pressure and rectal temperature were maintained within normal limits. A laminectomy (Th12-L1) was performed and animals were fixed in a stereotaxic frame. For the WR experiment, a projector lamp was focused on the blackened hind paw. The response latency and the threshold temperature were measured. After obtaining WR data, neuronal activity from a single WDR neuron with a receptive fields in the foot was recorded in the same animal. The response latency and the threshold temperature to activate the WDR neuron were measured and compared with WR latency and temperature. In addition, the maximum firing frequency(FF) produced by innoxious receptive field brushing and the heat evoked FF at the time point when WR occurred were compared. Student's t test was used for statistical analysis. Differences were determined to be significant with $P<0.05$. The latency of neuronal activity was significantly shorter than that of WR. The threshold temperature for neuronal activity was significantly lower than for WR. There was, however, no statistically significant difference between the maximum FF of WDR neurons evoked by the innoxious brush stimulus (26.07±5.20 spikes/sec) and the FF of WDR neurons evoked by the heat stimulus at the time point each animal showed a WR (22.26±4.15 spikes/sec). These results suggest that the FF of individual WDR neurons may not distinguish between noxious and non noxious stimuli at the time of WR. (Supported by N.I.H. NS-09871 for LMK)

130.9

COMPARISON OF THE NOXIOUS-EVOKED ACTIVITY OF DORSAL HORN MULTIRECEPTIVE NEURONS AND PAW WITHDRAWAL LATENCY IN THE LIGHTLY ANESTHETIZED RAT.
M. M. Morgan* and H. L. Fields. Depts. of Neurology and Physiology, Univ. of California, San Francisco, CA 94143.

Noxious cutaneous stimuli evoke intensity-dependent increases in the activity of nociceptive specific (NS) and multireceptive (MR) neurons in the dorsal horn of the spinal cord. Such stimuli also evoke spinally mediated withdrawal reflexes. The present study compared the activity of NS and MR neurons in the lumbar dorsal horn with the latency for paw withdrawal during application of noxious heat. In addition, changes in noxious-evoked neuronal activity and withdrawal latency produced by a distant noxious stimulus was assessed. Male Sprague-Dawley rats were anesthetized with halothane and a laminectomy was performed so as to record single unit activity from the lumbar enlargement. The latency to elicit a paw withdrawal reflex and the number of spikes occurring prior to the reflex were determined before, during, and after submersion of the distal third of the tail in 50 ^{o}C water. Placing the tail in hot water (or noxious ear pinch) caused a reduction in the evoked activity of almost all MR, and many NS neurons, but did not inhibit the paw withdrawal reflex. In fact, the latency of the reflex often was shortened by tail heat. These results demonstrate that the relationship between noxious-evoked activity of dorsal horn neurons and withdrawal reflexes is not simple. In certain circumstances, simultaneously activated descending input to the dorsal and ventral horns may result in inhibition of the evoked activity of MR neurons yet facilitation of withdrawal reflexes.

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130.10

IS THE RELATIONSHIP BETWEEN SUBJECTIVE PAIN SENSATION AND FLEXION REFLEX IN MAN INFLUENCED BY ELECTROACUPUNCTURE?
C.W.Y. Hui-Chan* and S. Mah. School of Physical and Occupational Therapy, McGill University, Montreal, Quebec, Canada H3G 1Y5.

The purpose of this study was to investigate whether the flexion reflex (a physiological measure) will vary with a psychophysical measure (the visual analog scale) in a systematic manner during three stimulating conditions: placebo-TENS, electroacupuncture to the contralateral leg (EA1), and electroacupuncture to the contralateral arm and leg (EA2).

Sixteen normal healthy subjects participated in the study. Electrical stimuli were delivered at maximally tolerable intensity to the sole of their right foot. Flexion reflex (FR) was recorded electromyographically in the ipsilateral biceps femoris, with subjects' estimates of perceived sensation registered on a visual analog scale (VAS) connected to a linear potentiometer.

A high *positive* linear correlation between VAS and FR area was observed for all three stimulating conditions in 56-63% of subjects showing a *decrease* in the FR area, the r value being 0.89 and 0.93 respectively for EA1 and EA2, in contrast to r=0.63 for placebo stimulation. Interestingly, a high *negative* linear correlation was noted in 37-44% of subjects showing an *increase* in the FR area, with r=-0.93 and -0.97 respectively for EA1 and EA2, as compared with r=-0.47 for placebo stimulation. In other words, electroacupuncture could modulate the (perceptual) output at the cortical level in the *same* or *opposite* manner from that at the spinal motoneuronal level (the FR), according to whether the latter output is inhibited or facilitated. A possible goal appears to be to generate an overall "subjective" perception of a decrease in pain sensation consequent to electroacupuncture, at least under the experimental paradigm investigated.

This research was supported by a grant from the Fonds de la recherche en Santé du Québec.

130.11

SENSITIZATION OF THE TAIL-FLICK REFLEX FOLLOWING EXPOSURE TO A SINGLE PROLONGED TEST STIMULUS A.E. Baldwin, E.J. Weihbrecht, J.G. O'Neill III, & J.T. Cannon*. Dept. of Psychology & Neuroscience Program, University of Scranton, Scranton, PA 18510-4596.

The tail-flick response to radiant heat has long served as a benchmark for research involving nociception in rodents. This reflex figured prominently in studies of stimulation-produced analgesia in which a common criterion for analgesia has been reflex inhibition to a "cutoff" latency (often twice the baseline, or more) at which time both the heat stimulus and the brain stimulation are terminated. It is not uncommon, at this point, for animals to emit a vigorous flick response, suggesting that the analgesic effects of stimulation are short lived and that the animal is now exposed to the sequelae of supra-threshold tail heating.

We examined the effects of a single supra-threshold tail-flick trial on subsequent responding. Male albino rats were individually housed with food and water freely available on a 12/12 hour light/dark cycle and tested during the dark phase. Anesthetized animals were tested beginning 40 min after pentobarbital injection (55 mg/kg, i.p.). Baseline tail-flick latencies (M = 3.7 sec) were obtained at 1 min intervals from 5 test spots separated by 2 cm beginning 2 cm from the tail tip. At the next test interval, animals (6/group) were either tested as before (control) or manually prevented from responding to heating of the tail for 5 or 7 sec or a hindpaw for 5 sec. Testing then continued as before for 30 min.

Compared to controls, tail-flick latencies were significantly reduced for both the 5 and 7 sec conditions, with no significant difference between them. This reduction occurred for all spots but was greatest for the spot which received supra-threshold stimulation. Reduced latencies were evident throughout the session. Stimulation of the hindpaw did not change tail-flick latencies. Animals in the 5 and 7 sec conditions were tested awake 1-3 days after the preceding and no differences between test spots were observed at this time.

Growing evidence suggests that test parameters defining tail-flick baseline latencies can alter the interaction between this reflex and a number of antinociceptive manipulations (e.g., Jensen & Yaksh, 1986; Ness & Gebhart, 1986). The current demonstration of long lasting sensitization after a single supra-threshold tail-flick trial suggests that other test parameters may also contribute to the dynamic interaction between the tail-flick reflex and antinociceptive mechanisms.

130.12

PHYSIOLOGICAL STUDIES ON MECHANISMS OF ACUPUNCTURE ANTINOCICEPTION IN THE RAT SPINAL CORD V.V. Romita,* K. Yashpal, C. Hui-Chan and J.L. Henry, Departments of Physiology & Psychiatry, and School of Physical & Occupational Therapy, McGill University, Montreal, Quebec H3G 1Y6

In a study of the neurophysiological and neurochemical mechanisms underlying analgesia produced by aversive sensory stimuli, male Sprague Dawley rats were anaesthetized i.p. with Na-pentobarbital (20 mg/kg) and chloral hydrate (120 mg/kg) and the effects of electroacupuncture were studied on the tail flick reflex. Electroacupuncture (20 x threshold for muscle twitch) was applied for 20 min to points *femur-futu* (ST-32) and *fengshi* (GB-31), approximated from charts localizing these points in humans. In control animals (n=7), analgesia began within 5 min of the start of the stimulation, with maximum effect occurring after 15 min. This effect lasted 30-40 min after the end of the stimulation. Each of the substance P receptor antagonist, CP-96,345 (5 mg/kg, s.c., n=6), and the opiate antagonist, naloxone (25 mg/kg, i.p.; n=8), attenuated the analgesia during the stimulation by 50-60%. Furthermore, each drug abolished the analgesia which persisted after the stimulation. In spinal transected rats (n=11) electroacupuncture had no effect within the first 2 days. However, in days 3-7 antinociceptive effects began to reappear. These results indicate the partial involvement of opioid and substance P mechanisms in the mediation of acupuncture analgesia. Among other possibilities, these data suggest that electroacupuncture may activate substance P-containing primary afferents which excite opioid-containing intrinsic spinal neurones. They also suggest recovery or plasticity of antinociceptive mechanisms following spinal transection. (Supported by the Fonds de la recherche en santé du Québec and the Canadian MRC)

VISUAL CORTEX: FUNCTIONAL CIRCUITS AND OSCILLATIONS II

131.1

OSCILLATORY DISCHARGES OF THE VISUAL CORTEX IN THE BEHAVING CATS. C. Lee*, J. Kim, J. Park, and S. Chung. Dept. of Psychology, Seoul Nat'l University, Seoul, Korea 151-742.

Synchronized oscillations in the stimulus-induced activities of neurons in the cat visual cortex have been implicated to encode global stimulus properties (Gray et als, Nature, 388:334, 1989). As a preliminary step toward understanding the behavioral significance of the synchronized oscillations, we examined the occurrence of the oscillatory discharge in the activities of the areas 17 and 18 of the alert cats implanted with scleral search coil for monitoring eye movements.

Periodic activities (20-120Hz) with varying magnitudes were observed in autocorrelograms derived from the stimulus-induced responses as reported previously (Gray et als, 1989) , while the eyes are fixed. Similar oscillations were also found in discharges obtained across wide range of behavioral states unrelated with encoding stimulus features: during saccadic eye movements, slow-wave sleep and REM sleep. These results indicate that the oscillatory discharge itself is not a sufficient conditon for encoding stimulus features. (Supported by Korea Science and Engineering Foundatin Grant 91-01-00-04).

131.2

Bursting Cells in Visual Cortex Exhibit Properties Characteristic of Intrinsically Bursting Cells in Sensorimotor Cortex

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Synchronous, 30-60 Hz oscillatory activity recorded *in-vivo* in visual cortex is often associated with burst firing in single cells (Gray *et al* 1990). Synchronous activity recorded *in-vitro* in sensorimotor cortex depends on the activity of intrinsically busting cells in layers IV and V (Silva *et al* 1991). These findings suggest a dependence on bursting cells for the synchronization of activity in visual cortex. In order to investigate the relationship between the two types of burst firing I have measured the amplitude and time dependent properties of burst firing in single cells recorded extracellularly in cat striate cortex. These cells exhibit the following properties: 1) bursts of spikes having 2-9 spikes/burst; 2) a large percentage of inter-spike intervals less than 3-4 msec in duration; 3) peak firing rates within a burst as high as 600 Hz; 4) three forms of spike modulation occurring within a burst over time: amplitude decrease; firing rate descrease; and spike width increase. Each of these properties, with the exception of peak firing rate, characterize the behavior of intrinsically bursting cells recorded in-vitro, and thus provide indirect evidence that burst firing in visual cortex is an intrinsic cellular property. A direct test of this hypothesis will require intracellular recording.

131.3

Stimulus-Induced Oscillatory Synchronization is Inhibited by Stimulus-Locked Non-Oscillatory Synchronization in Cat Visual Cortex: Two Modes That Might Support Feature Linking.

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Stimulus-induced oscillatory synchronizations (35-80Hz) were found in cat visual cortex. They were proposed to support feature linking in visual perception (Eckhorn et. al.1988, Biol. Cybern. 60:121). It was further suggested that stimulus-locked cortical synchronization supports feature linking in stimulus-dominated states. (Eckhorn et al. 1990; Neural Computation 2:293), and we found that stimulus-locked synchronizations can supress oscillatory activities completely. The present work concentrated on mechanisms and quantitative aspects of such depression. For this multiple microelectrode recordings were made from visual cortical areas 17 and 18. Oscillatory synchronizations were induced by constant velocity stimuli. Stimulus-locked synchronizations were evoked by random stimulus movements. A systematic change from oscillatory to stimulus-locked responses was achieved by superimposing random movements of different amplitudes upon the constant velocity. This caused a successive increase of synchronized stimulus-locked response power while oscillatory power was progressively suppressed, and the mean oscillation frequencies were slightly increased (grand average, N=104). Additionally, with our stimuli A18 showed on average higher levels of stimulus-locked power than A17. We propose that stimulus-locked activities suppress oscillatory synchronizations, (1.) because they activate inhibitory cortical neurons, that act on the oscillatory units and (2.) because they disturb the oscillatory cortical processes by their different time courses. We assume that feature-linking is supported by stimulus-locked synchronizations directly after ocular saccades or sudden object movements, while it is supported by oscillatory synchronizations during fixation and smooth ocular pursuit.

131.4

DYNAMICS OF COHERENT FIRING IN CAT VISUAL CORTEX.

C. Schwarz[1], A. Aertsen[2], and J. Bolz*[1]. [1]Friedrich-Miescher Labor der Max-Planck Gesellschaft, Spemannstr. 37-39, 7400 Tübingen, Germany and [2]Inst. für Neuroinformatik, Ruhr-Universität, P.O. Box 102184, 4630 Bochum, Germany.

Cross-correlation analysis in visual cortex has revealed that the most frequent pattern of neuronal interaction is synchronous firing - the so called 'common input'. To investigate whether the strength in coherent firing ('effective connectivity') might be used for encoding stimulus properties we recorded simultaneously from pairs of nearby single neurons or multi units in cat area 17 and 18 using various stimulus conditions. Combined variation in firing rates were estimated by the product of the peri-stimulus time histograms of the two sites (PSTprod). 'Effective connectivity' along the stimulus period (normalized peri-stimulus time coincidence histogram; NPSTCH) was assessed by computing normalized cross-correlograms (NCC) based on the joint peri-stimulus time histogram (JPSTH; A. Aertsen et al., J. Neurophysiol. 61: 900, 1989). Most cells in the same cortical column displayed narrower peaks in the NCC (range 1-6ms) as compared to cells in distant columns (range 5 - 12ms; C.Schwarz and J.Bolz, J. Neurosci. 11:2995, 1990). Whenever the NCC showed signs of synchronous firing, this synchrony was preserved under all stimulus conditions tested. However, NPSTCH's revealed that the 'effective connectivity' was enhanced as the stimulus moved across the receptive fields. Orientation tunings computed with NPSTCH's and PSTprod's showed in most cases similar orientation preferences. In several pairs NPSTCH tuning curves matched the selectivity of those measured with single PSTH's, but usually they were broader than those of PSTprod's. Similar results were obtained with cells in distant columns. In view of the weak interconnections usually observed among cortical cells, the modulations in 'effective connectivity' described here might be used to encode stimulus information in a form suitable for higher stages along the visual pathway.

131.5

THE EFFECTS OF BACKGROUND PHOTIC STIMULATION ON VISUAL CORTICAL HYPERSYNCHRONOUS BURSTING. E.W. Kinghorn, D.E. Fleming*, R.W. Rhees, Psychology and Zoology, Brigham Young University, Provo, UT 84602.

Photically evoked after-discharge (PhAD) bursting is initiated by retinal processes, entrained by the dorsal lateral geniculate nucleus (dLGN) of the thalamus, and modulated by input from the brainstem reticular formation. The current study was designed to investigate retinal processes, specifically the recovery time necessary for the initiation of PhAD following a conditioning light pulse. The Sprague-Dawley rat was used for the experimental procedures. A Grass PS22 photostimulator delivered photic brief pulses at intensity values of either 4, 8, or 16 on a scale of 1-16 as required by the experimental treatment. A second PS22 photostimulator was programmed to deliver a test photic pulse 50, 100 ,150, or 200 msec following the first (conditioning or background) pulse. The intensity level of the second photic stimulator was set at 8 on the 1-16 scale. Recording of the evoked cortical after-discharge was time-locked with the test (second) photic pulse. Examination of the data indicated that the intensity of the conditioning pulse had no significant effect on the occurrence of PhAD. On the other hand, the interval between the conditioning and the test pulses significantly impacted the production of PhAD. PhAD was significantly reduced in animals that received the test pulse 50, 100, or 150 msec following the conditioning pulse. At an interval of 200 msec between conditioning and test pulses PhAD bursting was not significantly different from control values. These data suggest that at the brightness values used in this study, a relatively long retinal time-constant exists for the recovery of cortical hypersynchronous bursting.

131.6

THRESHOLD PROPERTIES OF INDIVIDUAL MODEL NEURAL OSCILLATORS CONTRIBUTE TO RAPID NETWORK SYNCHRONIZATION AND MAY POTENTIALLY PLAY A ROLE IN PERCEPTUAL FEATURE BINDING. D. Somers* and N. Kopell, Department of Cognitive and Neural Systems and Department of Mathematics, Boston University, Boston, MA 02215

The synchronization properties of locally coupled neural oscillators were investigated analytically and by computer simulation. When coupled in a manner that mimics excitatory chemical synapses, oscillators having fast–slow interactions (relaxation oscillators) were found to approach synchrony using very different mechanisms than oscillators with a more sinusoidal waveform. The relaxation oscillators make critical use of fast modulations of their thresholds, a mechanism that leads to a rate of synchronization relatively independent of coupling strength within some basin of attraction; this rate is faster for oscillators that model conductance attributes than for neural caricatures such as the FitzHugh-Nagumo equations that lack such attributes. Computer simulations of one–dimensional network arrays show that oscillators in the relaxation regime synchronize much more rapidly than oscillators with the same equations whose parameters have been modulated to yield a more sinusoidal waveform. These results suggest that the emergent synchronization behavior of oscillating neural networks can be dramatically influenced by the intrinsic properties of the network components.

In the context of the reported synchronized oscillations in visual cortex (Gray et al., Nature, 338,1989), these results show that prior conclusions (Kammen et al., 1989) drawn about the neural connectivity required for rapid synchronization do not generalize as claimed; purely local connections may yield rapid synchronization. Furthermore, under the synchronized binding hypothesis of perception, selective modulation of intrinsic oscillator properties may permit a focal subpopulation of cells to synchronize (and thus become perceptually coherent) much more rapidly than the rest of the population and thus may potentially serve as an "attentional searchlight" mechanism.

131.7

A COMPARISON OF SINGLE NEURON AND NEURAL POPULATION DYNAMICS. Heather L. Read* and Ralph M. Siegel, CMBN, Rutgers University, Newark, NJ 07102.

When the retina is bombarded with periodic flash stimuli, primary visual cortical neurons become entrained to the stimulus rhythm to various degrees depending on the stimulus parameters (Siegel, 1990). Some of the essential features of periodically driven visual cortical neurons are observed in a periodically driven model of a population of neurons with intralaminar excitatory connections. Firing of neurons in this model is dictated by the Hodgkin-Huxely differential equations. In the present study, the dynamics of a single sinusoidally driven H-H model neuron were explored and the frequency-amplitude parameter space was mapped. Over much of the parameter space the model neuron displayed simple phase locking patterns. That is, action potentials occurred at a regular phase of the stimulus cycle so that the ratio of spikes to stimulus cycle was some rational value. Transitions from a given phase locked pattern to another with changes in stimulus frequency were abrupt, particularly when low amplitude stimuli were used. Phase locking and transition zones of the model neuron were similar to those observed in the normal squid axon but were considerably more compressed than those of the spontaneously oscillating squid axon (Gutman et al., 1980; Matsumoto et al., 1987). Unstable, quasi-periodic firing patterns were observed between regions of stable phase locking. In these regions, spikes no longer occurred at a fixed phase but instead occurred unpredictably. Interspike interval times for phase locked and quasi-periodic activity were exact or near integer multiples of the stimulus period. During quasi-periodic activity, the range of ISIs was extended in a manner similar to that of the population model and real visual neurons. The isolated H-H model neuron differed from the H-H neuron population in that: 1) the parameter space for evoking quasi-periodic activity was larger in the population and 2) the deviations of ISIs from multiples of the stimulus period were more prominent in the population. These results suggest that flash evoked visual cortical neuron activity patterns arise from the dynamics of the population rather than from the quasi-periodic behavior of the single neuron.

131.8

MEASURING NEURONAL INFORMATION TRANSMISSION WITH NEURAL NETWORKS. T. W. Kjaer, B. J. Richmond*, and J. A. Hertz. Nordita, Copenhagen, and Laboratory of Neuropsychology, NIMH, Bethesda, MD.

The content and utility of the signals carried by single neurons in the visual system is not always obvious. Thus, methods that help identify and quantify the contents of these signals are needed for making and testing hypotheses about neuronal function. We have used neural networks to decode signals from neurons in macaque primary visual cortex. The inputs to the net were the principal components of the responses elicited by a set of 16 1-dimensional Walsh-pattern stimuli. The network had 16 normalized-exponential output units, one for each stimulus pattern, and when the input was a signal produced by pattern number p, the target output pattern consisted of a 1 for unit number p and 0 for all other units. Back-propagation learning was used to minimize a cross-entropy cost function. The numbers of hidden units and training cycles were optimized by cross-validation on data not used in making the fit. Once this fit has been made, the average transmitted information was computed by sampling either the training or validation sets. We also tried networks that implement a Parzen-window fit similar to the one used previously by Optican and Richmond (1987) and by Optican et al (1991). The back-propagation neural network gave superior fits: The validation-set cross-entropy for it was always lower than that for the Parzen-window net, with an average difference of 0.17 bits. We computed the information transmitted about the stimulus pattern from the optimized model for each of the 9 cells, finding an average transmitted information of 0.74 bits, with a fraction ranging from 14% to 60% contained in the temporal variation of the signal. The systematic fit optimization using the validation-set error criterion permits greater confidence in the resulting information transmission estimates than was possible with previous calculations.

VISUAL CORTEX: ANATOMY OF EXTRASTRIATE CORTEX

132.1

LOCAL PATCH NEURONS AND CALLOSAL NEURONS COMPRISE SEPARATE, MORPHOLOGICALLY DISTINCT POPULATIONS IN THE UPPER LAYERS OF CAT AREA 18. D.M. Thejomayen*, J. Zhang, and J.A. Matsubara. Depts. of Anatomy and Ophthalmology, University of British Columbia, Vancouver, British Columbia, Canada, V5Z 3N9.

The pyramidal neurons in the upper layers of cat visual cortex participate in several anatomical systems, such as the patchy, intrinsic connections, the corticocortical and callosal projections. Earlier intracellular studies suggest that many upper layer pyramids possess a local axon collateral, which is often patchy in its arborization pattern, plus a descending axon trunk entering white matter. Thus, it would appear that at least some, if not all, of the local patch neurons in the superficial layers may network locally and participate in a projection pathway.

The morphology of callosal neurons in the upper layers was compared to the local patch neurons. Cells belonging to different projection pathways were identified by retrograde transport of fluorescent dextrans, followed by intracellular filling with lucifer yellow of prelabeled cells in 250 μm fixed sections taken in coronal and tangential planes. Cells were imaged with a confocal microscope (Biorad MRC 600).

Overall, the callosal and local patch cells form separate and distinct populations based on 1. somatic size, 2. dendritic branch pattern and 3. dendritic field dimensions. 1. The somatic areal measurements showed mean values which were quite distinct: local cells at 236 μm^2 (sd=99 μm^2) vs callosal cells at 379 μm^2 (sd=93μm^2). In the callosal population, there were more star pyramids, of all sizes, whereas only a few, small star pyramids were seen in the local patch sample. 2.Callosal cells possess a much more complex dendritic branch pattern, with numerous branch points. 3. Finally, callosal cells have a symmetric, circular dendritic field when viewed in the tangential plane, with the medial-lateral (ML) axis dimension equivalent to the anterior-posterior (AP), while local patch cells have a ML:AP ratio of 1.3. Thus, we await future studies to identify the output target(s), if any, of the local patch neurons. This work funded by MRC (Canada) 5-99150 (JM).

132.2

THE DISTRIBUTION OF CALLOSAL CELLS CORRELATES WITH DENSE CYTOCHROME OXIDASE STRIPES IN V2 OF THE MACAQUE MONKEY. J.F. Olavarria* and J. W. Lewis, Department of Psychology, University of Washington, Seattle, WA 98195.

In visual cortex, the patterns formed by inhomogeneities in the distribution of callosal connections are thought to be closely associated with spatial attributes of the underlying visual maps. For instance, callosal connections accumulate preferentially in regions representing the vertical meridian of the visual field. However, the possibility that callosal connections also participate in the preferential transfer of specific kinds of visual information has received less attention. We have investigated this possibility by correlating the distribution of callosal cells with the pattern of cytochrome oxidase (CO) stripes in V2 (CO-dense thick and thin stripes, and pale interstripes). Several lines of evidence suggest that these stripes relate to specialized functional streams in visual cortex.

Callosal cells were retrogradely labeled with the TMB-HRP technique after implants of acrylamide gel-HRP were placed in the transected corpus callosum. The patterns of callosal connections and of CO staining were revealed in series of sections cut tangentially to the surface of the physically unfolded and flattened cortex, and close correlation of both labeling patterns was facilitated by careful superimposition of these sections.

Data from three monkeys indicate that callosal cells accumulate in finger-like clusters which extend perpendicularly from the V1/V2 border up to 7 mm into V2. In addition, these clusters are in register with CO-dense stripes (thick and thin), with few cells in the interstripe regions. Statistical tests confirmed the significance of this correlation. Thus, it appears that the distribution of callosal connections in V2 is dictated not only by the topography of V2, but also by the arrangement of functional streams. By extending to interhemispheric pathways the notion of functional specificity currently associated mainly with visual *intrahemispheric* pathways, these results also open additional avenues for teasing apart the functional contributions of the CO subregions in V2.

132.3

COMPARTMENTAL DISTRIBUTION OF GABA NEURONS AND THEIR SUBPOPULATIONS IN MONKEY V2. R.K. Carder*, V. Wan and S.H.C. Hendry. Dept. of Anatomy and Neurobiology, College of Medicine, University of California, Irvine, CA 92717.

V2 is a mosiac of three compartments, thick, thin and pale cytochrome oxidase (CO) stripes, each displaying unique physiological and connectional properties. To determine the neurochemical features of these compartments we have focussed upon GABA neurons which comprise the major population of inhibitory interneurons within the cerebral cortex. In V2 of normal Old and New World monkeys, somata and terminals immunoreactive for GABA are unevenly distributed, forming stripes perpendicular to the V1/V2 border. Comparison with an adjacent section stained for CO demonstrates increased GABA immunostaining within both the thick and thin CO stripes. Neurochemically distinct subpopulations of GABA neurons labelled by two members of the calcium binding protein family, calbindin and parvalbumin, are also preferentially distributed within the CO stripes. These patterns within the CO stripes include calbindin immunoreactive neurons as well as parvalbumin immunoreactive neuropil. The distribution of GABAergic elements parallels the organization of monkey V2 into functionally distinct subdivisions, suggesting that intrinsic elements as well aspecific afferents may contribute to unique physiological properties within each of the compartments of V2. Supported by EY06344.

132.4

INTRINSIC CONNECTIVITY IN MACAQUE V2: EVIDENCE FOR INTERACTION BETWEEN FUNCTIONAL STREAMS. J.B. Levitt*, T. Yoshioka and J.S. Lund. Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Primate visual cortical area V2 contains three alternating stripelike compartments distinguished by their differential staining for cytochrome oxidase (CO) and Cat-301. These three compartments have different afferent and efferent connectivity, and contain neurons with predominantly different physiological properties. Since different types of visual information are relayed through closely-apposed parallel stripes of tissue in V2, this area might provide a locus for interaction of different visual channels.

We made focal (200-300 μm) microinjections of biocytin into V2 to determine whether there are specific interconnections between compartments. Alternate tissue sections were reacted for CO or Cat-301, to identify injected and labeled sites relative to compartments. Discrete patches of terminal label (250-300 μm diam) were found within both injected and neighboring stripes. Clusters were found up to 4 mm from an injection site, and were distributed in an elongated field orthogonal to the stripes. While a substantial proportion of each stripe's local afferent and efferent connectivity was within the same compartment, connections were also made with **both** the other compartments. As previously reported (Livingstone and Hubel, 1984), we found preferential connections between CO-rich compartments, and we have confirmed that pulvinar terminals in V2 are precisely coincident with the CO-rich stripes. However, pale stripes were equally connected to CO-rich or pale zones. Patchy connectivity within stripes, and interconnections between all compartments, suggests both compartment substructure and a substrate for channel interactions. Specific inter-compartment connectivity rules may not be necessary to explain these data; we are currently exploring whether they might reflect simple constraints on the range over which local connections may occur. (Supported by NIH grants EY05282, EY06275, EY08098, and ARVO/Alcon).

132.5

PHA-L STUDY OF CONNECTIONS FROM TEO AND V4 TO TE IN THE MONKEY VISUAL CORTEX. K.S. Saleem[1]*, K. Tanaka[1,2], and K.S. Rockland[3], [1]Lab. for Neural Information Processing and [2]Information Science Lab., RIKEN, Japan; [3]Dept. of Neurology, University of Iowa, USA.

TE is an anterior extrastriate area located at a latter portion of the ventral visual pathway. Cells in TE require complex object-features for activation, and cells with similar stimulus selectivity cluster in columns within TE (Fujita et al., Soc Neurosci Abstr, 1991; Vol 17, p 1283). In the present study, we analyzed the organization of afferents to TE by injecting PHA-L in two Japanese monkeys (*Macaca fuscata*). Iontophoretic injections were made in two sites in TEO in the first case and in two sites in V4 in the second case. The diameter of the injection sites was 0.7 to 1.0 mm.

After injections in TEO, labeled axons were distributed with 3 dense core regions in TE. In the core regions, labeled axons formed distinct clusters, which extended through all cortical layers. The core regions measured 250 to 400 μm along the mediolateral axis. Along the postero-anterior axis, one core measured 800 μm and the other two were split into two subregions, which measured 200 to 300 μm each and were separated by 120-160 μm. Beyond the core regions, labeled axons became less prominent in the supragranular layers, and, at the fringe of the projection zone, were more limited to layers 4, 5 and lower 3 (middle layers). After Injections in V4, labeled axons were more sparsely distributed in TE and more limited to layer 4 than TEO case.

Single axons reconstructed in the TEO case from regions just surrounding the cores have prominent branches reaching layers 1 and 2 (n=2), whereas those reconstructed from fringe regions have arbors limited to the middle layers (n=2). There were 3-5 arbors, each 100-250 μm in diameter. The total postero-anterior extent of the arbors of single axons ranged 0.64 to 3.6 mm. Axons reconstructed in the V4 case (n=3) also had arbors mostly limited to the layer 4. There were 2-3 arbors, each 100-250 μm in size.

We suggest that the specific and columnar projection from TEO to TE may constitute one of the bases of the functional columnar organization in TE.

132.6

PROJECTIONS TO VISUAL AREAS V4 AND TEO FROM TEMPORAL, PARIETAL, AND FRONTAL LOBES IN MACAQUES. H. Nakamura*, R. Gattass, R. Desimone, and L. G. Ungerleider. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

To investigate differential inputs to areas V4 and TEO, we injected them with different retrograde tracers and analyzed the distributions of labeled neurons in the temporal, parietal, and frontal lobes in flattened preparations of the cortex. In inferior temporal area TE, V4- and TEO-projecting cells showed a patchy distribution with overlapping and nonoverlapping regions extending forward to the rostral tip of the area. Whereas the labeled cells were predominantly in the infragranular layers in rostral TE, in caudal TE they had a bilaminar distribution. In parahippocampal area TF and perirhinal area 36, V4- and TEO-projecting cells were intermingled. In the intraparietal sulcus, labeled cells were seen in both LIPv and LIPd; V4-projecting cells were located mainly in the infragranular layers of LIPv, while TEO-projecting cells were located mainly in LIPd where they had a bilaminar distribution. In the prefrontal cortex, V4- and TEO-projecting cells were intermingled in the anterior bank of the arcuate sulcus (area 8) and extended rostrally into area 46, although there was a tendency for V4-projecting cells to be located more posteriorly than TEO-projecting cells. Thus, V4 and TEO receive similar, though not identical, inputs from temporal, parietal, and frontal areas.

132.7

ARBORIZATION PATTERNS OF INDIVIDUAL AXONS PROJECTING FROM CAUDAL TO ROSTRAL INFERIOR TEMPORAL CORTEX IN SQUIRREL MONKEYS. G. E. Steele* and R. E. Weller. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Based on patterns of cortical connections and architectonics, inferior temporal (IT) cortex of squirrel monkeys consists of rostral (ITR) and caudal (ITC) regions. ITR is defined as the projection zone of ITC (Weller & Steele, J. Comp. Neurol., in press). In macaque monkeys, the region of cortex comparable to ITR contains nontopographically organized neurons with large receptive fields that respond best to complex visual stimuli. These characteristics may be due to convergent and/or divergent input from ITC. In order to investigate these possibilities, the present study examined the terminations of individual axons in ITR labeled from iontophoretic injections of the neuronal tracer biocytin in ITC (4 cases) or from pressure injections of horseradish peroxidase into the white matter underlying ITC (4 cases). Axons in ITR terminate in layer IV and lower layer III. Individual boutons were evenly spaced along terminal branches, with approximately 3 boutons per 20 microns. Axons reconstructed to date have relatively limited terminal arbors, suggesting that the receptive field properties of neurons in ITR are not due to divergent projections from single neurons in ITC. Supported by EY07147 to REW and MH10070 to GES.

132.8

ORGANIZATION OF CORTICAL VISUAL PROJECTIONS TO THE DORSAL AND VENTRAL PARTS OF AREA TE OF THE INFEROTEMPORAL CORTEX IN MACAQUES. M.Yukie*, K. Hikosaka and E. Iwai. Dept. of Behav. Physiol., Tokyo Metro. Inst. Neurosci., 2-6, Musashidai, Fuchu-City, Tokyo 183, Japan.

In previous studies we have shown that the dorsal and ventral parts (TEd and TEv parts) of area TE of the macaque inferotemporal cortex are distinguished from each other by their connectivity with the amygdala and hippocampus CA1 area and have suggested that area TE may not be always an unitary area. However, it is unclear in what manners visual afferents into area TE are organized from the posterior visual areas TEO and V4. In the present study, to clarify organization of cortical visual afferents to the TEd and TEv areas, injections of retrograde tracers, WGA-HRP and fluorescent dyes, Fast Blue (FB) and Diamidino Yellow (DY), were made into different levels of area TE of Japanese monkeys (*Macaca fuscata*). Following WGA-HRP injections into the anterior and posterior TEd (TEad and TEpd) portions, predominant distribution of labeled cells was seen, respectively, in the TEpd and dorsal TEO (TEOd) areas and in the TEOd portion and part of area V4 located between the lunate and inferior occipital sulci. By contrast the anterior and posterior TEv (TEav and TEpv) received an abandunt projection from the TEpv and ventral TEO (TEOv) portions and from the TEOv and part of area V4 of the inferior occipital gyrus, respectively. Separate injection of DY and FB into the TEpd and TEpv in an animal produced discrete regions of DY and FB labeled cells in each of areas TEO and V4, with some small overlap of distribition. These results demonstrate that area TE is subdivided into the anterior and posterior TE areas by presence or absence of direct afferents from area V4 and that visual afferents to the TEd and TEv areas from areas TEO and V4 are differentiated roughly and suggest that the TEd and TEv areas receive major afferents, respectively, from the representation of the central visual field and from that of the peripheral visual field of areas TEO and V4.

132.9

ORGANIZATION OF CORTICAL PROJECTIONS TO SUPERIOR TEMPORAL POLYMODAL CORTEX OF THE RHESUS MONKEY: DOUBLE ANTEROGRADE LABELLING STUDIES. B. Seltzer*, M. Cola, M. Massee, C. Weldon, and C.G. Cusick. Depts. of Psychiatry & Neurology and Anatomy, Tulane Univ. Schl. of Med.; VA Med. Ctr., New Orleans LA 70112.

Previous studies showing multiple cortical projections to superior temporal polymodal (STP) cortex in the upper bank of the superior temporal sulcus (STS) involved tracing connections in multiple hemispheres. To examine how different afferent cortical inputs interrelate within STP cortex, we used a double anterograde labelling strategy. In 5 different cases, 1 cortical zone that projects to STP cortex, e.g. caudal inferior parietal lobule, was injected with tritiated amino acids and another, e.g. mid-superior temporal gyrus, with WGA-HRP. Alternate coronal sections of the hemisphere, processed for radio- and HRP-labelled terminal fibers, were then compared. Many afferents of different cortical origins are segregated, but there are also columns within STP cortex that receive directly converging input from 2 different regions. Both overlapping and nonoverlapping laminar patterns of termination are found. The different target zones correlate with cyto-, myelo- and chemoarchitectonic divisions of the STS. Most of the convergence takes place within areas "TPO" and "PGa" (Seltzer and Pandya, '78). Supported by the VA, NIH, and Tulane University.

132.10

PROJECTIONS OF THE CORTICAL LENS ACCOMMODATION AREA IN THE CAT. H. Maekawa, K. Ohtsuka* and M. Sawa. Dept. of Ophthalmology., Sapporo Medical College, Sapporo 060, Japan.

It is well known that the lateral suprasylvian (LS) area of the cat has important roles in the control of lens accommodation. Neurons in the LS area exhibit burst of discharge preceding the onset of accommodative responses, and microstimulation of the LS area elicite accommodative responses. However, anatomical connections of accommodation-related area in the LS area are not established. In the present study, we injected WGA-HRP into the accommodation-related area which was identified by microstimulation, and examined afferent and efferent connections of the cortical accommodation area in the cat.

Retrogradely labeled cells were found mainly in ipsilateral Brodmann's area 18 and 19, pulvinar, lateral posterior nucleus of the thalamus and the contralateral LS area. Axons arising in the accommodation area ran through the retrolenticular portion of the internal capsule and the pulvinar, and then terminated in the ipsilateral pretectum, superior colliculus, and pontine nuclei. Labeled terminals were also found in the ipsilateral lateral posterior nucleus of the thalamus, pulvinar and the contralateral LS area.

These findings suggest that the cortical accommodation area receives visual inputs from Brodmann's area 18 and 19, and projects accommodation-related signals to the superior colliculus, pretectum and thalamus.

VISUAL CORTEX: PHYSIOLOGY OF STRIATE CORTEX

133.1

RESPONSES OF CELLS IN THE CAT'S LGN AND AREA 17 TO RIVALROUS STIMULI. Frank Sengpiel[1], Richard Harrad[2] and Colin Blakemore[1]. [1]University Laboratory of Physiology, Oxford OX1 3PT, and [2]Department of Ophthalmology, Bristol BS1 2LX, U.K.

Current models of binocular rivalry, supported by recent psychophysical evidence, postulate the existence of reciprocal feedback inhibition between monocular neurons prior to binocular integration. Hence, the suppressive circuitry should be located in either the LGN or layer 4 of area 17.

We recorded from neurons in both LGN and area 17 of anaesthetized cats. Moving grating stimuli were presented to the two eyes separately on two CRT monitors. Binocular interaction was tested by presenting a constant stimulus of optimal orientation to the dominant eye, and varying stimuli, randomly interleaved, to the non-dominant eye.

Responses of cells studied in the A and A1 laminae of the LGN were either not affected by stimulation of the non-dominant eye, or were tonically inhibited, independent of the orientation or phase difference between the stimuli.

In area 17, many binocular units were transiently or tonically inhibited by gratings presented to the non-dominant eye, when the orientation of the grating differed by more than 30° from the preferred one. In some cells inhibition occurred in some trials, but was absent in others. The effect did not depend on the relative phases (disparity) of the gratings, in contrast to interactions found for orientation differences of 25° or less. No monocular cells found so far displayed inhibition of this type relayed through the silent eye.

Our data give the first evidence for a type of suppression in the primary visual cortex that might underlie binocular rivalry. It is observed at the level of binocular rather than monocular cells. - Supported by the MRC.

133.2

SPATIOTEMPORAL INTERACTIONS OF POSTSYNAPTIC FIELD POTENTIALS IN CAT VISUAL CORTEX: POSSIBLE INTRACORTICAL INTEGRATION OF VISUAL AFFERENTS. M. Kitano*, K. Niiyama, R. Kuroda and M. Ioku. Dept. of Neurosurgery, Kinki Univ. Sch. of Med., Osaka 589, Japan.

Cortical neurons in area 17 have extensive afferent and efferent connections between large cortical areas. We found these anatomical data did not reflect classical receptive fields of single units, but did "receptive fields" of postsynaptic potentials evoked within a small cortical mass (Soc. Neurosci. abst. 1991). In the present study, we simultaneously recorded local field potentials from two cortical sites within area 17 of the anesthetized and paralyzed cat using two pairs of bipolar electrodes. One pair of bipolar electrodes (tip separation, ~1mm) were placed at corresponding sites near the area centralis (P,5mm; L,1mm; D,1mm), the other was near the 15-deg periphery (P,1 mm; L,2mm; D,6.5mm). Field potentials were evoked by the pseudorandom temporal modulation of contrast reversal of gratings at 61 5-deg patches covering a 45-deg visual field centered on the area centralis. Each of the 61 patches was modulated simultaneously but independently. Responses corresponding to each of the 61 patches were extracted by a multi-input, non-linear systems analysis method.

With single-patch stimulation at least two types of postsynaptic components were identified: a fast retinotopic component and a slow non-retinotopic component with a very large receptive field. With all-patch stimulation only the retinotopically restricted fast component of field potentials was obtained. The disappearance of the non-retinotopic components with all-patch stimulation indicates the presence of non-linear spatial interactions. These findings confirm our early suggestion of the presence of long-range lateral interactions within visual cortex.

133.3

RESPONSES TO BROAD-BAND AND BAND-LIMITED NOISE IN V1: DEPENDENCE ON SPATIAL FREQUENCY, COLOR, AND ORIENTATION. E. Katz*, K. Purpura, B. Mao, C. Schroeder† and J. Victor Dept. of Neurology and Neuroscience, Cornell University Medical College, New York, NY 10021 and †Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

Responses to visual patterns were recorded from anesthetized and paralyzed monkeys using multi contact electrodes that spanned the depth of parafoveal V1. We used visual stimuli that were broad-band in space and time (based on M-sequences) to map the linear and non-linear inputs to the local field potential (LFP). In all cases these inputs were localized to a one deg. region, and often in a 20 min region.

We used band-limited Gaussian noise to define the spatial frequency and orientation tuning of the LFP at each of the 16 electrode contacts. On a scale of 150μ, neural assemblies showed well-defined spatial frequency preferences which correlated with their responses to checkerboards and gratings. In some cases spatial frequency tuning varied along the vertical penetration. Upper sites tended to show a preference for higher spatial frequencies. Substitution of chromatic isoluminant patterns for achromatic patterns shifted the spatial frequency tuning to lower frequencies. Many electrode sites showed evidence of orientation tuning. Orientation tuning was manifest by changes in LFP dynamics, but not necessarily amplitude.

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133.4

RESPONSES TO BROAD-BAND AND TEXTURE STIMULI IN V1: EVIDENCE FOR SPATIAL AND TEMPORAL INTERACTIONS. K. Purpura*, E. Katz, B. Mao, A. Canel, and J. Victor. Department of Neurology and Neuroscience, Cornell University Medical College, New York, NY 10021.

Grouping of spatial and temporal features in the visual world is an important aspect of early visual processing. We examined response properties of neural assemblies in monkey V1 to evaluate the extent of spatial and temporal interactions (nonlinearities) and their contributions to visual grouping.

Recordings were made in 5 anesthetized and paralyzed monkeys (*Macaca fascicularis*). Local field potentials (LFPs) were sampled simultaneously from many layers of parafoveal V1 with a 16-element electrode. The texture stimuli all shared the same spatial power spectrum but differed in higher-order correlation statistics (elements of form). Broad-band stimuli were generated by assigning regions of the visual stimulus to different taps into a binary M-sequence. Binary M-sequence values were mapped to either two luminances or orientations of a bar.

At all electrode penetration sites in V1, and in many cortical layers, differential responses were observed to the interchange between textures differing in form elements. Linear summation, rectification or energy computation cannot account for these results; a successful model requires cooperative interaction between spatial subunits. Responses to M-sequence stimuli also revealed interactions in space and time. There was a significant contribution to the LFP which depended on whether or not stimulus tokens (luminance or orientation) presented simultaneously to two neighboring regions matched. Similarly, there was a significant contribution to the LFP which depended on whether the tokens presented to a single region on successive frames matched. The nature of the LFP suggests that these interactions occur at the level of local neural assemblies in V1. Our data suggest that such interactions are widespread in monkey V1 and as such, V1 may perform simple spatial grouping operations.

Supported by the McDonnell-Pew Program (KP) and EY9314 (JV)

133.5

VERGENCE ANGLE MODULATES NEURAL ACTIVITY IN PRIMATE AREA V1.

Y. Trotter, B. Stricanne, S. Celebrini, S. Thorpe and M. Imbert*. IDN, Lab. Neurosc. Vision, Univ. Paris 6, France.

We recently demonstrated (Trotter et al, Neurosci. Abstract, 1991) in behaving monkeys that the visual responsiveness and the background activity of neurons in area V1 were clearly modulated by the viewing distance. Disparity sensitivity was often present only or sharpened at a given distance of fixation (20, 40, 80 cm) with no obvious change in peaks. An increase in the amount of spontaneous activity for a particular viewing distance was often associated but independent of the modulation of the visual responsiveness. The question now is to determine the origin of these modulations.

To emphasize these phenomena, additional experiments were repeated in a behaving monkey performing a fixation task by using random dot stereograms of different dot densities. In all nine neurons tested with RDSs of dot densities between 10 and 30% at different viewing distances, the modulation of disparity sensitivity and of background activity was shown to be independent of the dot pattern. Although some neurons preferred a particular dot density, the qualitative effect of the viewing distance was always maintained.

The question is raised as to whether vergence angle or accommodation or both are implicated in these phenomena. The preliminary data show that changing the vergence angle by using wedge prisms (from 4 to 10 dioptries), replicates the effects of real changes of the fixation distance. When the effects of distance on disparity sensitivity are present, they can be replicated by using prisms producing similar vergence angles. We conclude that vergence angle is implicated in the modulation of visual responsiveness of neurons in area V1, emphazing the hypothesis of an extraretinal control of neural activity in the primary visual cortex.

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133.6

MULTI-DIMENSIONAL RECEPTIVE FIELD PROPERTIES OF NEURONS IN V1 OF THE AWAKE MONKEY.

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Multiple single neuron responses were recorded from a single electrode in V1 of a monkey performing a fixation task. Drifting sinusoidal gratings were presented for 500 ms in the cells' overlapping receptive fields. The stimulus was varied along several visual dimensions: orientation, spatial and temporal frequency, and direction of motion. Several cells exhibited inseparability of tuning to these visual dimensions. The strength of the interactions varied among neurons, suggesting a continuum from fully separable to truly multi-dimensional receptive fields. In addition, several cells showed different temporal response dependences to variation of different stimulus dimensions. For example, a cell might respond with a change in average firing rate as a function of one dimension (e.g. orientation), and a change in depth of modulation to variations of another (e.g. spatial frequency). Thus neurons may simultaneously code for different stimulus dimensions, increasing their information-processing capabilities.

Although tuning of most simultaneously recorded cell pairs was similar, each of the dimensional preferences and bandwidths could differ considerably. Time-dependent cross-correlation analysis revealed that joint tuning for pairs of neurons was often narrower than for either of the two individual receptive fields.

A model is proposed, based on statistical wiring within the cortical column, and differential temporal response properties controlled by cell membrane potential. The model accounts for multi-dimensional response properties and the computation of a precisely tuned population response despite individual neuron variance.

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133.7

BINOCULAR COMBINATION OF CONTRAST SIGNALS IN THE STRIATE CORTEX OF MACAQUE MONKEYS.

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We used extracellular microelectrode recording techniques to examine the binocular combination of contrast signals in the monkey's striate cortex. Neuronal responses were measured for dichoptic sinusoidal gratings. The spatial frequency, orientation, and relative interocular spatial phase parameters were optimized for each cell. The dependent variable was the interocular contrast ratio. For each interocular contrast asymmetry, the threshold contrasts required to produce a criterion response were derived from contrast response functions and used to construct binocular interaction contours.

Simple cells, with few exceptions, combined contrast signals from the two eyes in a linear manner. Complex cells exhibited a range of binocular interactions. Complex cells that were sensitive to relative interocular spatial phase and some non-phase selective cells also demonstrated linear binocular interactions. As with simple cells, the slope of the binocular interaction contours for these complex cells varied with ocular dominance, and the nature of the binocular interactions, i.e., facilitation vs suppression. The remaining complex cells demonstrated non-linear interactions, which in some cases, resembled quadratic binocular summation.

133.8

DISTRIBUTION OF PROPERTIES OF ENVELOPE RESPONSIVE NEURONS IN AREA 17 AND 18

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Some neurons respond to envelope stimuli which consist of a stationary high spatial frequency luminance grating (carrier) with its contrast modulated by a moving low spatial frequency sinusoidal grating (envelope). For a given neuron, the dependency on carrier spatial frequency (SF) shows a narrow bandpass higher than the luminance passband of the cell; effective envelope SFs are lower than the luminance passband. A model involving two parallel streams has been proposed to explain the envelope and carrier SF dependencies (ARVO, 1992). One is the luminance response stream, a bandpass tuning mechanism. The other is the envelope response stream, a three-stage cascade: high SF bandpass subunits, a pointwise nonlinearity, and a low SF bandpass.

The distribution of optimal carrier SFs for different neurons suggests that the upper limit of the optimal carrier is the cutoff SF of retinal ganglion X-cells. The optimal carrier-to-luminance SF ratio covers a range from 4 to 30, among 28 envelope responsive cells. This ratio appears to be an even distribution over 4 to 20, with only one cell's ratio at 30. Furthermore, there is no correlation between the optimal carrier and luminance SFs. This distribution of optimal carrier and luminance SFs imposes constraints on the passband of the subunits in the envelope response stream for a given neuron.

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133.9

AXES OF REFERENCE FOR THE STUDY OF THE VISUAL SYSTEM AND VISUAL TOPOGRAPHY OF STRIATE CORTEX (V1) IN THE MEGACHIROPTERAN BAT, *Pteropus*.

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As a first step in the electrophysiological study of the coritcal and subcortical visual areas in megachiropterans, we defined the relationship between the horizontal (HM) and vertical (VM) meridians and the optic disc (OD), and explored the visuotopy of area V1. The representation of the visual field in area V1 was studied by multi-unit recordings in 5 flying foxes anaesthetised with thiopentone and paralysed with pancuronium bromide. Receptive fields (RFs) were recorded across the striate/prestriate border in both hemispheres, and the position of the VM was defined as the mean reversal point of the RF sequences across the border. The mean position of the VM was found to be 35º nasal to the projection of the OD. The ipsilateral invasion of RF borders did not exceed 5º in the central field, and 20º in the periphery. The HM was estimated by determining the visual streak, using ganglion cell counts in retinal flat-mounts, and was found to lie 4.5º below the centre of the projection of the OD. Perimetric studies revealed at least 110º of binocular overlap along the HM; the whole visual field encompasses approximately 260º. A precise map of the contralateral hemifield was observed in V1, with lateral sites yielding upper quadrant RFs, medial sites yielding lower quadrant RFs, and the HM bisecting V1 in nearly equal halves. Only the central 20-30º are represented on the dorsal surface. The remaining parts of the visual field are located along the medial wall and splenial sulcus. Typical receptive field sizes in V1 are about 1º in the central representation and 8-10º in the far periphery. Along the HM, the areal cortical magnification factor (ACMF) in the centre is at least 15 times as large as in the periphery. The ACMF falls towards the peripheral representation more steeply than the ganglion cell densities in the retina.

133.10

EVIDENCE OF A RELATIONSHIP BETWEEN ELECTROTONIC PROPERTIES AND MORPHOLOGY OF PYRAMIDAL CELLS IN TURTLE VISUAL CORTEX.

M. Fowler*, P. S. Ulinski, L. Smith & L. J. Larson-Prior. Dept. Org. Biol. and Anat., U. Chicago, Chicago, Il. 60637

The cerebral cortex of turtles contains neurons that respond to visual stimuli moving anywhere in the binocular visual field. Geniculocortical fibers course from lateral to medial across the cortex, establishing a series of overlapping isoazimuth lamellae which consist of cortical neurons responding to the same azimuth line of visual space. However, pyramidal cells within a lamella vary in their morphology and their spatial relationship to geniculocortical fibers. Pyramidal cells in the lateral part of visual cortex have extensive apical and basal dendritic fields. Geniculate axons synapse upon the distal tips of their apical dendrites. Pyramidal cells in medial visual cortex possess only apical dendritic trees. Geniculate axons synapse upon their proximal dendrites and somata. These differences suggest that there may be major differences in the functional organization of the geniculocortical synapses within lamellae. This study correlates the electrotonic structure of pyramidal neurons with their position along the isoazimuth lamellae.

We recorded intracellularly in current clamp from pyramidal cells in a reduced *in vitro* preparation. Analysis of voltage responses to subthreshold hyperpolarizing pulses allowed the calculation of the cells' membrane time constants (T_m) and two equalizing time constants (T_1 and T_2) which yielded a value for the electrotonic length (L) of each cell. The values of T_m, T_1, T_2 and L were plotted as a function of distance along the isoazimuth lamellae. T_m remained constant (Mean = 10±36) while T_1 (Mean = 30±14) and T_2 (Mean = 20±10) decreased with increasing distance along the lamella. Consequently, L (Mean = 2.3±1.0) decreased from about 2.8 to 2.2 along the lamella. This suggests that changes in the electrotonic structure of pyramidal cells along the isoazimuth lamellae compensate for differences in the synaptic position of geniculocortical afferents. Thus, geniculocortical afferents can have significant effects at the soma despite the major differences in the geometry of the geniculocortical system. Supported by PHS Grant EY 08251.

133.11

ELECTROTONIC MODEL OF GABAERGIC HORIZONTAL CELLS IN TURTLE VISUAL CORTEX. J. M. Nicolaus*, P. S. Ulinski and L. J. Smith. Department of Organismal Biology and Anatomy, University of Chicago, Chicago, IL 60637

Turtle visual cortex is a trilaminar nerve network involved in visual motion analysis. Its third layer contains GABAergic horizontal cells whose dendrites extend within the layer parallel to the ependyma. Their axons have collaterals within visual cortex and also project from the cortex to the lateral geniculate complex. Knowledge of the biophysical properties of these cells is important in understanding the microcircuitry of visual cortex. We combined morphological data from GABA-immunoreactive neurons in cortical wholemounts with physiological data from current-clamp recordings to construct a compartmental model of a small horizontal cell.

The morphological data were used to calculate compartmental lengths and areas (total area = 2,764 μm^2). Analysis of voltage responses to current pulses (-0.1 nA, 1 sec) by the method of peeled exponentials yielded a membrane time constant of 55 ms and a first equalizing time constant of 29 ms. The cell had an electrotonic length of 4.3 and a space constant of 31 μm. The model was used to estimate membrane parameters by fitting its responses to current injection to the actual value of total input resistance (106 MΩ) and the real neuron's charging function. Axial resistance was 400 Ω, specific capacitance was 0.8$\mu F/cm^2$ and specific resistance was 9,322 Ωcm^2. Attenuation of voltage response with dendritic distance indicates these neurons are not electrotonically compact, so inputs from many pyramidal cells would be required to fire them.

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133.12

INTRINSIC FIRING PATTERNS IN NEURONS OF THE TURTLE VISUAL CORTEX. L.J. Larson-Prior* and P.S. Ulinski, Dept. Org. Biol. and Anat., U. Chicago, Chicago, IL 60637

Intrinsic physiologic properties of neuronal membranes play a critical role in the transformation of received input to a specific output, defined by the temporal patterns of action potential generation. Neuronal firing patterns correlated with anatomical properties have recently been utilized to classify neurons in the cortex of several species (eg. McCormick et al., 1985; Conners and Kriegstein, 1986)

In order to develop a quantitative method by which variations in firing pattern could be compared, intracellular recordings were obtained in a turtle visual cortical preparation *in vitro* . Direct responses of neurons to intracellularly injected square current pulses (+0.1-1.5 nA, 400-1000 msec.) were used to assess intrinsic firing pattern. Neuronal firing patterns changed significantly as levels of injected current were increased. Therefore, a method was devised in which firing responses to all levels of current intensity could be used to quantify a given cell's firing pattern. Initial firing rate was measured as the slope of a line derived from the first two points in the cumulative firing curve, normalized to the time of firing of the last action potential generated at the highest current intensity. The degree of spike frequency adaptation was measured by comparing the initial firing rate of the cell to its firing rate later in the spike train. The line generated to determine the initial firing rate represents the cell's response if no frequency adaptation occurs. A third order polynomial fit to the response of a cell to steps of increasing current intensity yielded a response curve which-when subtracted from the linear response profile expected with no adaptation- represents the degree of adaptation present in that cell. The area between the line of initial firing rate and the polynomial was taken as an index of adaptation The firing rate was then plotted against this index of adaptation in order to generate a curve which depicts the firing rate pattern of that cell across a large range of current injection steps.

This analytical method introduces descriptive parameters that can be used to describe the behavior of any neuron to depolarizing current injection. Supported by NS 30759 (LLP) and EY 08352 (PSU).

133.13

CONTRAST SENSITIVITY IN CATS AFTER REMOVAL OF THE Y-INPUT. C. Trimarchi[1], C.A. Marzi[2], G. Tassinari[2] and S. Bisti*[1]. [1] Istituto di Neurofisiologia C.N.R., Pisa, I-56127 & [2] Istituto di Fisiologia Umana, Università di Verona, I-37100.

The spatial performance of the visual system is best described by its contrast sensitivity function. Visual information reaches the visual cortex mainly through two parallel and functionally distint pathways originating in the retina from two classes of ganglion cells, X and Y. Several hypotesis have been advanced of the possible role of the two system in the analysis of visual pattern. A direct evaluation of the role of the two pathways can be made by analyzing the contrast sensitivity after selective elimination of either X or Y input. A lesion on the ventral sub-pial portion of the optic tract causes a selective degeneration of the Y-axons with a partial involvement of W-axons while the X-axons remain largely unaffected. This surgical procedure was utilized in the present study with the purpose of analyzing the properties of visual cortical neurones in cat with Y-input removed on one side. We have recorded Visual Evoked Potentials (VEPs) in response to gratings of sinusoidal luminance profile that were modulated sinusoidally in time. Responses to stimuli of different contrast and spatial and temporal frequencies were compared between normal and Y-deafferented visual cortical areas 17 and 18 of the same cat. The amplitude of VEPs recorded in the deafferented visual areas was reduced at low spatial and temporal frequencies and this reduction becomes more evident at increasing values of contrast. Spatial and temporal acuities were similar in both normal and deafferented hemispheres.

Supported by EEC grant SC1000224

VISUAL CORTEX: ANATOMY OF STRIATE CORTEX

134.1

SUBCORTICAL PROJECTION PATTERNS OF INDIVIDUAL NEURONS IN LAYER V OF STRIATE CORTEX P. Stawinski, M. Conley and D. Fitzpatrick*. Dept. Neurobiology, Duke University, Durham, NC 27710.

Pyramidal neurons in layer V of striate cortex are the source of projections to a number of brainstem targets, including the superior colliculus, pretectum, pons and the ventral lateral geniculate nucleus. Retrograde double label experiments have shown that some layer V neurons have axons that branch to innervate more than one subcortical target, but the complete branching pattern of individual axons remains unknown. To address this issue we made small injections of biocytin into area 17 of tree shrew striate cortex and traced individual axons from layer V cells to their brainstem targets.

Layer V neurons display axonal branching patterns that vary from innervation of a single target to innervation of all four targets. Despite this variation, there seem to be certain rules that govern the patterns of axonal branching. For example, only the superior colliculus and the pons receive projections from neurons whose axons are restricted to a single target; all the projections to the ventral lateral geniculate nucleus and pretectum arise from neurons that send collaterals to other targets.

These results show that pyramidal neurons in layer V comprise several distinct populations that differ in their axonal branching patterns. It remains to be determined whether these populations can also be distinguished on the basis of other characteristics such as axon diameter, soma size, dendritic morphology or laminar position within layer V.

Supported by EYO6821 and EYO6661.

134.2

INTRINSIC AND EXTRINSIC PROJECTIONS OF NEURONS IN LAYER VI OF STRIATE CORTEX: ANATOMICAL EVIDENCE FOR SELECTIVE INFLUENCE ON PARALLEL GENICULOCORTICAL PATHWAYS. W.M. Usrey* and D. Fitzpatrick. Dept. of Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

Pyramidal neurons in layer VI of striate cortex are the origin of descending projections to the lateral geniculate nucleus (LGN) as well as a major source of axon terminals in the layers of striate cortex that receive LGN projections. Since the pathway from the LGN to striate cortex is organized into parallel streams that target different cortical layers, we asked whether layer VI neurons might also comprise separate classes that differ in their patterns of intrinsic projections and in their projections to the LGN. To address this question, we made small extracellular injections of biocytin into layer VI of tree shrew striate cortex and traced the intrinsic and extrinsic axonal projections of individual labeled neurons.

Analysis of single cell reconstructions reveals two classes of intrinsic axon arbors that arise as collaterals from layer VI projection neurons. One class terminates in layer IV, while the other rises through layer IV to terminate throughout layers III-I. Reconstructions of individual corticogeniculate axons also reveal two distinct classes. One class terminates preferentially in LGN layers 1,2, 4 and 5, with little involvement of layers 3 and 6; the other terminates preferentially in LGN layers 3 and 6 and gives rise to a collateral projection to the pulvinar nucleus.

The differences between the classes of intrinsic axons and geniculate-projecting axons are significant in light of the organization of parallel geniculocortical pathways in this species: LGN layers 1,2 4 and 5 terminate in layer IV, while LGN layers 3 and 6 terminate primarily in the supragranular layers. This raises the possibility that individual layer VI neurons could exert a selective influence over the activity in functionally distinct geniculocortical pathways at the level of the LGN as well as the striate cortex.

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134.3

CYTOCHROME OXIDASE AND CALBINDIN STAINING IN VISUAL CORTEX OF THE GROUND SQUIRREL. H.R. Rodman*, H.J. Karten and M.I. Sereno. Depts. of Neurosci. & Cog. Sciences, UCSD, La Jolla CA.

Ground squirrels are diurnal mammals with an expansive visual cortex containing many distinctive visual areas (Sereno et al., NS Abs. 1991). We used cytochrome oxidase (CO) and calbindin D-28K (CaBP) to aid in the parcellation of cortical areas in the California ground squirrel (*Spermophilus beecheyi*) and the thirteen-line ground squirrel (*Sp. tridecimlineatus*).

CO. In flat mounts through the middle layers of cortex, CO staining formed a web-like pattern in the *monocular* segment of V1, especially in the thirteen-line squirrel. Staining in both the binocular segment of V1 and in V2 appeared uniform and relatively dense, showing no obvious blobs or stripes. Moving laterally, the intermediate zone was moderately stained, areas ML and L were lightly stained, and densely myelinated area Tp stained heavily and somewhat patchily. In coronal sections, the binocular compartment of V1 is sharply demarcated by a thick CO-dense layer IV. The uniform staining in V2 gives way laterally to a dark band in layer V of cytoarchitectonic area 19.

CaBP. In flat mounts, CaBP produced a pattern complementary to that seen in CO in many regions of neocortex, as reported for some layers of monkey V1 by Celio et al. (Nature 323: 715, 1986). In standard planes the most salient features of the CaBP pattern are the heavy staining of layers I and II and the paucity of staining in thalamorecipient layers of V1 and other primary sensory areas. Both pyramidal and nonpyramidal neurons were labelled by CaBP and were especially dense in superficial layers of the binocular portion of V1.The results suggest that CO and CaBP are valuable for delineating compartments within and borders between cortical areas in these species and support the association of CaBP staining with extrathalamocortical processing.

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134.4

SEGREGATED PROCESSING STREAMS IN CAT VISUAL CORTEX? RELATIONSHIP OF PATCHY CONNECTIVITY TO AN EXTRASTRIATE AREA, CYTOCHROME OXIDASE STAINING, AND LOCAL CONNECTIONS. J.D. Boyd*[1] and J.A. Matsubara,[1,2]. Depts of [1]Ophthalmology and [2]Anatomy, University of British Columbia, Vancouver, British Columbia, Canada, V5Z 3N9.

Primate area 17 is compartmentalized into distinct processing streams which deal with different types of information, i.e., colour, form, motion. These compartments can be seen by cytochrome oxidase (CO) staining, by their different projections to extrastriate visual areas, and by local connectivity (same stream compartments interconnect). Recent examinations of Lateral Suprasylvian projections (LS; Shipp and Grant, 1991), serotonin receptors and zinc staining (Dyck *et al.*, 1991) and CO staining (Murphy *et al.*, 1991), all showed heterogeneity within cat area 17, suggesting it contains processing streams analogous to those in primates.

After making multiple large injections of 1% wheat germ agglutinin-horseradish peroxidase (HRP) into the LS (10-12 µL at 18 sites), we examined the labeling in flattened tangential sections through area 17. Neurons projecting to the LS were arranged in clusters that tended to form an irregular lattice with a spacing of roughly 0.6mm to 0.9mm. In two cases where serial sections were run for HRP or CO, the clusters of cells colocalized with the blobs of high CO activity visible at the layer 3-4 border. In two cases, injections of HRP in the LS were combined with focal injections of 0.02µl 1% cholera toxin subunit B (CTB) into area 17. In both cases, the clusters of locally projecting cells labeled with CTB were preferentially colocalized with the clusters of LS projecting cells labeled with HRP.

The alignment of CO staining, local, and LS connections suggests that the LS projection demarcates a segregated stream in cat area 17. Although segregation of streams within primary visual cortex may be common to cats and monkeys, the particular streams in feline visual processing are unlikely to match those in the primate. Funded by MRC 5-99150 (Canada) to JAM.

134.5

BLOB-LIKE PATTERN OF CYTOCHROME OXIDASE STAINING IN FERRET VISUAL CORTEX. H.S.Cresho[1], L.M.Rasco[1], G.H. Rose[1,2], and G.J.Condo[2*]. Neuroscience Prog.[1] and Dept. of Psychology[2], Bowdoin College, Brunswick,ME 04011

The organization and differential staining of cytochrome oxidase (CO) patterns in visual cortex is a useful marker for visual cortical modules. This study evaluated the pattern of CO staining in adult ferret areas 17 and 18. Cortices were reacted for CO activity after postfixing for 24-48hrs in 2%paraformaldehyde - 1.25% glutaraldehyde. Sagittal and frontal sections yielded a pattern of CO staining similar to that reported for other species: dense staining of layer IV, darkly reactive pyramidal cells in layer V, weak irregular periodic staining in layer VI, and regular periodic staining in layer III. The blob-like pattern seen in supragranular layers was more evident in flattened cortical preparations in both central and peripheral areas. CO blob-like areas in layer III of area 17 were lighter and more irregular in shape than blobs reported in primates or cats. At the area 17/18 border irregular stripe-like CO staining in supragranular layers of area 18 is evident perpendicular to the border. Last, within area 18 darkly reactive layer V pyramidal cells increase in relative frequency but not in soma size at the area 18/19 border relative to samples of layer V CO reactive pyramidal cells at the area 17/18 border.

134.6

THE DISTRIBUTION OF PARVALBUMIN AND CALBINDIN POSITIVE NEURONS IN VISUAL CORTEX OF THE TREE SHREW (*Tupaia belangeri*). M. A. Sesma*, School of Optometry, University of Missouri-St. Louis, St. Louis, MO 63121.

The calcium binding proteins parvalbumin and calbindin-D28K mark separate populations of GABAergic neurons in mammalian CNS. The role of these proteins is unclear but their differential distribution may be related to distinct processing channels. To better understand the microarchitecture of visual cortex in the tree shrew the distribution of neurons immuno-positive for parvalbumin and calbindin were compared with the distribution of GABA immunoreactive neurons using commercially available antibodies and conventional immunohistochemical methods (avidin-biotin-peroxidase) on aldehyde-fixed, vibratome sections.

Parvalbumin immunoreactive neurons were distributed uniformly throughout layers II-VI in area 17, 18 and Temporal Dorsal area (TD). Most were medium to large in diameter (>10 µm), even within layer IV of area 17. Calbindin immunoreactive neurons were distributed predominantly in the superficial layers (II, III) in small to medium size (<15 µm) neurons. In area 17, layer IV was densely stained while layer IIIc was calbindin-free allowing easy discrimination of the 17/18 architectonic border. Calbindin staining in the infragranular layers was uniform but quite sparse and in area 17, a population of medium size pyramids was seen in upper layer V.

The pattern of visual cortical staining with antibodies directed against parvalbumin and calbindin-D28K differs in some respects from the distribution described for primates and carnivores, especially in the dense staining in the granular layers of area 17. This may reflect basic differences in a visual cortex evolved to segregate ON- and OFF-center channels rather than M(Y), P(X) pathways.

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134.7

The calcium binding proteins (CaBPs), parvalbumin, calbindin D-28k, and calretinin define cytoarchitectural features of the monkey occipital cortex

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Previous studies have demonstrated that CaBPs are highly reliable anatomical markers in that they define clear patterns of regional and laminar specialization. We have systematically studied their distribution in two visual areas (V1 and V2) in the macaque monkey, using immunohistochemical methods, in order to further precise their cytoarchitectural features. Of the three CaBPs studied, PARVALBUMIN immunoreactivity (PV-ir) was the densest, and occurred preferentially in the thalamorecipient layers of V1. The neurons showed the typical features of multipolar interneurons. These morphological and laminar distribution patterns were maintained in V2. Only the projection lamella in the white matter (WM), related to V1, contained thick PV-ir fibers. CALRETININ immunoreactivity (CR-ir) was less abundant than PV, and ir-neurons occurred predominantly in the superficial layers of V1 and V2, including layer 1. Most CR-ir neurons were vertically oriented and resembled double-bouquet cells (more distinct in V2 than in V1). In V1 a narrow band of labeled neurons was present at the border of 4Cß/5A. CR-ir labeled cells were always visible in short association fibers in the WM. CALBINDIN D-28k immunoreactivity (CB-ir) was the less densest of the three CaBPs. In the superficial layers of V1 and V2, interneurons as well as pyramidal cells were stained; CB-ir neurons showed also a vertical organization (more distinct in V2). CB-ir and CR-ir were not colocalized. The cytoarchitectural patterns revealed by the three CaBPs demonstrate their complementarity in defining neuronal typology, and outlining functional systems. (PRH supported by Brookdale Foundation ; MRC by SNF 31-27979.89)

134.8

CHANGES IN CYTOCHROME OXIDASE AND NEURONAL ACTIVITY IN V1 INDUCED BY MONOCULAR TTX BLOCKADE IN MACAQUE MONKEYS. T. C. Trusk*, M. Wong-Riley, & E. A. DeYoe. Dept. of Cellular Biology & Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226.

To determine if changes in metabolic capacity revealed by cytochrome oxidase (CO) histochemistry are related to sustained changes in energy-utilizing neuronal activity, we recorded multiunit firing rates along nearly tangential penetrations of V1 before and after single injections of TTX into the left eye of 5 adult macaques. To ensure that changes in cortical activity reflect the effect of TTX alone, we matched adaptation conditions by occluding the left eye and exposing the right eye to 1 of 4 adapting fields: black, gray, white and textured. Five sec samples of multiunit spike rate were obtained after 2 min adaptation to each condition. Results were similar for these 4 conditions. TTX blockade began to reduce CO activity in left-eye columns of layer 4C within as little as 12 hrs but reached $^2/_3$ of maximal depletion only after 72 hrs. Activity recorded in left-eye columns of layer 4C following TTX averaged 48% of that in right-eye columns. Immediately after TTX injection, ongoing activity in layers 2/3 measured repeatedly at single sites in both left- and right-eye dominated interpuffs decreased up to 3-fold in less than 1 hr but stabilized within 3-4 hrs to an average of 50% ($p<.01$) of pre-TTX rates. Before TTX, spike rates in CO-rich puffs were 33% greater than in CO-pale interpuffs. These data support the notion that energy-utilization linked to sustained spike rates partially regulates CO levels, at least in layer 4, and suggest that changes in neuronal activity induced by retinal TTX precede the detectable reduction in CO activity in V1. (Supported by NIH grant EY05439.)

134.9

INTERPUFFS IN THE MACAQUE STRIATE CORTEX: QUANTITATIVE EM ANALYSIS OF NEURONS BEFORE AND AFTER UNILATERAL RETINAL IMPULSE BLOCKADE. M. Wong-Riley,*T. Trusk, W. Kaboord and Z. Huang. Dept of Cellular Biology & Anatomy, Med. Coll. Wisconsin, Milwaukee, WI 53226.

Our previous studies indicate that unilateral retinal impulse blockade causes a down-regulation of cytochrome oxidase (CO) in the supragranular puffs along the deprived eye column, and that the effect is more detrimental to certain classes of neurons than to others. The present study aimed at analyzing the effect of TTX on interpuff neurons. Three regions were examined: intra-normal row interpuffs (INI) from normal monkeys, and intra-control row interpuffs (ICI) and intra-experimental row interpuffs (IEI) from unilateral TTX-injected animals. We found the same three major neuronal types in interpuffs as in puffs: Type A neurons were small nonpyramidal and pyramidal cells with relatively low levels of CO activity; type B neurons were medium-to-large pyramidal cells that were moderately reactive for CO; and type C neurons were medium-sized nonpyramidal cells darkly reactive for CO. All three cell types contained much fewer darkly reactive mitochondria than those in puffs. Both types A and B received exclusively symmetrical axosomatic synapses, while type C received both symmetrical and asymmetrical axosomatic synapses. Two weeks of TTX blockade induced a significant downward shift in the size and reactivity of mitochondria in type C neurons, while types B and A neurons were much less affected. Thus, our present data support our previous findings that retinal impulse blockade is most detrimental to the metabolically most active neurons in the adult visual cortex. (Supported by NIH grant EY05439).

134.10

DIFFERENTIAL REGULATION OF $GABA_A$ RECEPTOR SUBUNIT mRNAS IN ADULT MONKEY VISUAL CORTEX BY MONOCULAR DEPRIVATION.

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The $GABA_A$ receptor is composed of a number of subunits that, in addition to GABA, contain binding sites for benzodiazepines and barbituates. These polypeptide subunits are structurally similar, and can be divided into distinct classes (α, β, γ, δ, ε), and subclasses (α1, α2, α3, α4, α5), based on sequence identity. Immunoreactivity for various $GABA_A$ receptor subunits shows a distinct yet overlapping distribution in the primate visual cortex (Huntsman et al., 1991, Neurosci. Abstr. 16, 115). Portions of monkey specific cDNAs of previously published sequences were cloned into the pBS transcription vector for the localization of mRNA in area 17 of the monkey visual cortex. The localization of mRNAs for one representative of each receptor class (α, β, γ) was identified. The distributions of α1 and γ2 subunit mRNAs are similar and their distribution is a reflection of geniculocortical terminations, with highest grain density in layers IV and II/III. The β1 subunit, however, displays no laminar specificity.

Neural activity has a profound effect on the regulation of genes for these subunits. Following monocular deprivation by tetrodotoxin injection, mRNAs for the α1 and γ2 subunits decrease in deprived ocular dominance columns, whereas, the mRNA for the β1 subunit is not affected. These data indicate that levels of mRNAs for the polypeptide subunits of the $GABA_A$ receptor are not only localized in a specific laminar distribution, but are also regulated in an activity-dependent manner. Differential localization of these subunits may give rise to variations in the pharmacological properties of $GABA_A$ receptors in different layers of the primate visual cortex. Supported by grants EY07193 and NS26748.

134.11

Inhibitory and Excitatory Amino Acid Receptor Distributions in Adult Human Visual Cortex. [1*]D.F. March,[2]A. E. Hendrickson and [1]C. Shaw. [1]Dept. of Ophthalmology, Univ. of British Columbia, Vancouver, Canada and [2]Dept. of Biological Structure, Univ. of Washington, Seattle.

In previous studies we have examined the characteristics and distributions of the inhibitory $GABA_A$ receptor complex (Jour. of Neurosci 1991,11:3943) and the excitatory NMDA and AMPA receptors (Soc. Neurosci Abstr. 1991,17:365) in the visual cortex of the macaque monkey. We have now extended these studies to human visual cortex. In order to use human postmortem material we have also completed parametric experiments on receptor degradation in postmortem rat cerebral cortex. At 4°C the receptor populations in this study were stable for at least 24 hours. In adult human visual cortex $GABA_A$/benzodiazepine receptors showed moderate to low binding in layers 1,5 and 6, high binding in layers 2/3 and the densest binding in 4C. AMPA receptors showed the densest binding in layers 1-3, with moderate binding in layers 5/6 and low binding in layer 4. NMDA receptors showed moderate binding in layers 1-3, low binding in layers 5/6, and high binding in 4C. These studies reveal similarities for $GABA_A$ and AMPA receptor distributions between human/monkey visual cortex and that of lower mammals. There are, however, profound differences between primates and lower mammals in the distribution and time of expression of NMDA receptors. Since the NMDA receptor population is often considered crucial for the modification of neuronal activity, these data may suggest caution in the use of some animal models (eg., cat) of visual cortex function, development, and plasticity.

134.12

IMMUNOCYTOCHEMICAL DETECTION OF $GABA_A$ RECEPTORS IN THE CAT VISUAL CORTEX. A.M. Rosier, G.A. Orban and F. Vandesande*. Lab. of Neuroendocrinology and Immunological Biotechnology, Lab. of Neuro- and Psychophysiology, Catholic Univ. Leuven, Belgium.

The purpose of this study was to raise specific monoclonal antibodies against a synthetic peptide selected from the β-subunit of the bovine $GABA_A$ receptor in order to identify distinct cells in the cat visual cortex, exhibiting $GABA_A$-receptor-ir. Solubilized and affinity-purified $GABA_A$ receptors were used to examine the selectivity of the antibody with SDS-PAGE and immunoblotting, and with immunoadsorption.

In the cat visual cortex, two major populations of $GABA_A$-receptor-ir cells were observed: neurons and astroglial-like cells. Positive neurons occurred in all layers (± 250-400 neurons/mm^2), with a minimum in layer I (± 50 neurons/mm^2). Expressing the number of positive neurons as a function of the total laminar neuronal population, yielded the highest density in layer V (±60%), compared to ±40% in layers III-IV-VI; ±25% in layer II; and ±10% in layer I. Double stainings using receptor- and GFAP-antibodies, confirmed that the observed glial-like positive cells observed both in cortical grey matter and in the white matter are astrocytes.

134.13

PATTERNS OF LATERAL CONNECTIONS IN MACAQUE VISUAL AREA V1 REVEALED BY BIOCYTIN HISTOCHEMISTRY AND FUNCTIONAL IMAGING. T. Yoshioka*, G.G. Blasdel, J.B. Levitt, J.S. Lund, Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261; Dept. Neurobiology, Harvard Medical School, Boston, MA 02115

We made small iontophoretic injections (diameter:100-200μm) of biocytin in layers 1-3 of macaque area V1, and sectioned the tissue tangentially to examine the intrinsic lateral connectivity. Alternate tissue sections were reacted for biocytin or cytochrome oxidase (CO) to correlate the locations of injections and transported label in relation to the CO subcompartments (blobs, interblobs, and blob edges). Anterograde and retrograde label formed oval or circular shaped patches with maximum mean diameter of 250μm regardless of the size or location of the injection in relation to the CO compartments, with an average of 10 labeled patches per injection. Injections in each compartment showed projections mainly but not exclusively to the same compartment. The emphasis on connectivity within similar compartments (Livingstone and Hubel, '84) was about 70%. The total area of clearly distinguishable patches derived from single injections averaged 2.3mm by 1.5mm (average anisotropy 1.6). In most cases, anisotropy was at right angles to CO blob rows and ocular dominance bands visualized by voltage-sensitive dye imaging. The patches from single injections were predominantly (about 75%) in domains of the same ocular dominance. Although these results are consistent with previous suggestions that these connections primarily link cells of like property, nonetheless there is a substrate for interaction between neurons of unlike property. (Supported by NEI grants EY05282, EY08098, EY06275, ARVO/Alcon; EY05403, Office of Naval Research.)

134.14

INTRINSIC CONNECTIONS IN HUMAN VISUAL CORTEX REVEALED BY EXTRACELLULAR BIOCYTIN APPLICATION IN-VITRO. G. Kenan-Vaknin*, G.E. Ouaknine, N. Razon and R. Malach, Neurobiology Dept. Weizmann Institute, Rehovot and Neurosurgery Dept. Ichilove Medical Center, Tel Aviv, Israel.

Studies of human cortical circuitry, although of extreme interest, are limited by the poor resolution of analysis available on post-mortem material. Here we report on a novel tract tracing approach, using the anterograde tracer biocytin, which provides unprecedented detail in the study of cortical axonal projections. Human cortical specimens were obtained from non-diseased cortical tissue removed from the occipital lobes during neurosurgical procedures to treat tumors or brain malfunctions. Coronal and tangential slices were iontophoretically injected with biocytin, incubated for additional 2 hours and then processed according to Horikawa and Armstrong (1988). The results show Golgi-like staining of axons carrying numerous boutons extending up to 4 mm from the injection sites. Dendritic arbors and spines could be clearly observed as well. In coronal slices, injections in supragranular layers revealed axon trunks descending vertically to the white matter with dense collaterals in supra- and infragranular layers. Few oblique and horizontal collaterals were seen in layer VI. Injections in infragranular layers revealed long horizontal collaterals and vertical and oblique ascending axons towards layer I. Long oblique and horizontal collaterals were found in the white matter. In tangential slices, axons radiated from the injection sites forming a dense halo. The results indicate that biocytin injections provide a powerful tool for systematic analysis of local circuits in the human cortex.

134.15

CORTICOCORTICAL FEEDBACK CONNECTIONS IN VISUAL CORTEX SYNAPSE SELECTIVELY WITH DENDRITIC SPINES. R. R. Johnson and A. Burkhalter. Dept. Neurosurgery, Washington Univ. Sch. Med., St. Louis, MO 63110.

Although the feedback pathway from extrastriate to striate visual cortex in the rat, as in other mammals, is known to provide a very prominent input to both upper and lower layers, its function is largely unknown. In order to understand the role of the feedback pathway, we have identified the targets of feedback axons in primary visual cortex at the synaptic level. Feedback axons are labeled by the anterograde tracer Pha-L. Sections containing the feedback pattern of projections are embedded, and feedback terminals are identified in serial thin sections by an electron dense DAB product. Postsynaptic profiles of dendritic spines, shafts and cell bodies are identified by standard ultrastructural criteria.

Although we predicted that many feedback terminals would synapse with inhibitory interneurons (Johnson and Burkhalter, 1991, Soc. Neurosci. Abstr. 17:844), examination of profiles postsynaptic to feedback terminals indicates that input to interneurons may be very minor. Of the first 59 feedback synapses identified in upper layers, all feedback terminals in layer 1 (19/19) and almost all in layer 2/3 (39/40) made synapses with dendritic spines. Only one terminal in layer 2/3 was found to synapse with a dendritic shaft. In this preliminary data, therefore, the postsynaptic targets of the feedback pathway are ~98% spines and ~2% shafts.

Interestingly, these results suggest that (1) the organization of feedback connections is more like association pathways across the corpus callosum (Cipolloni and Peter, 1983, J. Neurocytol. 112:713) than forward connections (Lowenstein and Somogyi, 1991, J. Comp. Neurol. 310:253) or lateral connections (McGuire et al., 1991, J. Comp. Neurol. 305:370), and (2) feedback to inhibitory interneurons is unlikely to be the mechanism for interareal synchronization of oscillations (Engel et al., 1991, PNAS 88:6048) as suggested by Crick and Koch (Cold Spring Harbor Symp., 1990, 55:953).

134.16

BOTH STEADY AND FLASHING DIFFUSE LIGHT INCREASE 2-DG UPTAKE IN GROUND SQUIRREL BUT NOT IN RAT VISUAL CORTEX. R.M. Cooper*. Behavioural Neuroscience Research Group, Dept. Psychology, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

Diffuse light significantly affects visual cortex activity in the diurnal primate but not in the nocturnal cat, rabbit or rat. Thus, "luxotonic" units, i.e. cells which respond to diffuse light, are readily detectable in recordings made from macaque striate cortex but are rarely encountered in cat and rabbit cortex (Kayama et al., J. Neurophysiol., 42: 1495, 1979). Similarly, diffuse light stimulation elevates 2-DG uptake in monkey (Tootell et al., J. Neurosci., 8: 1569, 1988) but not in rat (Cooper & Thurlow, Exp. Neurol., 113: 79, 1991) visual cortex. Cones play a more important role in the vision of the diurnal macaque than they do in the more nocturnal cat, rabbit and rat. As a further test of whether the degree of cone vision might be correlated with the degree of the cortical effects of diffuse light, 2-DG uptake was examined in the strongly diurnal ground squirrel (Richardson's), a rodent with an almost completely cone retina. The awake and freely moving squirrels were tested with one eye covered by a white diffusing mask and the other eye with an occluding black mask; hemispheric differences in 2-DG uptake in monocular visual areas were used to assess effects. In contrast to the rod-dominated rat, both steady diffuse light (illumination supplied by 500 w halogen lamps) and flashing diffuse light (10 microsecond flashes from Grass photostimulator set at 5 hz and ~1.5 million CP intensity) did elevate monocular area 17 2-DG uptake in the cone-dominated squirrel. Apparently then, even within the relatively closely related rodent group, whether or not diffuse light information is important to cortical activity may be related to whether or not an animal has a rod- or a cone-dominated retina. The cortical processing of diffuse light (or intensity) information may be more important to an animal retinally specialized to function visually under bright light conditions.

134.17

TOPOGRAPHICAL ORGANIZATION OF AXONS IN THE SPLENIUM OF THE RAT CORPUS CALLOSUM.

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While callosal fibers show at least a rough topographical organization depending on the cortical region of origin, the precise organization of axons in the rat splenium has not been investigated. In the present study, discrete injections of WGA-HRP were made into the 17/18a border of the visual cortex of adult Long Evans male and female rats. Our results indicate that posterior injections labelled the posterior end of the splenium, while anterior injections labelled fibers located slightly anterior within the splenium. Injection sites which included the anterior-most extent of the primary visual cortex suggest that visual callosal fibers project through approximately the posterior eighth of the corpus callosum. Injections restricted to the secondary visual areas and the temporal cortex will be made to map their callosally projecting axons within the splenium. The topography of axons within the splenium will also be examined at younger ages.

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INVERTEBRATE SENSORY SYSTEMS

135.1

DEVELOPMENTAL APPEARANCE OF PROCESSING UNITS IN THE OLFACTORY AND ACCESSORY LOBES OF THE AMERICAN LOBSTER. S. M. Helluy*, M. L. Ruchhoeft and B. S. Beltz. Bio. Dept., Wellesley College, Wellesley, MA. 02181.

The brain of *Homarus americanus* enlarges 500 times (from 0.05 to 25 mm^3) between hatching and maturity. The aims of this study are 1) to characterize the accompanying enlargement of the olfactory (OLs) and accessory (ALs) lobes in the deutocerebrum; 2) to document the changes in the number of processing units (glomeruli) in these lobes; and 3) to acquire base-line data for a study of the potential morphogenic effects of serotonin on the organization and maturation of the deutocerebral lobes.

Serial sections of brains at different stages of development were traced on a digitizing pad linked to a 3-D computerized reconstruction system which also yielded morphometric data. Between a hatchling and a one pound lobster the volume of the OLs and of the ALs increases by a factor of 300 (from 0.0025 to 0.7 mm^3) and 550 (from 0.001 to 0.55 mm^3) respectively. Preliminary estimates show that the number of olfactory glomeruli (which are columnar and radially arranged at the periphery of the lobes) reaches more than 100 per lobe at hatching, increases rapidly during larval life, and stabilizes at about 200 to 300 in juvenile and adult lobsters. Glomeruli in the ALs are spherical structures scattered throughout the lobes and are seen for the first time in larval stage 2. There are about 2000 glomeruli per AL in stage 4 and this number seems to remain constant during the life of the lobster. In both lobes, it is the size of the interglomerular spaces and of the glomeruli themselves that increases (mean diameter: 21 to 100 µm at the distal surface of the columnar olfactory glomeruli, 6 to 30 µm for the spherical accessory glomeruli). Therefore the data suggest that in both OLs and ALs most of the glomeruli have been generated when the lobster metamorphoses (stage 3 to 4) and switches from a pelagic to a benthic existence. (Supported by BNS-8718938 and 8958169, and NIH NS-25915).

135.2

A CONDITIONAL DENDRITIC OSCILLATOR IN A LOBSTER MECHANORECEPTOR NEURON. D. Combes, J. Simmers and M. Moulins* Lab. de Neurobiol. Physiol. Comp., Univ. Bordeaux I & CNRS, Place Peyneau, 33120 Arcachon, France.

Although many neurons of the CNS possess endogenous bursting properties, to date such non-linear behavior has not been demonstrated in peripheral neurons. Here we report oscillatory burst-generating properties in a primary sensory neuron and show that the oscillator is located in the dendritic membrane of the cell close to its distal receptor terminals. This element, the Anterior Gastric Receptor (AGR), is a unique mechanoreceptor neuron belonging to the stomatogastric nervous system of *Homarus gammarus*. Due to its large geometry and accessibility for multiple recording *in vitro*, we have been able to demonstrate (a), a spiking capability in the dendrites of AGR that is dependent on Na^+ ions (b), these autogenic dendritic impulses can occur in rhythmic bursts superimposed upon voltage-dependent oscillations in membrane potential (c), this non-linear property imparts considerable integrative complexity to the transfer of mechanosensory information from AGR's receptor terminals to its central axon, in that sensory coding is mediated by changes not only in the frequency of ongoing dendritic bursts but also in the duration of individual bursts and their inherent spike frequencies.

Furthermore, the expression of dendritic bursting in AGR is not invariant, but can switch spontaneously to autoactive tonic firing. Although the basis for this change in endogenous behavior is not yet known, local application of F1 peptide (10^{-7}M) to AGR's dendritic membrane can reversibly alter its activity pattern from tonic firing to a bursting mode. We are currently employing this FMRFamide-related peptide, which is known to be present in the lobster stomatogastric nervous system, to examine the functional significance of a conditional sensory oscillator in the control of its postsynaptic targets.

135.3

THE SINUS GLAND NEUROSECRETIONS MODULATE THE RESPONSE TO MONOCHROMATIC LIGHT IN THE COMPOUND EYE OF THE CRAYFISH. V. Inclán-Rubio* and R. Mora-Mora. Depto. de Fisiología, Fac. de Medicina, A.P. 70-250, 04510 México, D.F. MEXICO. It has been demonstrated the ability the monochromatic light has to evoke phase changes in the ERG circadian rhythm in crayfish. The retinular screening pigments (RSP) and the distal pigment light-adapting hormone (DPLH) participate in these responses. It is well known that the application of white light induces the depletion of DPLH content inside the sinus gland (SG). However, the relationship between the application of a specific wavelenght and the content of DPLH in the SG is unknown. In this work, we determined that LM application in crayfish induces modifications in the amount of DPLH contained in the eyestalk. We worked with 5 experimental groups of Procambarus bouvieri submitted to different light (30 s): I:465nm, II:565 nm and III:630nm, IV:white light) and V:darkness. After illumination, the eyestalks were removed to prepare extracts injected in crayfish previously dark-adapted. The effect was evaluated through glow area. Results: the extracts of crayfish that were submitted to darkness induced a reduction in the glow area of 48%; blue light-46%; and green light-33%. White and red light without changes. These results show: A) a selective effect of the LM used over the content of the SG neurosecretions that movilize the retinal screening pigments.

135.4

IONIC CURRENTS UNDERLYING RAPID SENSORY ADAPTATION IN THE COCKROACH TACTILE SPINE. Päivi H. Torkkeli and Andrew S. French*. Department of Physiology, University of Alberta, Edmonton, Alberta T6G 2H7, Canada.

The cockroach, *Periplaneta americana*, femoral tactile spine contains a single mechanosensory neuron. The neuronal soma is at the bottom of the spine lumen, and a sensory dendrite passes through the spine wall. Intracellular recordings were made from the neuronal soma by advancing glass microelectrodes (~40 MΩ, 3 M KCl) down the lumen of spines cut just above the sensory dendrite. Membrane currents during step changes in membrane potential were measured by two techniques: (1) a switching single-electrode voltage-clamp amplifier, and (2) a computer-controlled process in which the current through a balanced microelectrode was adjusted iteratively to give a voltage step.

Depolarizing steps of 1-5 mV from resting potential produced an inward current which decayed with at least two time constants. Preliminary evidence indicates that these are comparable to the time constants of ~100 ms and ~1000 ms with which the threshold has previously been shown to change during adaptation.

Supported by the Medical Research Council of Canada.

135.5

SEGMENTALLY HOMOLOGOUS NEURONS AND INFORMATION FLOW IN THE LOCUST CNS. G.S. Boyan*, Zoology Institute, Rheinsprung 9, CH-4051 Basel, Switzerland

This study focusses on the synaptic properties of an array of neurons which are among the progeny of neuroblast 7-4 in consecutive ganglia along the ventral nerve cord of the locust. Intracellular recordings have been performed from pairs of homologous neurons within the array, and between members of the array and other non-related pre- and postsynaptic cells. A neuronal network is described in which all the postsynaptic cells belong to the lineage of NB 7-4 in their respective segment. Information flow within the network is highly directional as shown by the fact that the segmental homologs receive common synaptic drive from one and the same presynaptic interneuron. Homologous neurons within one particular segment receive synaptic inputs from common sets of presynaptic cells; and this criterion may complement morphological features in identifying other putative progeny of the neuroblast in the adult. Despite their common inputs serially homologous members of the array output to different motor pathways. An analysis of the information flow to and from this neuronal network aims at a better understanding of motor activity along a segmentally distributed nervous system.

Supported by the Swiss Nationalfonds.

135.6

DEVELOPMENT OF FILIFORM HAIRS AND ITS SPECIFIC CONNECTIONS TO A PROJECTION INTERNEURONE IN LOCUSTS. H.J.Pflueger[1]*, A.Czizek[1] and M.Burrows[2]. Freie Universitaet, Neurobiologie, Koenigin-Luise Str. 28-30, D(W)-1000 Berlin 33, FRG[1] and Dept. of Zoology, Cambridge, CB2 3EJ, UK[2].

The development of the projection pattern of identified filiform hairs on the prosternum was studied from the first instar to the adult locust. In adults these hairs make direct, high gain, monosynaptic connections to two projection interneurones, one in each connective, which activate motor neurones of wing steering muscles. In adults eight filiform hairs on one half of the ventral prosternum connect only with the contralateral interneurone, and accordingly only project into the contralateral neuropile of the prothoracic ganglion. Two filiform hairs on one proepisternum connect only with the ipsilateral projection interneurone, and correspondingly these project into the ipsilateral neuropile only. The projection pattern of the two filiform hairs on each proepisternum changes only with respect to number of side branches and varicosities.

Identified filiform hairs on the ventral prosternum of first to third instar larvae project into the ipsi- and contralateral neuropile. However, from the fourth stage onwards, ipsilateral branches are retracted and contralateral ones extended and increased in size. In accordance with these morphological observations, direct synaptic connections of ventral prosternal hairs with the ipsilateral interneurone were only found up until the third instar but not in later instars. The synaptic gain of the ipsilateral connection was much less than that of the contralateral connection.

135.7

DEVELOPMENTAL CHANGES IN THE ELECTROTONIC STRUCTURE OF THE CRICKET NEURON MGI CONTRIBUTE TO CHANGES IN SYNAPTIC EFFICACY OF IDENTIFIED AFFERENTS. A.A.V. Hill*, R.K. Murphey, D.H. Edwards. Neuroscience and Behavior Program, Univ. of Massachusetts, MA 01003.

The amplitude of EPSPs from identified sensory neurons that make monosynaptic connections with MGI decreases during postembryonic development (Chiba et al., *Science*, **240**, 901, 1988). This change in EPSP amplitude could be due to a passive phenomenon such as the growth of the postsynaptic cell or an active phenomenon such as a retraction of the presynaptic terminals. In order to assess the effect of growth of the postsynaptic cell on EPSP amplitude we constructed compartment models of MGI at different developmental stages. The models were based on Lucifer Yellow fills of MGI imaged with a scanning confocal microscope and reconstructed 3-dimensionally. An important parameter, the specific membrane resistance was estimated from the MGI's time constant determined from its response to current injection. We simulated synapses of the same strength in models of MGI at various stages of development by modelling a synapse as a conductance change with a given waveform. We found that the growth of MGI leads to a decrease in EPSP amplitude that is significantly less than the decrease reported for identified sensory neurons. This suggests that the change in EPSP amplitude is only partially due to passive changes in the electrotonic structure of MGI.

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135.8

FUNCTION, PHYSIOLOGY AND AXONAL PROJECTIONS OF THE CHEMORECEPTORS OF DIPTERAN WINGS. S. Hannaford* and J. Palka, Dept. of Zoology, Univ. of Washington, Seattle WA 98195.

Most flies have a set of multiply innervated bristles on their wings, which appear to be contact chemoreceptors. We find that they are widely conserved among the Diptera, present in members of the most primitive as well as the most advanced families. Despite this they remain largely uncharacterized. We have been studying the function of these sensilla in *Drosophila melanogaster*. Stimulation of the wing with a drop of sugar solution elicits a proboscis eversion reflex (PER), the first step in feeding behavior. This response is concentration dependent and sugar specific. When a high concentration of NaCl is added to the stimulating sugar solution the PER is inhibited. These behaviors are similar to those elicited by stimulating the tarsal and labellar taste hairs. We have also examined a number of mutants in which the inhibiting effect of salt application to the tarsi and labellum is known to be defective; in all cases wing evoked PER is affected as well. These behavioral results suggest that the wing chemosensilla are responsive to both sugar and salt and are capable of modulating feeding behavior. Preliminary electrophysiological recordings confirm that the receptor neurons are sensitive to salt and sugar. Dye fills show that the central projections of the wing chemosensilla differ from those of the tarsal sensilla; they occupy a discrete area in the mesothoracic neuropil and fail to project to the subesophageal ganglion. We are now looking for possible functional organization of the wing neuropil.

135.9

THE DIFFUSION OF SMALL MOLECULES IS REGIONALLY RESTRICTED IN THE RETINA OF THE COMPOUND EYE OF THE BLOWFLY. M. Järvilehto*, M. Syrjäkallio-Ylitalo and M. Weckström. Depts. of Zool. and Physiol., Univ. of Oulu, 90570 OULU, Finland

Histologically the layer of the retinular cell bodies appears as a diffusionally uniform space. We studied the diffusion in the retina of white-eyed blowfly *chalky* with two different methods: Dye diffusion and deep pseudopupil (DPP) changes affected by intraretinally applied KI or $CaCl_2$ solution. For the diffusion test a small excised ventral or dorsal area of the eye was covered with a drop of test substance, i.e. Toluidine Blue (1% FW 306), Methyl Blue (1% FW 799.8), KI (1 M, FW 166), $CaCl_2$ (1 M, FW 111). The substances were allowed to diffuse passively into the retina from a continuous supply. The changes of the DPP were stored on a video tape. The DPP of the other eye served as a control. The diffusion isoprofiles (DIP) show that the test substances are unevenly diffusing throughout the retina. The effect clearly depends on the type of the test substance and its concentration. The DIPs show that the test substances diffuse always first to lateral parts of the eye and finally to the opposite dorsal/ventral central region.

135.10

A COUPLED OSCILLATORY OUTPUT FROM AN INSECT AUDITORY RECEPTOR. S.R. Shaw.* D. S. MacIntosh and K.M. Kokic'. Psychology and Neuroscience, Dalhousie University, Halifax, Nova Scotia, Canada.

Subgenual organs (SGOs) in insect legs evolved originally as extremely sensitive contact vibration detectors, but in cockroaches are bimodal, with an additional sensitive direct response to airborne sound, best around 2 kHz. The SGO is suspended in haemolymph from a thin acellular membrane between a tracheal air-sac and the wall of the tibia. An unusual feature is that the impulses from the ca. 26 sensilla appear to synchronize during each burst, and furthermore fire at a constant frequency f_R of ~300 Hz, regardless of sound intensity. The origin of this apparent response resonance is of interest as an early step in mechanotransduction. Electrical coupling is an unlikely cause, since neither sensilla nor axons contact each other initially. No change in the effective frequency f_R follows the replacement of air in the tracheae with $He\text{-}O_2$, excluding a Helmholtz resonance in the subgenual air-sac as a causal factor. It is possible that a common mechanical drive coordinates the responses, if the parts of the SGO vibrate in unison at 150 or 300 Hz, in the local fluid environment. The large numbers of microtubules in the cells of the SGO might supply the requisite stiffening, and topical injections of colchicine do lower f_R. But colchicine is effective only at high initial concentration (> 5 mM), high K^+ has similar effects, taxol has none, and the temperature coefficient (Q_{10}) of f_R is ~2, pointing away from a simple mechanical origin for f_R. A source of intrinsic oscillation at a similar frequency in different sensilla could also account for both synchronization and frequency invariance, if a common mechanical trigger supplied initial coordination of the responses. To clarify the mechanism, preliminary intracellular recordings were made, and correlate with impulses recorded from leg nerve 5. These recordings rule out frequency doubling at 150 Hz from opposing groups of sensilla to generate a 300 Hz response, but so far have failed to yield evidence of a voltage-dependent resonance. (Supported by NSERC A9593)

135.11

EFFECTS OF BANG-SENSITIVITY MUTATIONS UPON MECHANOSENSORY BRISTLE RESPONSE IN DROSOPHILA. J. E. Engel* and C.-F. Wu. Biology Dept., Univ. of Iowa, Iowa City, IA 52242.

Drosophila bearing mutations of the genes *bang-sensitive* (bas^1), *bang-senseless* (bss^{MW1}), or *easily-shocked* (eas^2), become paralysed when subjected to mechanical shock ("bang-sensitivity"). Because the mechanosensilla may be involved in the bang-sensitivity phenotype, we monitored the sensory neuron response to step deflections of the anterior postalar (APA) thoracic macrochaete in mutant and wild-type (Canton-S) flies. The results provide a first glimpse at a common physiological effect of these mutations in a class of identified neurons.

The response to a step stimulus is phasic, decaying to a tonic baseline. The phasic response may be described by $y = A \cdot t^{-k}$, where k characterizes the rate of decay (adaptation). In wild-type sensilla deflected strongly enough to produce a saturating, maximal response, k = 0.37. Adaptation rate (k) in mutant sensilla was similar. However, during the first 100 msec of a saturating response, wild-type sensilla produced 33.3 ± 5.5 spikes (X ± SD, n = 16). The maximal response was lower in mutants: eas^2 = 27.3 ± 5.0 (n = 7), bss^{MW1} = 19.6 ± 3.2 (n = 7), bas^1 = 18.1 ± 2.9 (n = 7). This was not likely due to prior fatigue, since two of the mutants fatigued normally following the initial response: given 1000 msec stimuli every 5 seconds, wild-type responses dropped to 84% at the second stimulus, and remained there for subsequent stimuli. eas^2 and bss^{MW1} also dropped to 84% and 83%, respectively. bas^1 did not fatigue, remaining at 100% of initial response. Thus, in these bang-sensitivity mutants, adaptation and (in eas^2 and bss^{MW1}) fatigue were normal, but the maximal response was deficient. This suggests deficits in a rather specific mechanism in these mutants, which may also act at other sites in the nervous system, and thereby lead to the bang-sensitivity phenotype.

135.12

A DIFFERENTIAL INCREASE IN VISUAL SENSITIVITY CORRELATED WITH BIOLUMINESCENT FLASHING ACTIVITY IN TWO SPECIES OF FIRELIES ADAPATED TO DIFFERENT PHOTIC NICHES. Abner B. Lall*. Department of Biology, Howard University, Washington DC 20059.

Based on the bioluminscent flashing activity, North American firelies can be divided into two groups: a) those which restrict their luminescent activity to a short period at twilight (twilight-active) and b) those which are active all night (dark-active). Thresholds for visual sensitivity in the compound eyes was monitored over long periods (24-48 hr) by recording ERGs elicited by constant intensity 550 nm flashes. In the dark-active firefly Photuris versicolor, the day-time responses were small (< 0.5mV) while the night-time responses were ~5-6X larger than their day-time counterpart. This nightly increase in the amplitude of the ERG amounted to an increase in visual sensitivity on average 4.1 log units(N=5). In the twilight-restricted firefly Photinus pyralis, the amplitude of day-time ERG responses were large (~10mV) and increased only 3-4 mV at twilight. This increase in the amplitude corresponded to on average 1.8 log units (N=5) increase of the visual sensitivity. Supported by Howard University Faculty Research Support and NSF grant # NSF 9013076.

135.13

ELECTRICAL PARAMETER CHARACTERISATION OF FLY PHOTORECEPTORS IN MUSCA DOMESTICA. S. Gagné*, M. Liu. Lab. of Vision and Logical Systems, Laval Univ. Elec. Eng. Dept., Québec, Qué. Canada G1K 7P4.

The so called R1-R6 photoreceptors of the fly (musca domestica), looking in the same direction of space, joined together in a closed structure (cartridge) in the first neuropile (lamina) when feeding, by numerous chemical synapses, two large monopolar cells (LMCs). It is believed they make electrical synapses between nearest neighbours while making their close encounter with LMCs. The nature of the return path for the receptor current is not completely characterized but it is taught to be through a high electrical resistance formed by the cartridge. It is possible to take advantage of the symmetrical 3D receptor arrangement of the resulting circuit and to evaluate, with relatively simple experiments, its electrical parameters. It appears, the cartridge offers a large electrical resistance for the return current when a single receptor is stimulated but, is considerably reduced when all receptors are responding, which is the normal case of operation. It seems this fact could be a valuable design principle, that is, built a high electrical barrier first and, let the current from many sources, control its final value. In this way, the building contingencies are less severe from the developmental phase to the final structural arrangement. Another well known effect for the barrier: its averaging and voltage reference point for operating the LMC synapses is also considered.

135.14

CENTRAL DISTRIBUTION OF THE TERMINALS OF PERIPHERAL SENSORY CELLS OF *APLYSIA*. Y. Xin, K. R. Weiss and I. Kupfermann*. Cntr. for Neurobiol. & Behav., Columbia Univ. and the NYS Psychiat. Inst., New York, NY 10032; and Dept. of Physiol. and Biophys., Mt. Sinai Sch. of Med., New York, NY 10029.

The nerves of all central ganglia contain thousands of very small axons which number considerably more than the number of central neurons, suggesting that the small axons may originate from chemosensory and perhaps mechanosensory cells whose cell bodies are located in the periphery. The central terminations of presumptive sensory cells was studied by exposing peripheral structures to S^{35} methionine or H^3 leucine. Following a 24 hr incubation that permitted synthesis and axonal transport of labelled proteins, the central termination of presumptive sensory fibers was determined by autoradiography on sectioned material. Axons from the upper lip region terminate bilaterally in the cerebral, buccal and pleural ganglia, with fibers extending into the pleural abdominal connectives. Axons from the lower lip region terminate in the cerebral and buccal ganglia. Axons from mid-lip, mid-anterior tentacle and lateral-anterior tentacle region project through 3 branches of the anterior tentacle nerve and terminate bilaterally within the cerebral ganglia, with slightly different distributions for the 3 branches. Axons from the rhinophore terminate bilaterally in the cerebral and pleural ganglia, with fibers extending into the pleural abdominal connectives. The rhinophore also send fibers into the ipsilateral eye nerve, suggesting that light sensitive receptors in the rhinophore may directly communicate with the eye. Fibers from the siphon terminate in the abdominal ganglion. The feeding command-like neuron CBI-2 was filled iontophoretically with Lucifer yellow before radiolabelling of the upper lip. Combined fluorescent microscopy and autoradiography revealed that processes of CBI-2 and axons from the upper lip are present in the same small region of the cerebral ganglion, suggesting that CBI-2 may receive direct inputs from peripheral sensory cells. The overall results suggest that peripheral sensory cells provide a major source of input, that is widely distributed throughout the nervous system.

135.15

SOMATOSTATIN IN THE BRAIN OF THE POLYCHAETE ANNELID, NEREIS DIVERSICOLOR. E.I. Pinkhasov and S.C. Feldman*. Depts. of Neuroscience and Anatomy, Cell Biology & Injury Science, New Jersey Medical School, Newark, N.J. 07103.

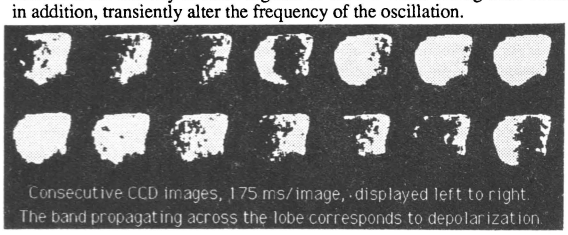
Somatostatin (SS) is a neurpeptide which has been shown by us to be present in several higher invertebrates and is primarily associated with sensory or integrative processing areas. Complex annelids such as the Polychaetes show, for the first time, ganglia which are believed to correspond to fore-brain, mid-brain and hindbrain regions.

In this study we examined the distribution of SS-immunoreactive (I-SS) neurons and fibers in the prostomium of the polychaete annelid Nereis diversicolor. I-SS was localized in the prostomia (heads) of 5 animals by immunocytochemistry using a well-characterized antiserum to the peptide. The prostomium is characterized by layers of presumed sensory neurons under the cuticle and dorsal to the eyes, as well as several central ganglia and extensive commissures and tracts. I-SS was identified in neurons in the dorsal epithelium, in a very few small cells in the optic ganglia and in several fiber tracts, including the optic nerve. The most striking feature was the presence of large numbers of I-SS neurons in the ciliated sensory cells just under the cuticle and in the large, pigmented cells in the epithelium directly covering the eyes. The presence of SS in these sensory layers and in the optic ganglia and tract suggest an early evolutionary association of the peptide with sensory function.

135.16

OLFACTORY INTERNEURON OSCILLATIONS: OPTICAL AND ELECTRICAL MEASUREMENTS OF INTRINSIC AND ODOR-MODULATED ACTIVITY. A. Gelperin*, R. Gervais, K. Delaney, M. S. Fee, J. Flores, D. W. Tank and D. Kleinfeld. Biol. Computation Research Dept., AT&T Bell Laboratories, Murray Hill, N.J. 07974

The intrinsic dynamics of the central network of rhythmically active olfactory interneurons in the terrestrial mollusc *Limax maximus* has been recorded with and without odor input using a nose-brain preparation. The spatially-averaged membrane potentials of cells and fibers in the procerebral (PC) lobe were measured optically after staining the preparation with Di-4-ANEPPS. The local field potential was recorded with a patch pipette. Phase differences in the peak depolarization of the oscillatory activity (T=1.4s) were apparent in multisite optical records. Sequences of CCD derived images revealed a wave-like propagation of depolarization, with velocity ≥3 mm/s, from apex to base of the PC coincident with the oscillation (Fig.) Mild odor stimulation transiently increased the activity in local regions of the PC while strong odor could, in addition, transiently alter the frequency of the oscillation.



135.17

BEHAVIORAL CONSEQUENCES OF REDUCING OLFACTORY INPUT TO THE BRAIN. M.A. Butler, M.A. Willis*, L.P. Tolbert, & E.A. Arbas. ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

Glomeruli are a common element of olfactory systems in a large number of animals, from humans to insects. They comprise spherical knots of nerve fibers where sensory axons and target neurons synapse. The aim of this study was to investigate the role of glomeruli in processing olfactory information, using behavioral and histological techniques. Previous studies have indicated that various degrees of glomerulus formation in the antennal lobes of the brain of the moth *Manduca sexta* can be effected by reducing the number of sensory axons that grow into the antennal (olfactory) lobe during development. Therefore, we surgically reduced the number of antennal segments in developing animals to produce animals with reduced numbers of glomeruli. The odor-stimulated behaviors of normal and experimental adult moths were observed and quantified in a wind tunnel, where the moths were challenged to fly upwind to the source of an attractive odor. Some of the moths that developed with reduced olfactory input flew upwind to the odor source. The flight behavior of these individuals was similar to the odor-mediated flight typically observed from normally developing moths. The histology of the moths' antennal lobes was then analyzed to determine the extent of formation of glomeruli. We found that the antennal lobes of more than half of the respondents were mostly aglomerular. There are a few isolated glomeruli, however, in at least one antennal lobe of each moth that developed with reduced antennal input and displayed odor-mediated behavior. This suggests that either a small number of glomeruli is sufficient to process the information necessary for this behavior or that glomerular organization may not be important for this fundamental odor-mediated behavior.

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135.18

ULTRASTRUCTURAL CHARACTERISTICS OF THE 5HT-IMMUNOREACTIVE NEURON IN THE ANTENNAL LOBE OF *MANDUCA SEXTA*. X. J. Sun*, L. P. Tolbert, and J.G. Hildebrand. ARL Div. of Neurobiol., Univ. of Arizona, Tucson, AZ 85721.

In each of the two antennal lobes (ALs), the primary olfactory centers, of the brain of *Manduca sexta*, one neuron displays 5-hydroxytryptamine (5HT)-like immunoreactivity, and this cell persists throughout the post-embryonic life of the insect. The morphology of this neuron was described by Kent *et al.* (*J. Neurobiol.* (1987) **19**:451-465): its cell body resides in the AL, and without further ramification in the AL, it sends processes into the ipsi- and contralateral protocerebrum and finally into the contralateral AL. The arborizations of the neuron in the contralateral AL are distributed in most or all of the glomeruli. What role(s) this neuron may play in processing of olfactory information is not clear. We report here the ultrastructural characteristics of the 5HT-immunoreactive neuron, in the AL of the adult female moth, revealed using peroxidase-antiperoxidase and immunogold labeling methods. The membrane of the cell body was highly indented and the cell body, very rich in endoplasmic reticulum, Golgi apparati, and clusters of dense-cored vesicles. No synapses were found on the unbranched neurite in the ipsilateral AL, although dense-cored and small clear vesicles were present. The neurites of the neuron in the contralateral AL, however, did participate in synapses. The branches within the AL glomeruli, among the finest in those neuropil modules, participated in synapses in the glomeruli at very low frequency: 66% of the documented synapses (29) were output synapses and 34% were input synapses, and among them, most appeared to be dyadic synapses in single sections. Our observations suggest that this neuron is a very active cell and may play a feedback regulatory role in the processing of olfactory information in the AL.

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135.19

MODULATORY EFFECTS OF 5-HYDROXYTRYPTAMINE ON INTERNEURONS IN THE ANTENNAL LOBE OF THE SPHINX MOTH *MANDUCA SEXTA*. P. Kloppenburg* and J.G. Hildebrand. ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

Among the ca. 1200 neurons in each antennal lobe (AL) in *Manduca sexta* is a single 5-hydroxytryptamine (5HT)-immunoreactive neuron. Each of these neurons has extensive ramifications in the ipsi- and contralateral protocerebrum and arborizations throughout the glomerular neuropil of the contralateral AL (Kent et al., *J. Neurobiol.* (1987) **19**:451-465; Homberg and Hildebrand, *Cell Tissue Res.* (1989) **258**:1-24). Combined immunocytochemical and ultrastructural studies suggest that this neuron may provide centrifugal (descending) input to the AL (Sun et al., accompanying abstract). We have begun to examine the physiological role of 5HT (serotonin) in AL interneurons by means of intracellular recording.

The responses of local interneurons induced by electrical stimulation of the antennal nerve (a depolarization sometimes leading to firing of action potentials) are enhanced by superfusion of the AL with 5HT (10^{-4}M). Preliminary results show low concentrations (10^{-8}M) of 5HT decrease the width of spontaneous spikes, whereas high concentrations (10^{-4}M) lead to spike broadening. In some neurons, electrical stimulation of the antennal nerve evokes a complex response that includes a brief hyperpolarization of the cell membrane. This hyperpolarization can be enhanced by a low concentration of 5HT (10^{-8}M). These effects of 5HT can be reversed by washing with saline for ca. 10 min. The spontaneous activity of AL neurons generally remains unaltered by 5HT.

The changes in membrane properties and the receptors that mediate these 5HT effects are currently under examination (Mercer et al., accompanying abstract).

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135.20

MODULATORY EFFECTS OF 5-HYDROXYTRYPTAMINE ON VOLTAGE-GATED CURRENTS IN CULTURED INSECT OLFACTORY NEURONS. A.R. Mercer, J.H. Hayashi, and J.G. Hildebrand*. ARL Div. of Neurobiol., Univ. of Arizona, Tucson, AZ 85721.

5-Hydroxytryptamine (5HT or serotonin) modulates the activity of antennal-lobe (AL) neurons in the brain of the sphinx moth *Manduca sexta* (Kloppenburg and Hildebrand, accompanying abstract). To understand better the mechanisms that underlie the actions of this biogenic amine *in vivo*, we have used whole-cell patch-clamp techniques to analyze the effects of 5HT on membrane currents in AL neurons grown in culture. AL neurons can be dissociated and cultured from animals at all 18 stages of adult development. A subset of neurons, identified on the basis of morphological characteristics, were found to respond to 5HT in a constant and predictable way. 5HT caused reversible reduction of both an A-type K^+ current and a more slowly activating K^+ current resembling the delayed rectifier. Modulation of K^+ currents may underlie the 5HT-induced increases in neuronal excitability and broadening of action potentials observed in some AL neurons *in vivo*.

In cells taken from pupae at stage 5 of adult development, 5HT modulates an inward Ca^{++} current that does not appear to be affected by 5HT in cells taken from pupae at a later stage in development (stage 9). The ability of 5HT to modulate specific membrane channels may play a role not only in olfactory information processing but also in the growth and development of AL neurons during metamorphosis of this insect.

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136.1

The Differential Laminar Distribution Of Cells In Prefrontal Cortex Projecting To The Mediodorsal Nucleus.

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Neurons in the deep layers of each area in the prefrontal cortex (PFC) project to the three subdivisions of the mediodorsal nucleus of the thalamus (MD). Horseradish peroxidase and retrograde fluorescent tracers were injected into MD to assess the laminar distribution of corticothalamic neurons in the different architectonic areas of PFC. The laminar distribution pattern of these cells follows the progressive architectonic differentiation of the PFC.

Although cortical projections to MD arise from cells in layers V and VI, the relative distribution of those neurons is not the same for each prefrontal area. Less differentiated areas of the PFC (Pro, areas 13, 14, 25 and 32) are weakly laminated, have a poorly granulated layer IV and have small pyramidal cells in layer III. MD projecting cells in these areas are concentrated in layers Vb and VI. PFC areas 9, 10, 11, 12 have intermediate cytoarchitectonic features including well defined laminae, modest granularity in layer IV and large pyramidal neurons in layer III. The corticothalamic neurons in these areas are located mainly in layer Vb, somewhat in layer VI and occasionally in layer Va. Areas 46 and 8 of the PFC are the best differentiated, with six well-defined laminae, clear layer IV and abundant large layer III pyramidal cells. Neurons projecting to MD in these areas are located mainly in layer Vb, regularly in layer Va and occasionally in layer VI. The variation in the laminar distribution of MD-projecting neurons in PFC parallels the progressive elaboration of cytoarchitectonic features of the PFC. Thalamic projections from the least differentiated areas of PFC arise from mainly from layers Vb and VI, whereas thalamic projections in the best differentiated areas arise mainly from layer Vb, Va and somewhat from layer VI.(Supported in part by NIH 16841).

136.2

ARCHITECTONIC SUBDIVISIONS OF THE VENTROLATERAL THALAMUS (VL) IN OWL MONKEYS (*AOTUS TRIVIRGATUS*): COMPARISON OF NISSL, ACHE AND CO PATTERNS.

I. Stepniewska*, T.M. Preuss, and J.H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

As part of investigations of thalamic connections of motor and premotor cortex, we used Nissl stains and histochemical procedures for cytochrome oxidase (CO) and acetylcholinesterase (AChE) to help identify nuclei in the ventrolateral thalamus of owl monkeys. Material was obtained from 15 brains cut in frontal, parasagittal or horizontal planes.

We were able to distinguish most of Olszewski's divisions of VL, although we use a modified nomenclature to designate them. These divisions were much more readily distinguished in AChE than in Nissl or CO. The anterior part of VL (VLo of Olszewski) contains darkly stained neurons of medium size arranged in characteristic clusters. VLo is also characterized by AChE-rich perikarya embedded in dark matrix of AChE-rich neurites. The posterior part of VL can be divided into three subdivisions. The principle portion, VLp (VPLo of Olszewski), is composed of large, darkly stained cells, sparsely distributed among bundles of fibers. VLp has a patchy pattern of neuropil that stains darkly for AChE and CO. More medially, VLx (area X of Olszewski) contains smaller, lighter and more uniformly distributed neurons. VLx stains lightly and evenly for AChE. CO staining in VLx is moderate, uniform, and similar to VLp. Dorsally, VLd (VLc of Olszewski) consists of densely packed neurons that are smaller than these in VLp and VLx. VLd stains darkly for both AChE and CO. (Supported by NS 16446)

136.3

OVERLAP OF THALAMO-ACCUMBENS AND THALAMO-AMYGDALOID PROJECTION NEURONS WITH PEPTIDERGIC FIBERS IN THE DORSAL MIDLINE THALAMUS OF THE RAT.

L. J. Freedman*[1] and M. D. Cassell[2]. [1]Neuroscience Program and [2]Department of Anatomy, University of Iowa, Iowa City, IA 52242.

Many of the afferents to the dorsal midline thalamus are peptidergic. Differences in the distribution of these fibers might suggest a topography whereby different neurochemically defined inputs influence groups of midline thalamic neurons projecting to different basal forebrain targets. We previously have shown that peptides are distributed in three basic patterns. Enkephalin and several other peptides are found in fibers in dorsal and ventral paraventricular thalamus (PV). αMSH and neuropeptide Y fibers are found in laterodorsal PV. Vasopressin fibers are found in medial PV.

To test how well these patterns of peptidergic fibers are aligned with the distribution of neurons projecting to specific basal forebrain targets, we combined retrograde tracing with fluorogold and red fluorescent microspheres with immunohistochemical localization of peptidergic fibers. We placed injections of these tracers in the core and shell of the nucleus accumbens and in the central amygdaloid nucleus. We observed that enkephalin fibers overlapped best with cells best projecting to the shell or core of the nucleus accumbens. αMSH fibers overlapped best with cells projecting to the shell of the nucleus accumbens. Vasopressin overlapped best with cells projecting to the central amygdaloid nucleus. This arrangement suggests a "labelled line" arrangement whereby specific inputs to the midline thalamus influence specific outputs to the basal forebrain.

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136.4

CHOLINERGIC INNERVATION OF THE HUMAN THALAMUS.

S. Heckers*, C. Geula, M-M. Mesulam. Harvard Medical School, Boston, MA 02215.

The cholinergic innervation of the human thalamus was studied with an antibody against the enzyme choline acetyltransferase (ChAT). All thalamic nuclei displayed ChAT-positive axons and varicosities. Only the medial habenula contained ChAT-positive perikarya. Intralaminar nuclei (central medial, central lateral, and paracentral), the reticular nucleus, midline nuclei (paraventricular and reuniens), some nuclei associated with the limbic system (anterodorsal nucleus and medially situated patches in the mediodorsal nucleus) and the lateral geniculate nucleus displayed the highest level of ChAT-positive axonal varicosities. The remaining sensory relay nuclei and the nuclei interconnected with the motor and association cortex displayed a lower level of innervation. The contribution of basal forebrain afferents to the cholinergic innervation of the human thalamus was studied with the aid of nerve growth factor receptor (NGFr) immunoreactive axonal staining. The anterior intralaminar nuclei, the reticular nucleus, and especially medially situated patches in the mediodorsal nucleus displayed a substantial number of NGFr-positive varicose axons, presumably originating in the basal forebrain. Rare NGFr-positive axonal profiles were also seen in many of the other thalamic nuclei.

These observations suggest that nuclei affiliated with limbic structures and with the ascending reticular activating system are likely to be under particularly intense cholinergic influence. While the vast majority of thalamic cholinergic input seems to come from the upper brainstem, some nuclei also appear to receive substantial cholinergic innervation from the basal forebrain.

136.5

THE THALAMIC PARAFASCICULAR PROJECTION TO THE SUBTHALAMIC NUCLEUS : A GLUTAMATERGIC EXCITATORY PATHWAY.

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A thalamic projection to the subthalamic nucleus (STN) arises from the intralaminar parafascicular nucleus (Pf). We recorded unit activity within the STN to electrical and/or chemical Pf stimulation in anesthetized (ketamine) rats in order to investigate the nature of the thalamo-subthalamic transmission. Electrical Pf stimulation (single square shock, 0,1 msec, 20-60 μA) elicited in the STN an initial single spike (latency : 3,7 ± 0,2 msec), an inhibitory period lasting for 6-9 msec, a secondary firing burst (2 to 5 spikes, latency 11,8 ±0,5 msec, discharge rate : 150 to 250 Hz) followed by a complete depression of the spontaneous activity.The initial excitation remains after section of cortico-subthalamic projection and/or pallidal lesion. Microinjection of he excitatory amino-acids receptors antagonist, kynurenic acid into the STN suppress the initial spike but not secondary burst in dose (0,035, 0,1 and 0,3 μg. in 200 nl) dependant manner. Long-lasting cholinergic stimulation of the Pf (microinjection of carbachol : 10 μg in 100 nl) elicited and maintained elevated firing rate of the STN (105±14%) for more than 30 min demonstrated during unit recording (n = 8) and/or 2-deoxyglucose experiments (n=5). These results suggest that the STN neurons are monosynaptically driven by the Pf efferents output ant that thalamo-subthalamic synaptic transmission may implicate excitatory amino acid glutamate.

136.6

INTRACELLULAR ANALYSIS OF A SLOW RHYTHM IN RETICULAR THALAMIC NEURONS.

R. Curró Dossi*, D. Contreras and M. Steriade. Lab. of Neurophysiology, Laval Univ. Sch. of Med. , Quebec, Canada G1K 7P4.

Thalamic relay cells oscillating at delta (1-4 Hz) or slower frequencies become synchronized by stimuli applied to cortical foci not directly related to the recorded cells (Steriade et al., J. Neurosci. 1991, 11:3200-3217). This finding led to the hypothesis that reticular thalamic (RE) cells are implicated in the synchronizing process. Here we report the results of intracellular recordings from RE neurons of urethane-anesthetized cats displaying a slow rhythm (≈0.3 Hz) similar to that observed in neocortical cells (Steriade et al., this meeting).

The slow rhythmicity observed in RE cells was constituted by long depolarizing plateaus and spike trains recurring periodically every 3 to 5 s. These plateaus were diminished and eventually blocked by hyperpolarization as well as by QX-314. The slow rhythm of RE neurons was closely correlated with EEG complexes, and transitions from synchronized to less synchronized EEG states were paralleled by the disappearance of RE-cells' oscillations.

Recordings of RE cell-couples revealed that simultaneously recorded RE cells may be synchronized within the 0.3 Hz rhythm. However, in other instances, RE neurons tonically fired at 10-40 Hz (see also Contreras et al. , this meeting), regardless of the EEG synchronization and of the slow rhythm in the simultaneously recorded cell.

We propose that RE cells, driven by the cortex , fire at the slow rhythm and , in turn, synchronously trigger long-lasting hyperpolarizations in thalamic relay cells. This would explain the periodic recurrence at 0.3 Hz of a hyperpolarization-activated intrinsic delta oscillation (1-4 Hz) in thalamic relay neurons.

Supported by MRC of Canada (grant MT-3689).

136.7

ELECTROPHYSIOLOGICAL PROPERTIES OF RETICULAR THALAMIC CELLS. D. Contreras*, R. Curró Dossi and M. Steriade, Lab. of Neurophysiology, Laval Univ. Sch. of Med., Quebec, Canada G1K 7P4.

Neurons of the reticular thalamic (RE) nucleus are implicated in processes of synchronization and rhythm generation within thalamocortical systems. To explore the intrinsic properties of RE neurons, we performed intracellular recordings in the anterolateral districts of the RE nucleus in urethane-anesthetized cats.

Upon injection of depolarizing current pulses, 82% (n=41) of the recorded cells responded with tonic firing at rest (≈-60mV) and with spike-bursts at a V_m more negative than -75 mV. Transition in firing pattern, from tonic to bursting, occurred gradually. During the pulse, the discharge frequency started to increase at -65 mV and reached a maximum of 250 Hz, with a peculiar burst morphology, at a V_m between -75 and -85 mV. The graded nature of spike-bursts in RE cells suggests that low-threshold spikes are generated in multiple dendritic foci and eventually summate, giving rise to somatic spike-bursts. In some cells, bursting behavior was seen as high-frequency dendritic spikes, with an amplitude of 3 to 7 mV, which could trigger somatic spikes or plateau potentials.

By contrast, 18% (n=9) of cells fired only in the single-spike repetitive mode. These non-bursting cells showed a high propensity for sustained regular firing (8-10 Hz) at rest, even during deeply synchronized EEG patterns, and increased their firing rates up to 40-50 Hz upon slight DC depolarization. The apparent lack of bursting behavior in this neuronal type can be related to a similar absence of spike bursts in ventral lateral geniculate neurons (Crunnelli et al., 1987) with which RE neurons share a common embryological origin.

Supported by MRC of Canada (grant MT-3689).

136.8

SIMULATED ENTRAINMENT OF THALAMOCORTICAL CELLS BY REPETITIVE CORTICAL SHOCKS. W. W. Lytton*, T. J. Sejnowski, and M. Steriade, University of Wisconsin, Madison, WI, 53792, The Salk Institute, La Jolla, CA, 92037, Laval University, Quebec City, Canada, G1K7P4.

Intrinsic properties and synaptic input both aid in determining the major oscillatory behaviors of thalamocortical relay cells: the 8-10 Hz spindle oscillations seen in early sleep and the 1-3 Hz slow oscillations seen in slow-wave sleep. We simulated the effects of cortical shocks, including both the initial excitatory component and feedforward inhibition. We were able to match intracellular recordings by placing both synapse types at sites about 100 microns out on the dendrites, with activation of $GABA_A$ feedforward inhibition at 30 ms following the EPSP and activation of $GABA_B$ at 50 ms. In order to obtain the smooth transition between EPSP and IPSP observed electrophysiologically, we had to activate multiple $GABA_A$ inputs over a 20 ms range of time intervals. A low-threshold spike followed the inhibition. Cortical shocks could reset the phase of either the spindle-frequency oscillations seen at resting membrane potential (V_m) or the slow waves occurring with D.C. hyperpolarization. The slower rhythm could also be entrained by rapid repetitive cortical stimulation to above 10 Hz in the model, though the responses were subthreshold for sodium spikes above 7 Hz. Cortical stimulation could not slow the frequency. Simulated repetitive cortical shock with the thalamocortical neuron model at resting V_m produced repeated damped oscillations at spindle frequencies.

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136.9

LAMINAR ORIGIN OF STRIATAL AND THALAMIC PROJECTIONS OF THE PREFRONTAL CORTEX IN RHESUS MONKEYS. E.H. Yeterian* and D.N. Pandya. Dept. of Psychology, Colby College, Waterville, ME 04901, E.N.R.M. V.A.M.C., Bedford, MA 01730, and Boston Univ. Sch. of Med.

Corticostriatal and corticothalamic projections of the prefrontal cortex have been shown to have specific, similar distributions. For example, the medial and orbital prefrontal regions are related to the medial portions of both the head of the caudate (HCN) and of the mediodorsal (MD) nuclei. To compare the laminar distribution of striatal and thalamic projection neurons, fluorescent retrograde tracers (Diamidino Yellow and Fast Blue) were injected into topograpically similar portions (e.g., medial sectors) of both nuclei in the same animal. Consistent with earlier studies, projections to common topographic sectors of the HCN and MD nuclei were found to arise from similar architectonic regions. The laminar origins of these projections, however, were distinctive. Although the projection neurons leading to the HCN and MD nuclei were located predominantly in infragranular cortical layers V and VI, with occasional cells noted in layer III, the bulk of striatal neurons was observed in layer Va, with fewer cells in layer Vb. In contrast, thalamic projection neurons were found mainly in layer VI, with a smaller number in layer V. Only occasional double-labeled cells were observed, suggesting segregation of striatal and thalamic projections. The differential laminar patterns of neurons projecting to the striatum and thalamus suggest that these structures receive independent rather than collateral forms of information from the prefrontal cortex. (Supported by the Department of Veterans Affairs, NIH grant 16841, and Colby College Social Science grant 01 2285.)

136.10

ENK+ AND SP+ STRIATAL NEURONS AND THEIR INTERACTION AT THE EM LEVEL. E.J. Karle*, K.D. Anderson and A. Reiner. Dept. of Anatomy & Neurobiology, Univ. of Tennessee, Memphis, TN, 38163.

Although information is available on the synaptic inputs to medium spiny striatal neurons, little is specifically known regarding enkephalin-containing (ENK+) versus substance P-containing (SP+) striatal neurons and the interaction between them. Therefore, we are investigating this issue in pigeons, in whom ENK+ and SP+ perikarya, dendritic shafts and spines, and terminals can be readily labeled.

Immunohistochemical single-label studies within the striatum revealed that most ENK+ and SP+ terminals formed symmetric synapses with unlabeled perikarya, dendritic shafts and spines. The ENK+ and SP+ input to spines showed noteworthy differences. ENK+ terminals were more likely than SP+ terminals to target spines. Of those terminals contacting spines, SP+ terminals typically made symmetric synaptic contact at the bases of spines, whereas ENK+ terminals often wrapped around the spines. Double-label EM study of ENK+ input to SP+ structures, using silver-intensified immunogold and diaminobenzidine as two distinct markers, showed that SP+ neurons received input from ENK+ terminals at their perikarya, dendritic shafts and spines.

These results show that the collaterals of ENK+ and SP+ striatal projection neurons form synaptic specializations with other neurons within the striatum, with many ENK+ terminals clearly contacting SP+ structures. Since we have previously shown similarities between basal ganglia in pigeons and in mammals, the present results should also be true of striatal medium spiny neurons in mammals. Supported by NS-19620 & NS-28721 (A.R.)

136.11

THE MARGINAL DIVISION IN THE NEOSTRIATUM OF THE CAT. S.Y.Shu*, X.M.Bao and Z.W.Xu. Dept. of Neurobiology, Zhu-Jiang Hospital, Guangzhou, 510282, China.

In our previous studies, a new subdivision, the marginal division(MrD) was found in both rat and monkey striatums(Shu et al, J.of Chemical Neuroanatomy, 1988. Shu and Bao, Soc Neurosci Abstr. Vol.16,1990). The division is located at the caudomedial extent of the striatum surrounding the rostrolateral border of the globus pallidus. It exhibits special cellular organization, immunohistochemical reactivity and fiber connections.

The present abstract reported our investigation on the marginal division in the cat striatum. Nissl staining,histochemistry and immunohistochemistry methods were used. A modified DAB-nickel method(Shu et al, Neurosci. Lett. 1988) was used to enhance the visualization of the immunohistochemistry reaction product.A band of scattering large fusiform neurons was observed at the medial border of the putamen surrounding the lateral edge of the globus pallidus in the cat striatum. Some of them are AchE positive, or Enk-, or SOM-, or CCK-immunoreactive. SP-, L-Enk-, 5-HT-, NT- and SOM-immunohistochemistry positive fibers and terminals were found more densely packed in this band than the rest of the striatum. The location of the band, the morphology and immunoreactivity of the neurons,and the immunoreactivity of the fibers and terminals in the band proved that there is also a marginal division in the cat striatum. This study indicates that the marginal division is probably an universal structure in the striatum of the vertebrate.

136.12

PALLIDOTHALAMIC AFFERENTS OF THE MEDIAL AGRANULAR CORTEX IN THE RAT: A COMBINED PHA-L AND CTB TRACING STUDY. S.T. Sakai*, K. Bruce and M. Park. Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

Pallidothalamic input to the supplementary motor area is thought to provide an important link in motor control pathways in primates. However, similar data in non-primate species are lacking primarily due to questions regarding the comparability of cortical and thalamic regions. Although the rat medial agranular cortex (AGm) is thought to contain the supplementary motor area, the source of the ascending inputs to AGm by way of the thalamus remains unclear. Our purpose was to determine if the AGm receives afferents originating from the entopeduncular nucleus (EP). The pallidothalamic and thalamocortical projections were determined using a double-labeling paradigm in which pressure injections of Cholera toxin subunit B (CTB) were made into different sectors of AGm. At the same time, iontophoretic injections of *Phaseolus vulgaris leucoagglutinin* (PHA-L) were made into EP. The tissue was processed using a sequential immunohistochemical protocol. The majority of retrogradely labeled CTB neurons were found in the ventral anterior lateral nucleus (VAL) and the ventromedial (VM) nucleus. Dense plexuses of PHA-L label were also observed in VAL, VM and the lateral habenular nucleus (LHb). The greatest coincidence of both CTB labeled cells and PHA-L anterograde label was in the anterolateral portion of VAL and in VM. This pathway may provide an anatomical substrate for the motor control functions of the AGm in the rat. (Supported by the College of Human Medicine BRSG).

136.13

THE CINGULOSTRIATAL PROJECTION IN THE MONKEY: TOPOGRAPHY AND RELATIONSHIP TO CHEMICAL ORGANIZATION. M.M.Mizobuchi* and S.N.Haber. Dept. of Neurobiology and Anatomy, Univ. of Rochester, Rochester, NY 14642

The ventral portion of the striatum is considered to receive input from structures related to the limbic lobe, the dorsolateral region receiving information from sensorimotor cortex. As part of a larger study to understand the organization of limbic-related striatal input, we injected tritiated amino acids and Lucifer yellow (LY) into areas 25, 32 and rostral 24 of the limbic-related cingulate gyrus and contrasted these striatal projections to ones arising from area 23, a motor-related part of the cingulate gyrus. Routine autoradiography and immunocytochemistry were carried out on 50 µm sections throughout the striatum. To compare the cortical terminal fields to acetylcholinesterase (AChE)-poor "striosomes", enkephalin (ENK)-positive, or calcium binding protein (CaBP)-negative patches, compartments adjacent to the autoradiography were processed for AChE, ENK or CaBP staining. A dense patchy distribution of labelled fibers from area 25, 32 is observed in the medial part of the caudate head, nucleus accumbens and rostromedial putamen. Caudally, label continues in the medial part of the body of the caudate. No terminal fields are seen in the caudal part of the putamen. The terminal fields from area 24 expand to more central part of the striatum than those from areas 25 and 32. The terminal fields from area 23 are in the dorsolateral caudate head and the central part and lateral edge of the putamen. There are no terminals in the nucleus accumbens. The results show an anterior-posterior cortical relationship corresponding to the medial-lateral alignment of terminal fields in the striatum. The distribution of each marker varies. Comparison between terminal fields and "striosomes" reveal inconsistent matches, however, in general the limbic-related cortices preferentially project the matrix compartment in AChE staining. A similar relationship is observed with other markers.
Supported by NIMH MH45573 and NIH NS 22511.

136.14

ORGANIZATION OF DOPAMINERGIC STRIATAL PROJECTIONS IN RELATION TO PEPTIDERGIC INNERVATION OF THE SUBSTANTIA NIGRA IN PRIMATE. E. Lynd-Balta*, and S.N. Haber, Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, New York 14642.

The dopaminergic neurons of the pars compacta that project to the striatum occupy a large area of the substantia nigra in the primate. A dorsal and ventral tier of cells have been identified with distinct characteristics. The substantia nigra is also heavily innervated by different neuropeptides, presumably due to striatal inputs. We have examined the organization of the dopaminergic neurons projecting to the striatum with respect to neuropeptide staining patterns in the substantia nigra.

The retrograde tracers horseradish peroxidase conjugated to wheat germ agglutinin and Lucifer yellow were injected into different areas of the striatum. Dual immunocytochemical staining for the tracer and specific peptides, i.e. Substance P and enkephalin, was performed on single sections to determine the relationship between neurons projecting to different functional areas of the striatum and the peptidergic innervation of the substantia nigra. For example neurons projecting to the ventral striatum are found in the dorsal tier of dopaminergic neurons, clearly separate from enkephalinergic or Substance P immunoreactivity. The results indicate that neurons projecting to distinct areas of the striatum may be influenced differently by striatal peptidergic inputs.
Supported by NIMH MH 45573 7NIH NS22511.

136.15

THE INTEGRATIVE ROLE OF THE SUBSTANTIA NIGRA IN BASAL GANGLIA CIRCUITRY. S.N Haber* and E. Lynd-Balta. University of Rochester School of Medicine & Dentistry, Rochester, New York 14642.

Several cortical- basal ganglia- thalamo-cortical circuits are thought to exist based on the topography of cortical inputs to the striatum, and striatal projections to the globus pallidus. The organization of the striato- nigro- striatal loop in relation to these different functional circuits has not been explored. This study was undertaken to determine the organization of dopaminergic neurons projecting to the striatumwith respect to the topography of inputs from distinct striatal areas in the primate.

A combination of retrograde and anterograde tracers were injected into different regions of the striatum. Adjacent or single sections were dual stained to visualize retrogradely labeled neurons and afferent terminals in the substantia nigra.

The results demonstrate that there are both reciprocal and non-reciprocal connections between the substantia nigra and distinct regions of the striatum. For example, when a retrograde and anterograde tracer were placed in adjacent regions of the dorsolateral striatum, terminal labeling and retrogradely labeled neurons overlapped extensively in the ventrolateral part of the substantia nigra. However numerous retrogradely labeled neurons were also found in the medial aspect of the substantia nigra, which is devoid of dorolateral striatal inputs. This medial region of the substantia nigra is known to receive inputs from the ventral striatum and the ventral pallidum. The combination of an anterograde and retrograde tracer in the ventral pallidum and dorsolateral striatum respectively, confirm this overlap of ventral pallidal inputs and dopaminergic neurons projecting to the dorsolateral striatum in the dorsomedial substantia nigra. The substantia nigra may allow different areas of the striatum to communicate via the organization of its afferent and efferent projections. Taken together these studies suggest that the ventral striatum may modulate the dorsolateral striatum via the connections of the substantia nigra.
Supported by MINH MH45573 7NIH NS22511.

BASAL GANGLIA AND THALAMUS II

137.1

BRAINSTEM CHEMOSPECIFIC AFFERENTS TO PRIMATE PALLIDUM. A. Charara, R. Boucher* and A. Parent. Centre de recherche en neurobiologie, Hôpital de l'Enfant-Jésus, Québec, Canada, G1J 1Z4.

Previous studies from this laboratory have shown that neurons of the substantia nigra pars compacta, pedunculopontine nucleus and dorsal raphe nucleus project to the pallidum in primates. The aim of the present study was to analyze the organization of projections from these brainstem nuclei to the pallidal complex in the squirrel monkey (*Saimiri sciureus*) by combining retrograde transport methods (cholera toxin B) with immunohistochemical techniques for tyrosine hydroxylase, calbindin D-28k, 5-hydroxytryptamine and choline acetyltransferase. After injection of cholera toxin B into the pallidum, retrogradely labeled neurons were found in the substantia nigra pars compacta, dorsal raphe nucleus and pedunculopontine nucleus. In the substantia nigra pars compacta, labeled neurons formed a compact group medially and a more diffuse group laterally. The majority of these neurons did not display immunoreactivity for calbindin. In the dorsal raphe nucleus, retrogradely labeled neurons occurred both centrally and dorsally. Many of these neurons displayed immunoreactivity for serotonin. In the pedunculopontine nucleus, retrogradely labeled neurons were found both ventrally and dorsally to the superior cerebellar peduncle. Some of them were also immunoreactive for choline acetyltransferase. These findings reveal that dopaminergic, serotoninergic and cholinergic neurons of the brainstem directly influence the output neurons of the basal ganglia at the pallidal level in primates. Investigations aiming at determining the exact distribution and proportion of these various brainstem chemospecific neurons projecting to the pallidum are presently under way. [Supported by MRC, FRSQ and FCAR].

137.2

BRAINSTEM CHOLINERGIC NEURONS EXPRESS GLUTAMATE-LIKE IMMUNOREACTIVITY IN THE SQUIRREL MONKEY. B. Lavoie* and A. Parent. Centre de recherche en neurobiologie, Hôpital de l'Enfant-Jésus, Québec CANADA G1J 1Z4

A previous study in rodents revealed that numerous cholinergic (NaDPH-d positive) neurons in the brainstem display glutamate (GLU) immunoreactivity (IR). In this study we used single and double immunohistochemical procedures with antibodies against choline acetyltransferase (ChAT) and GLU to localize these two types of neurons in the brainstem of squirrel monkey (*Saimiri sciureus*). Cholinergic neurons are largely confined to the pedunculopontine (PPN) and laterodorsal (LDT) tegmental nuclei, the parabigeminal nuclei, and the IIIth and IVth cranial nerve nuclei. Comparisons of adjacent sections immunostained for either GLU or ChAT revealed that the pattern of distribution of GLU-like neurons is similar to that of ChAT neurons, except that GLU-like neurons are scattered over a larger sector of the brainstem. GLU IR is mainly found in large neurons of PPN and LDT as it is also the case for ChAT IR. However, smaller GLU-like neurons are also present in this area. GLU IR is also found in neurons of the IIIth and IVth cranial nerve nuclei and in the parabigeminal nucleus. The analysis of double-immunostained sections reveals that both GLU and ChAT are colocalized in numerous neurons of the brainstem. However some neurons display only GLU or ChAT immunoreativity and the ratio of the three subpopulations varies from one cholinergic group to the other. Since PPN and LDT neurons are known to exert a powerful effect on various forebrain neuronal populations, our results suggested that this influence could be mediated by both acetylcholine and glutamate. [Supported by MRC]

137.3

ANATOMICAL ANALYSIS OF THE VENTRAL STRIATUM IN THE MACAQUE MONKEY. D.P. Friedman*, L.J. Porrino, and S. Vinsant. Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

The ventral striatal region (VSR) includes the nucleus accumbens, olfactory tubercle (OT) and a portion of the ventral putamen. Although this region has been distinguished from the dorsal striatum on a variety of anatomical and functional grounds, it has not yet been systematically described in the monkey. We have combined histochemistry, immunocytochemistry, and receptor localization in a first step to understand the regional anatomy of the VSR.

Both rhesus and cynomolgus monkeys were deeply anesthetized and either perfused with 4% paraformaldehyde or left unperfused before the brains were removed. Sections from perfused brains were incubated to reveal acetylcholinesterase (ACHE) or with antibodies against tyrosine hydroxylase (TH), calbindin (CALB), or neurotensin (NT). Sections from unperfused brains were incubated with [^{3}H] SCH23390 to reveal D1 dopamine receptors, or [^{3}H] sulpiride to reveal D2 receptors.

In the VSR, all markers revealed a narrow medial compartment that extended in a ventrolateral arc from the floor of the 3rd ventricle into the region of the OT. A larger, more lateral compartment could be distinguished from the dorsal striatum by relatively light TH immunoreactivity (IR) and a less dense population of D1 and D2 binding sites. Caudally, ACHE and TH and CALB-IR failed to distinguish the medial compartment from the OT.

These findings suggest that in the monkey the VSR is distinguishable from the dorsal striatum and that it consists of 2 compartments, a medial (shell) region that may include at least the caudal part of the OT, and a more lateral (core) region located largely in the ventral putamen.

137.4

CHEMICAL COMPARTMENTALIZATION OF THE RAT GLOBUS PALLIDUS. K. Elisevich*, N. Rajakumar and B.A. Flumerfelt, Dept.of Anatomy, University of Western Ontario, London, Ontario, Canada.

Striatal neurons projecting to EPN and SN contain SP and dynorphin colocalized with GABA, while those projecting to GP contain enkephalin (ENK) colocalized with GABA. Recent observations that two-thirds of striosomal neurons contain ENK/GABA, and that the content of ENK in SN and EPN is significantly low suggest a substantial projection from the striosomal compartment to GP. Therefore, it was undertaken to study the distribution of neurochemical markers in GP to identify separate areas that receive inputs from the two striatal compartments. Adult female Wistar rats were anasthetized with sodium pentobarbital and perfused with 4% PFA in PB (pH 7.4). Frozen transverse sections (40μ) were cut through the striatum and GP. Serial sections were immunolabeled for calbindin (CB) and adjacent sections through GP were also processed for single labeling with antibodies to SP, ChAT, leu- ENK or parvalbumin employing the indirect immunocytochemical technique using DAB, or double labeling using immunofluorescence. The results confirm a complete absence of immunoreactivity (IR) for CB in striosomes and in the dorsolateral part of the striatum. GP showed a moderate density of CB-IR. A continuous area devoid of CB-IR was seen extending throughout the rostrocaudal extent of GP, and was most conspicuous in the lateral aspect of the rostral part. The CB-negative area had parvalbumin containing neurons whereas the CB-positive area contained most of the ChAT- and SP-IR, and ENK was seen in both areas. The data indicate a compartmentalization of the GP that corresponds to the compartmental projections from the striatum. (Supported by MRC Canada).

137.5

DUALITY IN THE GABAERGIC OUTPUT SYSTEM OF THE BASAL GANGLIA IN THE RAT. B.A. Flumerfelt*, N. Rajakumar and K. Elisevich, Dept. of Anatomy, University of Western Ontario, London, Ontario, Canada.

The substantia nigra pars reticulata (SN-R) and the entopeduncular nucleus (EPN) form the main output centers of the basal ganglia, providing GABAergic projections to the thalamus, lateral habenula (LH), superior colliculus (SC) and pedunculopontine tegmental nucleus (PPN). Although many cells within the SN-R and EPN have been described as modality specific, the morphological similarity of the output neurons precludes their identification on a structural basis. Calcium binding proteins have been used successfully to verify neuronal heterogeneity in other regions of the brain. The present study was undertaken to investigate parvalbumin (PAR) immunoreactivity in the SNR and the EPN to provide a possible basis for characterizing their neurons. Adult Wistar rats were anesthetized with sodium pentobarbital and the retrograde tracer fluorogold or the anterograde tracer fluororuby was injected into the VL or VM nucleus of the thalamus, LH, SC, PPN, SN-R or EPN. After a one week survival period the rats were perfused with 4% PFA, and frozen transverse sections through the brain were cut (40μ) and processed with an indirect immunofluorescence method using antibodies to PAR or GAD. The results revealed that the majority of neurons in the SN-R and a significant number of neurons in the EPN contained PAR colocalized with GAD. Double-labeled neurons were found in the mid-portion of the EPN whereas they were distributed throughout the rostrocaudal extent of the SN-R except its medial part. The PAR immunoreactivity was seen in every output pathway of these centers. These results indicate the neurochemical heterogeneity of the basal ganglia output neurons and suggest multiple functions for each of these pathways. (Supported by MRC Canada).

137.6

ORGANIZATION OF THE EFFERENT PROJECTIONS OF NEOSTRIATAL COMPARTMENTS IN THE RAT. N. Rajakumar*, K. Elisevich and B.A. Flumerfelt, Dept. of Anatomy, University of Western Ontario, London, Ontario, Canada.

The striatum can be divided into neurochemically distinct striosome and matrix compartments that receive discrete projections from allocortical and neocortical areas respectively. Although different projections from the striosome and matrix have been ovserved, details concerning their organization are not available. In the present study the efferent connections of the striatal compartments are investigated to elucidate their functional organization. Adult female Wistar rats were anasthetized with sodium pentobarbital. The retrograde tracer fluorogold or the anterograde tracer fluororuby was iontophoretically injected into the dorsolateral or medial striatum, calbindin positive or negative parts of GP, rostral or caudal parts of EPN, SN-C, SN-R and medial or lateral parts of STN. After a one week survival period the rats were perfused with 4% PFA in PB (pH 7.4). Transverse sections were processed with an indirect immunofluorescence technique using antibodies to TH, calbindin (CB) or parvalbumin (PAR). The results confirm the existence of a striosome input to SN-C and a segregated projection from striosome and matrix compartments to habenulofugal neurons of the rostral EPN and thalamofugal neurons of the caudal EPN respectively. The dorsolateral striatum is reciprocally connected to the CB negative parts of GP whereas the medial striatum projects mainly to the CB positive areas of GP. The PAR-containing GABAergic neurons in the CB negative part of GP provide the main GP-EPN, GP-SN and GP-striatal projections whereas PAR negative GABAergic neurons in the CB positive part of GP provide the main GP-STN projection. The data suggest multiple levels of interaction in the efferent projections of neostriatal compartments. (Supported by MRC Canada).

137.7

COMPARTMENTALIZATION OF CHOLINERGIC INNERVATION OF THE HUMAN STRIATUM. D.J. Holt, S. de Lacalle, and C.B. Saper*. Depts. of Pharm. & Physiol. Sci. and Neurology, Univ. of Chicago, Chicago, IL 60637.

The striatum in humans, as in other mammals, may be divided into anatomically distinct compartments based upon the distribution of neurochemical markers. Diseases and pharmacological interventions in the human striatum that alter the dopaminergic system may change the expression of these neurochemical markers and therefore perhaps the compartmentalization itself. To investigate this possibility, it will be necessary first to determine the neurochemical compartmentalization of the normal striatum in humans. We examined the striatum from a series of human brains that were obtained at autopsy and fixed by perfusion through the carotid artery with a solution of 4% para-formaldehyde and 0.2% picric acid. Frozen sections were stained using an antiserum against human placental choline acetyltransferase (Hersh) with an immunoperoxidase and silver intensification method. We identified three distinct levels of density of cholinergic innervation in the human striatum, rather than the two (striosome and matrix) that have been reported in other species. In general, the compartment containing the lowest density of innervation filled much of the nucleus accumbens and extended into small, circumscribed areas in the putamen. The medium density compartment included small, circumscribed areas in the dorsal caudate nucleus and background regions in the putamen and ventral caudate. The heaviest density compartment accounted for much of the background in the caudate nucleus. Thus, the medium density compartment often appeared as "striosomes" in the caudate, but "matrix" in other regions of the striatum. Our observations indicate that the compartmentalization of the human striatum may be more complex than the two-compartment model used to describe the striatum in other mammals.

137.8

CO-LOCALIZATION OF DOPAMINE D1 AND D2 RECEPTOR mRNAs IN THE RAT NEOSTRIATUM. J. Lester[1], J.S. Fink[2], N. Aronin[3]*, M.DiFiglia[1]. Labs. of Cellular Neurobiology[1] and Molecular Neurobiology[2], Massachusetts General Hospital, Boston, MA 02114, and Dept. of Medicine[3], Univ. of Massachusetts. Med. Ctr., Worcester, MA 01655.

By ligand binding autoradiography, D1 and D2 dopamine receptors are highly concentrated in the striatum. Some physiological studies suggest that these receptors are co-localized and may functionally interact to mediate the effects of dopamine. However, other results based on mRNA localization have suggested that these receptor subtypes are contained predominantly in non-overlapping striatal output pathways. In order to determine more accurately the extent of cellular co-localization of D1 and D2 receptor mRNAs in the striatum, we used *in situ* hybridization in serial 4 μm frozen sections with ^{35}S labeled cDNA probes. Hybridized and emulsion-dipped sections were exposed for 12 weeks. After development, sections were photographed in dark field and 35 mm negatives were printed at 7x. In the photographs, fiber bundles were used as landmarks to accurately localize the position of the same labeled neuron in each section. Section pairs treated with the same cDNA probe were compared to those treated with different probes (D1 vs D2). Quantitative results based on the analysis of 1,775 cells showed that striatal neurons expressing D1 or D2 receptors were equally distributed throughout the caudate-putamen and that both receptors were co-localized in 26% (mean percents ranged from 11% to 36% per region examined) of the total neurons expressing one or the other receptor subtype. Results confirm previous reports that most striatal neurons do not appear to contain both receptors. Nevertheless, those neurons expressing both receptor subtypes represent a sizeable minority and it will be of interest to determine their neurotransmitter\neuropeptide content. (Supported by NIH NS 16367 to MD and NSF BNS8819989 to NA).

137.9

DISTRIBUTION OF NMDA RECEPTOR mRNA IN RAT NEOSTRIATAL NEURONS. P. Ge[1], H. Aizawa[1], J. Lester[2], E. Sapp[1], N. Aronin[3], J-P. VonSattel[1] and M. DiFiglia[2*]. Laboratories of Neuropathology[1] and Cellular Neurobiology[2], Massachusetts General Hospital, Boston MA 02114 and Dept. of Medicine[3], Univ. Mass. Med. Ctr., Worcester, MA 01655.

Glutamate, the neurotransmitter of the corticostriatal pathway, mediates its effects through multiple receptor subtypes in striatal neurons. Although previous studies using ligand binding and excitotoxic lesions with NMDA receptor agonists suggest that NMDA receptors may be widely distributed to most striatal cells, there is no direct evidence about the extent of neuronal distribution of this receptor subtype in the striatum. Using ^{35}S oligonucleotide probes, we examined the localization of NMDA receptor mRNA in the rat caudate-putamen using *in situ* hybridization. Frozen sections from the anterior striatum were hybridized with labeled oligomers directed to different regions of the predicted mRNA sequence of the NMDA receptor recently reported (Moriyoshi *et al.*, Nature, 1991). Sections treated with antisense probes showed specific labeling over medium and large-sized neurons throughout the striatum. Sense probes produced no specific hybridization signal. The density of labeled cells was determined with camera lucida by recording the location of cells in 1.6 mm^2 fields throughout the mediolateral and dorsoventral extent of the caudate-putamen. The density of labeled cells was compared to total striatal neurons recorded in adjacent counterstained sections which had not been used for *in situ* hybridization. Results from 141 fields counted in six sections showed that 88% of all neostriatal neurons express the NMDA receptor and that 88% of medium-sized cells and 49% of large neurons were labeled. Results provide evidence that the NMDA receptor is expressed by most striatal neurons. (Supported by NS16367 to JPV and MD).

137.10

PRE- AND POSTSYNAPTIC LOCALIZATION OF THE DOPAMINERGIC D1 RECEPTOR IN THE BASAL GANGLIA. D. Zhou[1*], Q. Huang[1], N. Aronin[2] and M. DiFiglia[1]. Laboratory of Cellular Neurobiology[1], Dept. of Neurology, Mass. General Hospital, Boston, MA 02114 and Dept. of Medicine[2], Univ. of Massachusetts Medical Center, Worcester, MA 01655.

Based on ligand binding and mRNA localization studies, D1 dopamine receptors are highest in the basal ganglia where they may play an important role in modulating the activity of D2 receptors and in mediating stereotypic behaviors. Although the subcellular distribution of D1 receptors is unknown, a variety of studies suggest that they may be involved in mediating the actions of dopamine both pre- and postsynaptically. Using a purified rabbit polyclonal anti-peptide antibody (see also Q. Huang *et al.*, this meeting), we examined the localization of D1 receptors at the light and electron microscopic level with the immunoperoxidase method. Results showed that in the neostriatum immunoreactive D1 was localized to 49% of neurons which included both medium spiny and medium aspiny types. Reaction product was seen in discrete patches primarily at postsynaptic sites within spines, spine necks, and dendritic shafts. Small axon terminals which formed synapses mostly with dendritic spines were also labeled. In the globus pallidus and substantia nigra, pars reticulata, D1 immunoreactivity was abundantly distributed to the axoplasm of myelinated and unmyelinated fibers and preterminal axon endings which were apposed to unlabeled axon terminals. In addition, small patches of immunoreactive product were found in cell bodies and at plasma membranes within dendrites. Results provide the first direct evidence that the D1 receptor is widely distributed to pre- and postsynaptic sites in all three areas of the basal ganglia. (Supported by NIH NS 16367 to MD and NSF BNS8819989 to NA).

137.11

VENTRAL PALLIDAL NEURONS PROJECT TO BOTH THE VENTRAL STRIATUM AND THE MEDIODORSAL NUCLEUS OF THE THALAMUS IN THE RAT. H. Kuo* and H.T. Chang. Dept. of Anatomy and Neurobiology, College of Medicine, The Univ. of Tennessee, Memphis, 875 Monroe Ave., Memphis, TN 38163.

Previous studies have shown that some neurons in the ventral pallidum (VP) project to the ventral striatum (VS) and some VP neurons project to the mediodorsal nucleus of the thalamus (MD). Since these two groups of projection neuron in VP appear to occupy similar locations and have very similar morphology, it is possible that the same individual VP neuron projects to both VS and MD. In order to study the relationship between these two types of VP projection neurons, two different retrograde tracers were used in double-labeling experiments. One retrograde tracer, FluoroGold (FG), was injected into VS, and the second retrograde tracer, cholera toxin B (CTB), was injected into MD in the same animal. Immunofluorescence reactions were performed on brain sections such that CTB-containing neurons were labeled by Texas Red conjugated secondary antibody. Our results showed that both FG and CTB labeled cells were found in VP. Careful examination revealed that some of the FG labeled cells were also labeled by CTB. This result indicates that some individual VP neurons project to both VS and MD.

(Supported by USPHS Grant AG05944)

137.12

THE CORTICONIGRAL PROJECTIONS IN THE RAT: A BIOTIN-DEXTRAN ANTEROGRADE TRACING STUDY. A. Naito and H. Kita*. Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee Memphis, Memphis, TN 38163 U.S.A.

The existence of corticonigral projections remains a debated issue. Several studies suggest the frontal cortex projects to the substantia nigra (SN) pars compacta, while others suggest this projection is not prevalent. We have studied this projection using a biotin conjugated dextran anterograde nervetracing technique. Biotin-dextran (2%) dissolved in 0.01M phosphate buffer (pH 7.2) was pressure injected into the cerebral cortex of 20 male Sprague-Dawley rats (280-390g). After a survival period of 5-8 days, rats were fixed with 4% paraformaldehyde and the brain was sectioned sagitally. Sections were processed for visualization of biotin-dextran containing fibers using the ABC-method with DAB-Ni reaction. The same sections were further processed for tyrosine hydroxylase (TH) immunohistochemistry for identification of the SN pars compacta area. A large number of the stained fibers with small boutons was found in both the compacta and the reticulata parts of the SN after injection in the frontal cortex, yet only a fibers were found after an injection in the other cortical areas. The present findings indicate 1) that the corticonigral projects exists; 2) that these terminations are not limited to the compacta part; and 3) that the main projection arises from the frontal cortex.

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137.13

SPECIFICITY IN THE EFFERENT PROJECTIONS OF THE NUCLEUS ACCUMBENS IN THE RAT: COMPARISON OF THE ROSTRAL POLE PROJECTION PATTERNS WITH THOSE OF THE CORE AND SHELL

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The efferent connections of the rostral pole of the rat accumbens were examined with PHA-L, for comparison with the projection patterns of the accumbal core and shell. Injection sites and transported PHA-L were evaluated with the aid of adjacent sections processed for substance P or calbindin immunoreactivities. Lateral rostral pole is more "core-like" and projects to the rostroventral globus pallidus, subcommissural ventral pallidum, entopeduncular nucleus and contiguous lateral hypothalamus, ventral tegmental area, dorsal pars compacta and, modestly, to the pars reticulata and lateral mesencephalic tegmentum. Medial rostral pole is more "shell-like" and projects to the subcommissural ventral pallidum, preoptic region, lateral hypothalamus, ventral tegmental area, dorsalmost pars compacta, retrorubral field, lateral midbrain tegmentum, pedunculopontine tegmental nucleus and central grey. The medial rostral pole contributes some projections to preoptic and sublenticular regions, but not to the bed nucleus of the stria terminalis. Concentrations of calbindin immunoreactive cells in the core and lateral rostral pole correlate with the origin of the "basal ganglia-like" projections, suggesting that core and rostral pole calbindin immunoreactive cells contribute principally to basal ganglia-like projections and that cells lacking calbindin immunoreactivity contribute more to the innervation of hypothalamus and midbrain tegmentum, i.e., that projection specificity, possibly in combination with topographic constraints, defines the patterns of projections from the accumbens. NIH NS-23805 & NS-17743.

137.14

VENTRAL PALLIDOSTRIATAL PROJECTIONS IN THE RAT: RECIPROCATION OF THE STRIATOPALLIDAL PROJECTIONS OF THE ACCUMBAL SUBTERRITORIES

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The nucleus accumbens comprises three subterritories: core, shell and rostral pole, which give rise to striatopallidal projections that define ventral pallidal subterritories. Shell projects selectively to a histochemically distinct ventromedial part of the subcommissural ventral pallidum (VPm). Core projects massively to a dorsolateral ventral pallidal subterritory (VPl) and less robustly to VPm and the rostromedial part of the globus pallidus (GP). The rostral pole projection involves primarily VPm but also, to a lesser extent, VPl and the rostromedial GP. We now report that components of the ventral pallidostriatal pathway (Kuo and Chang, Soc. Neurosci. Abstr. 17:453, 1991) reciprocate the striatopallidal projections from the accumbal subterritories. Immunohistochemically detected retrograde labeling was evaluated in the pallidal complex following injection of Fluoro-Gold (FG) into the core, shell or rostral pole. Substance P and calbindin 28 kD immunoreactivities were used to delineate subterritories in the ventral pallidum and accumbens. Following FG injections in the shell, FG cells were present only in VPm. When the injections were restricted to the core, FG cells were present in VPm, VPl, and the rostromedial GP. Rostral pole injections resulted in large numbers of FG cells in VPm and significantly fewer in VPl and the rostromedial GP. The divergent pathways from core and rostral pole, and their reciprocation by the neurons in VPm, VPl and GP, contrasts with the marked selectivity of the interconnections of the shell and VPm. These data are additional evidence that both specificity and topographic constraints contribute to projection patterns in the ventral basal ganglia. Supported by NIH NS-23805 & T32 NS-07254.

137.15

SUBTHALAMIC AND PEDUNCULOPONTINE INPUTS TO THE SUBSTANTIA NIGRA IN THE RAT: A DOUBLE LABEL EM STUDY. M. Damlama* and J.M. Tepper, Aidekman Research Center, Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ 07102.

Both the subthalamic (STN) and pedunculopontine (PPN) nuclei are considered to be important in the regulation of motor function, and both of these nuclei have been shown to project to the substantia nigra but the identity of their postsynaptic targets has not been demonstrated. Therefore, anterograde transport of biocytin combined with tyrosine hydroxylase (TH) immunohistochemistry was performed to investigate the relationship of these afferent inputs to dopaminergic neurons. The tissue was processed for sequential light and electron microscopic analysis.

At the light microscopic level, both the STN and PPN were seen to give rise to numerous fine, varicose axons and axonal boutons in the substantia nigra. Whereas the STN input was densest in the pars reticulata, that from the PPN was densest in the pars compacta. At the EM level, the terminal boutons of STN and PPN origin had an average area of 0.43±0.06 μm^2 (SEM) and 0.53±0.07 μm^2, respectively. The terminals contained small round vesicles, and 1-3 mitochondria. All boutons observed to form synaptic contacts formed asymmetrical synapses, the overwhelming majority of which were onto small TH negative dendrites of approximately 1 µm in diameter. The average length of the active zone was 0.36±0.05 µm for PPN, and 0.44±0.13 µm for STN terminals. Most of the terminals contained one active zone, but occasionally PPN terminals synapsed with more than one element. Although terminals of PPN and STN origin were quantitatively similar, PPN terminals were less tightly packed with vesicles than STN terminals, and unlike the latter, they contained some large dense core vesicles in addition to the more numerous small round vesicles. In summary, although numerous axonal boutons were seen in close apposition to TH+ material at the LM, the majority of synapses originating from STN and PPN were onto TH- dendrites at the EM level. Supported by MH45286.

137.16

CEREBELLO- AND PALLIDO-THALAMOCORTICAL PROJECTIONS TO THE PRIMARY MOTOR CORTEX: BODY MAPS. J.E. Hoover* and P.L. Strick. Research Service, VAMC and Depts. of Neurosurgery & Physiology, SUNY-HSC, Syracuse, NY 13210.

We injected a *retrograde* strain of herpes simplex virus type 1 (HSV-1) into the "face", "arm", and "leg" representations of the primary motor cortex of monkeys to label the origin of cerebellar and basal ganglia projections to these sites. We found neurons labeled by retrograde transneuronal transport in restricted regions of the dentate nucleus and the internal segment of the globus pallidus (GPi). Surprisingly, projections to the primary motor cortex originate from only 30% of the volume of the dentate and only 15% of GPi. Thus, the majority of the output from these two subcortical nuclei is directed to areas other than the primary motor cortex. The distribution of neuronal labeling in the dentate and GPi varied with the cortical injection site. Within the dentate, neurons labeled after virus injections into leg motor cortex were located rostral to those labeled by injections into arm motor cortex, whereas the neurons labeled after injections into face motor cortex were located caudal to those labeled from arm motor cortex. Within GPi, neurons labeled after virus injections into leg motor cortex were located dorsal to those labeled by injections into arm motor cortex, whereas the neurons labeled after injections into face motor cortex were located ventral to those labeled from arm motor cortex. Thus, these observations provide evidence for at least one map of the body in the dentate and one in GPi. Supported by VA Medical Research Service and USPHS 2957, 24328.

137.17

STRIOSOME / MATRIX AFFILIATIONS OF PREFRONTO-STRIATAL PROJECTIONS IN THE MONKEY. Frank Eblen* and Ann M. Graybiel, Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139

To identify the prefrontal afferents to the striosome and matrix compartments of the monkey striatum, intracortical injections of the anterograde tracers, [^{35}S]-methionine and HRP-WGA, extending through all layers were placed in the left prefrontal cortex of 6 adult female monkeys (*Maccaca fascicularis*). Polar, dorsolateral, lateral orbital and caudal orbito-proisocortical sites were injected, and tracer distributions in the ipsilateral striatum were studied in relation to striatal compartments marked by stains for acetylcholinesterase activity and enkephalin-like immunoreactivity.

The projection fields in all cases were patchy and topographically organized, with striatal labeling from dorsolateral injection sites more dorsolateral than that from ventral and orbital sites. The strongest labeling was in the head of the caudate nucleus and the anteroventral putamen. Predominant labeling of the matrix occurred with injections in the principal sulcus and anterolateral orbital cortex. Labeled projections from the frontal pole were also mainly to matrix along the medial edge of the caudate nucleus, but they showed some overlap with striosomes. In striking contrast, injections of caudal orbital cortex and proisocortex caudal to areas 12 and 13 led to predominant labeling of striosomes through most of the fields of labeling. Weaker matrix labeling occurred, especially in the ventromedial caudate nucleus and putamen.

These findings demonstrate that different parts of the primate prefrontal cortex project preferentially to striosomes and to matrix, and suggest that the caudal orbital cortex and contiguous proisocortex is one major source of projections to striosomes.

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137.18

INPUT-OUTPUT MODULARITY OF MOTOR AND SOMATOSENSORY PROCESSING IN THE PRIMATE BASAL GANGLIA. A. W. Flaherty[1,2]* and A. M. Graybiel[1]. [1]Dept. of Brain and Cog. Sci., M.I.T., Cambridge, MA 02139, and [2]Harvard Medical School, Boston, MA 02115.

In the extrastriosomal matrix of the striatum, cortical inputs and pallidal outputs are clustered in discrete zones ("matrisomes"). To determine the relationships between these input and output modules, we made electrophysiologically-guided injections of anterograde tracers (^{35}S-methionine and WGA-HRP) to label inputs to the striatum from body part representations in motor and somatosensory cortex, and in the same brains injected retrograde tracers (cholera toxin and WGA-apoHRP-gold) into the internal and external segments of the globus pallidus (GPi and GPe).

1) Divergent inputs to the striatum from body part representations in cortex can reconverge on specific locations in GPe and GPi. Anterograde and retrograde injections in corresponding parts of cortex and GP labeled multiple overlapping matrisomes. Dispersed sets of input matrisomes can thus act together as output matrisomes, and specific loci in GPe and GPi may reintegrate information from distributed sets of matrisomes concerned with the same body part. **2) GPe and GPi contain small input modules that may convey cortical information in parallel.** Matrisomes projecting to some regions of GPe and GPi are selectively avoided by sensorimotor cortical inputs, yet these GP regions are very near those receiving sensorimotor matrisome inputs. **3) Striatal outputs to GPe and to GPi are not rigidly segregated in different matrisomes.** Small retrograde injections in GPe and GPi each label multiple matrisomes, and these sometimes overlap. Within the areas of overlap, however, few striatal neurons contain both retrograde labels, suggesting that striatal projections to GPe and GPi are nonetheless distinct at the neuronal level.

Overall, the systematic convergence and divergence of matrisome connections may allow the putamen to sort cortical information into particular channels through both GPe and GPi. (NIH R0125529 and the Human Frontier Science Program.)

137.19

PALLIDAL TERRITORY OF THE MONKEY THALAMUS: CELL TYPES AND GABAERGIC STRUCTURES. H. Yi, I. Rachman, K. Kultas-Ilinsky* and I.A. Ilinsky. Dept. of Anatomy, Univ. of Iowa Coll. Med., Iowa City, IA 52242.

The densicellular part of the ventral anterior nucleus (VAdc) represents the major projection zone of pallidal efferents and projects itself to the premotor cortex. VAdc is characterized by clusters of varying size neurons but it is not clear how many cell types are present in these clusters and what is the ratio of individual cell types.

To elucidate this we made WGA-HRP injections in the area 6 of one hemisphere and applied the same tracer to the surface of the cortex at symmetric location in the contralateral hemisphere in two adult Rhesus monkeys. Thalamic neurons retrogradely labeled from both tracer locations (PN) were then compared as to their sizes, cytological features and immunocytochemical properties. For the latter, postembedding immunocytochemical procedure with antibody to GABA was carried out on the tissue processed for HRP and embedded in Epon.

PN displayed bimodal distribution of soma sizes with majority of cells being of small to medium size. These predominated in clusters. The mean size of neurons projecting to superficial layers of the cortex did not differ significantly from those of cells projecting to all cortical layers. The GABA positive cells represented distinct population: they were significantly smaller than PN, displaying minimal size overlap with the latter and did not become labeled from cortex. The distinct cell types differed also with respect to the density and distribution of GABAergic input as well as other ultrastructural features.

Our results suggest existense of three types of neurons in the pallidal territory of the thalamus: two types of PN, which differ in size, and some cytological properties but not cortical layers they project to, and small GABA containing cells which are similar to interneurons in other thalamic nuclei. The data suggest that synaptic and neuronal organization of the pallidal afferent territory of the thalamus differs from that of the nigral and cerebellar projection zones. Supported by RO1NS 24188.

137.20

THREE DIMENSIONAL REPRESENTATION OF BASAL GANGLIA AND CEREBELLAR PROJECTION ZONES IN THE PRIMATE THALAMUS. I.A. Ilinsky*, A.W. Toga• and K. Kultas-Ilinsky, Dept. of Anatomy, Univ. of Iowa Coll. of Med., Iowa City, IA 52242 and •Dept. of Neurology, UCLA Sch. of Med., Los Angeles, CA 90024.

The topographic relationships of nigral pallidal and cerebellar projection zones within the primate thalamus have been a subject of long existing controversy until recent unequivocal demonstration of their complementarity (Ilinsky and Kultas-Ilinsky, 1987, JCN, 262:331-364). The segregation of these subcortical afferent systems is clearly seen along the anterior-posterior axis in sagittal sections, whereas it is less obvious in the frontal section plane. One of the reasons for the latter is difficulty in making accurate comparisons and adjustments of frontal section planes from different experiments due to variations in cutting angles. The situation has been further aggravated by the fact that cytoarchitectonic distinctions within the motor thalamus are not always obvious and hence can not be routinely used as a reliable criterion. Therefore, it is important to reveal the spacial relationships of major subcortical afferent systems, since these may have major functional implications.

The purpose of the present study was to generate computer reconstructed images of nigral, pallidal and cerebellar projection zones in frontal plane of section using their outlines obtained in experimental studies utilizing sagittal sections (Ilinsky and Kultas-Ilinsky, 1987). The task of converting one section plane to another was accomplished by three-dimensional computer reconstructions from serial sections cut in one plane followed by re-slicing in the plane of choice (Toga, 1990).

The present study revealed more complex topographic relationships between nigral, pallidal and cerebellar afferent domains in frontal section plane as compared to those observed in the sagittal plane. In particular, the interdigitation of these domains was much greater than previously thought. This may have contributed to the mentioned above segregation vs. overlap controversy of topographic relationships between the major motor-related afferent systems.

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138.1

ORGANIZATION OF CONTACTS FROM VESTIBULOSPINAL AXONS ON THE DENDRITIC SURFACE OF DORSAL NECK MOTONEURONS IN THE CAT. P.K. Rose*, T. Corneil, R. Nirula and T. Jones, MRC Group In Sensory Motor Physiology, Queen's University, Kingston Ontario K7L 3N6

It is well established that the vast majority of synaptic contacts on spinal motoneurons are located on the dendritic tree. However, the arrangement of synapses from identified afferents on the dendritic tree is poorly understood. The goal of the present experiments was to describe the distribution of contacts made by axon terminals of vestibulospinal axons on the dendritic trees of individual spinal motoneurons. The method of anterograde transport of phaseolus vulgaris leucoagglutinin was used to visualize the boutons of vestibulospinal axons arising from the lateral or descending vestibular nuclei. The dendritic trees of dorsal neck motoneurons were stained intracellularly with horseradish peroxidase. All contacts consisted of a clearly discernible bouton that, at the light microscopic level, appeared to be a direct apposition with a dendrite. Despite the presence of over 5,000 boutons within the volume of ventral horn that contained dendrites of a single motoneuron, contacts were few, ranging from 12 to 26 on the three motoneurons examined in detail. These contacts were not randomly arranged, but instead, were either concentrated on proximal dendrites directed medially or laterally (1 motoneuron) or were evenly distributed along the proximal and distal regions of dendrites travelling rostrally or caudally (two motoneurons). Regardless of the medial, lateral, rostral or caudal orientation of the dendrites, most contacts were found deep in the ventral horn, within motoneuron nuclei. These results suggest that synapses on dorsal neck motoneurons from vestibulospinal neurons are arranged in specific patterns which may form a substrate for nonlinear interactions with other inputs received by dorsal neck motoneurons. (Supported by the Canadian MRC and MDA)

138.2

CENTRAL PROJECTION PATTERNS OF INTRA-AXONALLY-LABELLED TRIGEMINAL SECONDARY MUSCLE SPINDLE AFFERENTS. REVERS DONGA, DEAN DESSEM and RICHARD MESZLER.* Department of Physiology, School of Dentistry, University of Maryland, Baltimore, MD, 21201 USA.

Axons of jaw-muscle spindle afferents were labelled with HRP in the tract of the mesencephalic nucleus of the fifth nerve in rats anaesthetized with pentobarbital. Four axons with dynamic indices ≤ 20 imp/s during ramp and hold stretches that followed sine wave stretches were classified as secondary jaw-muscle spindle afferents. Incoming axons of all labeled secondary jaw-muscle spindle afferents bifurcated into the tracts of the mesencephalic nucleus and Probst and had somata throughout the rostro-caudal extent of the mesencephalic nucleus. These axons could be followed for approximately 4 mm. Most labelled somata were pseudounipolar although one possessed a clear dendritic process. The heaviest density of boutons was in the region dorsal to the trigeminal motor nucleus. All secondary spindle afferents had relatively fewer boutons in the fifth nerve motor nucleus. In addition, processes given off from the tract of Probst, at the level of the facial nucleus, had sparce boutons in the reticular formation. These results suggest that the strongest projection of secondary jaw-muscle spindle afferents is dorsal to the trigeminal motor nucleus with weaker but direct projections also to the trigeminal motor nucleus and the reticular formation. Supported by NIH DE10132.

138.3

IDENTIFICATION OF POSTSYNAPTIC TARGETS OF CORTICAL AFFERENTS IN THE RAT DORSAL COLUMN NUCLEI

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To test the hypothesis that sensorimotor cortex controls the activity of projecting neurons in the dorsal column nuclei (DCN) via GABAergic interneurons we studied the relationship between identified cortical terminals and their postsynaptic targets. WGA-HRP was injected bilaterally in the somatosensory cortex of 8 anesthetized Sprague-Dawley rats. Fifty µm thick Vibratome sections containing DCN were reacted with TMB/tungstate to reveal peroxidase, and studied under bright and dark field. For EM observations, selected sections containing the anterograde label were osmicated and wafer-embedded in Epon-Spurr. Thin sections from these were collected on nickel mesh grids and stained for GABA, using postembedding immunogold techniques and different antibody concentrations. The areas of the labeled terminals, postsynaptic dendrites, other terminals and dendrites, and glia processes were measured; gold particles were counted and their densities were calculated for each profile.

In the caudal medulla, the anterograde labeling was concentrated in the gracile, cuneate and external cuneate nuclei. At EM level, it was in the form of electron-dense crystals and amorphous deposits inside small dome-shaped terminals containing numerous clear round vesicles. These terminals were negative for GABA. The profiles postsynaptic to cortical terminals were predominantly small dendrites and dendritic spines with areas up to 1 μm^2. In about 50% of the cases these dendrites contained higher densities for GABA then the background. Thus, the preliminary results suggest that cortical afferents in the DCN contact GABAergic as well as non-GABAergic neurons.

138.4

NUCLEAR ORIGINS OF RETICULOTHALAMIC PROJECTIONS IN RATS

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Nuclear origins of reticulothalamic projections in rats were studied using WGA-HRP and Fluoro-Gold. Tracer injections were made into the intralaminar (IL), ventrolateral, mediodorsal (MD), and ventral posterolateral (VPL) nuclei. Counts of retrogradely-labeled cells were done on 19 select cases. The strongest reticulothalamic projections were to IL and lateral MD; the weakest to VPL. The strongest reticulothalamic projections arose from midbrain cell groups, including cuneiformis, subcuneiformis, and the pedunculopontine nucleus. Strong pontine projections arose from pontis oralis pars medialis, pontis caudalis pars beta, and the dorsomedial tegmental area. A medullary trans-nuclear field encompassing paragigantocellularis dorsalis and dorsal parts of gigantocellularis and parvocellularis contained many labeled cells. Reticulothalamic projections were ipsilateral from the midbrain, bilateral from the pons, and contralateral from the medulla. Within a given reticular nucleus, thalamic-projecting neurons were similar in their morphology to those projecting to the spinal cord, cerebral cortex, and cerebellum.

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138.5

A TOPOGRAPHICAL COMPARISON OF RAPHE NUCLEI PROJECTIONS TO ANTERIOR CINGULATE & AREA Fr2 OF PREFRONTAL CORTEX IN THE RAT. G. Behzadi, M. Janahmadi and N. Rajaee. Physiology Dept.,Shahid Beheshti Med. Sci. Univ., Tehran, IRAN. (Spon: Brain Research Association.)

Morphofunctionally, the anterior cingulate (ACg) is related to limbic system and area Fr2 is considered as a supplementary somatomotor cortex. Physiological studies indicate the implication of raphe-cortical projetions in the regulation of learning, memory and movement. To compare the abundancy of the raphe nuclei projections to ACg and Fr2 prefrontal regions on the basis of their site of origin, We used retrograde transport of WGA-HRP followed by TMB reaction. The injection of tracer in area Fr2 resulted in labeling of dorsomedian and ventromedian neuronal groups of anterior dorsal raphe nucleus (DR). But in the median level of DR, numerous labeled neurons were found in the lateral wing of DR. Median raphe nucleus (MnR) innervated more rostral part of Fr2 from its median part. Rostral and caudal linear raphe (RLi , CLi) contained a few scattered labeled cells. The raphe pontine (RPn) showed seldom labeled neurons. The rostral part of ACg was heavily innervated by ventromedian part of anterior DR and lateral wing of the nucleus in its median level. ACg received a strong innervation from median part of MnR comparing to area Fr2. the paramedian part of MnR send only a few terminals to ACg. The separate neuronal groups in rostral, ventral and lateral part of DR and median MnR project mostly to caudal ACg. RLi and CLi contained many labeled cells in comparison to their projections to area Fr2. Our data reveals that the strong connections of raphe nuclei (especially DR & MnR) to these specific prefrontal areas, arised mainly from separated neuronal groups and/or some of their common projections may occur via collateral axons.

138.6

PROJECTIONS FROM THE PERIAQUEDUCTAL GRAY TO THE SPINAL CORD. L. J. Mouton* and G. Holstege. Dept. Anatomy and Embryology, Faculty of Medicine, Rijksuniversiteit Groningen, The Netherlands.

Motoneurons represent the final output of the motor system. They are located in spinal cord and caudal brainstem. Recently it has become evident that the mesencephalic periaqueductal gray (PAG) takes part in the motor system. The question arises whether there exist direct projections from the PAG to the cat spinal cord and where such projections terminate.

In ten cats HRP was injected in the spinal cord each at a different spinal level. Prior to the injection a hemisection was made rostral to the injection site. In order to identify and count the retrogradely labeled PAG neurons every fourth 40 µm transverse section of the PAG was incubated using the TMB method. In the anterograde study injections of ^{3}H-leucine or WGA-HRP were made in the lateral PAG. The autoradiographic tracing and TMB incubation method were used to visualize the labeled fibers in the spinal cord.

The results show that there exist neurons in the lateral and ventrolateral PAG projecting throughout the length of the spinal cord. The average diameter of these neurons is 33 µm, which is about twice as large as the PAG neurons projecting to the caudal raphe nuclei. The PAG-spinal cord neurons are mainly located between A 1.6 and P 0.2. Their number varies from a few hundred (upper cervical cord) to a few single neurons (sacral cord). The neurons project to the medial part of the ventral cervical cord, containing inter and motoneurons innervating axial and proximal muscles. A small number project to the intermediolateral cell column of the upper thoracic cord. In all likelihood these PAG projections play a role in defensive behavior.

138.7

AFFERENT PROJECTIONS FROM THE SPINAL CORD TO THE PERIAQUEDUCTAL GRAY IN THE CAT. V.G.J.M. van der Horst* and G. Holstege. Dept. Anatomy, University of Groningen, The Netherlands.

The periaqueductal gray (PAG) not only plays an important role in nociception control, but also in motor activities, related to emotional behavior. The PAG is strongly influenced by higher limbic structures such as hypothalamus and amygdala, but it also receives input from the spinal cord. In order to obtain a precise description of the spinal cord-PAG projection in the cat, a combined retrograde and anterograde tracing study was done to determine the number and location of the spino-PAG neurons and their precise termination area in the PAG.

In the retrograde tracing study unilateral injections of WGA-HRP were made in the PAG. In order to identify and count the retrogradely labeled spinal cord neurons, all segments of the spinal cord were cut in transverse 40 μm thick sections and every fifth section was incubated using the TMB method. In the anterograde tracing study HRP was injected at different spinal levels after a hemisection was made just rostral to the injection site. The TMB incubation method was used to visualize the labeled fibers in the PAG.

The results show that the spino-PAG projections are bilateral with a clear contralateral preponderance. Spino-PAG neurons are not evenly distributed along the different segments. They are most numerous at upper cervical and sacral levels and to a lesser extent at upper and mid thoracic levels. At sacral levels they are mainly located in the area of the intermediolateral cell column. The anterograde study shows that fibers originating from neurons at different spinal cord levels project to different areas of the PAG, suggesting a somatotopical organization of spinal cord projections to the PAG.

138.8

PATHWAYS OF THE CORTICOSPINAL TRACTS PROJECTING TO LUMBAR CORD IN THE RAT. A. Chen, R. Melinek, J.-J. Kim[1], J.H. Kim*, The Miami Project To Cure Paralysis, Dept. of Neurol. Surg., Univ. of Miami, Miami, FL 33136 [1] Dept. of Anat., Chosun Univ., Kwangjoo, Korea

The majority of rat corticospinal tract (CST) axons project contralaterally to the ventralmost part of the dorsal column (DC) in the spinal cord and terminate in lamina III-VI of the dorsal horn (DH). The purpose of this study was to determine whether there are ventral and lateral CST projections to lumbar cord, and what is their terminal distribution. The anterograde tracer, PHA-L, was iontophoretically injected into the hindlimb area of the unilateral sensorimotor cortex of female Sprague-Dawley rats. Fourteen to 21 days later, the cortex and LC were sectioned and processed for ABC immunoperoxidase staining. As expected, the majority of labelled CST axons were located under the contralateral DC and in lamina III-VI of DH. In addition they were found in laminae I and II, as well as in the intermediate zone and lamina X. Some of the labelled axons were 2located bilaterally in the dorsolateral funiculi. A small number were also found in the ipsilateral ventral funiculus of LC. Interestingly, the ventral CST entered the ipsilateral ventral horn (VII), projected near large neurons (possibly motoneurons), and exhibited boutons *en passant* and *terminaux*.

For retrograde tracing, a radiofrequency lesion was made to the dorsal CST at the T8 level. One week later Fast Blue (FB) and Fluoro-gold (FG) were injected unilaterally into the cord, rostral and caudal to the lesion site. FB, FG and double labelled cells were observed bilaterally in the cerebral cortex. These results indicate that there are CST projections through the dorsolateral and ventral funiculi, and that single neurons in the cerebral cortex project to lumbar as well as cervical cord.(Supported by NIH Grant NS28059, The Miami Project Research Fund, and GE International Scholar Training Fund)

138.9

DIVERSITY OF FORM (AND FUNCTION?) OF COLLATERALS ORIGINATING FROM VESTIBULOSPINAL AXONS IN THE CAT. A.H. Donevan*, J.A. MacDonald, P.K. Rose. MRC Group In Sensory Motor Physiology, Queen's University, Kingston Ontario Canada K7L 3N6

Neurons in the upper cervical spinal cord are one of the primary targets of vestibulospinal axons. In the present experiments, the anterograde tracer, phaseolus vulgaris leucoagglutinin, was used to visualize the collaterals of single vestibulospinal axons in the upper cervical spinal cord of the cat. A total of 27 collaterals that arose from 23 different axons were examined in detail. All axons originated from cells in the medial and descending vestibular nuclei and travelled in the ipsilateral ventral funiculus. Almost half of the collaterals formed bushy arborizations. The boutons of these collaterals were usually widely distributed in the ventromedial nucleus, medial and central parts of lamina VIII, medial parts of lamina VII and occasionally the spinal accessory nucleus and lateral parts of laminae IV, V and VI. A small number of axon collaterals with bushy arborizations terminated in a single lamina, either VII or VIII. Bushy collaterals usually arose from large diameter (3.0 - 6.0 μm) axons located in the medial part or base of the ventral funiculus. Other collaterals had a string-like appearance with several short side branches. Some of these formed a line of terminals that extended from lamina IX to lamina V. Other string-like collaterals only terminated in one or two laminae, usually laminae VII, VIII or IX. Most of the string-like collaterals arose from small diameter (0.5 μm to 1.0 μm) axons that were found in the medial part of the ventral funiculus or just ventral to the base of the ventral horn.

The rich diversity of these collaterals suggests that vestibulospinal projections in the ipsilateral ventral funiculus are composed of multiple subdivisions. Each subdivision is distinguished by its innervation of specific interneurons or selected combinations of interneurons and motoneurons. (Supported by the Canadian MRC and MDA)

138.10

INTRA-CORD MAPPING OF FIELD POTENTIALS EVOKED BY EXTRAPYRAMIDAL SYSTEMS IN THE RAT. B.R. Park[1]*, J-H. Sohn, R. Melinek, M.S. Kim[1], A. Chen, J.H. Kim The Miami Project To Cure Paralysis, Dept. of Neurol. Surg., Univ. of Miami, Miami, FL 33136, [1] Dept. of Physiol., Won Kwang Univ., Iri, Korea

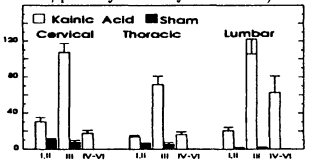
Somatosensory evoked potential (SSEP) and motor evoked potential (MEP) monitoring has been used to evaluate the integrity of ascending and descending pathways in the spinal cord (SC), and thus to assess pathways involved in spinal cord injury (SCI). However, several studies report a poor correlation between functional recovery of locomotion and electrophysiological assessment. The purpose of this study was: (1) to assess, electrophysiologically, conduction pathways of the extrapyramidal system in the rat and (2) to predict the pathways involved in SCI. The medullary reticular nucleus (RtN), lateral vestibular nucleus (VN), and red nucleus (RN) of adult Sprague-Dawley rats were stimulated using microelectrodes. Laminectomies were performed at the C3, T12 and L2 cord level. Field potentials (FPs) evoked by stimulation of each motor nucleus were recorded using a glass microelectrode filled with 2 M NaCl. To construct a cross-sectional map of the FPs, the recording was made in 7 tracks equally spaced across the SC. In each track, the FPs were recorded at seven equally spaced points from ventral to dorsal cord. Following VN stimulation, 1 or 2 short duration biphasic waves followed by a longer duration positive wave were monitored mainly in the ventral cord. Stimulation of RtN resulted in FPs with similar characteristics to those observed after VN stimulation, but their latency was much shorter. FPs generated by RN were monitored mainly in the dorsolateral funiculus and intermediate zone of the grey matter. The first wave component generated by each nuclei follows high frequency stimulation and is eliminated or significantly reduced by specific lesions placed above the recording site. Conduction velocities for the extrapyramidal systems were vestibulospinal tract, 37 m/sec, rubrospinal tract, 39 m/sec, and reticulospinal tract, 44 m/sec. (Supported by NIH Grant NS28059, The Miami Project Research Fund, and GE International Scholar Training Fund)

138.11

MAP OF SPINAL NEURONS ACTIVATED BY CHEMICAL STIMULATION IN THE VENTROMEDIAL MEDULLA OF THE AWAKE, DRUG-FREE RAT J. SANDKÜHLER* & K. BETT *II. Physiologisches Inst., Univ. Heidelberg, F.R.G.*

The ventromedial medulla (VMM) is a major source for medullospinal neurons modulating sensory input and motor output of the spinal cord. Here we used the expression of the proto-oncogene *c-fos* as a cellular marker of spinal neurons activated from the VMM.

Under deep pentobarbital anesthesia 13 S.D. rats were implanted with a guide cannula (.45 mm o.d.) to allow insertion of an injection cannula (.23 mm o.d.) into the VMM. For one week before and another week after the surgery the animals were daily allowed to adopt to the experimental environment and procedures. At the day of the experiment the animals were injected either with saline (0.9 %, 100 nl) or kainic acid (0.2 mmol in 100 nl). Five minutes before and 15 minutes after the injection the animals were placed into a Perspex chamber (40 x 40 cm) and their behavior war recorded on video tape. One hour after stimulation the animals were perfused with phosphate buffered saline and paraformaldehyde, the spinal cord was removed and postfixed overnight and cut in 25 μm cross sections through the cervical (C) and lumbar (L) enlargement and the mid-thoracic (T) cord. The c-FOS protein was detected by immunocytochemistry(avidin-biotin-method, primary antibody at 1:40.000).



In the figure the total numbers of FOS-positive cells per 5 sections are given. It is shown that stimulation in the VMM activates a large number of neurons throughout the spinal cord, predominantly in lamina III. *Supported by a grant from the DFG (SA 435).*

138.12

REDEFINING RED NUCLEUS: ANTEROGRADE PROJECTIONS FROM RED NUCLEUS TO THE INFERIOR OLIVARY NUCLEUS IN RAT. **D.Yu* and P.R.Kennedy. Neuroscience Lab, Georgia Tech, Atlanta, GA 30332.**

To confirm the retrograde labelling of red nucleus neurons from targets such as the inferior olivary nucleus (Yu et al., Soc. Neurosci. Abstr.,17(1)469, 1991), anterograde studies were undertaken.

Both WGA-HRP and PHA-L were injected stereotaxically into the red nucleus of adult female Long Evans rats. 5 to 15 nl of WGA-HRP were pressure injected while meniscus movement was observed. WGA-HRP or PHA-L were also iontophoretically injected using 2.5 uA monophasic cathodal pulses 100 ms in duration at 5 Hz for 10, 5 or 2.5 mins. Standard perfusion and tissue processing techniques were used.

Both tracers revealed terminal stippling contained within the boundaries of the inferior olivary nucleus (ION). Evidence of fibers in the brainstem was also seen. The very small injection sites associated with PHA-L injections within red nucleus resulted in terminal labeling confined to specific sub-divisions of ION.

These anterograde tracing studies corroborate the retrograde studies in providing evidence for the existence of a rubro-olivary projection in the rat. **Supported by NS grant 24602.**

138.13

CONNECTIONS OF TURTLE RED NUCLEUS AND CEREBELLAR NUCLEI STUDIED *IN VITRO* WITH NEUROBIOTIN AND BIOCYTIN. R. Sarrafizadeh*, J. Keifer, and J. C. Houk. Dept. of Physiology, Northwestern Univ. Medical School, 303 E. Chicago Ave., Chicago, IL 60611.

Neurobiotin and biocytin transport rapidly in both anterograde and retrograde directions in mammalian systems (Kita and Armstrong, *J. Neurosci. Meth.* 37: 141, 1991). Rapid transport of these tracers and the viability of the isolated turtle (*Chrysemys picta*) brainstem-cerebellum enabled us to study the afferent and efferent connections of the chelonian red nucleus (RN) and cerebellar nuclei (CN) *in vitro*.

Turtles were anesthetized with brevitol sodium (10 mg/kg i.p.) and perfused with heparinized oxygenated physiological saline. The midbrain and rostral spinal cord segments were then placed in oxygenated physiological saline, and 15-25 nl of neurobiotin or biocytin (4%) dissolved in 1M KCl and 0.25M TRIS buffer was pressure injected into RN or CN. Transport times ranged from 16-22 hours. Coronal sections were washed, incubated with ABC reagent, and reacted with DAB.

Neurobiotin and biocytin transport in both anterograde and retrograde directions. Retrogradely filled soma, dendrites, and axons were clearly visible, as were anterogradely labelled fibers and to a lesser extent terminals. RN injections resulted in dense retrograde label and some terminal label in the contralateral CN. A contralaterally descending rubrospinal tract and a smaller ipsilateral rubro-olivary pathway could also be seen. Cells in the ipsilateral superior reticular formation, several subdivisions of the contralateral trigeminal nucleus, and the dorsal column nuclei were retrogradely filled. Spinothalamic axons were visible ipsilaterally in the ventromedial spinal cord. Following CN injections sparse retrograde label and dense terminal label could be seen in the contralateral RN. Cells in the ipsilateral ventrolateral medulla (tentatively identified as the lateral reticular nucleus) and spinal nucleus of the trigeminal nerve, and in the contralateral inferior olive and dorsal column nuclei were also retrogradely labelled.

These data provide an anatomical basis for the concept of a turtle cerebellorubral recurrent network, in which positive feedback sustains bursting that is initiated by sensory inputs relayed in dorsal column nuclei and the spinothalamic system.

138.14

DISTRIBUTION OF THE SPINO-THALAMIC CELLS WITH COLLATERALS TO THE RED NUCLEUS IN THE CAT. Y. Padel*, M. Daadi, M. Gavioli and F. Condé. Equipe "Mécanismes Sensori-Moteurs", CNRS, 13009 Marseille, France

The cells at origin of the rubrospinal and corticospinal pathways receive a short latency somesthetic input through a ventral spinal pathway. The present study was to check if the afferents to the red nucleus (RN) are collaterals of fibres which terminate in the thalamus.

A double labelling neuroanatomical method was used: fluorescent markers were stereotaxically injected in the RN (the fast blue (FB) which labels the cytoplasm of afferent cells) and in the caudal part of the thalamus (the diamidino yellow (DY) which colors the nuclei).

Three groups of retrogradly labelled neurones were observed in the spinal cord : spinothalamic, spinorubral and doubly labelled cells. 80 % of the total population of FB labelled cells are located bilaterally in the four uppermost segments, in the VIIth and VIIIth Rexed lamina. They are mostly on the contralateral side in the cervical and lumbar enlargements.

These data lead to the conclusion that the spino-rubral input is transmitted for a large part (at least 56 %) through collateral branching of spino-thalamic fibres. This organization suggests that the same somatic information is sent in parallel to the two motor structures at the origin of the "lateral descending system" of Lawrence and Kuypers (1968) which controls limb motricity i.e. the red nucleus and the motor cortex.

138.15

CELLS OF ORIGIN AND FUNICULAR COURSES OF PROPRIOSPINAL AXONS DESCENDING IN THE TURTLE HINDLIMB ENLARGEMENT. A. Berkowitz* and P.S.G. Stein, Department of Biology, Washington University, St. Louis, MO 63130.

Sherrington and Laslett found descending propriospinal axons spread throughout the spinal white matter of the dog in 1903 (J. Physiol. 29:58-96). Nevertheless, it has been popularly believed in recent decades that such fibers are concentrated in *fasciculi proprii* in the inner zones of the lateral and ventral funiculi (see A. Brodal, *Neurological Anatomy*, 1981). We have recently recorded from many descending propriospinal axons throughout the lateral funiculus and dorsal funiculus of the turtle (*Abstr. Soc. Neurosci.* 17:123, 1991). We now describe retrograde labeling of spinal neurons with descending axons, following injections of horseradish peroxidase into portions of each funiculus in the middle of the hindlimb enlargement of the turtle spinal cord.

Following each injection, labeled cell bodies were found in the ipsilateral dorsal horn, intermediate zone, and ventral horn, as well as the contralateral intermediate zone and ventral horn, in the 7 mid-body and rostral hindlimb enlargement segments examined. Quantitatively, however, injections in each region of white matter gave rise to a characteristic distribution of labeled cells. Injections in either the dorsal funiculus or the dorsomedial part of the lateral funiculus labeled cells predominantly in the ipsilateral dorsal horn and intermediate zone. Injections in the lateral part of the lateral funiculus labeled mostly ventral horn cells bilaterally. Ventral funiculus injections labeled mainly contralateral cells, in the ventral horn and intermediate zone. Thus, there are consistent patterns in the funicular distributions of descending propriospinal axons. These distributions are much wider than other accounts suggest. Supported by NSF Grant BNS-8908144 to PSGS.

138.16

ASSESSMENT OF CERVICAL SPINAL CORD FUNCTION USING MAGNETIC MYOTOMAL MEPs. **LR Ray*, YP Zhang, RD Linden, CB Shields and JR Johnson. Depts. of Anatomical Sciences & Neurobiology, Orthopedic Surgery, and Surgery (Neurological), U of L, Louisville, KY 40292.**

The objective of this study is to determine whether myotomal motor evoked potentials (MEPs) may be reliably elicited in normal subjects and in spinal cord injured patients.

Transcranial MEPs were elicited following stimulation with a Cadwell Magneto-Electric Stimulator (MES-10) using a prototype skullcap coil. Stimulus intensity was set at 20, 40, 60, and 80% of the stimulator output (peak magnetic flux = 2 Tesla, pulse width=70 μsec). MEPs were recorded bilaterally from the sternocleidomastoid (SCM), deltoid (DEL), brachioradialis (BRR), flexor carpi ulnaris (FCU), abductor pollicis brevis (APB), and first dorsal interosseus (FDI) muscles. These muscles were selected because their innervation is primarily from a single spinal cord level: SCM=C4, DEL=C5, BRR=C6, FCU=C7, APB=C8, and FDI=T1. Single responses were replicated. The bandpass filter was 3-3,000 Hz and the timebase was 100 msec with a 10 msec pre-stimulus delay.

Normative data was obtained from 10 adult volunteers. Suprathreshold transcranial magnetic stimulation reliably elicited compound muscle action potentials in all muscles tested. At 80% output of the stimulator, the mean onset latencies of the MEP responses were SCM= 7.38 ± 2.54 ms, DEL= 11.64 ± 1.11 ms, BRR= 16.07 ± 3.28 ms, FCU= 16.36 ± 1.93 ms, APB= 21.66 ± 1.50 ms, and FDI= 22.38 ± 1.75 ms. The maximum peak-to-peak amplitudes were SCM= 3118 ± 1458 μV, DEL= 1148 ± 726 μV, BRR= 1192 ± 393 μV, FCU= 1517 ± 748 μV, APB= 5142 ± 2339 μV, and FDI= 6298 ± 2996 μV. Clinical data was obtained from 3 cervical spinal cord injured patients. The presence of myotomal MEPs correlated well with the clinical assessment of the level of the lesion, as well as the laterality of neurological deficit.

This study demonstrates that myotomal MEPs are easy to elicit and are reproducible. Myotomal MEPs may aid in the precise localization of the level of motor deficit following spinal cord injury.

SPINAL CORD AND BRAINSTEM II

139.1

EVIDENCE THAT SYNAPTIC TRANSMISSION IN LUMBAR PROJECTION NEURONES IS SUPPRESSED DURING ACTIVE SLEEP. P.J. Soja*, & J.-I. Oka. *Faculty of Pharmaceutical Sciences, UBC, Vancouver, BC, Canada V6T 1Z3.*

Despite numerous studies describing the central mechanisms controlling lumbar spinoreticular (SRT) and spinothalamic tract (STT) neurones, little is known regarding the excitability of these cells across naturally occurring behaviors, e.g., sleep vs. wakefulness (see monograph by Willis & Coggeshall, 1991). The present study was performed in the chronic, unanaesthetized cat to provide an overall assessment of the cellular excitability of lumbar spinal cord projection neurones as a function of behavioral state. Axons of SRT/STT projection neurones form a tight compact bundle which is located in close proximity to the facial motor nucleus (MotVII). The amplitude of the orthodromic field potential elicited by stimulation of the contralateral sciatic nerve was compared with the amplitude of the antidromic motoneurone field potential recorded within MotVII (see Soja, *Soc. Neurosci. Abst., 17: 644, 1991*) during wakefulness (W), quiet sleep (QS), and active sleep (AS).

The peak amplitude, latency-to-peak, and half width of the orthodromic field potential elicited by sciatic nerve stimulation (0.05msec, 0.5mA, 0.5Hz) during W was 0.78mV, 27.5msec and 29.5msec, respectively. These waveform parameters did not differ significantly between W and QS; however, the amplitude was reduced by 77% to 0.18 mV throughout AS when compared to preceding episodes of QS or W. The amplitude of the antidromic response was also reduced by 46% during AS when compared to QS or W. During W or QS, glycine was iontophoretically applied to the site where both field potentials were recorded and the effects of the drug were then assessed on each type of potential. Glycine markedly suppressed the antidromic response and exerted no effect on the orthodromic response during this state.

The present results suggest that the orthodromic response was recorded from lumbar neuronal axons of passage rather than MotVII somata, and that the AS-related suppression of the orthodromic response occurs at the lumbar level and may be due to the activation during AS of descending inhibitory influences which impinge on the somata and/or primary afferent input of lumbar projection neurones including the SRT and STT. Supported by MRC.

139.2

INTRACELLULAR STUDIES OF LUMBAR NEURONES IN THE CHRONIC CAT. J.-I. Oka*, & P.J. Soja. *Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada V6T 1Z3.*

Little is known about the activity of individual lumbar sensory neurones across the sleep/wakefulness cycle. The present report describes the first stage of ongoing studies designed to address this issue, namely the feasibility of performing intracellular analyses of lumbar neurones in the chronic, unanaesthetized cat during drowsy wakefulness (W) or quiet sleep (QS).

Two adult cats were prepared for chronic intracellular recording of lumbar neuronal activity using methodologies developed previously (*Physiol. Behav., 27: 355-362, 1981*). Bevelled glass micropipettes filled with 2M K^+-citrate were lowered into the spinal cord. The electrodes were lowered in tracks 0.75-1.0mm medial to those where the sciatic motor pools were first located. In these medial tracks, a 3-6mV negative going field potential (latency-to-peak: 3.3msec, half-width: 1.7msec) could be recorded in response to sciatic nerve stimulation (0.05ms, 0.3-0.5mA, 0.3Hz). Fifteen neurones were recorded intracellularly at spinal cord depths corresponding to the presence of this field potential. The membrane potential and spontaneous action potential amplitude of the impaled cells ranged from -45 to -55mV and 50 to 68mV, respectively. Graded sciatic nerve stimulation evoked complex polysynaptic EPSPs leading to action potential bursts and/or IPSPs; however, no antidromic spikes were evoked in any of these cells. Current pulses (200μsec) injected intracellularly revealed post spike afterhyperpolarizations consisting of a fast early component followed by a slower sustained component unlike those recorded from motoneurones in the same preparation (see *J. Neuroscience, 11: 2804-2811, 1991*). High-gain DC records revealed the marked presence of spontaneous synaptic activity during QS or W.

These results corroborate previous work performed in acute preparations; they also suggest that the neurones recorded were not motoneurones, but rather sensory tract neurones or propriospinal neurones. Further studies designed to identify the axonal projections of recorded neurones may reveal the presence of AS-related modulating influences on sensory tract pathways (see Soja and Oka, 1992, these proceedings). Supported by MRC.

139.3

ORDER OF REM SLEEP FIRING RECRUITMENT CORRELATES WITH SOMA SIZE OF PONTINE RETICULAR FORMATION NEURONS: INTRACELLULAR RECORDING AND LABELING IN THE NATURALLY SLEEPING CAT.

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Acute intracellular labeling studies in our laboratory have examined the projections of giant cell field pontine reticular formation (FTG) neurons. Type I neurons had primarily reticulo-spinal projections, relatively few collateorals, and most had soma diameters $\geq$ 47.5 µm. Type II neurons projected to brainstem reticular sites, often had collaterals, and had soma diameters < 45.7 µm. The present study used intracellular recording and intracellular labeling (PhAL, biocytin, or neurobiotin) in naturally sleeping, chronic cats to determine if the "lead time" of onset of markedly increased discharge rate prior to REM was correlated with soma size. Soma diameter was determined from digitizer measurements of cross-sectional area (inter- and intra-rater variation < 5%), and done blind to the physiology. RESULTS. Diameters of the 14 labeled neurons ranged from 42.9 µm to 87.9 µm. Within this size range there was a strong positive linear correlation (Pearson's $r = 0.64$, $p < 0.02$) between diameter and "lead time", which ranged from 0.25 min to 5.88 min. That larger neurons had longer "lead times" was contrary to our expectation that smaller neurons, which might be Type II neurons with abundant reticular projections, would show earlier recruitment. It was also expected from the inverse size dependence of recruitment order in alpha motoneuronal pools. Earlier recruitment of larger neurons may reflect a stronger cholinergic excitatory input.

139.4

EFFECT OF TRACHEOSTOMY UPON RESPIRATION CYCLE DURING ELECTRICALLY INDUCED VOCALIZATION IN DECEREBRATE CATS.

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To determine the functional role of laryngeal and vagal afferents upon the coordination of laryngeal and ventilatory muscles during vocalization induced by a pontine electrical stimulation (0.2ms, less than 60 µA, 60Hz, for 5-10s), tracheostomy was performed to bypass respiratory airflow.

Under halothane nitrous gas anesthesia, the cats were decerebrated at the precollicular-postmammilary level, and balloon catheter was inserted into the opening of a T-shaped tube, which was inserted into the trachea, to open and close the T-shaped tube at various timing of respiration. The same catheter was employed to monitor the subglottic pressure.

During continued tracheostomy, the respiratory cycle became shorter, although the pontine stimulation induced coordinated activation of laryngeal and ventilatory muscles. The sudden opening of prepared tracheostomy during expiration decreased the subglottic pressure and terminated the vocalization, however, the expiration phase continued until a termination of lung deflation. This suggested that the feedback inputs from the laryngeal mucosa or other upper airway tract were less important than the vagal inputs to determine a respiratory rhythm during vocalization.

139.5

GABA- AND SEROTONIN-ERGIC INNERVATION OF MEDULLARY POSTINSPIRATORY NEURONS IN THE CAT.

S.W. Schwarzacher†, M. Maschke†, T. Rudolph and D.W. Richter*. Center of Anatomy† and Center of Physiology and Pathophysiology,Univ. Göttingen, W-3400, FRG.

In mammals, the respiratory rhythm is generated by a network of medullary respiratory neurons. Postinspiratory neurons (PINs) seem to act as general inhibitors establishing a third, postinspiratory phase in the rhythm *(Richter et al 1982)*. In the anaesthetized adult cat we HRP-labeled bulbar PINs intracellularly in the caudal medulla. These PINs were not antidromically activated by vagal nor by spinal cord stimulation. The somata of 11 labeled PINs were located in the ventral respiratory region in or in close vicinity to the ambigual nucleus. Cell bodies were multipolar and showed extensive dendritic branching. Axons travelled dorsomedially and crossed midline ventral to the central canal. Identified axon-collaterals preferentially terminated along the ipsilateral ambigual nucleus. They terminated with boutons, which in EM proved to represent axo-dendritic and axo-somatic synapses on multipolar neurons. Immunocytochemistry revealed a dense network of 5-hydroxy-tryptamine-immunoreactive (5HT-IR) fibers in the ventrolateral respiratory region. Fine 5HT-IR terminals with multiple small boutons terminated as close appositions to proximal and distal dendrites of HRP-labeled PINs. At cell bodies of PINs, however, 5HT-IR terminals were only sparsely detected. In contrast, postembedding immunocytochemistry showed multiple GABA-IR-boutons on the somata of PINs.

Our findings verify intensive synaptic interconnections of PINs within the ventrolateral medulla, thus providing the structural basis for their proposed role as functionally important interneurons of the respiratory network. The dense GABA-ergic innervation corresponds with their pronounced post-synaptic inhibition by chloride inward currents through GABA-A-receptors *(Haji et al 1986, Richter et al 1986)*. The 5HT-innervation of PINs explains parts of the modulatory serotoninergic influence on central respiration. *S. b. DFG*

139.6

CARDIOVASCULAR NEURONS MEDIATING SOMATOSYMPATHETIC REFLEX IN ROSTRAL VENTROLATERAL MEDULLA

Y.S. Goo, S.J. Kim◆ and J. Kim◆* Dept. of Physiology, College of Medicine, Chungbuk National Univ., Cheongju 360-763 and Seoul National Univ. ◆, Seoul 110-460, Korea

Neurons having a close functional association with cardiac rhythm were found in the rostral ventrolateral medulla(RVLM). In the present study we intended to demonstrate that some of these cardiovascular neurons (CVN) can mediate the somatosympathetic reflex (SSR). Sixty-one neurons, showing spontaneous discharge, were identified as CVN in cats anesthetized with α-chloralose by using post R-wave spike histogram and spike-triggered averaging of the sympathetic nerve discharge. Since peripheral nerve stimulation could orthodromically activate these neurons to elicit SSR with a resultant change in arterial blood pressure (ABP), simultaneous measurements of the spike frequency (SF) and ABP were successfully made in 12 neurons while stimulating the sciatic nerve. Stimulation with C-intensity (10 mA, 0.5 ms) at a high frequency (20-50 Hz) increased SF of 10/12 neurons with a concommitant rise in ABP, while that of 2/12 neurons decreased. Although stimulation with Aδ-intensity (1 mA, 0.1 ms) at a low frequency (1 Hz) did not always significantly decrease ABP, an increased SF was observed in 7/12 neurons during depressor response. Some (2/12) neurons showed a decreased SF with depressor response and 3/12 neurons showed no measurable change in both ABP and SF. These results suggest that SSR can be mediated by functionally different classes of CVN present in the RVLM: the increased activity of pressor and depressor neurons facilitating, and respectively, inhibiting the reflex.

139.7

THE EFFECT OF TOOTH MECHANORECEPTOR STIMULATION ON TRIGEMINAL MOTOR UNIT ACTIVITY

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Motor unit firing in the muscles of mastication was recorded while graded forces were applied to maxillary teeth to examine the reflex effects of mechanical stimulation on various trigeminal motoneurons. Responses were recorded while horizontal or vertical forces (0.05N to 4N) were applied to the maxillary canine tooth in four cats anesthetized with pentobarbital or decerebrated. The afferent volley produced by mechanical stimulation was monitored via an electrode placed at the brainstem entry point of the trigeminal nerve. The reflex effects of ipsi- and contralateral tooth displacement were observed in twenty-two masseter, temporalis or medial pterygoid motor units using histogram analysis. Peristimulus histograms (100-500 sweeps; 1ms binwidth) were generated from the spontaneous firing of jaw-elevator motor units during the application of force. Motor unit firing was inhibited at forces less than 0.5N and this inhibition increased as the magnitude of force applied to the tooth was increased to 4.0N. Using these forces a jaw-opening reflex was never produced. Contralateral as well as ipsilateral jaw-elevator motor unit firing was inhibited for periods up to 45ms. The reflex effects on contralateral motor unit firing occurred at higher forces with slightly longer latencies. Motor units tested with both horizontal and vertical forces showed higher thresholds for modulation with vertical forces. Motor unit responses were modified by intravenous strychnine (0.35mg/kg) suggesting a glycinergic component to this reflex pathway. These results imply that the reflex effects produced by applying small forces to the teeth have different synaptic strengths to different jaw-elevator motoneurons. This reflex diversity may contribute to the precise control of jaw movement. Supported by DE10132

139.8

A COMPARISON OF THE DISTRIBUTION AND MORPHOLOGY OF NEURONS IN RAT TRIGEMINAL NUCLEUS ORALIS PROJECTING TO FACIAL, HYPOGLOSSAL AND TRIGEMINAL MOTOR NEURONS.

D. J. Sohn, W. M. Falls* and L. A. Smith. Dept. Anatomy, Michigan State University, East Lansing, MI 48824.

Retrograde HRP transport was used to examine and compare the overall distribution and morphology of neurons in rat trigeminal nucleus oralis (Vo) projecting to facial (VII), trigeminal (VMO) and hypoglossal (XII) motor nuclei. Injections into VII and VMO labeled neurons in ventrolateral (VL) and dorsomedial (DM) subdivision throughout the middle third of Vo. Labeled VL neurons outnumbered those in DM by a 2:1 ratio. VL and DM neurons projecting to VII had spherical somata with an average cross-sectional area of 239µm^2. VL and DM neurons projecting to VMO had large and small spherical-shaped somata with average cross-sectional areas of 407µm^2 and 175µm^2, respectively. Injections in XII labeled nearly equal numbers of VL and DM neurons in the middle third of Vo. Neurons with spherical-shaped cell bodies were found in each subdivision and had average cross-sectional areas of 185µm^2. Scattered VL and DM neurons with pyramidal-shaped cell bodies were observed following all three injections. These results suggest that there are morphologically distinct populations of neurons in the middle third of VL and DM projecting to VII, VMO and XII. VL projects densely to VII and VMO with VL and DM providing equal innervation of XII. Vo involvement in complex orofacial reflexes is suggested. Supported by NIH/BRSG to C.O.M.

139.9

WHOLE-CELL RECORDINGS OF IONIC CURRENTS UNDERLYING THE FIRING PATTERN OF NEURONS OF THE GUSTATORY AREA OF THE RAT NUCLEUS TRACTUS SOLITARII. F.J. Tell and R.M. Bradley*. Sch. of Dentistry, Univ. of Michigan, Ann Arbor, MI 48109.

Four groups of neurons within the rostral nucleus tractus solitarii (NTS) have been previously distinguished (Bradley and Sweazey, J. Neurophysiol., 67, 1992). Depolarizations induced a repetitive discharge in Group I, II and III neurons and a burst of spikes in group IV neurons. Moreover, after hyperpolarizations Group I neurons produced irregular firing and a long delay occurred in the initiation of discharge in Group II neurons. Hyperpolarizing pulses often elicited burst of spikes in Group I, III and IV neurons. Voltage clamp recordings of rostral NTS neurons in rat brainstem slices revealed that Group II neurons posses an early transient outward current which is turned on by negative voltage step commands. Application of 4-aminopyridine (1-2 mM) significantly reduced this current. For neurons exhibiting a burst of spikes following hyperpolarization, application of cobalt (2-4 mM) strongly reduced both its duration and frequency. In addition, a small inward current was elicited after negative step commands from rest. Results suggest that Group II neurons posses an A-like potassium current the activation of which delays the initiation of action potentials. An inward calcium current may underlie the bursting responses of the other neurons groups. Physiological activation of these conductances might play an important role in the processing of taste information by the rostral NTS.

N.I.H. Grant DC000288

139.10

HYPOGLOSSAL SENSORY FEEDBACK MODULATES MOUTH OPENING DURING FEEDING IN TOADS. K. C. Nishikawa* and C. Gans. Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011.

High-speed videography, electromyography, deafferentation, and electrical stimulation experiments were used to investigate how sensory feedback from the tongue, which enters the brain through the hypoglossal nerve, influences mouth opening during feeding behavior in the marine toad (Bufo marinus). Behavioral observations demonstrate that bilateral transection of the hypoglossal nerves prevents mouth opening during feeding. Before transection, the mandibular depressors become active 87 ms before the mandibular depressors. After transection, the depressors and levators become active simultaneously. Electrical stimulation experiments showed that hypoglossal sensory feedback inhibits both tonic and phasic activity of the jaw levators. This feedback system appears to coordinate jaw and tongue movements during feeding in toads. Similar experiments on other anuran species show that hypoglossal sensory feedback is a derived feature of anuran feeding behavior. Supported by NSF grant #BNS 8909937 to KCN.

139.11

AUDITORY INPUT TO THE PEDUNCULOPONTINE NUCLEUS. N.B. Reese*, E. Garcia-Rill and R.D. Skinner. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR

Previous studies have shown that the P1 potential, a middle latency auditory evoked response induced by click stimuli in the intact cat, is blocked by lesions of the cholinergic pedunculopontine nucleus (PPN) and by administration of the muscarinic antagonist scopolamine (Buchwald et al. 1986, 1989). We recently showed that this middle latency potential is evident at the level of the PPN in the decerebrate cat and rat (Reese, 1991). The present studies were conducted on precollicular decerebrate, paralyzed cats. Auditory click stimuli were delivered by earphones attached to hollow ear bars and depth auditory evoked potentials (3 Hz - 3KHz bandpass) and single unit responses were recorded. Recording sites were localized by Fast Green deposits in the region of histochemically (NADPH diaphorase positive) identified cholinergic PPN neurons. A total of 26% of PPN neurons were found to respond to click stimuli at a similar latency to the P1 potential (15.1± 3.8 msec), and 19% were found to respond at a shorter latency (8.0±3.2 msec), while 55% showed no auditory responses. A series of cats (n=6) and rats (n=4) underwent similar procedures except that the P1 was recorded only from the surface of the inferior colliculus during auditory click stimulation at 0.2 Hz for 2 hrs. The brains were then fixed and processed for immunocytochemical labelling for c-fos early gene expression and histochemically for NADPH diaphorase labelling of cholinergic PPN cells. Between 40% and 73% of cholinergic PPN neurons also were c-fos positive. Unstimulated (surgical) controls showed minimal labelling.

These studies suggest that some neurons in the PPN respond to auditory stimulation at a latency similar to that of the P1 and long-lasting auditory stimulation induces c-fos expression in a significant proportion of PPN cells. In addition, preliminary evidence suggests that the auditory input to the PPN is modulated during episodes of spontaneous fictive locomotion.

Supported by NIH grant NS20246 and NSF grant RII-8922108.

139.12

RETICULOSPINAL NEURONS AND DESCENDING INITIATION PATHWAYS FOR LOCOMOTION IN THE LAMPREY. A.D. McClellan* and G.R. Davis, Div. of Biol. Science, Univ. of Missouri, Columbia, MO 65211

In the lamprey, the lateral spinal tracts are important for the descending initiation of locomotion (McClellan, 1988). In this study the pathways in which reticulospinal neurons project were examined using a more sensitive tract-tracing method than previously employed in our laboratory. In larval lamprey, various partial lesions of the spinal cord were made either at the level of the third or fifth gill. After 5-7 days the behavioral capabilities of the animals were tested. At 7-10 days post-lesion, which in control experiments was found to be sufficient time to allow cut axons to seal, HRP was applied at 25% body length, and after 12-14 days the brains and spinal cords were removed and processed using the Hanker-Yates protocol developed by M.E. Selzer. Focal application of HRP to the spinal cord indicated that descending axons usually remained in the same spinal tracts after leaving the brain.

In control animals without partial lesions, a total of about 1200 labeled brainstem neurons were distributed in one diencephalic, two mesencephalic, three isthmic, two bulbar, one lateral, and three vagal cell groups. Animals with spinal hemisections were able to locomote, and Mauthner cells and brainstem neurons in subdivisions of the isthmic and vagal nuclei had crossed descending axons. The remaining brainstem neurons had ipsilateral axons. Animals with both lateral spinal tracts lesioned usually could not initiate locomotion, and mostly large Muller reticulospinal neurons and some mesencephalic and vagal neurons were labeled. Animals with medial spinal lesions were able to locomote, and most brainstem neurons other than Muller cells were labeled, particularly in the isthmic, bulbar, and vagal cell groups. These results support the notion that reticulospinal neurons in the isthmic, bulbar, and vagal cell groups with axons in the lateral spinal tracts are important for the initiation of locomotion. In addition, axons of neurons in several cell groups appear to descend in intermediate areas of the spinal cord including both lateral and medial subdivisions of the spinal cord.

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139.13

STUDY OF RETICULOSPINAL NEURONES AND THEIR SENSORY INPUTS DURING ACTIVE AND FICTIVE SWIMMING IN A LAMPREY SEMI-INTACT PREPARATION. S.Coghlin*, N.Bussières and R.Dubuc. Dép. Kinanthropologie, U. du Québec à Montréal H3C 3P8 and Centre de Recherche en Sciences Neurologiques, U. de Montréal H3C 3J7, Canada.

Reticulospinal (RS) neurones in lampreys show rhythmic membrane potential oscillations during fictive locomotion and this modulation originates at least partly from spinal cord locomotor networks (Dubuc and Grillner, *Brain Res* 1989 *483* 196). To study the rôle of movement related feedback and sensory inputs to RS neurones under more natural conditions, we have used a semi-intact preparation of young adult *Petromyzon marinus*. The rostral one-third of decerebrated animals (12-14 cm) was dissected isolating the brain and spinal cord with their underlying cranium and notochord. The remaining two-thirds were left largely intact with only a medial incision to expose the dorsal surface of the spinal cord. The rostral part of the preparation was then pinned down to the Sylgard bottom of a double depth recording chamber where the tail had unrestricted movement in the deeper reservoir filled with cold Ringer. Bouts of swimming (20-30 cycles) occurred spontaneously or after pinching the tail. Ventral roots (fictive swimming) and EMG (active swimming) were recorded and the pattern of activity in both was well coordinated. Intracellular recordings of RS cells showed rhythmic membrane potential fluctuations which required the presence of distinct bouts of fictive locomotion in the rostral ventral roots. Cutaneous electrical stimulation evoked large synaptic responses in RS neurones which consisted of a mixed excitation and inhibition. Mechanical stimulation of the skin in a preparation where the animal was eviscerated opened along the ventral midline and pinned flat, elicited excitatory responses in RS cells with greater responses in the large Müller neurones. This robust preparation provides an opportunity to examine more closely the response of RS cells to sensory and proprioceptive inputs. (Supported by MRC Canada, FCAR and FRSQ Québec, and FCER USA to SC)

139.14

ANATOMICAL AND PHYSIOLOGICAL STUDY OF VESTIBULOSPINAL NEURONES IN LAMPREYS. N.Bussières* and R.Dubuc. Dép. Kinanthropologie, U. du Québec à Montréal H3C 3P8 and CRSN, U. de Montréal H3C 3J7 Québec.

Supraspinal control in lamprey originates from two main systems: the reticulospinal (RS) and the vestibulospinal (VS) systems. RS neurones are known to play a key rôle in the control of locomotion but less is known about VS neurones which are located in three nuclei: the posterior, the intermediate and the anterior octavomotorii nuclei (OMI n., OMP n. and OMA n.). This study was aimed at characterizing the morphology of VS cells, their axonal projections as well as some of their inputs. Anatomical data were obtained using retrograde tracers such as cobaltlysine (*in vitro*), HRP (*in vivo*), as well as with intracellular filling with Lucifer Yellow. Our results show that VS cells of the OMI n. (50-60 cells; 15-60 μm) are anatomically segregated, the smallest being found at the rostral and the caudal poles of the nucleus. Most of these cells have a dendritic tree projecting laterally within the alar plate. They have several axonal branches projecting rostrally in the basal plate and one caudally, reaching the ipsilateral spinal cord where it travels either within the intermediate or medial tract. The OMP n. contains between 20 and 30 VS cells (25-80 μm), many of which are deprived of dendrites. Their axon projects to the contralateral basal plate where it bifurcates: one or two branches project rostrally and one or two caudally, with a larger branch projecting to the medial tract of the contralateral spinal cord. The spinal projection of some VS cells of the OMI n. and OMP n. reaches further caudally than the gills as demonstrated by HRP injections. Cells of the OMA n. project only to the very rostral contralateral spinal cord and the caudal brainstem. VS neurons were recorded intracellularly within the OMI n. and the OMP n. They receive mixed chemical and electrotonic synaptic inputs from vestibular afferents; the chemical component is blocked using kynurinate (4mM) suggesting EAA transmission. Other inputs from dorsal and/or lateral columns are presently under study. (Supported by MRC Canada, FCAR and FRSQ Québec).

139.15

GLUTAMATE METABOTROPIC RECEPTORS MODULATE SYNAPTIC INPUTS TO RETICULOSPINAL NEURONES OF THE LAMPREY. S. Alford and R. Dubuc* Dép. Kinanthropologie, U. du Québec à Montréal, H3C 3P8 and CRSN, U. de Montréal, Montréal, H3C 3J7, Québec.

Reticulospinal (RS) neurones are important for the initiation and control of locomotion. They receive inputs from several sources, the spinal locomotor networks and the periphery. Application of the glutamate receptor agonist N-methyl-D-aspartate significantly reduces the excitatory transmission from vestibulospinal inputs to RS neurones (Bussière *et al* 1992, Brain Res. in press). This study was extended to investigate the mechanisms involved and the possible rôle of this depression. Whole-cell patch clamp recordings were made from RS neurones with patch pipettes containing principally Cs^+ Methane sulphonate in isolated brains of *Petromyzon marinus*. Stimulation of the brainstem alar plate over axons of vestibular neurones activated a compound EPSC in the RS neurone. The application of NMDA (50 μM) caused a depression in amplitude (to 56 ± 5 % sem of control n=12) of the EPSC, which was unaffected by the application of the competitive NMDA antagonist 2-amino-5-phosphonopentanoate (AP5) or the non-competitive antagonist ketamine, suggesting a possible presynaptic effect of NMDA. Pretreatment of the brainstem for 24 hrs with pertussis toxin (A compound that inactivates certain classes of G protein) eliminated the this depressant action of NMDA indicating that NMDA acts at a metabotropic site in this case. Application of the glutamate metabotropic receptor agonist 1S-3R ACPD (20 μM) also caused a depression of the RS EPSC (to 67 ± 5 % sem of control) in addition to causing an increase in synaptic noise recorded from the neurone. On washout an enhancement of the EPSC amplitude was then recorded. To test for a physiological role of these receptors paired pulse stimuli were used. Responses to the second stimulus were depressed up to 30 s following the initial stimulus. Application of NMDA (50 μM) eliminated this depression by more effectively depressing the first as compared to the second pulse. In conclusion we propose that excitatory synaptic transmission onto RS neurones is subject to modulation by a glutamate autoreceptor on presynaptic terminals. This will lead to a frequency dependent modulation of transmission to these neurones.

Supported by the Canadian MRC and Fondation de l'UQAM.

139.16

INTERACTION BETWEEN MAUTHNER NEURONS AND THE SWIMMING PATTERN GENERATOR IN *XENOPUS* TADPOLES. M. T. Lee* and A. M. Olin. Dept. of Biology, Carleton College, Northfield, MN 55057.

The Mauthner (M) cells are paired reticulospinal neurons of fish and amphibians. Their firing triggers a short-latency body bend that is the earliest component of an escape behavior. We have studied the effects of M cell firing on later motoneuron activity in the isolated CNS of *Xenopus laevis* tadpoles at NF stages 51-58. M axons were stimulated in the spinal cord with intracellular microelectrodes, while motor activity was recorded via suction electrodes on ventral roots (VRs).

The brain and spinal cord in vitro spontaneously produce episodes of rhythmic VR activity, in which bursts of impulses occur alternately on opposite sides of the cord. Typical episodes last for several seconds, consist of approx. 5-10 cycles, and repeat at intervals of 15-30 sec. Longer episodes (20-300 cycles) have also been recorded. Cycle periods range from approx. 100 to several hundred msec; the shorter cycle periods overlap with the range of swimming periods in intact tadpoles at the same stages. We refer to these episodes as fictive swimming. Similar episodes are also generated by the isolated spinal cord. When the cord is separated into right and left halves (with the brain still attached), alternating VR activity is replaced by long barrages of spikes that occur synchronously in left and right VRs.

Direct activation of either M axon in the isolated CNS consistently elicits a large compound action potential (CAP) in ipsilateral VRs at a latency of 2-5 msec, followed within 15-40 msec by an episode of fictive swimming that resembles spontaneous episodes. The first burst in an M cell-evoked episode nearly always occurs in contralateral VRs; a brief period in which motoneurons are activated bilaterally is sometimes interspersed between the ipsilateral CAP and the contralateral burst. Stimulation of an M axon during an ongoing episode can increase the intensity of subsequent bursts and reset the rhythm of the episode. The ability of M cells to elicit fictive swimming is retained in the isolated spinal cord, implying that at least one pathway between the M cells and the swimming pattern generator lies within the spinal cord itself.

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139.17

EFFECTS OF INHIBITORY AMINO ACID AGONISTS AND ANTAGONISTS ON LOCOMOTOR RHYTHMICITY IN THE RAT SPINAL CORD *IN VITRO*. K.C. Harder*, B.J.Schmidt. Dept. of Medicine and Physiology, University of Manitoba, Winnipeg, Canada R3E 0W3.

Little is known about the role of inhibitory neurotransmitters in mammalian locomotor networks, although recent studies by Kudo et al. (Neurobiol. Basis of Human Locomotion 1991) suggest glycinergic systems are required for side-to-side coordination in fetal rats. In the present series, the effect of GABA and glycine receptor activation and blockade during pharmacologically induced locomotion was studied using isolated spinal cords of 1-7 day old rats. Rhythmic activity was recorded bilaterally from flexor (peroneal) and extensor (tibial) nerves.

The $GABA_A$ agonist muscimol (5-15 uM), $GABA_B$ agonist baclofen (1-10 uM), and glycine (1500 μM)abolished NMDA and ACH-induced rhythmic activity. In the absence of NMDA or ACH, antagonists of $GABA_A$, $GABA_B$, and glycine (bicuculline 20 μM, hydroxysaclofen 1400 μM, and strychnine 20 μM) failed to induce sustained rhythmic activity, although responses to afferent stimulation were markedly enhanced. Locomotor rhythm was unaffected by $GABA_B$ antagonists (hydroxysaclofen 400 μM, phaclofen 2000 μM), but bicuculline (4-20 μM) and strychnine (4-20 μM) enhanced phasic discharge and converted left-right and flexor-extensor alternating patterns into highly synchronous rhythms, the frequency of which depended on the concentration of NMDA or ACH. AP5 (30 μM) abolished the activity. Single isolated lumbar hemi-segments also demonstrated strychnine and bicuculline enhanced rhythmic bursts. Bilateral synchrony persisted after splitting the lumbar cord by mid-sagittal section, even when only 1 supra-lumbar spinal cord segment remained bilaterally intact. The results suggest that inhibitory amino acid transmission is not essential for establishing rhythmic oscillation in spinal networks but may mediate reciprocal antagonism that coordinates inter and intralimb locomotor patterns. (Supported by MRC Canada)

139.18

NEURAL ACTIVITY IN THE ANTERIOR MIDBRAIN CORRELATED WITH HINDLIMB EXTENSION INITIATED BY LOCOMOTOR STIMULATION OF THE HYPOTHALAMUS OF THE ANESTHETIZED RAT. D.R. Epuru, A. Salyapongse, H.M. Sinnamon*. Neuroscience. & Behavior Prog., Wesleyan University, Middletown, CT 06457.

To identify neurons that might mediate locomotor initiation, the anterior midbrain was mapped for responses to ipsilateral stimulation (3-4 sec trains of 30-50 uA pulses) of the perifornical area which produced bilateral hindlimb stepping by Nembutal-anesthetized rats. We varied pulse frequency to make the first hindlimb extension occur at different latencies. Type I neurons, considered the best candidates for an initiation role, had peak responses correlated in time with the onset of extension. Type II neurons had peak responses early in the train that were uncorrelated with the extension onset. Of Type I responses, the ratio of increases to decreases was 9:1; for Type II, the ratio was 2:1. To date, with more than 450 sites tested, Type I neurons (n=36) were most common in a 1-mm wide band that included the red nucleus and the tegmentum lateral to the central gray. Type II neurons (n=30) were found throughout the anterior midbrain. Few responses of any type were found in the VTA.

139.19

CONVERGENCE OF EXCITATORY Ib AND DESCENDING LOCOMOTOR PATHWAYS IN THE CAT. R.M. Brownstone*, B.R. Noga, & L.M. Jordan. Dept. Physiology, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3.

During fictive locomotion, oligosynaptic excitation from selected extensor Ib afferents to extensor motoneurones replaces the usual autogenetic inhibition (see Gossard et al, SFN Abst 1990). Stimulation of these Ib afferents has profound effects on locomotion, resetting the rhythm and initiating an early extensor phase, likely by exciting the extensor component of the spinal locomotor network (extensor half-centre). Stimulation of the mesencephalic locomotor region (MLR) produces locomotion and short-latency PSPs in motoneurones which are normally modulated through the step cycle, giving EPSPs during extension and IPSPs during flexion in extensor motoneurones (Shefchyk & Jordan, J. Neurophysiol. 1985). If these short-latency PSPs and Ib EPSPs are effected via common interneurones, spatial facilitation should be demonstrable with appropriate stimulation. The current study was undertaken to test this hypothesis.

Fictive locomotion was initiated in decerebrate cats by stimulation of the MLR, with intracellular recordings from motoneurones (see Brownstone et al, 1992). A condition-test protocol was employed with electrical stimulation of the MLR and ipsilateral group I (likely Ib) afferents from plantaris.

The short-latency MLR PSPs in ipsilateral extensor motoneurones were facilitated by Ib stimulation both during extension and particularly during flexion. Whereas a short-latency IPSP from the MLR is usually seen during the flexor phase, an EPSP was instead observed with the addition of Ib stimulation.

It can be concluded that there are populations of interneurones involved in the central generation of the extensor phase of locomotion which receive excitatory input from both the MLR and Ib afferents. These Ib interneurones are those involved in the recently described Ib excitatory pathway, rather than those of the classical autogenetic inhibitory pathway.

139.20

DIFFERENTIAL RECOVERY OF LOCOMOTION AFTER COMPLETE HEMISECTION D. M. Basso*, K. Ziegler, M. E. Goldberger. Dept. of Anatomy & Neurobiology. Medical College of Pennsylvania, Philadelphia, PA 19129

Cats with spinal cord hemisections sparing the dorsal columns recover segmental (bipedal) and supraspinal (overground) locomotion (McBride & Goldberger, Society for Neuroscience, 1989). We extended these studies by examining the effect of complete hemisection (dorsal column included) on recovery of these types of locomotion. Two cats were trained to perform bipedal and overground locomotion and their spinal cords were then hemisected at T-13/L1. Five weeks of postoperative training began the day after surgery. A high speed Motion Analysis (TM) system generated pre- and postoperative hindlimb angular excursions and phase plane diagrams ipsilateral to the hemisection which were used to examine recovery of locomotion in that limb. Complete hemisection had a more deleterious effect on bipedal than on overground locomotion as noted by: 1) a longer time course of recovery; 2) persistent deficits in swing; 3) greater decomposition of ankle movement; and, 4) hypermetria in knee and ankle flexion. The results suggest that recovery of segmentally-mediated locomotion is more impaired by axotomy of the ascending collaterals of dorsal root ganglion cells than is recovery of supraspinally-mediated locomotion. (Supported by NS16629 & NS24707).

140.1

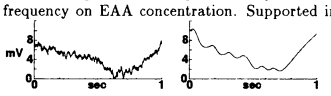
A SINGLE ACTION POTENTIAL IN A RETICULOSPINAL NEURON CAN RESET THE FICTIVE SWIMMING RHYTHM IN GOLDFISH. J.R. Fetcho*. Dept. Neurobiology and Behavior. SUNY at Stony Brook, NY 11794.

Fish can produce escape movements in the midst of swimming, indicating that the neural networks mediating escape and swimming must sometimes interact. I studied these interactions in goldfish. Fictive swimming was elicited by stimulation of the midbrain of decerebrate, paralyzed goldfish and the motor pattern was monitored by recording extracellularly from branches of ventral roots. Escape movements in fish are initiated by the firing of an identifiable reticulospinal neuron, the Mauthner cell (M-cell). An intracellular microelectrode was used to fire a single action potential in the Mauthner axon (M-axon) at different times during the swimming rhythm to activate part of the escape network in the midst of swimming. Firing the M-axon produced a burst of activity in an ipsilateral ventral root regardless of whether that root was in an active or silent phase of the swimming rhythm. The single M-axon spike sometimes reset the swimming rhythm dramatically, so that swim bursts following the M-axon spike were shifted in different trials by roughly 20 to 50 % of a cycle relative to when they would be expected to occur based upon the pre M-axon burst pattern. These results indicate that the M-axon can override the output from the swimming network and also has a very powerful influence on the central pattern generator for swimming . Supported by NIH NS26539.

140.2

NEURAL NETWORK SIMULATION OF LOCOMOTION IN LAMPREY. J.T. Davis* and L.E. Moore. Dept. of Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77555-0641

Neurons of the lamprey central pattern generator have been shown (Grillner et al Ann. Rev. Neurosc. 14:169 1991) to display a periodic activity that maintains a constant phase relation to the onset of the ventral root burst. During fictive swimming some fraction of the neurons in the circuit remain subthreshold. These subthreshold membrane potentials were signal averaged over twenty or more oscillations to obtain representative waveforms of a single cycle for different excitatory amino acid (EAA) agonists. In kainate induced fictive swimming the estimated synaptic input of a neuron was correlated with the shape of its subthreshold oscillation. In NMDA or glutamate induced fictive swimming some features of the subthreshold record could not be accounted for by synaptic input. It is postulated that these features are due to membrane mechanisms induced by activation of the NMDA membrane receptor. This hypothesis was tested using simulations of kainate, glutamate, and NMDA induced fictive locomotion. Simulations were done using a modified form of the neuronal model developed by the Grillner group (Brodin et al J Neurophysiol. 66:473, 1991; Ekberg etal Biol. Cyber. 65:81, 1991). The simulations show that in kainate induced fictive swimming all of the subthreshold shape features can be attributed to the synaptic input. In contrast, in NMDA or glutamate induced fictive swimming, membrane mechanisms generate additional shape features in the subthreshold waveform. In addition to generating the waveform of subthreshold oscillations, the simulations also reproduce the experimentally determined dependence of the oscillation frequency on EAA concentration. Supported in part by DHHS-R01-MH45796.



Comparison of archetype of interneuron in kainate induced swimming and simulation of kainate induced swimming.

140.3

In larval lamprey the brainstem and spinal cord interact to produce a novel motor output A.H. Cohen, R. Pitts, J.C. Presson* and L. Guan, Department of Zoology, University of Maryland, College Park, MD 20742.

In adult lampreys, brainstem neurons can activate the spinal pattern generator for locomotion; after locomotion begins, the brainstem neurons themselves become periodically active, apparently being driven by the bursting spinal neurons. In larval lampreys, this interaction is likely but has not yet been shown. In the larval lamprey, we tested the effect of the brainstem-spinal cord interaction, since fictive locomotion in the larval spinal cord is unstable making it likely to be sensitive to the potential positive feedback between brainstem and spinal cord.

Without the brain, the isolated larval lamprey spinal cord, stimulated by bath applied D-glutamate, produces a sequence of motor patterns: a slow often irregular rhythm, followed by disorganized activity lasting about 45 minutes, often ending in relatively regular rapid bursting qualitatively similar to adult fictive locomotion. With the lower brainstem present, after 30-40 minutes D-glutamate elicits a novel pattern. Rostral and caudal segments burst at different frequencies, only episodically becoming phase locked at a single stable frequency. Two hours after drug application, the segments can still be seen to display this pattern. After removing the brainstem, the spinal cord stabilizes like controls to a single fast frequency 30-45 minutes after drug application. Thus, in the larval lamprey the brainstem and spinal cord strongly interact during generation of locomotion. The brainstem is not simply maintaining the rostral segments at a faster frequency as has been suggested by others. The comparison to adult lamprey, the details of the interaction, and the possible mechanisms remain to be determined. Support by grants NIMH #MH44809 and NSF #BNS9009570 to AHC.

140.4

Evidence for long-distance coupling between segmental oscillators is inferred from the intrinsic variability of fictive swimming in the lamprey *Ichthyomyzon unicuspis*. T. Kiemel[1], N. Mellen[2], and A. H. Cohen[3*,1]Mathematical Research Branch, NIDDK, NIH, Bethesda MD, 20892; [2] Department of Psychology, Cornell University, Ithaca NY 14853;[3] Department of Zoology, College Park MD 20742.

Data from 16 locations along 50 segments of isolated spinal cord of the lamprey *Ichthyomyzon unicuspis* have been analyzed to infer properties of the functional anatomy of the central pattern generator (CPG) for locomotion. Our analysis suggests that long-distance connections between segmental oscillators play a role in the coordinating system. The relative phase model of the lamprey CPG (Cohen, Holmes and Rand, 1982) with noise has been used to interpret the data. This approach associates each recording location with an oscillator whose state is given by its absolute phase. Coupling between oscillators is a function of relative phase, linearized around a travelling-wave solution. Noise has been introduced into the model using independent autocorrelated stochastic processes to force the oscillators. A maximum-likelihood estimation of parameters in the model has been performed based on experimental burst onset times. The results indicate that the model requires long-distance connections in order to best account for the experimental data.
Supported by NIMH grant no. MH44809 to AHC.

140.5

THE ROLE OF APAMIN SENSITIVE POTASSIUM CHANNELS IN THE LAMPREY LOCOMOTOR NETWORK. R. H. Hill, J. L. Schotland*, T. Matsushima and S. Grillner, Nobel Institute for Neurophysiology, Karolinska Institute, Stockholm, Sweden.

Several factors have been identified which control burst duration in the lamprey locomotor network. One important mechanism is spike frequency adaptation in network interneurons which is dependent on the slow afterhyperpolarization (sAHP) mediated by calcium dependent potassium channels (K_{Ca}).

Apamin, which is a highly specific blocker of small conductance K_{Ca} channels, sharply reduced the sAHP in all lamprey spinal neurons observed. This is similar to the effect of 5-HT in this and previously reported studies (Wallén et al., 1989; Matsushima and Grillner, 1992). Thus, apamin sensitive K_{Ca} channels contribute to the sAHP and 5-HT may block, directly or indirectly, the same type of K_{Ca} channel.

Bath application of excitatory amino acids to the lamprey spinal cord can induce rhythmic bursting which alternates between left and right similar to the motor output during swimming (Wallén and Williams, 1984). Under these conditions spike frequency adaptation associated with the sAHP in interneurons is thought to have a key role in burst termination. Apamin was applied to spinal cords during fictive locomotion induced by kainate. This resulted in a clear decrease in the frequency of ventral root bursting. 5-HT application had similar results on burst frequency during fictive locomotion. During fictive locomotion induced by NMDA (150 μM), in which other burst terminating factors also contribute, apamin reduced the average frequency of bursting, but to a lesser extent.

Thus, regulation of K_{Ca} channels may be an important mechanism for control of the locomotor network. 5-HT is an endogenous transmitter of the lamprey spinal cord and likely exerts some of its effects on the network by this mechanism (Wallén et al., 1989; Christenson et al., 1989). Activation of $GABA_B$ receptors has been shown to reduce calcium entry and diminish the sAHP (Matsushima et al., 1992) and may represent another endogenous system utilizing a similar mechanism.

140.6

ROLE OF THE LATE AFTERHYPERPOLARIZATION IN FICTIVE SWIMMING IN THE LAMPREY. J.T. Buchanan* and D.P. Meer. Dept. of Biology, Marquette University, Milwaukee, WI 53233.

The role of the late after-spike hyperpolarization (l.AHP) in the firing properties of lamprey spinal neurons and in fictive swimming was tested by bath application of apamin, a selective blocker of the sk calcium-activated potassium current. Membrane potentials of identified classes of interneurons and motoneurons were recorded intracellularly in the isolated spinal cord. Apamin (0.2 to 1μM) reversibly reduced the l.AHP of all four neuronal classes tested [74±19% (±SD) reduction, n=24] without affecting resting potential or other aspects of the action potential. The interneurons tested were two classes involved in fictive swimming (lateral interneurons and CC interneurons) and a class of intraspinal stretch receptors (edge cells). The reduction of the l.AHP increased the steepness of the firing frequency vs. input current (F-I) relationship. During fictive swimming, however, apamin (1 to 10μM) had no significant effect on ventral root burst rate, burst intensity, or intersegmental phase lag (n=7).

It was also shown in the present experiments that motoneurons and interneurons exhibit l.AHPs during fictive swimming in the absence of apamin. Intra-axonal recordings of neuronal firing during fictive swimming revealed that most cells tend to fire at frequencies below 10Hz, which is a region of the F-I curve unaffected by apamin. These results suggest that the l.AHP has little role in fictive swimming in the lamprey due to the relatively low rates of neuronal firing, and that mechanisms other than spike adaptation must account for burst termination in the lamprey swim cycle.

140.7

DOPAMINERGIC MODULATION OF FICTIVE SWIMMING IN LAMPREYS. D.McPherson* and C.Kemnitz. Dept. of Biology, Marquette Univ., Milwaukee, WI 53233.

We have used an *in vitro* preparation of the spinal cord to test dopamine (DA) as a potential modulator of the central pattern generator for swimming in lampreys. The motor program for swimming was elicited by bath application of D-glutamate or N-methyl-DL-aspartate (0.2-0.5 mM), and activity was recorded extracellularly from spinal ventral roots through suction electrodes. Bath application of dopamine at low concentration (0.1-1 μM) consistently increased the swim cycle rate by about 20%, while 10 μM dopamine produced cycle rates similar to control values. Higher concentrations of dopamine (31.6-1000 μM) caused dose-dependent reduction of cycle rate, with about 50% reduction at 31.6 μM. These data suggest that dopamine has two distinct modulatory effects, accelerating swimming at low concentration, and slowing it at higher concentrations. The general features of fictive swimming, such as left-right alternation, rostral-caudal phase lag, and the ratio of burst duration to cycle period, were relatively unaffected by low to moderate [DA] (0.1-31.6 μM). In rectified ventral root recordings, the mean amplitude of bursts during swimming was slightly depressed by low [DA] (0.1-10 μM), and significantly augmented by higher concentrations (31.6-100 μM). Immunocytochemistry with antibodies to DA indicates it is present in cells and processes in the spinal cord, providing a source for dopaminergic modulation *in vivo*.

140.8

NEUROCHEMICAL MECHANISMS FOR GENERATION AND TRANSMISSION OF LOCOMOTOR RHYTHM *IN VITRO* A. Lev-Tov[1], J.C. Smith & B. I. Schmidt*. Systems Neurobiology Lab, Dept. of Physiological Science, UCLA, Los Angeles, CA 90024-1527. [1] Dept. of Anatomy, The Hebrew University Medical School, Jerusalem, Israel.

Neurochemical mechanisms involved in generation of locomotion were studied in the *in vitro* isolated spinal cord, or spinal cord-hindlimb preparation from 0-4 day old rats. Locomotor patterns were characterized by suction electrode recordings from homologous lumbar ventral roots, EMG recordings from hip and ankle flexor and extensor muscles, and sharp-electrode intracellular recordings from lumbar motoneurons. Locomotor patterns could be induced by bath application of NMDA; glutamate + the glutamate uptake inhibitor DHK or DHK alone; dopamine; acetylcholine + edrophonium (choline esterase inhibitor) or edrophonium alone. Although there were differences in the detailed patterns of motoneuron and muscle activation with each of the neurochemicals tested, alternating rhythmic patterns characteristic of quadruped stepping were induced in homologous ventral roots as well as in flexor and extensor muscles, in all cases. Intracellular recordings from lumbar motoneurons showed that each of the neurochemicals induced an initial tonic activity accompanied by membrane depolarization (10-15 mV) which was then transformed into rhythmic activity with large rhythmic depolarizing drive potentials (5-15 mV) imposed on the repolarized resting membrane potential. Block of NMDA and non-NMDA receptors with APV and CNQX, respectively, or activation of AP4 receptors, reduced the depolarizing rhythmic drive to subthreshold levels in a dose-dependent manner without affecting the cycle timing. Our findings indicate that multiple neurochemical mechanisms can generate an organized locomotor pattern. We suggest that activation of excitatory amino acid receptors is required for transmission of the rhythmic locomotor drive to motoneurons, but not for its generation. Supported by NIH NS-28805 and HL-02204.

140.9

STRIATO-THALAMO-CORTICAL CIRCUIT FOR HALOPERIDOL ACTION IN VIVO IN HUMAN BRAIN: PET/FDG ANALYSIS., C.A. Tamminga* H.H. Holcomb, E. Gastineau, D. Medoff. University of Maryland, MPRC, P.O. Box 21247, Baltimore, MD 21228

Haloperidol (HAL) is a potent antipsychotic drug, whose action is mediated by D_2 receptor blockade. HAL actions at the postsynaptic DA receptor produce its functional effect, first on that postsynaptic neuron, then on subsequent projection neurons, eventually influencing entire neuronal circuits. PET with FDG is a technique which can reflect regional functional changes in neuronal activity produced by a drug at all sites where they occur, integrated over the incorporation period of the ligand. Eleven schizophrenic subjects were studied on (4 weeks) and off (4 weeks) HAL using PET/FDG. Images were volumetrically reconstructed and resampled along the same planes; then analyzed using traditional ROIs; scaled data from the two scans were compared in the data analysis. Results show a significant HAL-induced increase in the regional cerebral metabolism of glucose (rCMRglu) in caudate and putamen; then in the striatal projection area, the globus pallidus, rCMRglu was decreased by drug. In thalamus, the next projection area, HAL increased rCMRglu. And in two cortical areas, the frontal cortex and cingulate, the drug decreased rCMRglu. These data are not readily understandable by postulating a primary HAL effect in each of these sites. Rather, we suggest that HAL has its primary and direct functional effect in the striatum, ie caudate and putamen. Thereafter, HAL action is mediated through basal ganglia and thalamus, to neocortex in a predictable fashion, through the described cortical-subcortical circuits (TINS 13:266, 1990). These data suggest the potency of these striato-thalamo-cortical pathways, in information transmission to cortex.

140.10

THREE-DIMENSIONAL IMAGING OF THE CEREBELLORUBRAL NETWORK OF TURTLE USING ANATOMICAL DATA FROM *IN VITRO* NEUROBIOTIN AND BIOCYTIN TRACER STUDIES. L. He*, R. Sarrafizadeh, T. Skimina and J. Houk. Department of Physiology, Northwestern University Medical School, Chicago, Illinois 60611-3008.

Neurobiotin and biocytin can be used in an *in vitro* turtle brainstem-cerebellum preparation to characterize recurrent feedback connections thought to comprise the cerebellorubral network (Sarrafizadeh et al. this meeting). Because of the small molecular size of these tracers good retrograde cell fills together with detailed information concerning axon locations and dendrite arborization patterns may be obtained. Our goal is to three-dimensionally reconstruct the cerebellorubral interconnections by imaging serial sections from anatomical studies performed in an *in vitro* turtle (*Chrysemys picta*) brainstem-cerebellum preparation.

Images are captured by light field microscopy with a CCD camera, after which the section boundaries are manually traced and cell, axon, and dendrite positions are located. Sections are aligned using anatomical landmarks and the third dimensional coordinate is assigned to each section. Using the method of triangulation, a three dimensional model of the tissue surface is constructed. Each cell is modelled with a sphere marking its center and axon bundles and dendritic processes are recreated with curves which follow their trajectories in space. The boundaries of different nuclei are marked with polyhedrons which form a convex hull covering all labeled cells in that nucleus. Realistic three-dimensional images of the tissue are subsequently created using surface rendering techniques. These three-dimensional images may be created to reflect the rotation of the tissue in space giving the observer different perspectives of the anatomical data.

This technique may be used to reconstruct the three-dimensional structure of the different postulated components of the cerebellorubral network, namely the red nucleus, the lateral cerebellar nucleus, and a nucleus in the lateral cerebellar zone. Furthermore, reconstruction of the recurrent connections between these nuclei may be used to demonstrate global and local neuroanatomical information which would be difficult to grasp from serial sections alone.

140.11

SYNCHRONIZED EARLY-INSPIRATORY PHRENIC UNIT ACTIVITIES IN DECEREBRATE CATS. W.-X. Huang*, M.I. Cohen, R. Barnhardt and C.N. Christakos. Dept. of Physiol., Albert Einstein Col. Med., Bronx NY 10461.

Synchronization of phrenic (PHR) nerve and unit activities was studied in 8 decerebrate, paralyzed, and vagotomized cats, by simultaneously recording discharges of two or three PHR single fibers (n = 151) and of the left and right PHR nerves. Short-time-scale (2-ms bins) cycle-triggered histograms (CTH) always showed a distinct burst in whole PHR activity at the onset of neural inspiration (I). This burst arose mainly from the summation of the first spikes of early-I (onset delay < 80 ms) PHR units (80/151). 60 of these 80 units showed an oscillation in the CTH that was locked to the onset of I. In 40 of a total of 71 pairs of early-I PHR units, each unit showed such an early-I oscillation. For spectral analyses, the I phase was divided into two halves. 1) First half of I. 31/40 pairs had significant coherence peaks in the range of medium frequency oscillation (MFO) (14.4 ± 6.4 S.D. Hz, coherence value 0.17 ± 0.11 S.D.). The coherent frequency was usually close to the initial rates of the rhythmic and augmenting unit discharges. By comparison, only 15/40 pairs showed coherence in the range of high frequency oscillation (HFO) (70.4 ± 19.5 S.D. Hz). 2) Second half of I. The oscillations in the CTHs that were locked to the start of I became weaker or disappeared; and only 13/40 pairs showed significant MFO coherences, which were lower (0.08 ± 0.03 S.D.) than in the first half. In contrast, a larger proportion (24/40) of significant HFO coherences was observed than in the first half. Thus, the rhythmic firing of many early-I PHR units is locked to the start of I, resulting in significant coherences in the MFO range between unit pairs; and this transient synchrony of PHR unit spikes near the start of I can account, in part, for MFO coherences (unit-PHR and PHR-PHR) that are sometimes observed. (Christakos et al., J. Neurophysiol. 66, 674-687, 1991).
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140.12

STATE-DEPENDENT SYNCHRONIZED FAST RHYTHMS IN NEURAL NETWORKS (INSPIRATORY AND SYMPATHETIC). M.I. Cohen*, C.N. Christakos, R. Barnhardt, W.-X. Huang and W.R. See. Dept. Physiol., Albert Einstein Col. Med., New York, NY 10461.

Fast rhythms in unit and population discharges have been observed in various neural systems, such as the inspiratory (I) and sympathetic (symp). The properties of such rhythms (amplitude, frequency, phase) may reflect network interactions, as suggested by spectral, coherence, and crosscorrelation analyses applied to I and symp activities.

I motor nerve (e.g., phrenic) and medullary I unit discharges exhibit, in addition to the respiratory rhythm, faster rhythms in the range 50-100 Hz (high-frequency oscillations, HFO). a) There are high coherences between HFOs in different I nerves, indicating a common brain-stem origin of these rhythms. b) The great majority of medullary I neurons have HFO, which are highly coherent with I nerve HFOs. c) Different types of I neuron have different phase relations of unit to I nerve HFOs. d) HFO properties vary with time in the I phase, with respiratory afferent inputs, and with state (e.g., between respiration and fictive vomiting). These properties suggest that the system HFOs are due to interactions (possibly via excitatory and inhibitory loops) between neurons of the inspiratory pattern generator.

Symp nerve discharges also have fast rhythms, albeit on a slower time scale: spectral peaks occur in two ranges, 2-6 Hz and 9-11 Hz. There are high coherences between different symp nerve activities as well as between medullary unit and nerve activities. These rhythms also vary with time in the central respiratory cycle, as well as with state (e.g., baroreceptor activation). These features suggest that symp rhythms arise from interactions in sympathetic-generating networks. (Supported by N.I.H. Grant HL-27300.)

140.13

An Electrophysiological Topography of the Rat Corticostriatal Projection. G.E. Glynn[1]* & B.K. Yamamoto[2]. [1]Parke-Davis, Ann Arbor, MI 48105. [2]Dept. of Psych. Case Western Res. Univ. Clev.,OH 44106.

Anterograde degeneration, amino acid transport and striatal biochemical changes due to cortical ablations indicate that a corticostriatal topography exists. The aim of this study is to examine the physiological significance of these projections using quantitative electrophysiological techniques. Bipolar stimulating electrodes delivering a biphasic pulse (800-1000 μA, 0.2 msec) were implanted in the frontal, motor, hindlimb, auditory, visual, occipital and somatosensory areas of the rat cortex. Multiple unit activity was measured in 7 dorsal and 7 ventral areas of the striatum for a period of 25 msec prior to and after the delivery of a stimulus to a cortical site. Stimulation of each cortical site produced a unique regional pattern of striatal activation. The degree of activation (max = 800 ± 300% s.e.m.) was also stimulation site dependent. All areas except the frontal ctx produced a greater dorsal than ventral activation. A rostrocaudal topography was evident. These are the first results to show a quantitative 3 dimensional corticostriatal topography.

140.14

FAILURES OF ANTIDROMIC INVASION OF EMBRYONIC RAT DRG-SOMATA DURING HIGH FREQUENCY STIMULATION.

Chr. Lüscher*, J. Streit and H.-R. Lüscher, Dept. of Physiology, University of Berne, Berne, Switzerland

Impulse conduction along branching axons might be an integrative instead of a simply transmittive process, since it has been shown that impedance mismatch can lead to failure of impulse propagation. In order to investigate the presence of such a process in spinal cord circuits, we have examined the reliability of antidromic invasion of action potentials from central unmyelinated axons into the somata of DRG cells in organotypic rat embryonic spinal cord cultures. Somatic membrane potentials were recorded during extracellular stimulation of the central axons of DRG cells. By localized stimulation at various sites along the axons the conduction velocity was measured to be 0.2-0.6m·s^{-1}; the chronaxie 150μs. With stimulation at 1 Hz four possible responses were observed in different cells: action potentials (AP), complete failure and two intermediate responses indicating propagation failures at different sites along the axons. In cells with reliable conduction propagation failures could be induced by hyperpolarizing or depolarizing the cells through current injections. The reliability of conduction was increased when the extracellular Ca^{++} concentration was lowered from 5mM to 1mM., but was decreased by Cd^{++} (1.5mM). Failures again appeared after several stimuli at 10 Hz but never when applying paired stimuli 100ms apart. The frequency dependent propagation failures were more pronounced in high extracellular K^+ (10mM). These findings show that the safety factor for impulse propagation in embryonic DRG axons is low.

140.15

A NEW MULTI-CONDITIONAL STATISTICAL MEASURE FOR DETECTING SPATIO-TEMPORALLY CORRELATED FIRING PATTERNS IN MULTIPLE SPIKE TRAINS. D. C. Tam.* Dept. of Biological Sciences, University of North Texas, Denton, TX 76203.

A multi-conditional statistical measure is developed to extract the conditions under which firing in neurons occur in recorded spike trains. It is well-known that firing of action potentials in a neuron could depend not only on a single input but also on multiple inputs to the neuron. Thus, specifically designed statistical measures are needed to correlate the specific timing relationship of the multiple inputs to a neuron with its firing probability. This multi-conditional statistic measures the firing probability of the next spike for a neuron in relation to the firing times of spikes in other spike trains. Thus, this statistic detects the spatio-temporal conditions under which a neuron will fire. The probability distribution function (p.d.f.) of firing is estimated by the statistics relative to the pre-firing interspike and cross-intervals. Once the multi-conditional probability distribution function (c.p.d.f.) is established, this statistic can be used to detect what spatio-temporal firing patterns of other neurons are related to the firing probability of a neuron. Since precise synaptic inputs to a neuron cannot be easily established experimentally in multi-unit recordings, this conditional statistic can be used to detect which of the n recorded neurons contribute to the combinatorical firing probability. Thus, the combinatorics can be used to isolate which sub-groups of neurons are correlated with the firing probability of a neuron and which are not. It can also provide statistical inference on the interactions in firing of spikes in the sub-group of correlated neurons that may be considered as a functional unit. The ability to detect neural functional units and their firing relationships is crucial in the interpretation of physiological functions performed by neuronal networks using multi-unit spike train analysis. (Supported by ONR N00014-90-J-1353 and USPHS RR05425-30)

140.16

MULTI-UNIT SPIKE DISCRIMINATION USING WAVELET TRANSFORMS. G. Zouridakis*† and D. C. Tam‡. †Dept. of Electrical Engineering, University of Houston, Houston, TX 77204-4793, and ‡Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

In multi-unit extracellular recordings, action potentials from different neurons can be identified by their characteristic waveforms recorded at each electrode. We employed wavelet transforms (WTs) to extract unique features of the spikes corresponding to individual neurons, and then used these features for spike discrimination.

In analogy to the Fourier transform, where a signal is expressed as a weighted sum of sines and cosines, the WT represents a signal by a weighted sum of basis functions, called wavelets. The basis functions are obtained by scaling and time shifting a single prototype (a spike, in our case). The WT not only provides information about the frequency content of a spike at every time instant (like the spectrogram), but also allows both the time and the frequency resolutions to vary. Thus, typical features associated with spikes from different neurons can be identified more readily in the WT domain.

Spike discrimination starts with spike detection, which is accomplished by setting criteria in the time domain (such as amplitude-threshold crossing and peak detection) or in the WT domain. Then, feature extraction is performed in the WT domain, where typical patterns are determined by computing the ratios of the weighting coefficients that correspond to individual spikes. Different patterns in the WT domain correspond to different spikes in the time domain. These "signatures" are then used to separate spikes into distinct classes that correspond to different neurons.

The performance of the method is evaluated using both simulated data and extracellular recordings from actual neurons.

(Supported by ONR N00014-90-J-1353 and USPHS RR05425-30)

140.17

USING THE ASYMPTOTIC KOLMOGOROV ENTROPY TO IDENTIFY DETERMINISTIC ORDER IN MULTI-UNIT SPIKE TRAINS. G. T. Kenyon* and D. C. Tam Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

We describe a method for testing the hypothesis that, within a neural population, the sequence of firing times is deterministic (chaotic), characterized by a finite Kolmogorov entropy, K, and thus that such sequences may encode behaviorally significant information. We contrast this with the alternative hypothesis that the sequence of firing times is stochastic, characterized by an infinite K, implying that behaviorally significant information can only be encoded in the firing rate. To compute K, an ensemble of digitized firing patterns is first obtained by overlaying the multi-unit spike trains with a series of spatial-temporal grids, each aligned along a different reference spike, and denoting the occurrence of one or more events in a grid square by a 1 (0 otherwise). The spike train entropy, H, is estimated by the average over the logarithms of the occurrence frequencies of all digitized firing patterns in the ensemble. K is then given by the asymptotic value of the rate of the marginal increase in H as the grid resolution and pattern size are increased. Starting from an explicit expression for a train of uncorrelated events, we show that for any reasonably smooth stochastic process, K grows logarithmically with increased temporal resolution (for a sufficiently large, fixed pattern size). A sufficient criterion for identifying an underlying deterministic order is therefore that K asymptotes to a finite value as the temporal resolution is increased. We also consider renormalized spike trains, obtained by selecting every nth event from each reference spike and rescaling the time axis by the same factor. The renormalized spike train can be used to investigate temporal structures at longer time scales, such as burst patterns or slow oscillations. Stereotypical results are illustrated through the analysis of computer generated spike train data. A parallel algorithm for extracting the spike train entropy, which on the CM-2 exhibits near linear scaling with both ensemble size and number of processors with over 99% of the execution time devoted to parallel operations, is described. (Supported by ONR N00014-90-J-1353).

140.18

WORKING MEMORY IN SMALL INHIBITORY-FEEDBACK NEURAL NETWORKS: NOISE, BISTABLE NEURONS AND CODING OF SCALAR VALUES. A.B. Kirillov, C.D. Myre* and D.J. Woodward. Biographics, Inc., and Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

We reported earlier that an inhibitory-feedback network inspired by neostriatal circuitry may exhibit bistable neuronal firing and spontaneous switching phenomenon. In the presence of excitatory noise, some neurons switch "on" and generate streams of impulses while other neurons remain quiescent. In time, the existing "on" neurons spontaneously switch "off" and other neurons switch "on". In this work we examined the nature of bistability and memory phenomenon using simple models consisting of several (2 to 7) totally interconnected neurons with symmetrical inhibitory synapses. A network with spontaneously active neurons and minimal noise settles in a specific pattern of "on" and "off" neurons, and the active neurons establish stationary phase relationships. A pattern of "on" and "off" states can be set by excitatory stimulation. The network then sustains the pattern, thus working as a memory register. Whereas the two-neuron network acts as a common flip-flop binary device, networks with several neurons can be made to store a scalar value in the form of a summed subset of neurons in "on" state. Neuroanatomy suggests that neostriatal circuitry is designed to operate in this mode. Increased noise causes switching which washes out this form of memory trace or allows expression of long term memory patterns stored as synaptic strengths. Our analysis shows that the ability of the network to exhibit memory properties depends on the complex of physiological parameters, including duration of afterhyperpolarization, strength and the time duration of inhibitory post-synaptic potentials and the temporal pattern of noise. Our hypothesis is that inhibitory-feedback networks may operate as a form of local temporary scalar memory in a motor control system. *Support from the Texas Adv. Res. Prog., Biol. Humanics Found., MH44337, DA02338 and AFOSR 90-146.*

140.19

PHYSIOLOGICAL PROCESSING STRATEGIES OF SINGLE NEURONS W.E. Faller*, S. Shah and M.W. Luttges. Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80309-0429.

Sampling biases, for large neurons, are especially worrisome for the analysis of simultaneous single-unit recordings where the results are commonly assumed to infer network information processing strategies. Since a majority of the cells recorded are large, it is unclear whether the results from such analyses yield an accurate picture of the network function. To address this problem we developed a method for determining the size of each individual neuron obtained using paired-electrode sets. The technique assumed that recorded spike amplitudes decreased with the inverse of distance squared while spike angles (widths) increased linearly with distance from the cellular source to the recording electrodes. Starting with the recorded spike amplitudes and angles for each cell a numerical algorithm was iterated to estimate the true value of the amplitude and angle minus these distance effects. The resolved amplitudes were a direct, consistent estimate of the size of each recorded neuron. Using this technique simultaneous single-unit recordings from 57 neurons were analyzed. The results indicated that the ratio of small to large cells was roughly 2:1. Histologically, however, a ratio of small to large cells on the order of 10:1 would have been expected. A sampling bias for the recording of large cells was apparent. Since the technique determined the size of each individual cell, however, neither individual cell nor population studies were effected by such sampling biases. The population statistics for the small and large cell groups were calculated for the mean firing frequency, standard deviation of the firing period and both the number of "functionally" inhibitory and "functionally" excitatory connections per cell. Small and large cells were shown to make different contributions to the processing of information in this network of neurons. Small cells on average showed a slightly lower mean firing rate, more than twice as many functionally inhibitory connections with other cells and fewer functionally excitatory connections.

LIMBIC SYSTEM I

141.1

ELECTROPHYSIOLOGY AND MORPHOLOGY OF RAT SUBICULAR NEURONS *IN VITRO*. A.J.R. Mason*. University Department of Pharmacology, Oxford, OX1 3QT, UK.

Although the subiculum is an important output structure of the hippocampus, the membrane properties of its neurons have received little attention. Horizontal slices of the hippocampus and para-hippocampal region were maintained at 34 °C in an interface chamber. Intracellular recordings were made with conventional, sharp micro-pipettes. A metal, bipolar, stimulating electrode on the slice surface was used to evoke synaptic potentials. Analysis of the data from 23 neurons gave values for resting potential of -64 +/- 6 mV (mean +/- SD), input resistance of 28 +/- 9 MΩ, and membrane time constant of 12.7 +/- 3.6 ms. Action potentials (APs) had peak amplitudes above rest of 102 +/- 7 mV, and widths at base of 1.2 +/- 0.1 ms. Nineteen of the cells responded to depolarising current pulses with an initial burst of 2-4 APs riding on a depolarising wave and followed by a marked hyperpolarisation. Depolarising pulses of sufficient strength and duration evoked trains of single APs after the initial burst. Burst-firing was resistant to blockade of excitatory synaptic transmission with a mixture of CNQX (10 μM) and AP5 (50 μM) but was abolished by maintained depolarisation to just below firing levels, suggesting that burst-firing is an intrinsic membrane property. Stimulation of CA1 oriens/alveus or the molecular layer of the subiculum evoked a biphasic synaptic potential. The depolarising component was abolished by CNQX and AP5, leaving a hyperpolarising response which could be reversed by membrane hyperpolarisation. Intracellular injection of biocytin into 10 neurons revealed pyramidal morphology with spiny dendrites. Most basal dendrites projected into the white matter and most apical dendrites extended across the molecular layer to the hippocampal fissure.

141.2

BLOCKADE OF EXCITATION REVEALS INHIBITION IN DENTATE HILAR NEURONS IN RAT HIPPOCAMPAL SLICES. H.E. Scharfman*. Neurology Research Center, Helen Hayes Hospital, West Haverstraw, NY, 10993-1195, and Departments of Pharmacology and Neurology, Columbia University, NY, NY.

To understand the relative contributions of excitation and inhibition to dentate hilar activity, hilar cells were recorded intracellularly in rat hippocampal slices and their spontaneous and evoked activity were analyzed pharmacologically. Despite evidence of GABA-immunoreactive terminals on hilar neurons, and the ability to evoke robust IPSPs in other neurons in the same slice, both spontaneous activity and evoked responses in most hilar neurons were solely excitatory. The AMPA/kainate receptor antagonist CNQX blocked spontaneous potentials and responses to perforant path stimuli of all spiny hilar neurons as well as most aspiny hilar neurons. The NMDA receptor antagonist APV had relatively little effect, even when $[Mg^{2+}]_o$ was lowered to 0.5 mM. When combined CNQX and APV application blocked hilar and granule cell excitation, IPSPs were revealed, but only after raising stimulus intensity. These IPSPs were mono- or biphasic, and reversal potentials were near -65 and -80 mV, respectively.

These results suggest that, at least in slices, hilar cells are ordinarily excited and after excitation is blocked inhibitory influence is unmasked. The hyperpolarizing inhibition that is revealed appears to be similar in mechanism to the type of inhibition exerted on hippocampal and dentate principal cells. The neurons that may be responsible for hilar cell inhibition are the local GABAergic interneurons that have dendrites in the molecular layer, and/or the somatostatin/neuropeptide Y-immunoreactive hilar cells with axons in the molecular layer, although it is not yet proven that the latter are inhibitory.

The balance of excitation and inhibition is fundamental in allowing normal signal processing while preventing seizure activity. A possible example of inappropriate emphasis on excitation appears to exist in the hilus of the rat fascia dentata, with the possible consequence of predisposing the dentate and hippocampus to epileptiform behavior.

141.3

INTRACELLULAR RECORDINGS FROM MEDIAL SEPTAL PACEMAKER NEURONS DURING HIPPOCAMPAL THETA RHYTHM. E. S. Brazhnik and S. E. Fox*, SUNY Health Sci. Ctr., Dept. of Physiology, Brooklyn, NY 11203.

Medial septal bursting cells project to the hippocampus and pace the theta rhythm. Many of the septohippocampal (S-H) cells are cholinergic, others are GABAergic. Two classes of S-H rhythmically bursting neurons have been identified by their sensitivity to muscarinic antagonists (e.g. atropine). The rhythmicity of one class is abolished by atropine, the other is unaffected. This study provides evidence that the atropine-sensitive S-H neurons are cholinergic.

Rats were anesthetized with urethane (1.0 g/kg, i.v.). A 3 mm hole was made in the skull and the septum was exposed by aspiration. The cavity was filled with mineral oil and capped with wax to seal around the shaft of micropipets filled with K-acetate (R_E: 50-100 MΩ). Hippocampal macroelectrodes recorded the theta rhythm.

Recordings from 24 septal cells showed resting potentials greater than 40 mV ($\bar{x}$ = 58 mV). Nine rhythmic neurons were recorded during theta. Of these, four had short action potential (AP) durations, consistent with those of noncholinergic septal cells (Markram & Segal, Brain Res. **513**: 171, 1990). These neurons fired rhythmic bursts of 5 to 20 spikes on each theta cycle. Such rapid bursting is typical of the atropine-resistant S-H cells. In the other 5 rhythmic neurons recorded during theta, AP durations were longer, as were afterhyperpolarizations. These properties are consistent with those reported for cholinergic S-H cells. Like the atropine-sensitive S-H neurons, these usually fired only 1 to 3 spikes/cycle. One of these cells was tested with scopolamine (0.5 mg/kg i.v.), whereupon its rhythmic bursting ceased. These data support the hypothesis that muscarinic antagonists abolish the rhythmicity of cholinergic S-H neurons, leaving the GABAergic S-H neurons unaffected. (Supported by NIH NS17095.)

141.4

ORIGINS OF THE HIPPOCAMPAL THETA RHYTHM: ENTORHINAL AND SEPTAL CONTRIBUTIONS. S. E. Fox, J. Brankack and M. Stewart*, SUNY Health Sci. Ctr., Depts. of Physiology and Pharmacology, Brooklyn, NY 11203.

Single unit data from all hippocampal subfields, entorhinal cortices, and septal nuclei suggest a picture of EEG generation that accounts for the theta-related firing throughout the hippocampal formation. Excitatory lateral entorhinal inputs: 1) generate much of the phasic current sinks located in str. lacunosum/moleculare of CA1 and the distal molecular layer of the dentate (accounting for the EEG negativity at the fissure), and 2) modulate granule and pyramidal cell excitability, as tested via apical synaptic activation. Excitatory medial entorhinal (MEC) inputs: 1) generate a phasic mid-molecular layer sink in the dentate (peaking on a phase nearly opposite to the LEC-generated current sinks), and 2) drive dentate granule cells, forming the first segment of the tri-synaptic pathway. Dentate neurons drive the CA3/4 pyramids, which activate CA1 pyramids, completing the tri-synaptic pathway. The absence of phasic MEC-generated current sinks in CA1 and CA3/4 indicate that theta-related firing by pyramidal cells is *not* due to direct MEC afferents.

Medial septal afferents entrain entorhinal and hippocampal projection cells with rhythmic excitatory (cholinergic) and inhibitory (GABAergic) inputs to interneurons. In walking rats, however, hippocampal interneuron firing is driven, in addition, by potent recurrent and feed-forward activation from projection cells. Here, rhythmic septal GABAergic inputs are most important for synchronizing interneurons (hence the atropine-resistance of the walking theta rhythm). In urethane anesthetized rats, where rhythmicity of projection cells is notably less, the rhythmic excitation via cholinergic septal afferents dominates interneuron firing (hence the atropine-sensitivity of the urethane theta rhythm, and the differences in hippocampal projection cell and interneuron firing phases). [Supported by NIH grants NS17095 and NS09175.]

141.5

EVIDENCE FOR SUPRAMAMMILLARY CONTROL OF THE FREQUENCY OF RETICULARLY-ELICITED HIPPOCAMPAL RHYTHMICAL SLOW-WAVE ACTIVITY (THETA). I.J. Kirk*, B.J. Logan and N. M°Naughton. Department of Psychology and the Neuroscience Research Centre, University of Otago, Dunedin, New Zealand.

The present study assessed the role of ascending systems in the production of hippocampal rhythmical slow-wave activity (RSA). RSA was elicited in urethanized and freely-moving rats by high frequency (100 Hz.) stimulation in the reticular formation (RF). Procaine hydrochloride (0.5 µl., 20% w/v) was microinfused at various caudo-rostral extents between the pontine RF and the medial septum/ diagonal band (MS/DB), and its effect on the frequency and amplitude of reticularly-elicited RSA determined by spectral methods. It was found that, in the region of the medial forebrain bundle or in the MS/DB, procaine reduced the amplitude of elicited RSA but had no effect on its frequency. In the RF, up to the level of the caudal diencephalon, procaine principally reduced the frequency of elicited RSA. In a relatively restricted zone in the supramammillary (SUM) region, procaine reduced both the amplitude and frequency of reticularly-elicited RSA. These results suggest that the frequency of reticularly-elicited RSA may be encoded in the SUM, rather than in the MS/DB as has previously been suggested.

141.6

ALPHA$_2$ MODULATION OF TYPE 1 AND TYPE 2 HIPPOCAMPAL THETA IN THE FREELY MOVING RAT. R. S. Sainsbury* and L. A. Partlo. The University of Calgary, Calgary, AB, Canada T2N 1N4.

Changes in both type 1 and type 2 theta were examined following infusions of detomidine (alpha 2 agonist) or procaine (local anaesthetic) into one of three brain sites in the freely moving rat: the hippocampus, median raphe nucleus (MRN) and the locus coeruleus (LC). Detomidine infused into the hippocampus had an attenuating effect on type 1 theta, while type 2 theta remained unchanged. Infusions of detomidine into the MRN produced no apparent effects on type 1 theta, while a release/driving of type 2 theta was noted. No changes were observed following detomidine infusions into the LC. Procaine infusions into all three brain sites had no effect on type 1 or type 2 theta. The results of this work suggest the hippocampus as being the site of action responsible for the attenuation of type 1 theta following detomidine infusions. The MRN is likely responsible for the release/driving of type 2 theta.

141.7

EFFECTS OF Mk-801 ON HIPPOCAMPAL EEG SPIKES IN THE RAT. M. Oguri,* M. Takita and Y. Sugiyama Department of Biometrics Faculty of Science, Toho University, Tokyo 253, Japan.

In the normal rat, highly conspicious spikes are superimposed on spontaneous hippocampal EEG activity during behaviors not accompanied by rhythmical slow activity such as immobility and slow wave sleep. From the point view that hippocampus contains much glutamic acid, NMDA receptor might take part in inducing hippocampal EEG spikes. The present study was undertaken to investigate the effects of MK-801, an antagonist for NMDA receptor of Glu on the spontaneous EEG spikes in the CA1 region of rat hippocampus. Bundles of guide tubing and macroelectrode wewe placed in the CA1 region of rat hippocampus, together with electrodes to record cortical EEG, nuchal EMG and eye movements. MK-801 were microinjected through Hamilton syringe with Gazel infusion pump. Microinjections and electrophysiological recordings were conductedunder the head-restrained condition. MK-801 injection suppressed distinctively the appearance of hippocampal EEG spikes in awake state, while the hippocampal EEG in slow wave sleep still showed conspicious spikes. It is suggested that the effects of antagonistic function of MK-801 on NMDA receptors might be different among sleep states.

141.8

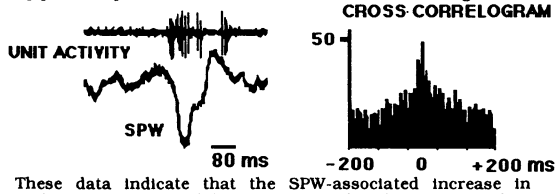
Axon arborization of CA3 pyramidal cells in vivo: an intracellular labeling study. X-G. Li, J. M. Tepper*, G. Jandó, G. Buzsáki Center for Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ 07102

Twenty CA3 pyramidal cells in 60 rats were physiologically characterized and filled with biocytin in urethane anesthetized rats. CA3 pyramidal cells arborized most extensively in CA1, followed by CA3, in line with previous observations. Bouton density was similar in both regions (mean interbouton distance: 4.89 µm). The axon length of a single neuron exceeded 200 mm. Based on axon length and bouton density, we estimate that a CA3 pyramidal cell can contact 20,000-30,000 neurons. Two cells, with horizontal dendrites and numerous thorny excresences at the CA3c-hilus border ("mossy-like" cells) were also labeled. These neurons also projected to CA3 and CA1. All neurons had axon collaterals in the hilus and some of the terminals reached the border of the granule cell layer. Three CA3c pyramidal cells in the ventral hippocampus had extensive projections to the inner third of the dentate molecular layer (!), and to CA3 and CA1. Based on these findings, we suggest that (a) CA3 pyramidal cells have wider projections than hitherto suspected, (b) there is a bidirectional communication between hippocampal CA3 and the dentate gyrus, and (c) CA3 projections in the dorsal and ventral hippocampus are qualitatively different.

141.9

HIPPOCAMPAL-RETROHIPPOCAMPAL INTERACTIONS DURING SHARP WAVES. J.J. Chrobak*, R. Urioste and G. Buzsaki. Ctr.for Mol. and Behav.Neurosci. Rutgers University, Newark, NJ 07102.

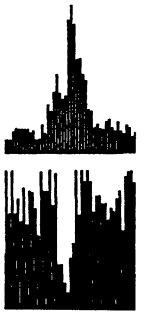
Hippocampal sharp waves (SPW) may play an important role in memory consolidation processes. These hippocampal field potentials reflect excitatory potentials in the CA1 dendritic region driven by CA3 input. During SPW's, there is a relatively synchronous activation of CA3, CA1 and subicular neurons. The present study examined the activity of retrohippocampal (subiculum, presubiculum, parasubiculum and entorhinal cortex) neurons during SPW's. Retrohippocampal neurons were observed that exhibited a transient increase in activity concurrently with hippocampal SPW'S (see figure).



These data indicate that the SPW-associated increase in intrahippocampal excitability is associated with a sequential or concurrent increase in the excitability of retrohippocampal neurons. The emerging picture suggests a transient increase in the activity of a network of neurons from CA3 through to the entorhinal cortex. This network event could provide the synaptic drive to potentiate synaptic connections within this temporal lobe circuitry and may represent ongoing memory consolidation processes.

141.10

PHYSIOLOGICAL CLASSIFICATION OF HIPPOCAMPAL FEEDBACK AND FEEDFORWARD INTERNEURONS. **R. Urioste*, J. J. Chrobak, G. Buzsáki, Ctr. for Mol. and Behav. Neurosci., Rutgers University, Newark, NJ 07102.**



Analysis of the network interactions between the principal and non-principal hippocampal neurons reveals important intrinsic and extrinsic control. Particularly important are the feedback (recurrent) and feed forward inhibitory influences of interneurons on pyramidal cells. Extracellular analysis of interneuron activity and its correlation with spontaneous sharp waves (SPW) is a physiological way of classifying interneuronal subtypes. We examined the possible correlations between interneuronal firing and SPW-associated population bursts of pyramidal cells in the awake rat. Three patterns of interneuron activity were observed: (i.)interneurons excited during SPWs (via recurrent CA1, or feedforward CA3, pyramidal activity); (ii.) a subclass of interneurons exhibiting relationship to the population activity; (iii.) a small subset of interneurons inhibited during SPWs (possibly by extrinsic, subcortical input or by lateral inhibition mediated by neighboring interneurons). These findings underscore the importance of normal physiological activity in the classification of hippocampal network elements participating in neuronal cooperativity.

141.11

The dentate spike: a new network pattern reflecting interneuronal synchrony. A. Bragin, G. Jando, Z. Horvath and G. Buzsáki* Center for Molecular anf Behavioral Neuroscience, Rutgers University, Newark, NJ 07102

Field potentials and unit activity was recorded in the dentate area of the rat using single and multiple microelectrodes. During behavioral immobility and drowsiness large amplitude (2-4 mV), short duration (<25 ms) field spikes were observed. Two-dimensional potential maps of the dentate spike (DS) showed largest amplitude positivity in the hilar area with a reversal in the outer molecular layer. This depth profile was distinctly different from the simultaneously recorded potentials evoked by stimulation of the perforant path. Simultaneous recordings from left and right hili revealed that DSs occurred with zero time lag in the two hemispheres. Recordings with multielectrode arrays spanning several mm along the longitudinal axis of the hippocampus showed very high coherence of DSs. Cross-correlation histograms of unit firing in the hilus showed prominent peaks during DS, while simultaneously recorded CA3c pyramidal cells and putative mossy cells were inhibited. To date, we did not find a reliable relationship between DSs and hippocampal sharp waves which also occur during behavioral immobility. We hypothesize that DSs are due to synchronous discharge of hilar inhibitory interneurons. The mechanism of interneuronal synchrony has yet to be discovered.

141.12

NEURONAL FIRING AS DIFFERENTIABLE MANIFOLDS EMBEDDED IN NEURONAL FIRING RATE SPACE. James B. Ranck Jr.* Dept of Physiology, SUNY-Brooklyn, NY 11203.

If the discharge of a single neuron is converted into a firing rate, the possible firing of a whole class of neurons can be described as an n-dimensional space, where "n" is the number of neurons in the class. In addition to anatomical and biochemical criteria, part the current definition of a neuronal class, is that the neurons fire under similar circumstances. Therefore, the actual firing of a class of neurons in not n-dimensional, but rather a smaller dimension, "m". If there is a clean relation between the firing of the neuronal class and the external world, the firing can be expressed mathematically as an m-dimensional differentiable manifold embedded in an n-dimensional firing rate space. For instance, the firing of head direction cells is a one dimensional manifold without boundary, topologically equivalent to a circle. The firing of an ideal hippocampal place cell is a 2-dimensional manifold with boundary. The firing of visual and somatosensory neurons up to primary sensory cortex is a more complicated topological structure and requires a bundle structure. This approach allows us to consider many different topological structures embedded in firing rate space, some of which have behavioral consequences. There is an algorithm by which one can determine the dimension of a manifold from the firing of many neurons of a class recorded simultaneously, independent of the relation of the firing to the external world.

Under certain restricted, but fairly general conditions (which include consideration of persistence of effects) maps can be written between manifolds which express how the inputs to a class of neurons cause the firing of the output. By composing these maps the relation of the firing of a class of neurons to the outside world can be written. These composed maps show that a differentiable manifold is not only a description of the firing of a class of neurons, but also can reflect causal relations in brain. This analysis shows that the brain is a dynamical system, which can be analyzed with with this approach. (Supported by NIH NS14497.)

141.13

A CURRENT SOURCE DENSITY (CSD) ANALYSIS OF STIMULUS INDUCED FIELD POTENTIALS IN RAT DENTATE GYRUS SLICES FOLLOWING ENTORHINAL LESION. R. Nitsch*(1), H. Clusmann (2), and U. Heinemann (2). (1) Centr. of Morphology, Univ. Clinic Frankfurt, 6000 Frankfurt/M. 70, FRG and (2) Inst. of Neurophysiol., Univ. Cologne, 5000 Köln 41, FRG

Following ipsilateral entorhinal lesion sprouting of remaining afferents (eg. commissural fibers) into the hippocampal termination zones of the degenerated perforant path fibers has been reported suggesting a compensatory replacement of excitatory synaptic input. Conversely, peripheral dendrites of inhibitory interneurons retract from these zones, which might result in altered inhibition. In order to study the functional consequences of adaptive changes following lesion we analyzed field potential responses to stimulation of the inner and middle molecular layer and to stimulation of mossy fibers in the hilus. In addition we performed a current source density analysis (CSD) of the stimulus induced field potentials in slices obtained from normal and animals 55 days post lesion. Stimulation of the inner molecular layer caused large field EPSPs with small if any generation of population spikes in the granule cell layer both in normal and lesioned animals. In lesioned animals responses evoked by stimulation of the middle molecular layer cause smaller field potentials than in normal animals. The CSD analysis revealed a shift of current sinks induced by stimulation of either the inner or the middle molecular layer to a common site. Activation of mossy fibers does not reveal alterations of evoked potentials following lesion. These results suggest that the postlesional reorganisation, i.e. sprouting of remaining afferents is not effective in functionally compensating for the loss of entorhinal input.

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141.14

ANATOMICAL AND ELECTROPHYSIOLOGICAL EVIDENCE FOR CONVERGENCE OF MONOSYNAPTIC AND DISYNAPTIC ENTORHINAL INPUT TO CA3. Theodore W. Berger* and Mark F. Yeckel, *Dept. of Biomedical Engineering, and Program in Neural , Informational, and Behavioral Science, University of Southern California. Los Angeles, CA 90089.*

We have demonstrated previously that stimulation of entorhinal axons results in monosynaptic excitation of dentate granule cells, and CA3 and CA1 pyramidal neurons. In addition, CA3 and CA1 pyramidal neurons are excited at longer latencies consistent with polysynaptic activation through the trisynaptic pathway. Based on these findings we have proposed that excitatory input from the entorhinal cortex initiates a two-phase feedforward excitation of pyramidal cells, with the dentate gyrus providing feedforward excitation of CA3, and with both the dentate and CA3 providing feedforward excitation of CA1. An implicit assumption of this model is the convergence between mono- and multisynaptic entorhinal input to any one subpopulation of pyramidal neurons. Therefore, anatomical and electrophysiological experiments were performed using New Zealand white rabbits to examine the extent to which the topography of monosynaptic entorhinal input to CA3 parallels that of disynaptic input via mossy fiber axons.

Two general strategies were attempted with both methodologies: i) afferent pathways were examined individually and the spatial distribution of monosynaptic and reconstructed multisynaptic circuitry were compared, ii) connectivity was examined as a function of transsynaptic transport or propagation of activity, and compared to monosynaptic innervation. Anatomical experiments utilized WGA-HRP with enhanced sensitivity for the detection of transsynaptic transport. Electrophysiological characterization included analysis of the latencies for evoked responses, laminar profiles, and pharmacological manipulation of cellular responsivity. In general, the results of these experiments demonstrate a considerable overlap for mono- and disynaptic input to CA3 from the entorhinal cortex; however, differences in topographies for the direct and indirect pathways to CA3 also indicate that the extent of convergence varies as a function of the septotemporal axis and CA3 subfield (CA3a-c). These findings indicate that the number of cells receiving convergent input will be influenced by the degree of synchronous discharge among granule cells.

141.15

FUNCTIONAL THREE-DIMENSIONAL DISTRIBUTION OF ENTORHINAL PROJECTIONS TO DENTATE GRANULE CELLS OF THE IN VIVO RABBIT HIPPOCAMPUS. Andrew J. Nowak* and Theodore W. Berger, *Dept. of Behavioral Neuroscience, Univ. of Pgh, Pittsburgh, PA 15260; Dept. of Biomedical Engineering and Program in Neural, Informational and Behavioral Sciences, Univ. of Southern California, Los Angeles, CA, 90089.*

Anatomical mapping studies have indicated that the distribution of entorhinal projections to dentate granule cells is extensive along both the septotemporal and transverse axes of the hippocampus (see review by Amaral & Witter, 1989, Neurosci, 31), in contrast to electrophysiological studies which have emphasized an organization primarily oriented with respect to the transverse axis and a restricted septotemporal distribution (Andersen, et al., 1971, Exp. Brain Res., 13). To examine the three-dimensional extent of the granule cell field excited by perforant path fibers, we stimulated the perforant path through electrodes in the angular bundle of halothane-anesthetized rabbits and recorded simultaneously the resulting extracellular field potentials generated along the septotemporal and transverse axes of the dentate gyrus using multiple electrode arrays. Laminar profiles of field potentials and current source density analyses revealed widespread activation of granule cells and systematic changes in population spike latency and amplitude across both axes of the hippocampus. Additionally, results indicated the activation of two distinct subpopulations of granule cells as judged by the existence of bimodality in the distribution of population spike latencies.

To minimize stimulation of multiple subpopulations of perforant path fibers, additional experiments utilized focal stimulation of layer II and III cells in the entorhinal cortex. Present results show systematic changes in the gradient of entorhinal activation of dentate granule cells in a septal-temporal direction, as revealed by: 1) a decrease in the peak latency and an increase in peak amplitude of somatic EPSPs; and, 2) decrease in the slope of dendritic EPSPs. (Supported by MH18273 to AJN and by ONR, AFOSR, MH00343 to TWB).

141.16

FUNCTIONAL EVIDENCE FOR GREATER $GABA_A$ RECEPTOR-MEDIATED INHIBITION IN VENTRAL VERSUS DORSAL *in vitro* HIPPOCAMPAL DENTATE GYRUS. Choi Choi* and Theodore W. Berger, *Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pgh., PA 15260, and Dept. of Biomedical Engineering, and Program in Neural, Informational, and Behavioral Sciences, Univ. of Southern California, Los Angeles, CA 90089.*

Interneurons within the hippocampal dentate gyrus containing γ-aminobutyric acid (GABA) provide feedforward and feedback inhibition of dentate granule cells. The time course of this inhibition overlaps with the time course of processes which facilitate granule cell responses to perforant path input. In the *in vitro* slice, facilitative processes predominate. Previously, we evaluated the interaction of inhibitory and facilitative effects using nonlinear systems analysis.

Slices of 600 μm thickness were cut transverse to the longitudinal axis of the hippocampus from the dorsal and ventral thirds of the hippocampus. The perforant path projection to dentate granule cells was stimulated with a train of electrical impulses having randomly determined interimpulse intervals. The relationship between the interimpulse intervals and amplitudes of the evoked population spikes were expressed as the kernels of an orthogonalized functional power series. The first order kernel represents the average population spike amplitude to all impulses in the train. The second order kernel represents the facilitation and suppression of the population spike amplitude due to any preceding impulse with interval delta (Δ).

In the previous study, we found that second order kernels for dorsal slices showed a larger facilitation at short Δ values than those from ventral slices. In the present study, we used identical experimental and analytical procedures to test the hypothesis that dorsal slices have less inhibitory input on the granule cells. We compared the second order kernels of both dorsal and ventral slices with and without 10 μM bicuculline in the bathing media. The responses from ventral slices showed a greater change in second order kernel facilitation after the addition of bicuculline than responses from dorsal slices. This result suggests that greater GABAergic feedforward and/or feedback inhibition exists in the ventral dentate gyrus. Supported by ONR, AFOSR, MH45156, and MH00343.

141.17

STIMULATION OF THE CONTRALATERAL DENTATE DISINHIBITS PERFORANT PATH INPUT TO HIPPOCAMPAL GRANULE CELLS

T. A. Blanpied* and T. W. Berger *Dept. Behav. Neuroscience, Univ. Pittsburgh, Pgh, PA 15260, and Dept. of Biomedical Engineering and Program in Neural, Informational, and Behavioral Sciences, Univ. Southern California, Los Angeles, CA 90089*

Stimulation of the glutamatergic perforant path (PP) input to the dentate gyrus initiates EPSPs in granule cells which are truncated by IPSPs resulting from feedforward and/or feedback excitation of local GABAergic interneurons. GABA released after a stimulus can serve to reduce subsequent inhibition of granule cells by acting on terminal autoreceptors. Because stimulation of the contralateral dentate gyrus also excites ipsilateral dentate GABAergic neurons, we have investigated whether this separate, commissural input disinhibits granule cell responses to PP activation.

Using rabbits anesthetized with halothane, the PP was stimulated with paired suprathreshold impulses (20 ms interval) which inhibited a population spike response to the second stimulus. Preceding contralateral stimulation affected the first and second responses over separate contralateral–PP interval ranges. The first population spike was greatly inhibited with intervals of 5–40 ms, but was facilitated with intervals of 50–500 ms. The second population spike was facilitated with intervals of 5–40 ms (i.e. paired pulse inhibition was eliminated) and also could be facilitated with an interval of 200, but not 75 or 400 ms. Next, short bursts of stimulation subthreshold for the induction of LTP were applied to the PP either alone or preceded by 200 ms by contralateral stimulation (1–30 impulses, 1–10 Hz). No LTP was induced. However, longer trains of contralateral stimulation facilitated the induction of LTP. Thus, these experiments demonstrate functional interactions of the GABAergic systems activated by PP and contralateral stimulation. Possible mechanisms underlying this disinhibition include activation of presynaptic $GABA_B$ receptors or reduction of the driving force on PP stimulus-evoked $GABA_A$ inhibition. (Supported by an NSF Predoctoral Fellowship to TAB, and by ONR, AFOSR, MH45156, and MH00343)

141.18

FIMBRIAL INPUT TO HIPPOCAMPAL CA3 PYRAMIDAL CELLS STUDIED USING A COMBINED ANATOMICAL AND ELECTROPHYSIOLOGICAL APPROACH.

D.A.Henze*, W.E.Cameron & G.Barrionuevo. *Depts of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260*

It has been previously shown that the induction of LTP at the mossy fiber/CA3 cell synapse is associated with LTP of fimbrial inputs converging onto the same CA3 pyramidal cell (Bradler & Barrionuevo, 1989, 1990). However, the anatomical and electrotonical location of fimbrial synapses onto CA3 cells has not been well characterized. This study examined the anatomical and electrotonic locations of these synapses in transverse slices (300-400 μm) prepared from rat hippocampus. Intracellular recordings from CA3 pyramidal cells were obtained using neurobiotin-filled micropipettes. Neurobiotin was iontophoresed into the cell after measuring input resistance, time constant, and rise time and decay time of fimbrial EPSPs. A double-barreled pipette was used to deposit a small quantity of biocytin into the fimbrial stimulus site. Labeled cells and fimbrial fibers (and their synaptic swellings) were reconstructed in three dimensions using the Eutectic Neuron Tracing System. The electrotonic length of the dendrites for each cell was calculated from geometry and specific membrane resistance. The locations of individual fimbrial synaptic swellings in the *s. oriens* were determined relative to the average electrotonic distance from the bottom of the CA3 cell body layer. Results showed that the CA3 cell basilar dendrites reach their peak branching complexity at a distance of 80 to 100 μm from the soma. This anatomical distance corresponds to an electrotonic distance of 0.1 to 0.2 length-constants. This region of the *s. oriens* exhibiting the greatest pyramidal cell dendritic surface area is coextensive with the region of highest number of fimbrial fiber synaptic swellings. Supported by NS01196,NS24288,& HD22703.

141.19

STIMULATION OF PREFRONTAL CORTEX FACILITATES CA1 AND DENTATE POPULATION SPIKES BUT NOT EPSPS.

N. Coniglio, B.Y. Yang, T.W. Berger and D.J. Weisz.* Departments of Behavioral Neuroscience and Neurological Surgery, University of Pittsburgh, Pittsburgh, PA 15260

In electrophysiological studies the influence of prefrontal cortex on population responses elicited in the hippocampus were investigated in rabbit. In the first set of experiments stimulation was applied to many sites within the prefrontal cortex prior to the elicitation of a granule cell population spike or EPSP in the dentate gyrus. Facilitation of the spike but not EPSP was observed at stimulation sites along the medial wall of the prefrontal cortex. The same basic results were obtained when prefrontal stimulation preceded the elicitation of spikes and EPSPs in CA1 pyramidal cells.

These results suggest that medial structures in the prefrontal cortex facilitate dentate granule cell and CA1 pyramidal cells by acting at the cell body layer.

(This work was supported by NIMH Grant MH45156)

141.20

PHYSIOLOGICAL EFFECTS OF PULSED, FOCUSED ULTRASOUND ON THE RAT HIPPOCAMPAL SLICE.

C.Georgiades, P.C.Rinaldi*, J.P.Jones, L.Price and F.Reines. Departments of Neurosurgery, Radiological Sciences and Physics, University of California, Irvine, CA 92717.

Pulses of focused ultrasound have been found to cause physiological changes in mammalian Peripheral and Central Nervous Systems (CNS). Specifically in single nerve as well as population responses, ultrasound was found to cause a change in the magnitude of the evoked response and a reduction in the stimulus-response delay. In this study the rat hippocampal slice was employed to study the effects of pulsed, focused ultrasound on the mammalian CNS. The magnitude of evoked field potentials as well as response latency were studied. Emphasis was placed on eliciting a reversible enhancement or reduction in the response magnitude, toward a goal of using pulses of focused ultrasound to stimulate or inhibit mammalian CNS cells in a non-invasive manner. In 500 micron slices, classical field potentials were recorded during ultrasound irradiation and compared to control responses. Pulsed, focused ultrasound caused a 30-80% reduction in the antidromic cell population spike and a 20-60% reduction in the orthodromic cell population spike. Complete recovery was noted for most cases with partial recovery for the remainder. Enhancement was also observed in a number of experiments, however reliable reproduction of these results has not yet been achieved.

Two previous studies from our group utilizing different ultrasonic transducers noted the following: first, a 10-25% reduction with complete recovery and second, a more significant reduction with only partial recovery. In conclusion, ultrasound has the ability to significantly alter the physiology of mammalian CNS cells in a reversible manner. Furthermore, the reversibility and direction of response magnitude change show a dependency on the parameters of ultrasound, such as center frequency, pulse rate and power.

141.21

DURATION OF LINDANE-INDUCED CHANGES IN DENTATE EVOKED RESPONSES DEPENDS ON INPUT PATH.

K.-S. Dai, D.E. Woolley*, L. Zimmer and Z.A. Hasan. Dept. of Animal Physiology, Univ. of Calif., Davis, CA 95616.

Limbic electrodes were positioned under surgical anesthesia in the rat for later study in the freely behaving state, in order to relate behavior and evoked potentials. After recovery from surgery a single dose of lindane was administered and the time course of the resulting minimal seizure activity was compared with that for changes in evoked potentials recorded in the dentate gyrus (DG). Seizure activity peaked between 45-120 min. Amplitude of the dentate population spike (PS) evoked by stimulation of the medial-dorsal perforant path (DPP) was increased prior to onset of seizures, was maximally increased at 1 h, was still increased at 4-6 h, but was within normal limits by 8 h. PS amplitude was increased in the absence of a change in the initial slope of the population slow wave (SW), as if EPSP-spike (E-S) coupling was enhanced, perhaps because of a reduction in feedforward inhibition. Amplitudes of the first and second SWs evoked in the DG by paired-pulse stimulation (48 ms interstimulus interval) of the prepyriform cortex (PPC) were also increased before seizures appeared and were near maximal values by 1 h; thereafter, however, amplitudes, especially of the second SW, remained high with only a slight decline during the 48 h of the study. The long-lasting increase in amplitude of the PPC-evoked DG response after administration of lindane appears to represent a form of LTP and contrasts with the relatively rapid return to normal of the DPP-evoked dentate PS amplitude.

142.1

INVAGINATION OF PYRAMIDAL CELL DENDRITES BY AXONS AND OTHER DENDRITES IN THE ADULT HIPPOCAMPAL CA1 REGION. N. L Desmond* and C. A. Scott. Dept. of Neurosurgery, Univ. of Virginia, Charlottesville, VA 22908.

Serial section 3-D reconstruction of normal adult hippocampal CA1 s. moleculare has revealed nonsynaptic invaginations of pyramidal cell dendrites by protrusions from dendrites and unmyelinated axons. These dendritic and axonal protrusions are completely engulfed by the invaginating dendrite. Two normal adult rats were perfused with a mixed aldehyde fixative, and hippocampal sections were prepared conventionally for electron microscopy. Serial thin sections (silver to gray) of CA1 s. moleculare were photographed. Whorled membranes and clustered vacuoles were occasionally identified in dendrites and were reliably associated with a protruding axonal or dendritic finger in adjacent sections. These fingers were thin (ca. 50-100 nm) and were in clear continuity with the parent axon or dendrite on only 1 or 2 serial sections. The protruding fingers were, however, quite long (ca. 1 μm). In the case of protruding unmyelinated axons, the axon sometimes also formed a normal appearing synapse on the engulfing dendrite. The protruding dendritic fingers appeared to contain actin and were often associated with ribosomes, endoplasmic reticulum, spine apparatus, and/or mitochondria. Interestingly, pairs or triads of dendrites protruded reciprocally into one another. The functional significance of these structures is unknown. Interesting possibilities include growth of distal pyramidal cell dendrites in the mature hippocampus and nonsynaptic communication between a pair of dendrites or an axon and a dendrite.

Supported by NIH NS26645 to NLD and NS15488 to W. B Levy.

142.2

SPINY HILAR NEURONS OF THE RAT IN VIVO. I. Soltesz* and M. Deschênes. Centre de Recherche en Neurobiologie, Université Laval, Quebec, Canada.

The importance of the hilus in regulating the input-output operations of the hippocampus both in health and excitotoxic diseases has been recognized, and correlated *in vitro* studies provided important information about the physiological features of hilar neurons. However, we need to learn more about the *in vivo* characteristics of these cells.

Intracellular recordings were obtained from six spiny hilar neurons in the anaesthetized rat using electrodes filled with a K-salt and biocytin. One multipolar cell, which has been reconstructed, was located 5.2 mm posterior from the bregma (P=5.2). Its membrane characteristics, as well as the high level of spontaneous activities mainly consisting of EPSPs, were similar to those reported for mossy cells by Scharfman et al. The high background activity resulted in some degree of jitter in the intracellular membrane potential's phase relation to the EEG theta rhythm. The cell had giant spines on its proximal dendrites and simple spines on its distal dentrites. One dendrite entered the molecular layer and ended in its outer region. Some axon collaterals displayed boutons in the hilus, others entered the granule cell layer and within 200 µm of the soma 3 main branches could be seen studded with boutons in the inner one third of the molecular layer, running parallel with the granule cell layer. Axons in the molecular layer were present up to P=6.2 towards the temporal pole and to P=3.8 towards the septal pole of the hippocampus. It is suggested that spiny hilar neurons might influence a number of other cells both in the same hippocampal 'lamella' (Andersen et al, '71) and considerable distances away in the septotemporal axis (Amaral & Witter, 1989).

142.3

THE DENTATE AREA OF THE DOMESTIC PIG. I.E. Holm*. Inst. of Neurobiology, Univ. of Aarhus, DK-8000 Aarhus C, Denmark.

The hippocampal region of rodent brains is widely used as experimental model in studies of ischaemic brain damage and temporal lobe epilepsy. In order to enhance the clinical relevance of such studies, we are currently attempting to establish an experimental model involving the domestic pig. Since no comprehensive description of the pig hippocampus was available we have studied the Timm staining pattern, the distribution of somatostatin- (SS-li), neuropeptide Y- (NPY-li), cholecystokinin- (CCK-li), met-enkephalin- (ENK-li), and substance P- (SP-li) -like immunoreactivity. The present report deals with the dentate area which was seen to be relatively large in comparison with other species and possess a complex cytoarchitecture with a clear lamination of the dentate hilus. This lamination was reflected in the Timm staining pattern by a particularly intense staining of the superficial part of the hilus. NPY and CCK-li cell bodies were present in the deepest part of the granule cell layer and in the hilus, whereas SS-li cell bodies were only seen in the hilus. SS and NPY-li fibers were present in large numbers forming particularly dense fiber plexus in the molecular layer and in the superficial part of the hilus. SS, NPY, and CCK-li puncta were present in the molecular layer. Except for a few SP-li cell bodies in the hilus, ENK and SP-li cell bodies were very faintly stained and were rarely encountered. ENK-li puncta were present in large numbers forming an intensely stained layer in the inner third of the molecular layer, but were otherwise absent. SP-li puncta were present in abundant numbers in the superficial part of the hilus surrounding the hilar neurons. Considerable septotemporal variation was observed for all neuropeptides. The distribution of SS, NPY and CCK-li in the pig hippocampus largely resembles that of other mammals, whereas the distribution of ENK and SP-li differs markedly from that observed in other species.

142.4

PERFORANT PATHWAY PROJECTIONS TO THE AMMONS HORN AND THE SUBICULUM IN THE RAT. AN ELECTRON MICROSCOPICAL PHA-L STUDY. M.P. Witter*, B. Jorritsma Byham and F.G. Wouterlood. Department of Anatomy & Embryology, Vrije Universiteit, Amsterdam, The Netherlands.

Light microscopical studies have provided evidence that the perforant pathway (PP) distributes to all subfields of the hippocampal formation. Electron microscopical (EM) analysis has shown that PP fibers make synaptic contacts in the dentate gyrus (DG) and CA3, but failed to find such evidence in CA1. The PP projection to the subiculum has not been studied at the EM level. Since the terminal distribution of the PP in the latter two fields shows a marked topography, the negative observations in CA1 might be the result of a sampling problem. We therefore reinvestigated these PP projections at the EM-level in female Wistar rats using the sensitive anterograde tracers Phaseolus vulgaris-leucoagglutinin and biotinylated dextran. As a reference, the projections to CA3 were also analyzed.

In all subfields, labeled fibers had rather small diameters of approximately 0.4 μ, whereas many unlabeled fibers showed diameters up to 0.8 μ. Labeled boutons were observed in the molecular layers of all hippocampal subfields and without exception, they formed asymmetrical synapses predominantly with spines. The largest diameter of labeled boutons measured approximately 0.8 μ.

The present results show that the PP indeed terminates in all hippocampal subfields and that in a previous study the terminals in CA1 simply may have been missed because of the topography of the PP. We further conclude that the projections to the DG and CA3 on the one hand and to CA1 and the subiculum on the other, although they originate from different cell layers in the EC, do not differ with respect to the gross morphology of their terminals.

142.5

RECIPROCAL CONNECTIONS BETWEEN THE SUBICULULM, FIELD CA1 AND THE ENTORHINAL CORTEX IN THE RAT Nobuaki Tamamaki* and Yoshiaki Nojyo, Dept. of Anatomy, Fukui Medical School, Fukui 910-11 Japan

In the formation of short term memory and its conversion into long term memory, the entorhinal cortex relays information from the neocortex to the hippocampus and from the hippocampus to the neocortex. In order to obtain a perspective on the communication, fluorescent tracer, DiI was applied into the entorhinal cortex closest to the rhinal sulcus, and labeled neuronal elements were investigated in the dorsal hippocampus. DiI showed the same anterograde labeling pattern revealed by other tracers in the dorsal hippocampus. When the injection of DiI involved deep layers of the entorhinal cortex, the anterograde labeling of the perforant path was accompanied by retrograde labeling of the subicular neurons and the CA1 neurons. Distribution of labeled cells completely correspond to the distribution of labeled fibers. The labeled cells might receive input from the labeled fibers in the str. lacnosum-moleculare or in the molecular layer. I conclude that the output from a point of the entorhinal cortex to the subiculum and field CA1 converges on the subiculum and then returns to the same cortical column of the entorhinal cortex.

142.6

PATTERNS OF CONNECTIVITY IN THE HIPPOCAMPAL FORMATION OF THE RAT. D.Chan*, D.Al Dulaimi & J.O'Keefe. Dept. of Anatomy, University College London, Gower St., London WC1E 6BT, UK

The retrograde fluorescent tracers Fast Blue and rhodamine microspheres were used to map the topography of four pathways in the hippocampal formation of the rat, namely the CA3→CA1 and CA3→CA3 pathways and the direct projections from the entorhinal cortex to CA3 and CA1. Injections were delivered under electrophysiological control and the hippocampus was dissected and straightened along the septotemporal (ST) axis before sectioning.

The pattern of backfilling in CA3 following CA1 injections indicates that this projection is organized as a series of bands diagonal to the ST axis of the hippocampus. In contrast the CA3→CA3 association pathway is organized in bands parallel to the ST axis. Taking into account the organization of the mossy fibre pathway orthogonal to the ST axis, these results suggest a lattice arrangement for the intrahippocampal pathways similar to the crossing fibre arrays proposed by Tamamaki and Nojyo (1991).

Different populations of cells in the entorhinal cortex project to the CA3 and CA1 fields. While the former arises from cells across the entire extent of the entorhinal cortex and is organized topographically with respect to the ST axis, the latter arises from a delimited population of cells around the rhinal fissure and shows no obvious organization.

142.7

ULTRASTRUCTURAL RELATIONS OF SEPTAL TERMINALS WITH NEUROPEPTIDE Y-CONTAINING NEURONS IN THE DENTATE GYRUS.

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The ultrastructural relations of terminals arising from the medial septal and diagonal band nuclei (i.e., the septal complex) with neurons containing neuropeptide Y (NPY) was examined in the rat dentate gyrus. The septal complex of adult male rats was injected with 1% ibotenic acid (150 nl). Following a 2 - 4 day survival, the hippocampal formation was processed for the immunocytochemical demonstration of NPY. Electron microscopic analysis revealed that terminals with the morphological characteristics of anterograde degeneration, in particular an increase in osmiophilia, were most abundant in the hilus of the dentate gyrus. In this region, degenerated terminals (n = 105) were mostly small (0.2 - 0.4 μm in diameter) and formed both asymmetric and symmetric synapses with small dendrites. The degenerated terminals were associated with (1) one unlabeled perikaryon or dendrite (48%); (2) one NPY-containing perikaryon or dendrite (20%); (3) the same unlabeled or NPY-labeled perikaryon or dendrite as an NPY-containing terminal (10.5%). The remaining degenerated terminals were either directly apposed without glial intervention to unlabeled and NPY-immunoreactive terminals (10.5%) or lacked associations with any neuronal processes in the plane of section analyzed (11%). These findings provide cellular substrates for direct synaptic regulation as well as presynaptic modulation of hippocampal NPY-containing neurons by septal terminals. (Supported by MH42834 and HL18974.)

142.8

COLLATERAL PROJECTIONS FROM THE RAT HIPPOCAMPAL FORMATION TO THE LATERAL AND MEDIAL PREFRONTAL CORTEX.

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The subiculum and field CA1 of the hippocampal formation (HF) have a direct projection to the ipsilateral medial prefrontal cortex (mPFC). Indications exist that the lateral PFC (lPFC) also receives fibers from these hippocampal areas. With respect to the functions of the PFC it is important to know the distribution of the cells of origin of these fibers and whether collateral connections exist. Rats received an injection of diamidino yellow into the mPFC and of fast blue into the lPFC. The distribution of cells projecting to the mPFC was similar as found previously by Swanson (Brain Res., 217 (1981) 150). The cells sending axons to the lPFC were mainly found in the pyramidal layer of CA1 and subiculum. They were located in the posterior septal part, the entire intermediate part and the anterior temporal part of HF. The numbers of cells having collateral projections and of those projecting to the lPFC only were comparable. In the splenial and temporal HF the total number of cells projecting to lPFC was considerably lower than that of the neurons innervating mPFC. The septal part hardly contained double labeled cells, while neurons projecting to the lPFC were slightly more numerous than cells connecting the mPFC. The hippocampal projection to the frontal cortex may be more diverse than is generally assumed.

142.9

CONVERGENCE OF LIMBIC INPUT TO THE CINGULATE MOTOR CORTEX IN RHESUS MONKEY.

R.J. Morecraft* and G.W. Van Hoesen. Depts.of Anatomy and Neurology, Univ. of Iowa, Iowa City, IA. 52242

Limbic system influences on motor behavior are widespread, ranging from the initiation of action to the motivational pace of motor control. Motor abnormalities are also a common feature of psychiatric illness. Several subcortical limbic-motor entry points have been defined in recent years but cortical entry points are understood poorly, despite the fact that a part of the limbic lobe, the cingulate motor cortex, contributes axons to the corticospinal pathway. Using fluorescent tracers in monkeys, we investigated the limbic input to area 24c and adjacent area 23c. We summarize here several intrinsic limbic sources that have as a target the cingulate motor cortex. Cortical input to both areas 24c and 23c arise from cingulate areas 24a, 24b, 23a and 23b, retrosplenial cortex and temporal areas 35, TF, TH and TPO. Areas 24c and 23c were also interconnected strongly. The dysgranular part of orbitofrontal cortex and insula project primarily to area 24c while the granular part of orbitofrontal cortex and insula project primarily to area 23c. Cortical areas 25 (the anterior cingulate gyrus), 28 (the entorhinal cortex) and 38 (the temporopolar cortex) project only to area 24c. Subcortical telencephalic afferents to areas 24c and 23c originate from the claustrum, nucleus basalis and lateral basal nucleus of the amygdala. In addition, a few neurons in the cortical, accessory basal and medial basal nuclei of the amygdala project to area 24c. Since our observations indicate that a variety of telencephalic limbic structures project to areas 24c and 23c, and it is known that areas 24c and 23c project to the spinal cord, red nucleus and primary and supplementary motor cortices, it seems reasonable to conclude that cortex lining the lower bank and fundus of the cingulate sulcus forms a strategic cortical entry point for limbic influence on the motor system. (Support: NS 14944)

142.10

CONNECTIONS OF RETROSPLENIAL CORTEX (CINGULATE GYRUS) IN MACACA FASCICULARIS STUDIED WITH WGA-HRP.

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The retrosplenial cortex (RSC, areas 29 and 30) forms the caudal extension of the cingulate cortex. Reported data relate RSC to memory, emotion and attention through connections with the frontal, temporal and parietal cortices. Some of these connections are established through more rostral portions of the cingulate cortex. We made a series of experiments to get a comprehensive view of the RSC connections in the monkey.

Five monkeys were anesthetized, the medial surface of the hemisphere exposed and 200-300 nl. of a 1% WGA-HRP solution injected with an air pressure system. 48 h later, animals were deeply anesthetized and perfused. Serial, 50 μm sections through the brain were collected and reacted.

Preliminary analysis shows reciprocal connections with association cortices of the dorsolateral (areas 10, 46, 9) and orbital (areas 11, 12 and 13) portions of the frontal lobe, temporal lobe (dorsal bank of the superior temporal sulcus, layer V of caudal entorhinal cortex and TF), cingulate cortex (areas 32, 24, 25 and 23), posterior parietal cortex (area 7) and cortex of the rostral calcarine sulcus adjacent to area 17.

142.11

PATHWAYS MEDIATING THE CHOLINERGIC INNERVATION OF THE RETROSPLENIAL CORTEX AS REVEALED BY ChAT IMMUNO-CYTOCHEMISTRY.

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Previous studies from our laboratory using retrograde tracers provided evidence for a septal innervation of the retrosplenial cortex (RSC) via the fornix pathway. In the present study we have used choline acetyltransferase (ChAT) immunocytochemistry to demonstrate that this innervation is cholinergic in nature. We studied the pattern of cholinergic fiber innervation in the RSC of normal rats, rats with cingulum lesions, and rats with fornix lesions. In normal rats we found that the RSC exhibited two patterns of fiber orientation, one was perpendicular to the cortical surface, the other was parallel to the cortical surface. The fibers that ran perpendicular in direction originated from cholinergic interneurons found in layer IV. The fibers that ran parallel to the cortical surface formed a distinct laminar pattern with the highest density of ChAT positive fibers in layers I and IV, moderate staining in layers III and V/VI, and light staining in layer II. Cingulum lesions alone reduced the density of staining but did not alter the general laminar pattern. Fornix lesions, however, dramatically abolished all of the parallel fibers and the laminar pattern of innervation leaving a few scant perpendicularly oriented fibers. We conclude that the fimbria-fornix, in addition to the cingulum bundle, is also involved in mediating the cholinergic projection from the medial septal nucleus and the diagonal band of Broca to the RSC. (Supported by NIH RO1-NS-24464).

142.12

PROJECTIONS FROM THE ANTERODORSAL AND ANTEROVENTRAL NUCLEUS OF THE THALAMUS TO THE RETROSPLENIAL AND HIPPOCAMPAL CORTICES.

T. van Groen* and J.M. Wyss. Dept of Cell Biology, University of Alabama, Birmingham, AL 35294

Previous studies suggested that the anterodorsal (AD) and the anteroventral nuclei (AV) of the thalamus projected to the retrosplenial granular cortex (Rg); but, preliminary evidence from our retrograde tracing studies suggests that many AD and AV axons extended beyond Rg. In this study we used anterograde tracers to characterize the projections of AD and AV in detail. Injections into the middle part of AD reliably labeled axon terminals in Rgb, Rga, subicular, and caudal entorhinal and perirhinal cortices. In contrast, injections into AV consistently labeled terminal fields only in Rgb, Rga, and subicular cortices. Both the AD and AV projections were topographically organized, such that rostral parts of each nucleus projected to caudal parts of each cortical area, whereas caudal parts of each nucleus projected to rostral parts of cortical areas. Further, the axons of AD and AV terminated in distinct layers in each cortical region. In Rgb and Rga, AD axons ended in layers I and III-IV, but AV axons terminated in layers Ia and IV. In postsubiculum AD terminals were in layers I, II/III, and V, whereas AV terminals were in layer I and V. In presubicular and parasubicular cortices both AD and AV terminals were predominantly distributed in the deep layers (i.e., layers V-VI); however, AV terminals were also in layer I. The AD projection to the caudal entorhinal and perirhinal cortices ended in the deep layers (i.e., layers V-VI). These results demonstrate that the cortical projections from AD and AV are very similar, but the differences in laminar organization suggest that each nucleus innervates a distinct group of dendrites in each cortical region.

142.13

DEVELOPMENT OF DENDRITIC BUNDLING IN THE RETROSPLENIAL GRANULAR CORTEX IN THE RAT. J. M. Wyss*, M. Liumsiricharoen and T. van Groen. Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294.

Apical dendrites in layer II of the retrosplenial granular cortex (Rg) of the rat bundle together tightly in near in layer Ic and Ib and then unbundle in layer Ia, where they arborize receive their primary input, i.e., the axonal terminals of the anteroventral thalamic nucleus. In the present study, the developmental time course of the bundling has been characterized in brains in which the retrograde tracer fluorogold was injected into the contralateral cortex. Both Nissl and fluorogold material suggest that the neurons reach layer II of Rg between postnatal day (P) 1 and 3. By P5 the first presumptive bundles are present, and in these bundles, 10-20 apical dendrites group together in layer Ic and Ib, but few of these dendrites reach layer Ia. Also on P5 many of the apical dendrites of the layer II neurons bifurcate in layer Ic. Between P9-P12, apical dendrites that compose the dendritic bundles begin to reach layer Ia and initially arborize in this sublayer. Around P15 these apical dendrites begin to arborize extensivcly in layer Ia, and the interbundle spaces become much more obvious. Finally, by P21 the dendritic bundles are morphologically mature and not structurally different from those in adult Rg. At this stage, very few, layer II apical dendrites lie outside of the dendritic bundles, almost none of the dendrites branch in layer Ib or Ic, and virtually all dendritic spines on these apical dendrites are located in layer Ia. These results suggest that the initial development of the dendritic bundles in Rg is coincident with the arrival of the anteroventral thalamic nucleus input to these dendrites, suggesting that common forces underlie the development of both.

142.14

ELECTROCHEMICAL DETECTION OF MONOAMINES IN THE HIPPOCAMPUS. A.K. Wiser* and G.M. Rose, Medical Research, VAMC, and Dept of Pharmacology, UCHSC, Denver, CO.

The purpose of this study was to investigate the composition and distribution of monoaminergic signals in the hippocampus of pentobarbital anesthetized Sprague Dawley rats. Carbon fiber (35 micron diameter) electrodes were Nafion-coated to minimize the contribution of monoamine metabolites or ascorbate to the electrochemical signals. Releases were evoked by local microejection of an iso-osmotic calcium/potassium (2.5 mM/70 mM) solution or by electrical stimulation of the commissural path. Signals were detected at two different potentials, 0.25 V and 0.5 V, in an attempt to discriminate between the noradrenergic and serotoninergic contributions to the evoked signals.

Responses were obtained to both types of stimulation; those following electrical stimulation were smaller than the potassium-evoked signals. Potassium evoked responses were reproducible at intervals of 15 minutes or greater. Signals obtained at 0.5 V were consistently larger than the ones obtained at 0.25 V, indicating that the former contained both noradrenergic and serotoninergic components. Further studies using selective neurotoxins to confirm this conclusion are in progress. Only small differences in signal amplitude were found as the electrode was lowered from the stratum pyramidale to the stratum radiatum of the CA1. No obvious relationship was found between electrochemical signals and the occurrence of long term potentiation following high frequency stimulation of the commissural path.

142.15

MESOLIMBIC DOPAMINERGIC INNERVATION OF THE HIPPOCAMPUS IN THE RAT. C. Pacitti, E. Campana, R. Innocenzi, A. Gasbarri*. Lab. of Human Physiology, Sch. of Med., 67100 L'Aquila, Italy.

The present study offers a description of the projections from the ventral tegmental area (VTA-A10) to the hippocampus (HPC) in the rat. In the first part of our research we used Phaseolus vulgaris-leucoagglutinin (PHA-L) and retrograde fluorescent tracers. Following injections of PHA-L into VTA anterogradely labeled fibers were mainly found in the ventral subiculum, adjacent CA1 field and dorsal subiculum. Few labeling was also observed in the CA3 field and dentate gyrus. The distribution of VTA neurons projecting to the HPC was also examined by injecting retrograde fluorescent tracers (Fluoro-Gold, Fast Blue and Nuclear Yellow) in several hippocampal areas. In the second part of this study we combined the retrograde neuronal tracer technique with the tyrosine hydroxylase (TH) immunocytochemistry to evaluate the percentage of dopaminergic (DA) VTA cells projecting to the HPC. We injected Fluoro Gold (FG) in several areas of either ventral or dorsal HPC (CA1, CA3, subiculum, dentate gyrus). Sections containing retrogradely FG labeled neurons were either mounted directly as controls or incubated with TH antiserum revealed with IgG-TRITC. The quantitative evaluation of retrogradely labeled and TH stained cells showed that the VTA-HPC projection was partially DA (about 15%) and partially crossed (about 10%). Our results show that the most abundant VTA-HPC projections originate from the upper and lower edges and the lower half of the VTA. The terminal fields in the HPC i.e., ventral subiculum, adjacent CA1 and dorsal subiculum, also contain the major quantitivity of DA cells and match with the hippocampal areas projecting towards the nucleus accumbens (Acb). Since Acb and its DA innervation would play an important role in the locomotor initiation and goal-directed behaviors, the input from the HF is considered to be able to influence this behavioral phase.

142.16

NEURAL CONNECTIONS FROM HIPPOCAMPUS TO NUCLEUS ACCUMBENS TO SUBPALLIDAL AREA MAY CONTRIBUTE TO LOCOMOTOR ACTIVITY C. T. Tsai* Department of Biology, National Changhua University of Education, Changhua, Taiwan, ROC.

Neural connections of hippocampus-Nucleus Accumbens-Subpallidal area have been demonstrated by behavioral technique. Locomotor activity recorded in an automated activity cage was increased substantially by unilateral injection of carbachol, a cholinergic agonist, into the dentate gyrus of hippocampus; this increase of activity was reduced significantly after the injection of glutamate antagonist, GDEE into the ipsilateral nucleus accumbens. In the other hand, this hyperactivity elicited by the injection of carbachol was also reduced by injection of GABA into subpallidal area. In another observation, the increase of locomotor activity was recorded following the injection of dopamine into nucleus accumbens; then this increase of locomotor activity indcued by dopamine was attenuated by the injection of GABA into subpallidal area. These observations suggest that neural connections from hippocampus to nucleus accumbens to subpallidal area may contribute to locomotor activity.

142.17

CALCIUM-CALMODULIN-DEPENDENT PROTEIN KINASE TYPE II mRNA EXPRESSION IS ALTERED BY RECURRENT LIMBIC SEIZURES. K.D. Murray,*§ C.M. Gall,§ D.L. Benson¶ and P.J. Isackson,# §Dept. of Anatomy and Neurobiology, University of Calif., Irvine, CA 92717, ¶ Dept. Neurosci., Univ. of Virginia Sch. of Med, Charlottesville, VA 22908 and #Dept. of Biochemistry and Molecular Biology, Mayo Clinic, Jacksonville, Fla, 32224

Calcium-calmodulin-dependent protein kinase type II (CAM II kinase) is a ubiquitous forebrain protein which has recently been shown to be modulated by neuronal activity. Specifically, CAM II kinase mRNA is increased in visual cortex of monocularly deprived monkeys (Benson et al., 1991) and hippocampus of Alzheimer's diseased brains, both expected to have reduced levels of physiological activity. To further investigate the regulation of CAM II kinase by activity, *in situ* hybridization of a riboprobe complimentary to 350 bases of monkey CAM II kinase mRNA was examined in rat forebrain following recurrent seizures. In adult rats an electrolytic lesion was placed in the dentate gyrus hilus, this triggers ≈ 10 hrs of intermittant limbic seizures. At 12hrs following lesion placement, CAM II kinase mRNA levels were dramatically decreased in the dentate gyrus granule cells and pyramidal cells of region CA1, bilaterally, and remained below control levels as long as 30hrs postlesion. Decreased mRNA content also was detected in superficial layers of piriform cortex and neocortex. These results provide evidence for the negative regulation of CAM II kinase expression by physiological activity and reinforce the interpretation that decreases in activity underlie increased CAM II kinase expression in Alzheimer's brain. (Supported by NIHAG00538)

142.18

DOSE-DEPENDENT EFFECTS OF INTRACEREBROVENTRICULAR KAINIC ACID ADMINISTRATION ON SUBCORTICAL GLUTAMATE CONCENTRATIONS. M. E. Bardgett*, C. T. Wrona, J. W. Newcomer, J. G. Csernansky. Psychiatry Dept., Wash. Univ. Sch of Med., St. Louis, MO 63110.

Glutamate concentrations and/or monoamine turnover in the rat dorsal hippocampus, corpus striatum (CS), and nucleus accumbens (NA) were quantified one week after intracerebroventricular (ICV) administration of the excitotoxin, kainic acid (KA). Three doses of KA were employed (1.4, 3.8, and 5.2 nM) which have been shown previously to produce progressively greater levels of hippocampal cell loss. Adult male rats were sacrificed one week following bilateral ICV KA infusion, brain regions dissected, and glutamate/monoamines assayed by HPLC-EC. Exposure to the high KA dose reduced glutamate concentrations in the dorsal hippocampus. When compared to the low KA dose, the moderate dose was also found to decrease glutamate levels in the dorsal hippocampus. Glutamate levels, as well as dopamine and serotonin turnover, were not altered in the NA and CS of KA-treated animals. The results indicate that basal glutamate concentrations are altered in the dorsal hippocampus at one week post-KA infusion, while basal glutamate/monoamine levels in other subcortical brain regions remain unchanged. We are currently examining other indices of subcortical neurochemical functioning at one week following ICV KA administration and at other post-infusion time points.

142.19

INTRINSIC CONNECTIONS OF THE HIPPOCAMPAL FORMATION IN THE HUMAN BRAIN. C. Lim*, J. Kordower, E. Mufson and C.B. Saper. Depts. of Pharm. & Physiol. Sci. and Neurology, Univ. of Chicago, Chicago, IL 60637 and Dept. of Neurology, Rush Medical College, Chicago, IL 60642.

The hippocampal formation plays a critical role in the consolidation of long-term memory, especially in humans. The intrinsic connections of the hippocampal formation have received intensive study in rodents, but rather less attention in monkeys, and virtually none at all in humans. We therefore have studied the intrinsic connections of the human hippocampal formation using a lipid soluble fluorescent tracer, dil and several related compounds. Either crystals or injections of 15-45 nl of a 1% solution of tracer in EtOH, DMSO or dimethylformide were placed into various hippocampal fields in formalin-fixed blocks of post-mortem human brain. Following incubation of 3-12 months at 45° C. tissue was sectioned on a freezing microtome, mounted, and coverslipped with a water-based mounting medium (Dako).

We observed several pathways that have not previously been reported in non-human material. Injections placed into the granule cell layer of the dentate gyrus labeled fibers that ran through the alveus and stratum oriens of the CA3 field, to a terminal area in the outer part of the pyramidal layer of CA2. Injections into the molecular layer of the dentate gyrus labeled fibers in the classical perforant pathway, which penetrated the subiculum/distal CA1 to innervate the superificial part of the dentate and CA fields molecular layer. A large contingent of fibers, however, appeared to turn anterolaterally, running through the deep stratum lacunosum of CA1 before before turning into the plane of section and joining the fibers in the superficial molecular layer. These observations suggest that the intrinsic pathways of the human hippocampal formation may differ from the classical conceptualization based on data in other species.

142.20

MORPHOLOGICAL EVIDENCE FOR LEFT-RIGHT ASYMMETRIES IN THE HUMAN HIPPOCAMPUS. Dahlia W. Zaidel*, Margaret M. Esiri, and John M. Oxbury. Dept. of Psychology, UCLA, and Dept. of Neuropathology, Radcliffe Infirmary, Oxford.

Neuronal densities in surgically removed left or right hippocampal tissue of temporal lobe epilepsy patients were determined in CA1, CA4, and the dentate gyrus. We examined 14 left and 13 right hippocampi. Mean counts showed no statistically significant left-right asymmetry in CA1 and the dentate gyrus but did show previously unreported lower densities in the right CA4 than in the left. Another new finding is the asymmetry in regional intercorrelations: Positive (high and significant) r values among the 3 subfields were obtained only in the left hippocampus. In addition we confirmed presence of positive r value between CA1 and age of onset of habitual epilepsy, on either side. With respect to the subfields studied here, the findings suggest regional interdependence within the left hippocampus and regional independence within the right hippocampus.

142.21

A MOLECULAR APPROACH TO HIPPOCAMPAL PHYLOGENY. S. Shafqat*, A.D. Roses and J.R. Gilbert. Departments of Neurobiology and Medicine (Neurology), Duke University Medical Center, Durham, NC 27710.

In brain evolution, the hippocampal formation first appears in higher order vertebrates. In order to recognize its phylogenetic forerunners, therefore, additional markers of hippocampal identity must be developed that are independent of conventional morphology. We have decided to approach this question by (i) isolating genetic transcripts preferentially expressed in the human hippocampus and (ii) characterizing their molecular homologs in a diverse range of species. A human hippocampal subtraction library was prepared from hippocampal and neocortical (post-central gyrus) cDNA pools using a protocol based on aqueous hybridization and biotin-avidin extraction. Subtracted cDNAs were directionally cloned into phagemid pSPORT (BRL) to yield an initial library of ~600 clones. Screening of this library with subtracted, unsubtracted and control probes has highlighted 43 clones that are putatively hippocampus-specific. Preliminary analysis of four 'best-signal' clones by Northern blot and dideoxy sequencing has revealed a hippocampus-enriched species with partial homology to the mRNA for the human dopamine D4 receptor. This and other transcripts confirmed to be overrepresented in the hippocampal formation will be used as in situ hybridization probes to identify hippocampal homologs in submammalian brains.

COMPARATIVE NEUROANATOMY

143.1

ENKEPHALINERGIC AND TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN THE PRETECTAL AREA AND VENTRAL MESENCEPHALON OF CARTILAGINOUS FISHES. C.A. Meade, S.L. Stuesse*, and W.L.R. Cruce. Neurobiology Dept., Northeastern Ohio Universities College of Med., Rootstown, OH 44272.

There are two pathways between basal ganglia and tectum by which visuomotor control may be regulated in vertebrates: via a pretectal area characterized by enkephalin positive (ENK+) cells or via the substantia nigra (SN). The pretectal pathway is present in several nonmammals including geckoids and snakes (Medina and Smeets, 1991). The SN pathway is in mammals, birds and several reptiles. The SN in mammals has two divisions: pars compacta (TH+ cells) and pars recticulata. The pars reticulata projects to the tectum. The presence of a SN or of ENK+ cells in the pretectal area may be indicative of ventral or dorsal mesencephalic pathways, respectively. The distribution of ENK+ and TH+ cells in the midbrain of cartilaginous fishes was determined using antibodies against TH or ENK. Two sharks, *Squalus acanthias* and *Heterodontus francisci*, two rays, *Myliobatis californica* and *Raja* sp., and a holocephalian, *Hyrdrolagus colliei*, were studied. The holocephalian is in a sister group to other cartilaginous fishes. *Hydrolagus* had pretectal ENK+ cells but no TH+ cells in the ventral mesencephalon. The other cartilaginous fishes had TH+ cells in the ventral mesencephalon, SN and ventral tegmental nucleus, but lacked ENK+ cells in the pretectal area. Thus *Hydrolagus* may have a visuomotor pathway through the dorsal mesencephalon while the other cartilaginous fishes may have a pathway through the ventral mesencephalon (NIH grant NS25895).

143.2

SOME CONNECTIONS OF THE TORUS LONGITUDINALIS IN THE OSTEOGLOSSOMORPH TELEOST, Xenomystus nigri. M.R. Braford, Jr.*, A.A. Jensen and R.W. Russell. Biology Department and Neuroscience and Biopsychology Program, Oberlin College, Oberlin, OH 44074

In X. nigri (but not in all teleosts) the torus longitudinalis (TL) comprises three cell layers--from ventral to dorsal--A, B and C. HRP and/or DiI injections of the TL, tectum, valvula and corpus of the cerebellum, and specific portions of the diencephalon and telencephalon revealed different patterns of connectivity for the three layers. Major sources of input to the TL arise from tegmental cell groups including nucleus Q and a nucleus we have designated as S that lies ventrolaterally adjacent to the granule cell layer of the cerebellum near the junction of the valvula and the corpus. Q and S both project heavily to layer B. Cell bodies of layers B and A are retrogradely labeled after injections in the tectum. Cell bodies of layer C are retrogradely labeled after injections of a caudal nucleus (PGp) of the preglomular complex of the thalamus. Injections of the TL that include layer C result in anterograde labeling of terminals in PGp. Injections of PGp also result in anterograde labeling of terminals in a parvocellular group (DLa) within the lateral part of the area dorsalis of the telencephalon. Injections of DLa retrogradely label cell bodies in PGp. These data suggest that a toro-thalamo-telencephalic pathway is present in X. nigri. Support: NSF 8820858 & Oberlin College.

143.3

Acoustic Areas in the Hindbrain and Midbrain of the Goldfish. C.A. McCormick*, D.V. Hernandez, M.R. Braford, Jr. Dept. Biology, Oberlin College, Oberlin, OH 44074

The projections of each inner ear endorgan and of various medullary and midbrain areas were determined in the goldfish, Carassius auratus, using horseradish peroxidase. Otic endorgan projections were largely similar to those in the related otophysan Ictalurus. In both species the medial portion of the descending octaval nuc. extends dorsally towards the cerebellar crest. In Carassius, these dorsomedial cells do not reach the ventricular surface, as they do in Ictalurus. The dorsomedial descending nuc. in Carassius receives afferents from the saccule and lagena and projects to a higher-order, presumably acoustic population which lies at the ventricle medial to the facial nerve tract. Both this ventricular population and the dorsomedial descending nuc. project to a superficial, medial zone of the torus semicircularis which is largest at caudal levels. The primary lateral line nucleus, nuc. medialis, projects to a toral population which lies partly ventral, and partly lateral, to the superficial medial toral zone. The anterior octaval nuc. contributes few, if any, axons to the torus semicircularis. The efferents of a previously identified superior olive (Echteler, S.E. [1984] J. Comp. Neurol. 230) may not be restricted to the presumed acoustic zone of the torus. Supported by NSF BNS 8820098, NSF BNS 880858, and the Department of Biology, Oberlin College.

143.4

THE OCULOMOTOR SYSTEM OF A BLIND CAVE FISH. S.E. Fish* and P. Ghosh. Department of Anatomy and Cell Biology, Marshall University School of Medicine, Huntington, WV, 25755-9350.

The Mexican Characin, *Astyanax jordani*, has a greatly reduced eye commensurate with evolutionary adaptation to a cave environment. The vestigial optic cyst is buried beneath the skin suspended by the usual set of six oculomotor muscles. In order to determine the extent to which the extraocular motor system is intact, the muscles were examined in paraffin sections of the cave fish's head, and CNS motor neurons projecting to the muscles were traced with HRP. For comparison, similar experiments were performed in the cave fish's direct evolutionary ancestor, the normally sighted river fish, *Astyanax mexicanus*.

Cave fish extraocular muscles contained slightly reduced numbers of muscle fibers. Muscles of both fish consisted of partially segregated populations of large and small fibers, identified elsewhere as white and red types, with a shift toward an increased distribution of smaller ones in the cave fish. Patterns of muscle innervation from brainstem nuclei were the same as in most other vertebrates studied. Within limitations of the HRP technique, extraocular motor neurons in the brainstem of the two fish were comparable in number and gross morphology.

The contrast between a greatly reduced eye and a relatively intact oculomotor system has implications for theories of regressive evolution in caves. These findings could be explained by several mechanisms, including the possibility that there is greater selection pressure against an eye. (supported by NIH R15 NS29566 and USFWS Endangered Species Office, Section 6, Job 2-1).

143.5

CENTRAL PROJECTIONS OF THE SPLANCHNIC NERVE IN THE CATFISH, *ICTALURUS PUNCTATUS*. L.E. Goehler* Dept. C&S Biology, U. of Colorado Sch. of Med., Denver, CO 80262.

Vertebrate splanchnic nerves provide both autonomic (sympathetic) and sensory innervation of the coelomic viscera. In mammals and birds, splanchnic preganglionic neurons lie in the lateral horn or pericentral grey of the thoracic and upper lumbar spinal cord. The location of preganglionic neurons in fish has not been determined. Splanchnic sensory nerves in mammals terminate in laminae 1 and 5 of the spinal dorsal horn. These areas receive convergent visceral and somatic sensory information, an arrangement that complicates characterizing splanchnic sensory information. This experiment used the neuroanatomical tracer, dil, to determine the location of splanchnic motor neurons and the area of termination of splanchnic visceral sensory fibers in channel catfish. After dil was placed on the splanchnic nerves, labeled neurons occurred both in paired ganglia situated at the junction of the celiaco-mesenteric artery and the aorta, and in the dorsal root ganglia of the first three spinal segments. In the spinal cord, labeled neurons lie in the pericentral grey and dorsal part of the ventral horn at the level of the second through fourth spinal segments. Except for the rostral location, these neurons are situated in a similar position as preganglionic neurons in mammals and birds. The processes of labeled neurons of the dorsal root ganglia enter the spinal cord with the dorsal roots, and travel in a tract adjacent to the lateral border of the dorsal horn, to terminate in a nucleus of the ventromedial dorsal horn. This nucleus recieves few somatic sensory inputs, suggesting that in contrast to mammals, visceral and somatic sensory information in catfish are processed in separate regions of the dorsal horn.

Supported by NIH grant DC 00147 to T.E. Finger.

143.6

DISTRIBUTION OF SECONDARY OLFACTORY FIBERS IN THE CHINOOK SALMON. S. Matz* Institute of Neuroscience, University of Oregon, Eugene, OR 97403

Juvenile salmon "imprint" to their homestream odor prior to their migration out to the ocean. During their return journey, they use this olfactory information to locate their natal stream. The aims of this study were to localize forebrain areas that receive input from the olfactory bulb in juvenile chinook salmon (*Oncorhynchus tshawytscha*). We injected a concentrated solution of a carbocyanin dye, DiI, unilaterally into the olfactory bulb.

The results of this study are consistent with that reported in the literature. Labeled fibers travelling in a dorsolateral tract terminated in Dl and Dp and coursed through the anterior commissure to the contralateral hemisphere. Labelling was also present in Vv, Vd, Dc, Dm and in the preoptic area (POA). The presence of labeled fibers in Dm and in the POA is of particular interest. Fibers in the POA course near neurons that contain hypothalamic releasing factors. Although we have not determined synaptic contact between olfactory fibers and hypophysiotropic neurons, the possibility exists that the activity of these neurons may be modulated by olfactory input.

The existence of labeled fibers in Dm was enigmatic since olfactory projections to this region had not previously been described. Furthermore, the projections are to very discrete parts of Dm, which raises the possibility of a topographical projection pattern. In order to confirm the origin of the labeled fibers in Dm, DiI was injected into this region and retrogradely labelled cell bodies were found in the olfactory bulb. This work was supported by NIH Systems Physiology Training Program (GM07257).

143.7

THE LOCUS COERULEUS IN THE SUNFISH: NEURONAL NUMBER, NEURONAL MORPHOLOGY AND PROJECTION PATTERN. P.M.Ma* Dept. of Neurobiology, Harvard Medical School, Boston MA 02115.

In fish, the locus coeruleus (LC) is a group of large noradrenergic neurons located in the brainstem isthmus. The number of neurons and their morphology were studied with immunocytochemistry using antibodies directed against tyrosine hydroxylase or dopamine-β-hydroxylase. In the Sunfish (*Lepomis spp.*), each LC contains an average of 12 neurons. The number of neurons ranges between 8 - 17 among fish, but the number between the two sides is similar for each individual. The average diameter of LC cells is approximately 40 μm. They form a compact cluster below the floor of the 4th ventricle. The dendritic arbors of LC neurons are oriented primarily dorsomedially and ventrolaterally. Axons from these cells appear to be braided and leave the LC ventrolaterally, bifurcate, and project both rostrally and caudally. Some of the rostral projections can be traced to the nucleus isthmus, the optic tectum, and the telencephalon. The relatively large size, consistent location, and characteristic projection pattern of LC neurons in the Sunfish provide a possible model system for electrophysiological studies of central noradrenergic function. LC neuronal number was also studied in several other species of fishes. In general, each species has a characteristic average number of neurons. The number ranges from about 24 neurons in the goldfish to about 6 neurons in the zebrafish. (Supported by NIMH grant MH-47368).

143.8

AN HRP STUDY OF PATHWAYS LINKING THE SUPRACHIASMATIC NUCLEUS TO BASAL FOREBRAIN NUCLEI IN HYLA CINEREA. J.D. Allison* and W. Wilczynski. Dept. of Psychology, University of Texas, Austin, TX. 78712.

In frogs, the preoptic area (POA), ventral hypothalamus (VH), and striatum (St) receive auditory input related to reproduction. To investigate the pathways by which the photoperiod cues involved in reproduction might reach these nuclei, HRP was iontophoretically injected into either the optic nerve (ON, n=3), POA (n=8), VH (n=10), or St (n=3) of male green treefrogs (Hyla cinerea). After a 4 day survival, the injected animals were sacrificed under anesthesia and the brains extracted, fixed, cut at 40 μm, mounted, and reacted with standard techniques using TMB as the chromogen. ON fibers coursed dorsally from the optic chiasm, entered the ventromedial region of the ipsilateral SCN, and terminated within the nucleus. A number of cells in the ipsilateral periventricular portion of the SCN were well labeled after POA injections. In contrast, HRP deposits in VH labeled SCN cell bodies in more dorsolateral portions of the nucleus. Finally, striatal HRP injections resulted in labeled cells in the ventrolateral corner of the rostral SCN. Thus, in treefrogs, the SCN receives a direct retinal input and separate suprachiasmatic areas may relay this retinal information to basal forebrain nuclei which also receive biologically important auditory input. (Supported by NIMH grant R01 MH45350).

143.9

HYPOTHALAMIC AND EXTRAHYPOTHALAMIC DISTRIBUTION OF ARGININE VASOTOCIN IMMUNOREACTIVITY IN A URODELE AMPHIBIAN. C.A. Lowry*, L.J. Miller, L.E. Muske, and F.L. Moore. Dept. of Zoology, Oregon State University, Corvallis, OR 97331-2914.

Using immunocytochemical techniques, we determined the distribution of arginine vasotocin immunoreactive (AVTir) cell bodies and fibers in the brain of rough-skinned newts (*Taricha granulosa*). In males collected in March, AVTir cell bodies were found in the following hypothalamic and extrahypothalamic areas: olfactory bulb, medial pallium, caudal ventral cellular prominence, caudal striatum, amygdala pars medialis, anterior preoptic area, ventral preoptic area, magnocellular preoptic area, posterior preoptic area, ventral thalamus, ventral hypothalamus, nucleus visceralis superior, and nucleus posterior tecti. Highest densities of AVTir fibers were found in the medial pallium, lateral pallium, amygdala pars medialis, all regions of the preoptic area and hypothalamus, isthmic tegmentum, and interpeduncular nucleus. Moderate densities of AVTir fibers were found in the striatum, nucleus accumbens, amygdala pars lateralis, ventral thalamus, dorsal thalamus, habenula, tegmentum dorsale mesencephali, nucleus tuberalis posterioris, eminentia subcerebellaris tegmenti, nucleus visceralis superior, corpus cerebelli, tegmentum trigemini, tegmentum facialis, and nucleus motorius tegmenti. This distribution of AVTir cell bodies and fibers is more widespread than previously reported in other amphibians and is consistent with the conclusion that, in addition to its hormonal function, AVT functions as a neurotransmitter within the brain to regulate multiple behavioral responses. Supported by NSF BNS-8909173.

143.10

PRESUMPTIVE HOMOLOGUE OF THE MAMMALIAN STRIATONIGRAL GABAERGIC PATHWAY IN THE FROG BRAIN. I.Kratskin*, J.Pierre, J.Reperant and J.P.Rio. Lab. of Neuromorphology, INSERM U-106, 75651 Paris, France; Smell and Taste Center, Univ. of Pennsylvania, Philadelphia, PA 19104, USA.

A large number of striatopallidal neurons in amniotes use gamma-aminobutyric acid (GABA) as a neurotransmitter and project to the substantia nigra. The question of the possible existence of similar GABAergic projections in an anamniote brain remains open. In the current study, double labeling with horseradish peroxidase (HRP) retrograde axonal tracing (diaminobenzidine-cobalt technique) and GABA immunohistochemistry (avidin-biotin method) and postembedding GABA immunogold staining of the tegmentum have been used. After unilateral iontophoretic application of HRP into the rostral mesencephalic tegmentum of the frog, Rana temporaria, numerous retrogradely labelled GABA-immunoreactive (GABA-IR) cell bodies were observed in the dorsal and ventral striatum. In thin sections of the tegmentum, there were many GABA-IR myelinated fibers and axon terminals in the region of the anterodorsal, posterodorsal and superficial isthmal reticular nuclei. These axon terminals contained small pleiomorphic and large dense core synaptic vesicles and established symmetrical synaptic contacts with GABA-immunonegative dendrites. This study suggests that GABAergic striatomesencephalic projection system is an ancestral feature of the brain in terrestrial vertebrates.

This work was supported by a grant from INSERM to I.K.

143.11

PHYLOGENETIC, ONTOGENETIC AND EXPERIMENTAL EVIDENCE SUGGESTING THAT INNER EAR EFFERENT CELLS ARE REROUTED FACIAL MOTONEURONS. B. Fritzsch* and D. H. Nichols. Div, Anat., Creighton Univ., Omaha, NE 68178.

An efferent innervation exists in all vertebrate inner ears except hagfish. In lampreys and frogs cells of origin are ipsilateral to the ear and adjacent to the facial motoneurons (Fritzsch et al., Neurosci. Lett. (1989) 96:241). In other vertebrates, efferent perikarya to the inner ear are bilaterally distributed but remain located in proximity to the facial nucleus. In addition, the pathways of the axons of both facial and efferent populations show parallel variation (presence or absence of a genu). During development of chickens and mice the facial motoneurons and efferents to the ear are coextensive in the same rhombomere before they simultaneously migrate to their final positions. Thus proximity in original position, similarity of fiber pathways and timing of migration are consistent with the interpretation of efferents being redirected facial motoneurons. After transplantation of the otocyst in Xenopus into the dorsal fin a few spinal motoneurons could be backfilled from this ear. This is experimental evidence consistent with an evolutionary scenario in which formation of an otocyst may have caused motoneurons of the facial nucleus to project to the ear instead of nearby somites. Supported by the NIH.

143.12

SEQUENCE AND ATLAS OF LHRH mRNA AND PEPTIDE IN DEVELOPING AND ADULT *XENOPUS*: EVIDENCE FOR CONSERVED DOMAINS IN GnRH-ASSOCIATED PEPTIDE (GAP) AND DELAYED LARVAL ONSET. W.P. Hayes*, S. Wray, Y.P. Loh and J.F. Battey. Labs. of Dev. Neurobiology (NICHD) & Neurochemistry (NINDS), NIH, Bethesda, MD 20892.

To identify mechanisms responsible for the diversification of neuronal fate in brain, we are using neuropeptide genes as phenotypic markers and analyzing cell-cell interactions underlying their expression onset in frog embryos. In *Xenopus, in situ* hybridization showed that the adult-like brain patterns of POMC and TRH cells arise locally in the neural tube before brain nuclei form (Hayes and Loh, 1990, *Development* **110**:747). Similarly, in mammals, the first GnRH cells were found to be born in the nasal placode from which they migrate into forebrain (Wray et al. 1989, *PNAS* **86**:8132).

To ultimately determine if removal of the placode eliminates all or part of the GnRH system, the full-length GnRH cDNA was cloned for *Xenopus* using the RACE protocol. Expectedly, the 396-base mRNA, comprising 5'-UTR, signal peptide, prohormone and 3'-UTR, showed the amino acid sequence of GnRH was identical to the mammalian decapeptide; however, unlike the GnRH cDNA reported recently for fish (Bond et al. 1991, *Mol. Endo.* **5**:931), frog also showed high homology in the flanking GAP region, with conserved domains yielding up to 42% identity to mammals, but only 4% to fish.

Serial section *in situ* hybridization and immunocytochemistry showed 250-350 GnRH cells in adult forebrain, with over 70% in the anterior preoptic area, and massive projections to the median eminence. Further, this gene was not expressed by midbrain cells shown by others to be immunoreactive for the fish form of LHRH, and only a single mRNA could be detected by Northern blotting. Interestingly, neither GnRH mRNA nor peptide was found until the late larval period, indicating that, unlike in mammal, this gene may be suppressed during early frog development. Studies are in progress to show where and how this neuropeptide system arises in *Xenopus*.

143.13

GNRH IMMUNOREACTIVITY IN THE BRAIN OF THE BROWN ANOLE, *ANOLIS SAGREI*, L.S. Demski* and D.E. Wright. Div. of Natl. Science, New College of the Univ. of S. Florida, Sarasota, FL 34243; Dept. of Anatomy and Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY 40536.

Reproduction and sexual behavior have been well studied in lizards (Crews, J. Exp. Zool., 4:164-166), yet a clear understanding of the neuroendocrine systems underlying these behaviors is lacking. In particular, the neuropeptide gonadotropin-releasing hormone (GnRH) has been examined in only a limited number of reptiles, and the results have been contradictory. For this reason, we have examined GnRH systems in the lizard, *Anolis sagrei* using antisera that recognize several GnRH forms. Populations of GnRH-immunoreactive (ir) neurons were observed in four different areas: the ventral periventricular preoptic area, septum, posterolateral anterior hypothalamus, and midbrain tegmentum. Dense ir-fibers were present in the septum, medial and dorsal cerebral cortex, ventral hypothalamus, infundibulum, tegmentum, cerebellum, dorsal and ventral medulla. Fewer ir-fibers were observed in the optic tectum and spinal cord. Ir-neurons and fibers were absent in the olfactory nerve, bulb, and rostroventral forebrain. Thus, our data supports the view that reptiles lack a terminal nerve, at least in relation to GnRH systems. Moreover, preoptic and midbrain GnRH neurons in the anole appear similar to those observed in other vertebrates, while the presence of GnRH neurons in the posterolateral hypothalamus may be uniquely shared only with birds (Millam et al., Soc. Neurosci. Abst. 257.12)

143.14

ABNORMAL DEVELOPMENT OF GnRH NEURONAL SYSTEMS IN THE STERILE EYELESS MUTANT AXOLOTL. T.J. Maccagnan and L.E.Muske*. Biology Dept., Franklin & Marshall College, Lancaster PA 17604.

A recessive allele, "eyeless" induces a sterile and eyeless phenotype in the axolotl, *Ambystoma mexicanum*. To evaluate whether sterility of mutants is linked to deficits in GnRH development, paired mutant and phenotypically normal siblings were sacrificed at 6 week intervals between ages 4 and 12 months and their brains processed in tandem for immunocytochemistry, using anti-mammalian GnRH 635-5 (courtesy Dr. L. Jennes) and a commercial avidin-biotin-HRP secondary antibody system. We examined GnRH immunoreactivity (ir) in: (1) the medial septum/POA, locus of GnRH cell bodies that arise from the olfactory placode and nervus terminalis (TN); (2) the hypothalamic-hypophyseal (H-H) tract, locus of GnRH axons to the median eminence and pituitary; (3) the posterior tubercle (TP) of the caudal diencephalon, locus of GnRH neurons that develop independently of the TN and placode. In 4 month old juveniles, there are GnRHir cell bodies in the septum/POA and a few axons in the H-H tract, and no detectable differences between normals and mutants. Between 6 and 12 months, ir-GnRH increases dramatically in the H-H tract of normals, but not mutants. Normal axolotls have about twice the number of GnRHir cell bodies in the TP as mutants at all ages examined. Results support the interpretation that early stages of GnRH development, including centripetal migration of GnRH neurons from the placode, occur normally in mutants, but that maturation of GnRH neuroendocrine pathways, and normal development of the TP GnRH system, is blocked.
Supported by Franklin & Marshall College Committee on Grants

143.15

DISTRIBUTION AND INNERVATION OF ELECTRORECEPTORS IN A SOUTH AMERICAN TYPHLONECTID CAECILIAN. R.G. Northcutt* and K.C. Catania. Neurobiology Unit, SIO, and Dept. of Neurosci's., Sch. of Med., UCSD, La Jolla, CA 92093-0201.

The morphology and distribution of lateral line organs in juvenile and adult *Typhlonectes natans* were determined from flat mounts of skin from the head and trunk and from scanning electron microscopy. These methods confirm earlier observations that this caecilian species lacks neuromasts but does possess approximately 200 ampullary organs on each side of the head (Fritzsch and Wake, J. Herpetol.,'86). These organs are not distributed randomly but form distinct lines of receptors, which can be homologized to the supraorbital, infraorbital, oral, otic and preoperculomandibular lateral lines of other amphibians and fishes. The innervation of the ampullary organs was determined by gross dissection, Sudan Black staining of the cranial nerves in cleared specimens, and serial transverse sections of whole heads. Anterodorsal, anteroventral, and otic lateral line nerves can be recognized. The anterodorsal nerve innervates the ampullary organs of the supraorbital and infraorbital lines; the anteroventral nerve innervates the receptors of the oral and preoperculomandibular lines; and the otic lateral line nerve innervates the organs of the otic line. These results indicate that *Typhlonectes* does not possess postotic receptor lines or the three additional lateral line nerves that occur primitively and are seen in other caecilians, such as larval ichthyophids, and in many larval salamanders. Supported in part by NIH NS24669.

143.16

THE PECULIAR VASCULARIZATION OF THE NEURAL COMPLEX OF THE MESENCEPHALIC TRIGEMINAL NUCLEUS IN MAUREMYS CASPICA (TURTLE).

Paz Doel* R., Fernández E., Gcia. Cordovilla R., Fdez. Soriano J. Departamento de Biología Celular. Facultad de Biología. Universidad Complutense, Madrid. Spain.

A Neural Complex can be identified in the mesencephalon of Mauremys caspica by using optic and electron microscopy. This Neural Complex is placed along the sagittal ventricular tectal ridge and consists of both the medial neuronal population of the mesencephalic trigeminal nucleus and the deeply modified underlying ependyma. One of the most relevant features of this neurohemal formation is its peculiar vascular network provided, in some specific regions, of sinusoids and fenestrate capillaries in very close relationship with the neurons and the cerebro spinal fluid. The peculiar vascularitation of this Neural Complex is very similar to that of the circumventricular organs.

143.17

REPTILIAN POST-SYNAPTIC DORSAL COLUMN SYSTEM. M.B. Pritz* and M.E. Stritzel. Div. Neurol. Surg., Univ. Calif. Irvine Med. Ctr., Orange, CA 92668

Both mammals and birds possess a postsynaptic dorsal column system (PSDCN) which requires at least one synapse in the spinal cord before the dorsal column nucleus (DCN) is reached. We asked whether a PSDCN system was present in reptiles, Caiman crocodilus.

Several experiments were performed to identify the PSDCN in Caiman. First, a cytoarchitectonic analysis of the spinal cord was undertaken to identify its laminar organization utilizing a schema similar to that described by Rexed. Second, terminations of dorsal root fibers in the dorsal portion of the upper cervical cord and in the brachial and lumbar enlargements were investigated by anterograde HRP and orthograde degeneration techniques. Dorsal root fibers terminated in lamina I to VI in these regions. Third, placements of HRP into the dorsal cord revealed a fiber path that entered the dorsal funiculus to terminate in the DCN. Fourth, HRP injections into the DCN revealed retrogradely labeled neurons in lamina IV and lamina V in the cervical, thoracic, and lumbar dorsal cord.

These data document a PSDCN system in Caiman which represents a second path whereby non-facial somatosensory information can reach the DCN. The presence of a PSDCN system in Caiman suggests that this neural circuit is phylogenetically ancient and may well be a feature common to all amniotes.

143.18

LOCALIZATION OF SPINAL CORD MOTONEURONS INNERVATING SUBUNITS OF THE PECTORALIS MUSCLE IN THE ALLIGATOR. J.A. Peterson, C.K. Haun*and I. Tan. Dept. of Anatomy & Cell Biology, USC School of Medicine, Los Angeles, CA 90033.

Recent studies of neuromuscular organization in birds and mammals suggest that muscles consist of discrete subunits innervated by topographically distinct populations of motoneurons. This study investigates whether neuromuscular subunits/compartments occur in a lower tetrapod, Alligator mississippiensis. Specifically, the study examines the organization of spinal cord motoneurons innervating discrete subunits of the pectoralis muscle, which were previously determined by histochemistry, architecture, electromyography and nerve stimulation. Motoneuron cell bodies were identified by retrograde labeling with HRP or Fluoro-Gold. The anterior and middle/posterior subunits of the muscle are innervated by neurons extending from the caudal portion of spinal nerve 8 to caudal 9 and from cranial 9 to caudal 10, respectively. This result provides further evidence that muscular compartments occur in lower vertebrates. The topographic organization of the pectoralis is similar to that described previously for birds, suggesting that it may be possible to: i) establish compartment homologies among species, and ii) frame evolutionary hypotheses in terms of selection on neuromuscular compartments.

We gratefully acknowledge financial support from the Medical Faculty Women's Association of the USC School of Medicine.

143.19

DISTRIBUTION OF PEPTIDE IMMUNOREACTIVE CELL BODIES IN THE HYPOTHALAMUS OF THE PIGEON. M.L. Berk* and S.E. Smith. Dept. of Anat. and Cell Biol., Marshall Univ. Sch. of Med., Huntington, WV 25755.

Immunocytochemical data can provide information on the organization of avian hypothalamic cell groups, and serve as a basis for comparison to mammalian cell groups. The present survey of the distributions of cholecystokinin (CCK), calcitonin-gene related peptide (CGRP), leucine-enkephalin (LENK), α-melanocyte stimulating hormone (α-MSH), neuropeptide Y (NPY), neurotensin (NT), somatostatin (SS), substance P (SP), and vasoactive intestinal peptide (VIP) immunoreactive cell bodies was undertaken to achieve this goal. A routine immunoperoxidase avidin-biotin technique was used to identify peptide containing cell bodies in colchicine treated pigeons.

The medial preoptic area has many CGRP, LENK, NT, and SP immunoreactive cells. The periventricular preoptic area has SS immunoreactive cells. The lateral preoptic area contains several NT and VIP immunoreactive cells, and some LENK and SS cells. The anterior lateral hypothalamic area has many VIP, LENK, and NPY cells. The tuberal nucleus is principally composed of numerous α-MSH, NPY, and NT containing cells, while fewer LENK, SP, VIP, CCK, and CGRP cells are located in this nucleus. The ventral part of the posterior medial hypothalamic nucleus has several α-MSH, NPY, and NT immunoreactive cells, and the posterior lateral hypothalamic nucleus contains several CCK, SP, and VIP cells. The stratum cellulare internum in the caudal hypothalamus is composed of many CGRP and VIP immunoreactive cells. The lateral mamillary nucleus contains many CCK cells. The presence of specific populations of peptide immunoreactive cells will aid in the demarcation of avian hypothalamic cell groups. Supported by NSF R11-8922106.

143.20

COMPARISON OF TYROSINE HYDROXYLASE(TH) VS. DOPAMINE (DA) SPECIFIC ANTIBODY PROCEDURES FOR MAPPING DA-CONTAINING PERIKARYA THROUGHOUT THE CHICK BRAIN. W.J. Kuenzel, J. Kirtinitis and W. Saidel*. Poultry Sci. Dept., Univ. of Maryland, College Park, MD 20742.

A polyclonal antibody to TH (dilution 1:200) was compared to a monoclonal antibody against DA (dilution 1:2000). Beginning at the caudal region of brainstem and moving rostrally, the n. tractus solitarius (S)/vagal complex showed immunoreactivity (ir). With TH, many perikarya immunostained within the S. In contrast none was found using an antibody against DA, however many fibers were immunostained. A small chain of perikarya were found that immunostained with DA directly ventral to the origin of the IXth and X nerves. The n. reticularis subtrigeminalis immunostained. Moving rostral to the midbrain, the n. tegmenti pedunculo-pontinus, pars compacta (substantia nigra) had large numbers of perikarya ir to TH. In contrast, fewer perikarya were found ir to DA in that n. The most intense staining for DA was found in the area ventralis of Tsai (AVT). A few scattered neurons within the central gray immunostained with both TH and DA. The sixth nucleus that immunostained with both TH and DA was found at the caudal extent of the hypothalamus just dorsal to the supramamillary decussation, the n. intramedialis (nI). Both dorsal and ventral projections were observed the latter appeared to travel to the median eminence. The last group of DA neurons (cerebrospinal fluid contacting neurons) was found within a circumventricular organ, the paraventricular organ. Supported by USDA grant #90-37240-5506 and MAES competitive grant.

143.21

EARLY SEXUAL MATURATION INDUCED IN CHICKS BY NEUROPEPTIDE Y: BIOGENIC AMINE AND NEUROANATOMICAL EFFECTS.
G.S.Fraley
and W.J. Kuenzel*. Poultry Science Dept., University of MD; College Park, MD 20742.

Neuropeptide Y (NPY) was administered intracerebroventricularly to immature male chicks to determine its effects on sexual maturation. Male broiler chicks were cannulated within the lateral ventricle at three weeks of age. Chronic injections of 5.0 ug NPY were injected for four consecutive days during the fourth week and repeated the fifth week of age. At six weeks of age sexually mature respondant birds were selected on the basis of advanced secondary sex characteristics and compared with groups of nonrespondant and control animals. Birds were then injected with 100 mg/kg body weight hydroxybenzylhydrazine, an amino acid decarboxylase blocker, to ascertain levels of L-DOPA as well as other biogenic amines. After thirty minutes, the brains were removed and prepped for HPLC analysis. Micropunches of the preoptic area (POA), paraventricular nucleus (PVN), bed nucleus of the pallial commissure (nCPa), and median eminence (ME) were analyzed. Results showed an increase in dopamine (DA) and L-DOPA in both the ME and POA, but a decrease in DA within the PVN. Also, an increase in DA metabolites were seen in the PVN and nCPa. These results are interpreted as an increase in dopaminergic activity within the ME and POA suggesting a localized stimulatory affect on gonadoytropin releasing hormone (GnRH) neurons, while a decrease in dopaminergic activity in the PVN and nCPa. Anatomical studies support the previous knife-cut data from surgically induced precocious puberty (Fraley et al, 1991). Thus, NPY could be an initiator of cascading events including the localized stimulatory action of DA on GnRH neurons. Supported by USDA Grant #90-37240-5506.

143.22

DISTRIBUTION OF CHAT IMMUNOREACTIVE PERIKARYA AND FIBERS IN THE PIGEON BRAIN. L. Medina* and A. Reiner Dept. Anat. & Neurobiol., Univ. of Tennessee, Memphis, TN 38163.

In order to provide a complete description of the cholinergic systems of the pigeon (White Carneaux) CNS, brain sections were processed for choline acetyltransferase (ChAT) immunohistochemistry using a rabbit anti-chicken ChAT primary antiserum (kindly donated by Dr. Epstein, Univ. Wisconsin).

Within the telencephalon most ChATi neurons are confined to the basal regions, such as striatum, globus pallidus, ventral pallidum, diagonal band nucleus and septum. Further caudally, ChATi cell bodies are present in the preoptic region, several hypothalamic nuclei, the thalamus, the medial habenula, the pretectum (adjacent to the posterior commissure), tectal layer 10, central gray, nucleus of Darkschewitsch, ventral tegmental area, parvocellular and semilunar isthmic nuclei, nucleus dorsalis lemnisci lateralis, pontomesencephalic tegmentum, reticular formation, descending trigeminal nucleus, medial vestibular nucleus, nucleus cuneatus externus, and all cranial nerve nuclei. Cholinergic fibers and varicosities are widespread over the brain. Prominent plexi are seen in the hyperstriatum accessorium, striatum, intrapeduncular nucleus, all retinorecipient neuropiles, and lateral spiriform, intercollicular, and interpeduncular nuclei. The ChAT distribution in the pigeon brain largely resembles that of other vertebrates, indicating that the cholinergic systems have been highly conserved during evolution.
Funded by Spanish Ministry of Education to L.M. and NS-19620 to A.R.)

143.23

IMPLICATIONS OF NEURON REDUCTION IN CETACEAN AND SIRENIAN CORTEX: T.Deacon,*[1] R.Reep,[2] C.Marshall[2] & J.Moore[1] Anthrop., Harvard U., Cambridge, MA 02138 [1] & Physiol., U. of Florida, Gainsville, FL 32610 [2]

Neuronal cell counts with respect to surface area in the cerebral cortices of four cetacean and one sirenian species are compared with those of six terrestrial mammal species. The four cetacean species (2 mysticetes and 2 odontoctes) and the one sirenian species all have cell numbers reduced to approximately 40% of the corresponding cell numbers in the cortices of the terrestrial mammals (including a marsupial). Two possible developmental causes of this cell reduction are 1) underproduction of cortical plate neurons from germinal cells or 2) high post-mitotic cell death of cortical plate neurons. No direct evidence is available for either mechanism, however, the cetacean cortical plate is unusually thin for brain size, whereas the sirenian cortical plate appears unusually thick, compared to terrestrial mammals. Sirenian brains also exhibit a clearly differentiated layer IV in some regions, whereas cetacean brains do not. This suggests that the cetacean pattern may be a consequence of reduced germinal cell production, whereas sirenian pattern may be a consequence of selective cell death. Truncation of cortical plate neurogenesis would have significant consequences for cell types, axon-target affinities, and laminar designation within the cerebral cortex, since the timing and order of cell production in the cortical plate determines both target position within the mature cortex and also the laminar specificity of function and connectivity. If the generation of cortical plate cells is somehow shut off after generating only 40% of the normal complement, then there may be no cells that would otherwise differentiate to express the characteristics of cells in upper cortical layers: the targets for specific thalamic afferents and the sources and targets for the majoritiy of corticocortical connections. This would severely impact cortical function and may reflect a lower limit on cortical cells necessary for essential functions. Because cell numbers in the opossum cortex are comparable to those in other terrestrial mammal brains, cell reduction in these aquatic species is derived, and not a retained primitive feature. Convergence on this cortical cell reduction pattern by these independent, long aquatically adapted lineages may reflect an oxygen conservation adaptation.

143.24

DOLPHIN PERIPHERAL VISUAL TRACT IN CHRONIC UNILATERAL OCULAR ATROPHY: COMPLETE DECUSSATION APPARENT. R.J. Tarpley, J.B. Gelderd*, S. Bauserman & S. H. Ridgway. Depts. of Vet. Anat., Anat. & Med. Neurobiol., Pathology, Texas A&M University, College Station, TX 77843 and NRaD., San Diego, CA. 92152

Components of the peripheral visual pathway were examined in two bottlenose dolphins *Tursiops truncatus*, each with unilateral ocular degeneration of three years or more duration. In both dolphins, the optic nerve associated with the blind eye (right eye in Tg419 and left eye in Tt038) appeared translucent upon gross examination. This translucency was also evident in the optic tract contralateral to the affected eye. Many axons, primarily myelinated, were identified in the left optic nerve of Tg419 while essentially none were seen in the right optic nerve. In Tt038, axons were found in association with the right optic nerve and left optic tract but were essentially absent in the left optic nerve and right optic tract. The optic chiasm of Tt038, examined by light microscopy in serial horizontal section, appeared near its center to be arranged in segregated, alternating pathways for the decussation of right and left optic nerve fibers, while the exterior portions of the chiasm were dedicated ventrally to fibers from the left optic nerve and dorsally to fibers from the right optic nerve. Because of this architectural arrangement, the right and left optic nerves appeared to overlap as they crossed the optic chiasm with the right optic nerve coursing dorsal to the left optic nerve. At light and electron microscopic levels, optic nerves and tracts lacking axons were well-vascularized and dominated primarily by astrocytes. Although extensive postmortem change precluded accurate fiber counts, the essential absence of axons in one of the optic tract pairs (right in Tt038 and left in Tg419) appears to support the concept of complete decussation of right and left optic nerve fibers at the optic chiasm in the bottlenose dolphin. Supported by NOSC grant #91M3773

143.25

NEUROANATOMIC VARIABILITY OF RAT BRAINS
J.R. Absher, A.W. Toga, P.K. Banerjee, R.C. Collins*, and E.M. Santori,
Laboratory of Neuro Imaging, Dept of Neurology, UCLA School of Medicine Los Angeles, CA.

We report an analysis of intersubject variability in Spraque-Dawley rats and compare the "average" rat brain from 5 subjects to the Paxinos and Watson rat brain atlas. Coronal planes were obtained after in situ freezing, and sagittal planes were obtained from the 3D data set. The Paxinos and Watson atlas data set was reconstructed from the individual images, corresponding exactly to the sagittal planes taken from each subject. Eleven brain regions were traced on each plane. Region area, perimeter, and percent overlap (of subject and atlas brain regions) were determined for all brain regions. A 3D model was derived by triangulation, and surface area, volume, and center of mass were determined. Translational and rotational "correction factors" were calculated to fit the individual data sets to the the Paxinos and Watson atlas data set. "Average" translational and rotational correction factors were also derived. The results reveal substantial intersubject variability in location (center of mass) and orientation (rotation about the center of mass) for most brain regions.

143.26

TOPOGRAPHY OF CORPUS CALLOSUM FIBERS IN THE RAT. M.F. Novotny* and D.P. Crowne. Department of Psychology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

The topographical relationship between callosal fibers and their cortical origins in the adult rat were examined using a lipophilic tracer. DiI crystals were inserted into the frontal cortex of whole fixed brains, and onto discrete sectors of the corpus callosum. The results confirm the rostral-caudal sectoring of the corpus callosum, with axons in the anterior callosum originating from the corresponding frontal cortex. Commissural projections displayed a vertical band organization, mainly occupying layers III and V. The results may provide insights for anatomical and behavioural studies of interhemispheric communication.

144.1

EVIDENCE FOR A UNIQUE POPULATION OF PYRAMIDAL NEURONS IN BROCA'S AREA. T.L. Hayes* and D.A. Lewis. Depts. of Behav. Neurosci. and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15216.

In the left hemisphere, Brodmann's regions 44 and 45 (Broca's area) are believed to support motor speech, whereas in the right hemisphere, the role of these regions remains unknown. Hemispheric differences in the characteristics of the layer III pyramidal neurons of those regions have been reported, including greater dendritic arborization (Scheibel 1984) and larger neuron size (*Soc Neuro Abstr* 17:1021, 1991) in the left hemisphere. In a sample of 11 cases, we have confirmed our previous finding that the cross-sectional area of the largest layer III pyramids is greater in left than in right area 45 ($p<.0001$). In every case the mean size of these neurons in the left hemisphere was greater than that in the right, and in eight cases this difference was statistically significant (7-38% greater in the left than in the right). This difference was specific to the largest pyramids, as there was no hemispheric difference in the mean size of all layer III pyramidal neurons in area 45. Immunohistochemical studies have demonstrated that large pyramidal neurons in area 45 contain non-phosphorylated neurofilament protein (NFP). However, no hemispheric differences were found in the size of the largest NFP-positive layer III pyramids. Comparisons of the Nissl and NFP findings suggest that the largest pyramids in left area 45 may be morphologically and chemically distinct from those in the homotopic region in the right hemisphere. Other studies to examine the chemical specificity of these neurons are underway. These findings suggest that area 45 of the left hemisphere contains a unique population of large pyramidal neurons, which may be specialized for the language functions mediated by this region.

144.2

BROCA'S REGION: ANATOMICAL AND FUNCTIONAL ASYMMETRIES. S.F. Witelson* and D.L. Kigar. Dept. of Psychiatry, McMaster Univ., Hamilton, Ont., Canada, L8N 3Z5

Frontal cortical regions related to language production were measured for their extent and right-left asymmetry and the relationship of these anatomic measures to psychological measures of hemispheric functional asymmetry. Previously, we demonstrated that variation in gross structure in posterior language gyri surrounding the Sylvian fissure (SF) was associated with handedness and, by inference, with functional asymmetry (Witelson & Kigar, Abst. Soc. NSc, '90; '91). The structure-function relationship was observed in men; not women. We focused on frontal regions in this study for two reasons: (1) they are very asymmetric functionally in the mediation of speech and this could be represented by an asymmetric structure; (2) handedness may be associated in women with frontal region morphology. There have been a few studies of right-left asymmetry of inferior frontal regions (variably defined) which, in general, indicate nonsignificant asymmetry. We defined Broca's region as the region bounded by the precentral sulcus, the anterior horizontal ramus of SF, the inferior frontal fissure and SF, and studied this region in 67 postmortem brains (24♂ and 43♀), whose hand preference [consistent-right-hand (CRH) or nonCRH] was tested prior to death. Statistical analyses revealed that Hemisphere was not a significant main factor. Handedness was, with nonCRH having greater frontal regions. This relationship held only for men. In addition, in men, Handedness interacted with direction of asymmetry. These results are consistent with the bilateral differences found in posterior brain regions between CRH and nonCRH men, and again indicate a perplexing lack of relationship of handedness with structural variation in women compared to men. Implications for the neural substrate of varying patterns of cognition will be discussed. Supported by US NINDS.

144.3

THREE DIMENSIONAL RECONSTRUCTIONS OF THE CAUDAL SUPERIOR TEMPORAL REGION IN HUMANS SHOW NO ASYMMETRIES IN CORTICAL SURFACE AREA W.C. Loftus, C.E. Thomas, M.J. Tramo†, R.L. Green††, R.A. Nordgren and M.S. Gazzaniga*. Ctr for Neurobiology, University of California, Davis, CA 95616, †Dept of Neurobiology, Harvard Medical School, Boston, MA 02115, ††Dept of Psychiatry, Dartmouth Medical School, Hanover, NH 03755.

In order to consider whether left hemisphere specialization for language is associated with hemispheric asymmetries in gross cortical morphometry, the surface area of the caudal superior temporal region was measured from magnetic resonance scans of 10 young, normal, right-handed subjects. Planar map measurements of the full extent of the infrasylvian cortical surface caudal to Heschl's gyrus were first obtained by summing the length of the convolutions across contiguous coronal sections. A leftward asymmetry was found (mean left=7.18, mean right=5.57, $p<0.007$). This region was then measured using 3D reconstructions that take into account the intrinsic curvature of the cortical surface and the oblique portions of the posterior sylvian fissure. No asymmetry was found (mean left=6.91, mean right=6.67, $p=0.719$). Finally, the 3D model allowed us to measure the combined surface area of Heschl's gyrus, caudal superior temporal gyrus, and adjacent parainsular cortex. Again, no left-right asymmetry was found (mean left=29.81, mean right=29.29, $p=0.678$).

These findings indicate that: 1) measurements of the caudal superior temporal region from serial coronal sections that do not explicitly model the tilt of the ascending sylvian ramus with respect to the rostrocaudal axis are biased in favor of leftward asymmetry; and 2) three dimensional reconstructions of the caudal superior temporal region reveal no left-right asymmetry of cortical surface area. Supported by ONR N00014-89-J-3035, NIH/NINDS P01 NS17778-10, the McDonnell-Pew Foundation and 1 K08 DC00071 (MJT).

144.4

THE FIBER COMPOSITION OF THE HUMAN CORPUS CALLOSUM. F. Aboitiz, A.B. Scheibel* and E. Zaidel. Brain Research Institute and Department of Psychology, UCLA, Los Angeles CA 90024.

The densities of fibers of different sizes were calculated in ten regions of the corpus callosums of twenty human brains (ten females, ten males, ages 25-68) under light microscopy. Large fibers (>3um in diameter) are least dense in the anterior corpus callosum (genu), and increase in density posteriorly towards the middle and posterior midbody, where somatosensory and auditory areas apparently project, respectively. Towards the posterior corpus callosum (splenium), the density of large fibers decreases, but in the posterior pole of the callosum, where visual areas project, there is a local increase in their density. So far, the highest density of large-diameter fibers was in the region presumably connecting auditory areas. Thin fibers (>0.4 um in diameter) show a pattern exactly complementary to that of large fibers, being most dense in the genu and decreasing their density toward the posterior midbody. Their density increases when entering the splenium, to show a local decrease in the posterior pole of the callosum. Across subjects, the density of callosal fibers had no significant correlation with callosal area and therefore an increased callosal area indicated an increased total number of fibers crossing through. No sex differences in the fiber composition of the corpus callosum were found.

144.5

LATERALIZED DIFFERENCES IN MORPHOLOGICAL COLUMNS OF AREA Tpt. D. Buxhoeveden* and E. Armstrong, University of Chicago, Chicago, IL 60637 and A.F.I.P. Washington, D.C., 20306.

Selected algorithms from a method that analyzes the size and shape of cell columns in the cortex was used to study laterality in the posterior language area, Tpt. Gross anatomical differences exist between the two hemispheres here, but little is known about asymmetry at the histological level. Comparisons were made between 7 human, 5 chimpanzee and 4 rhesus monkey brains. All were normal adult or young adult. Nissl stained coronal sections were digitized on a computer imaging system.

In human brains, the morphological columns on the left side are farther apart and there is slightly more non-neuronal space at the periphery of individual columns than on the right. The cell arrangement is less vertically oriented in the left hemisphere than in the right in lamina III, but is more vertical in all the other lamina. The chimpanzee brains show no laterality in these features. The rhesus brains have an asymmetry in lamina III that runs counter to that of the human Tpt. Cell columns in the rhesus left hemisphere are more vertically oriented and are closer together than in the right. No difference in the peripheral space was observed.

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144.6

WHAT IS ANOMALOUS IN THE DYSLEXIC PLANUM? C. M. Leonard*[a,b], K.K.S. Voeller[b], L.J. Lombardino[c], J.C. Honeyman[d], and O.F. Agee[d]. Depts. of Neuroscience[a], Psychiatry[b], Communication Processes[c] and Radiology[d], University of Florida, Gainesville, FL 32610.

Post mortem studies of dyslexics have revealed symmetry of the planum temporale due to increased growth on the right. We asked which bank of the planum was responsible. Computer-based measurements of average planar length were made on thin sagittal images from MR scans of 12 controls [(C): ages 14-52]; 9 dyslexics [(D): ages 15-65]; and 10 unaffected relatives of the dyslexics [(R): ages 6 - 63]. All three groups had leftward asymmetry in the temporal bank {T-P/[(T+P)*.5]= 17%, 48%, and 40%, respectively} and rightward asymmetry in the parietal bank (-18%, -67%, and -45%, respectively). We confirmed Galaburda's finding of an anomalous right planum, although, in our subjects, the anomaly was restricted to the parietal bank. The dyslexic group had a significantly larger proportion of tissue devoted to the parietal bank: [*intrahemispheric* T/P asymmetry = 81%, 2% and 19%, respectively (D vs C: $p < 0.02$, R vs C: $p > 0.10$, post-hoc Duncan)]. There were no significant differences on the left (T/P asymmetries of 108%, 101%, and 98%, respectively). When the data on anomalous intrahemispheric asymmetry were combined with data on left and right sided perisylvian anomalies (extra or missing gyri), 7 dyslexics, 2 relatives and no controls were found to have bilateral anomalies. *Interpretation:* Visuospatial functions are localized in the parietal lobe while auditory functions are localized in the temporal lobe. The reversed T/P asymmetry found in dyslexic families could reflect cognitive biases for spatial strategies that are not suited to the sequential analysis of language. The risk of dyslexia increases with additional left-sided perisylvian anomalies that interfere with normal language processing.

144.7

VISUAL ATTENTION DEFICITS IN AUTISTIC ADULTS WITH CEREBELLAR AND PARIETAL ABNORMALITIES. J.Townsend*, E.Courchesne,B.Egaas.(UCSD Psychiatry, Neuroscience Depts.) Children's Hospital, Neuropsychology Research Lab, 8001 Frost St., San Diego, CA 92123.

A model of visual-spatial attention developed by Posner and Petersen (1990) proposes that brain systems involving posterior parietal cortex, the lateral pulvinar nucleus, and the superior colliculus guide the orienting of visual attention in space. Studies using a behavioral test that follows attentional cuing with a simple visual target, have shown patterns of deficits in orienting of spatial attention in humans with specific brain lesions, suggesting involvement of the posterior parietal cortex in the disengagement of attention; the superior colliculus in the moving of attention; and the lateral pulvinar in the engagement of attention. We tested autistic subjects from a group with cerebellar and, in a subset, parietal abnormalities, using several variations of the Posner spatial task. Autistic patients showed a unique pattern of responses with two important findings. First, compared to controls and other autistic patients, autistic patients with parietal abnormalities were extremely slow to respond to targets when their attention had been cued to the location opposite the target (a deficit similar to that seen in patients with parietal injury), **with a strong correlation between the level of behavioral impairment and MRI quantitative estimates of parietal damage.** In the second major finding, both groups of autistic subjects had significantly slowed responses when the cue to target delay was short (100 msec), but in the context of normal attention-related response patterns (i.e. faster response to validly than invalidly cued targets). This time-related delay has not been reported for other patient groups (e.g. parietal, frontal, midbrain, thalmic lesions), but is similar to time-related delays in attention shifts reported in patients with autism and with acquired cerebellar damage.

144.8

PARIETAL LOBE ABNORMALITIES DETECTED BY MAGNETIC RESONANCE IN PATIENTS WITH INFANTILE AUTISM. E.Courchesne*, G.Press, R.Yeung-Courchesne. (UCSD Dept. Neuroscience) Children's Hospital, Neuropsychology Research Lab, 8001 Frost St., San Diego, CA 92123.

Autism is a neurologic disorder that severely disrupts the development of higher cognitive functions. Cerebellar maldevelopment beginning between prenatal and early postnatal life has been detected by autopsy and radiologic analyses in autistic patients. Increased cell packing density in limbic structures reflecting developmental arrest by 3 years of age has also been seen at autopsy in several cases. Whether the cerebrum is affected is unknown. The MR scans of 21 healthy autistic subjects (ages 6-32 years) were examined for evidence of cerebral abnormality. Cortical volume loss in the parietal lobes was seen in 7 autistic patients; in 4 cases, cortical volume loss extended either into the adjacent superior frontal or occipital lobe. Additional abnormalities detectable by MR included white matter volume loss in the parietal lobes (3 patients), and thinning of the posterior body of the corpus callosum (2 patients). Abnormalities were bilateral in all but one patient. Three also met neuroradiologic criteria for cerebellar hypoplasia (as previously reported by our group). The mesial, lateral and orbital regions of frontal lobes, temporal lobes, limbic structures, basal ganglia, diencephalon and brainstem were normal in all autistic subjects. These results indicate that the parietal lobes are reduced in volume in a portion of the autistic population. The observed focal parietal volume loss may be by consequence of a progressive age-related decline. If so, then to our knowledge this would be the first example of such a phenomenon. A contrasting possibility is that the cerebral abnormalities we observed might be part of a broader pattern of altered CNS development possibly related in time and/or causality to the cerebellar hypoplasia known to occur in many cases of autism.

144.9

SELECTIVE ATTENTION AND THE ANTERIOR CINGULATE: A DEVELOPMENTAL NEUROANATOMICAL STUDY. B.J. Casey, J. Giedd, Y. Vauss, C.K. Vaituzis and J.L. Rapoport*. Child Psychiatry Branch, NIMH, Bethesda, MD 20892.

Selective and divided attention measures were correlated with MRI measures of the anterior cingulate in 14 right-handed normal children between the ages of 8 and 16 years. Parasagittal slices from a 3-D T1 weighted (TR=400, TE=12) data set (1 X 1 X 1.5 mm) were used to obtain whole brain and cingulate measurements. A dual visual and auditory detection task and a visual discrimination task were used to assess divided and selective attention. Selective attention measures were correlated with age ($r = -.76$, $p < .005$, $r = -.81$, $p < .0005$) as was the anterior cingulate measure ($r = .58$, $p < .05$), but not whole brain area. There were significant correlations between the selective attention measures and the anterior cingulate measures ($r = -.66$, $p < .01$, $r = -.74$, $p < .005$), especially the right anterior cingulate ($r = -.62$, $p < .025$, $r = -.75$, $p < .0025$) The results are consistent with recent PET findings of activation in the right anterior cingulate during selective attention and provide additional support for proposed structure/function relationships in the anterior attention system.

144.10

DEVELOPMENT OF PLACE MEMORY IN CHILDREN AS MEASURED IN A DRY MORRIS MAZE. **W.H. Overman*, L. Carter, S. Thompson, Dept. of Psychology, U.N.C.at Wilmington, Wilmington, N.C. 28401.**

The Morris maze has been used to measure place learning and memory in rodents (Morris, R.G.M., 1980, Learn. & Motiv., 12, 239-260). We adapted this maze for use with children by filling a large pool with plastic chips to a depth of 60 cm. Children, ages 2 to 12 years, spent 5 trials per day, for 3 days, searching for a candy filled box that was always hidden in the same location. Results were that latency to find the goal box decreased significantly across three test days for each age group and the latency had an inverse relation to age of subject. The same result was found when the dependent measure was distance traveled to find the goal. By the third day, older children followed direct paths to the goal. After their last trial children were asked (in another room) to locate the goal position in a scale model of the test room and maze. On this task, children below age 7 were significantly inferior to older children. Taken together these data suggest that cognitive mapping ability continues to mature up until at least age 7.

144.11

OBJECT AND LOCATION RECOGNITION MEMORY IN PRETERM AND FULLTERM INFANTS. T. Wilcox, L. Nadel* and R. Rosser. Dept. of Psychology, Univ. of Arizona, Tucson, AZ 85721.

This study (1)investigated the relative development of the object vision and location vision systems (inferior temporal and posterior parietal areas; Underleider & Mishkin, 1982); and (2)determined the effect of alterations in early experience on their development. 18 healthy pre-term (PT) infants and 21 fullterm (FT) controls were tested on an object recognition task (Visual Paired Comparision) and a location recognition task (Visual Search). The mean chronological age for the PT infants was 3.9, 5.9 & 7.9 mos; for the FT controls it was 2.5, 4.5 & 6.5 mos. For both tasks delay intervals of 5, 10, & 30 secs were used according to the age of the subjects.

Both PT and FT infants evidenced location recognition memory at all ages; there were no differences between the groups. However, the PT infants performed better than the FT infants on VPC. They evidenced a stronger preference for novelty at each age tested. This finding can be explained by the older chronological age of the PT infants. The fact that the PT infants did not also perform better on VS suggests that location recognition abilities may be more affected by premature birth than object recognition abilities. This is consistent with converging neural and behavioral evidence that the spatial ("magno") system may be a more rudimentary and earlier developing system than the object ("parvo") system.

144.12

NCTR OPERANT TEST BATTERY (OTB) PERFORMANCE IN CHILDREN: CORRELATION WITH IQ. M.G. Paule*, D. J. Blake, R.R. Allen and P.H. Casey. C.A.R.E., Dept. of Pediatrics, Arkansas Children's Hospital, Little Rock, AR 72205 and Div. of Neurotoxicology, National Center for Toxicological Research, Jefferson, AR 72079-9502.

In order to determine whether aspects of operant performance are related to subject IQs, OTB data obtained from 109 6 1/2 year old children were compared to their IQ scores obtained at age 5. Tasks in the nickel-reinforced, multiple schedule OTB and the specific function(s) they are thought to model include: Progressive Ratio (PR), motivation; Conditioned Position Responding, color and position discrimination; Incremental Repeated Acquisition (IRA), learning; Delayed Matching-to-Sample (DMTS), short-term memory and attention; and Temporal Response Differentiation (TRD), time perception. Endpoints included response rates (RR), accuracies (ACC), and percent task (PTC) completed. Highly significant correlations were noted between several OTB measures (e.g., CPR ACC, $r=0.60$, $p<0.0001$) and IQ, whereas other OTB measures (e.g., PR endpoints) did not correlate at all. These data demonstrate that: a) certain aspects of OTB responding are associated with subject IQ; b) certain OTB measures are not reflective of IQ and thus apparently measure other aspects of brain capacity. Since operant methodology is easily used for assessing the behavior of animal subjects, it should now be possible to quantitate aspects of 'animal IQ' using the NCTR OTB.

144.13

PLASMA COBALAMIN LEVELS ARE ASSOCIATED WITH INFORMATION PROCESSING SPEED IN A LONGITUDINAL STUDY OF HIV-1 DISEASE. G Shor-Posner*, R Morgan, F Wilkie, RS Beach, E Mantero-Atienza, and MK Baum. Univ. of Miami Sch. of Med., Miami, FL 33101

Our recent studies indicate that HIV-1 infected individuals with low plasma cobalamin (vitamin B_{12}) levels (<240 pg/ml) demonstrate significantly poorer performance on measures of information processing, as compared to those with adequate vitamin B_{12} status (Arch Neurol; 1992, In press). The present longitudinal study examined the impact of change in vitamin B_{12} status, over 12 month intervals, in relationship to the speed of accessing highly overlearned name codes as measured by the Posner Letter Matching Test in HIV-1 infected homosexual men (n=84, CDC Stage II, III, IV) evaluated for up to 30 months. Autoregression analysis indicated that normalization of vitamin B_{12} status, from deficiency to adequacy was associated with faster processing speed, while change from adequacy to deficiency, in contrast, was associated with slower reaction time ($p < 0.002$). These findings, which are independent of CD4 status and zidovudine treatment, suggest that vitamin B_{12} deficiency may be an important cofactor in cognitive changes observed during HIV-1 infection. Clinical trials are needed to confirm that restoration of adequate plasma vitamin B_{12} levels improve information processing speed in HIV-1 disease.

144.14

NEUROCOGNITIVE AND NEUROIMAGING CHARACTERISTICS OF HIV-1 INFECTION. R. C. Verma,* J. O. Harker, M. P. Gan, L. R. Bennett, F. D-L. Jones, G. Mathisen, F. Kioumehr, M. A. Mandelkern, W. H. Blahd, J. Ropchan. Olive View-UCLA Medical Center, Sylmar, CA 91342

This study was undertaken to determine the frequency and correlation of neurocognitive (NC) and neuroimaging (NI) abnormalities in HIV-1 infection.

HIV-1 seropositive subjects (27 in Group A, symptomatic, and 14 in Group B, asymptomatic) and 14 high risk seronegative subjects (Group C) underwent NC testing (n=55), CT/MRI (n=44), I-123-iodoamphetamine SPECT cerebral perfusion imaging (n=48), and F18-flourodeoxyglucose PET brain scans, FDG, (n=19). Subjects with focal neurological lesions were excluded. NC tests included 22 measures of memory, reasoning, motor function, and cognitive flexibility.

Global NC impairment (GNCI) score was computed by expressing the number of abnormal (±1.5SD) NC tests as a percent of the total tests given to each subject. Cortical atrophy on CT/MRI, cortical perfusion deficits on SPECT, and striatal FDG metabolism on PET brain scans were graded on a scale of 5 to 8, 5 being normal and 8 being marked abnormality.

The mean ±SD of CD4 cell count/cmm and NC and NI measures were:

	CD4	GNCI	Atrophy	↓Cort. Perf.	↑Str. Perf.	↑Str. FDG
A	225±136	36%±29	6.3±0.8	6.5±0.8	5.4±0.6	5.8±1.0
B	771±179	5 % ±7	5.5±0.8	6.8±1.0	5.2±0.7	4.7±1.0
C		9 % ±7	5.4±0.7	6.2±0.8	5.0±0	5.2±0.9

Group A demonstrated a significantly higher GNCI ($p< 0.001$), cortical atrophy ($p< 0.012$) and ↑striatal perfusion ($p< 0.028$) than Group B or C or B and C. A modest correlation was demonstrated between CD4 cell count and GNCI ($r = - 0.44$), cortical atrophy ($r = - 0.39$) and striatal FDG ($r - 0.58$). Overall, there is a significantly higher frequency of neurocognitive impairment, cortical atrophy and striatal hyperperfusion in subjects with symptomatic HIV-1 infection and these measures correlate with disease severity as measured by CD4 status.

144.15

MRI STUDY OF WHITE MATTER CHANGES IN CYSTINOSIS. Barbara L. Hodge, John R. Hesselink+, Doris A. Trauner*. Departments of Neurosciences and +Radiology, University of California at San Diego, San Diego, California.

In an attempt to learn more about brain-behavior relationships, we used MRI scans to correlate structural changes in brains of children with cystinosis, with the known cognitive deficits associated with this disorder. Children with cystinosis, an autosomal recessive disorder of cystine metabolism, have a specific visual processing deficit on a background of normal IQ. Previous MRI studies have reported cortical atrophy in people with cystinosis.

MRI scans were done on nine children with cystinosis (age range: 5 to 14 years) and on nine age- and sex-matched normal controls using a G.E. Signa 1.5-Tesla unit. The radiologist who examined the scans was blind to the status of each of the subjects.

Six of the nine children with cystinosis had evidence of subcortical abnormalities. Four had changes consistent with defective myelination and two had white matter volume loss. All of the controls had normal scans.

The metabolic defect in cystinosis may preferentially affect white matter. No other conditions were present that might have caused these changes. The visual processing deficits seen in children with cystinosis may thus result from the disruption of the subcortical connections between different brain regions.

144.16

WEIGHTED LINEAR ESTIMATOR PROCEDURES FOR NEUROMAGNETIC SOURCE RECONSTRUCTION I. Gorodnitsky, J.S. George*, H.A. Schlitt and P.S.Lewis. Los Alamos National Laboratory, MS: M-715, Los Alamos, NM, 87545

The goal of neuromagnetic source reconstruction is high resolution 3-D mapping of the current distribution within the brain. A useful computational strategy for current reconstruction involves a 'basis matrix' which specifies the signal at each sensor produced by a set of orthogonal unit current vectors associated with each volume element (voxel) in the reconstruction space. Given such a matrix, the forward calculation is a simple linear combination of the basis vectors associated with each of the component currents. However, expressed in this manner, the neuromagnetic inverse problem is ill-posed and typically underdetermined. The Moore-Penrose pseudoinverse provides a linear algebraic inverse calculation that simultaneously minimizes chi-square and the Euclidean norm of the component currents. Such 'minimum norm' reconstructions often produce diffuse and superficial current distributions because voxels nearer the sensor array can account for more power in the data with less current than deeper voxels. Our approach overcomes the superficial bias of minimum norm procedures by using weights chosen to compensate for the distance dependence of magnetic signal strength. In our procedure, the Euclidean norm of the set of basis vectors associated with each source voxel is normalized, and the weighted basis matrix is used for the reconstruction. We also apply a Bayesian weighting strategy in an iterative pseudoinverse computation, to address the bias of the linear estimator procedure toward diffuse solutions. At each successive iteration the previous solution is accepted as *a priori* knowledge and is used to weight the reconstruction. This strategy produces a progressively more focal current distribution while accomodating distributed current sources, and appears to effectively reduce the problems associated with the under-determined linear system. Procedures based on singular value decomposition, hieararchical reconstruction strategies and anatomical constraints can be employed in the context of this algorithm to handle problems associated with noisy data, and modeling arrors associated with the discrete volumetric grid used for current reconstruction. The weighting strategy also provides a natural mechanism to incorporate estimates of source location based on other analyses or other functional imaging techniques.

144.17

SUSTAINED ATTENTION DEFICITS AND WHITE MATTER CHANGES (MRI) IN PATIENTS WITH PHENYLKETONURIA J. Pietz, C. Benninger*, U. Meyding-Lamade Childrens Hospital University of Heidelberg, D-6900 Heidelberg, INF150, FRG

Phenylketonuria (PKU) is an autosomal recessive disorder of amino acid metabolism. By the early institution of a phenylalanine restricted diet the severe impairment of brain development can be prevented. We investigated 32 early treated PKU patients (mean age 19.8 years) with cranial magnetic resonance imaging (MRI) and with a continuous performance task (CPT) to determine the level of sustained attention. In addition IQ testing (WAIS-R) was performed. All 32 patients showed lesions (high signal intensities in T2-weighted images) in the white matter of the central nervous system. A subgroup of 6 patients were found to be severely affected with extension of the lesions to the frontal, temporal and subcortical white matter. These patients showed distinct deficits in the CPT and had lower IQs in comparison to the other patients. Within the patients with a mild (N=15) or moderate (N=11) degree of MRI changes the extent and/or location of these lesions (mainly within the posterior and parietal hemispheres) was not correlated with CPT and IQ results.

144.18

CEREBRAL ACTIVATION STUDIED WITH PET AND (O-15)-BUTANOL: METHODOLOGICAL CONSIDERATIONS M. Ingvar,* L. Eriksson, T. Greitz , S. Stone-Elander, C. von Euler, and P. af Trampe The Karolinska Hospital/Institute, Karolinska Pharmacy and the University of Stockholm, Stockholm, Sweden

A total of 32 cerebral blood flow (rCBF) studies have been performed in four healthy volunteers with the aim of investigating methodological aspects of brain activation studies. Four rCBF studies while reading aloud and four studies while reading silently were performed in each subject. During a period of three minute, the volunteer read words from a semantically balanced list presented on a screen in front of him. Each word was presented every 1.5 s, starting 20 s before the bolus injection of 30 mCi (O-15)-butanol. The uptake of the flow tracer was followed for 100 s with time frames of 10 s using a Scanditronix (GE) PC2048-15B positron camera system. The arterial concentration of the flow tracer was monitored every second for two minutes with a Scanditronix automatic blood sampling system.

A computerized atlas was adapted to the individual rCBF images. By using transformations that are inverse to those used to adapt the atlas, the individual rCBF images were reformatted to the atlas coordinate system. Average and error images were generated for the group as well as for each individual allowing intra- and inter-individual comparisons. Maps of the difference between the two activation states divided by the error were calculated both for the individual cases and the group. Areas of robust activations, for ex Brodman area 04, with "reading silent" as the reference state and the frontal cortex with "reading aloud" as the reference state, were found for the group and for each individual. This is in agreement with findings of DH Ingvar.[J. Human Neurobiol 4,127(1983)]. Our results indicate that intra-individual averaging is a powerful tool for reducing noise in activation studies.

145.1

ATTENTION TO LOCATIONS IN SPACE: THE NEUROPHYSIOLOGY OF EARLY SELECTION S. Johannes, H.C. Hughes* & G.R. Mangun. Medizinische Hochschule Hannover, 3000 Hannover, Germany; Dartmouth College, Hanover, NH & University of California, Davis, CA.

Previous studies of spatial attention have shown that attended visual stimuli produce amplitude enhancements of sensory event-related potentials. This has been interpreted as a facilitation of visual processing. We investigated whether attention more effectively modulates the processing of weak sensory signals, as well as whether these attention-sensitive ERP components (P1 & N1) represent serially dependent visual processes, or instead parallel processing channels.

Stimuli consisted of low and high luminance bars which were randomly flashed 5 deg. to the right and left of fixation. Subjects attended to stimuli at one location while ignoring those at the other location.

The overall amplitude of the occipital P1 component was affected by both luminance and attention, but neither the absolute magnitude of the attention effect, nor the peak latency differed as a function of luminance. In contrast, for the occipital-temporal N1 component, luminance affected the latency of the N1 peak, but not the amplitude or latency of the attention effect observed in this time range. These data suggest partial dissociations between sensory processes and the effects of attention on early ERP components. Further, they suggest that the P1 and N1 components are differentially affected by attention and that they may reflect activity in parallel processing streams.

145.2

ORIENTING ATTENTION IN SPACE: ELECTROPHYSIOLOGICAL STUDIES OF CONTROL MECHANISMS. G.R. Mangun*, Department of Psychology and Center for Neurobiology, University of California, Davis, CA 95616.

The mechanisms of attentional orienting and selective sensory processing were examined in a trial-by-trial spatial cuing task. Subjects were cued by a left or right pointing arrow to orient covert attention to a lateral field location in order to discriminate a target stimulus. Event-related potentials (ERPs) were recorded in response to both the cue and the target. During the cue-target interval, two effects were observed. The first was in the latency range of 250-350 msec post-cue and was manifest as a relative negativity over the parietal-temporal scalp contralateral to the direction of the cue. The second effect was observed only over the right hemisphere between 300-500 msec latency; the response to the left cue was more negative than the response to the right cue. This effect was composed of an early negativity (300-400 msec) over right anterior scalp regions, and a later negativity (400-500 msec) over right occipital-temporal scalp sites. The target stimuli elicited enhanced occipital P1 (100-140 msec) and N1 (160-200 msec) peaks when the target appeared at the cued location.

These findings suggest that lateralized executive orienting mechanisms are activated in the response to the attention-directing cues, as first shown by Harter and colleagues (1987). Further, the present data indicate a specialization of the right hemisphere for some aspects of the orienting process. One result of such mechanisms is the preparation of cortical sensory areas for the differential processing of attended and ignored stimuli, thereby leading to improved perceptual representations of, and responses to the target stimuli.

145.3

THE SPATIAL FREQUENCY CONTENT OF THE STIMULUS PATTERNS DOES NOT EXPLAIN FACE-RESPONSIVE COMPONENTS IN VISUAL EVOKED POTENTIALS

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In previous studies face-responsive components were found in man and primate visual evoked potentials (EP's). Photographs of faces, tools, houses, flowers, other parts of the body etc. were used as stimuli (slide projection).

Differences in face-responsive EP-components were also found when adult human-faces, human-baby-faces, adult animal-faces and animal-baby-faces were used as stimuli. Fourty slides of each category were projected in recording sessions with 16 subjects (9 female and 7 male, EEG-recordings from F3, T5, Cz, Oz, T6, F4, reference: linked mastoids).

The most prominent and significant differences were found in the EP's recorded through Cz-T6. The greatest differences at this recording site occured between EP's aroused by human-baby-faces and adult animal-faces as well as between human-baby-faces and animal-baby-faces. The differences in EP's evoked by human-baby-faces and adult human-faces were smaller but still significant. There were no significant differences in the EP's related to adult animal-faces and animal-baby-faces.

The two-dimensional spatial frequency distributions of the stimuli (Fourier transforms) were computed and averaged for the 4 stimulus categories. The spatial frequency content (nearly identical for the four stimulus categories) can not explain the differences in EP's.

(Supported in part by a grant of the Deutsche Forschungsgemeinschaft, Gr 161).

145.4

SHIFTING OF VISUOSPATIAL ATTENTION: AN EVENT-RELATED POTENTIAL STUDY S. Yamaguchi*, H. Tsuchiya, S. Kobayashi, T. Tsunematsu, Third Div. of Internal Medicine, Shimane Med. Univ., Izumo 693, JAPAN

Distinct neural systems are involved in sustained and phasic attentional mechanisms. Both hemispheres may separately contribute to phasic attentional allocation while the right hemisphere has superiority in tasks demanding voluntary sustained attention. The current study was designed to clarify the nature of voluntary attention shifts by recording event-related potentials (ERPs) during a visual discrimination task. Five visual stimuli were sequentially presented one at a time in a straight line from the periphery through the fovea to the opposite periphery while subjects fixed their eyes to the fovea. The first four stimuli were identical in color and shape. The fifth stimuli was presented with a different color or shape from the preceding four stimuli, or without any changes. In some trials, the fifth stimuli was presented at the spot shifted from the point expected from the four preceding stimuli. Subjects pressed a button when the fifth stimuli changed in color and were obliged to shift their attention from the expected spot to the remote spot immediately after the shifting stimuli were presented.

The fifth stimuli generated a positive-going resolution of the CNV for all stimulus types. An additional positive component (P260) was observed in the ERP elicited by the shifting stimuli. This component had a maximal amplitude at the frontal scalp site and was preceded by a small negative component (N180) maximal at the parietal-temporal site. The P260 amplitude depended on the degree of shifting distance and direction. Stimuli with larger shifting generated larger amplitudes associated with shorter latencies. Left-to-right shifting of stimulus spot generated amplitudes in the right hemisphere and vice versa. The N180 amplitude was maximal at the posterior temporal site contralateral to stimulus side. These results suggest that N180/P260 potentials may index the shifting of overt visuospatial attention, and support the notion of separate contribution of each hemisphere on phasic attentional shifting across visual field.

145.5

ESTIMATING NEURAL GENERATOR POWER AND FIELD CLOSURE FROM INTRACRANIAL CURRENT SOURCE DENSITY (CSD) PROFILES. C.E.Tenke*, M.Steinschneider and C.E.Schroeder, Dept. Biopsychology, NYS. Psychiatric Institute,NY,NY and Depts. Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY

CSD profiles computed at low spatial sampling resolution provide a useful preliminary description of the neural populations and temporal activity patterns underlying surface-recorded event-related potentials (ERPs). Although localizing capacity improves as spatial resolution is increased, the CSD also becomes more sensitive to sharply localized activity that may not be reflected in the ERP recorded at the scalp. We have reported that closed field contributions may be separated from volume conducting activity by comparing CSD profiles computed using two differentiation grids (Tenke and Schroeder, 1990; 1991). The present study extends the applicability of this approach by quantifying overall activity generated in cortical and subcortical sensory structures in the monkey. Three measures of effective amplitude were examined: root mean square (RMS), standard deviation (SD) and mean absolute value. Findings for the Flash-ERP of the lateral geniculate nucleus (LGN) include the following: 1) peak power generation is coincident with the surface N25 (14-29ms), and suffers little cancellation locally; 2) a lower amplitude, sustained response includes oscillatory activity (25-60ms), which is cancelled locally in the LGN. While the standard deviation waveform has the advantage of allowing direct statistical comparisons, all methods produce similar results if the mean CSD waveform is approximately zero. This approach may provide an additional means for determining the relative contributions of the activity patterns within specific neural structures to the surface-recorded ERP. (Supported by MH36295, MH06723 and DC00657)

145.6

SPATIAL AND TEMPORAL PATTERNS OF HUMAN BRAIN ACTIVITY DURING VISUAL SPATIAL ATTENTION. G.V Simpson* and J. Foxe. Depts. of Neurology & Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

We examined the brain regions and their temporal activation patterns underlying visual processing in a spatial attention task. Event related brain potentials (ERPs) were recorded from 50 scalp sites while subjects performed a visual spatial attention task. Small white rectangles were randomly presented at a rapid rate to the left and right of fixation. Subjects attended to the stimuli in one visual field and responded only to the rare smaller rectangles on that side while ignoring all stimuli in the other visual field. Current source density (CSD) maps were calculated and a spatiotemporal generator analysis (BESA) was used to estimate the locations and temporal activation patterns of the active brain regions generating the ERPs. MRIs of each subject's head and brain were used to maximize the accuracy of the spherical volume conductor model used in BESA, and to visualize and evaluate the active brain regions corresponding to the BESA results. CSD maps revealed multiple foci during the P1 and N1 ERP components. The major foci were: at lateral-occipital scalp (contralateral to the eliciting stimulus), followed by a similar focus on the ipsilateral side, a dorsal frontal focus, then dorsolateral posterior foci on both sides. BESA/MRI results revealed the generator complexity underlying the CSD foci and provided an estimate of the active brain generators and their temporal patterns of activation. BESA/MRI results support sequential and overlapping activation of the following brain regions: contralateral (to the stimulus) occipital extrastriate cortex, then ipsilateral extrastriate cortex, anterior cingulate/medial frontal cortex, bilateral dorsolateral parietal cortex and medial parieto-occipital cortex. In those subjects with large enough responses preceding the P1 (60-90 msec), BESA/MRI and CSD suggest a striate source. These spatial and temporal patterns of activation provide some evidence for both serial and parallel flows of processing in cortical areas ranging from striate to frontal and parietal regions during visual spatial attention.

145.7

THE EFFECT OF PREPARATORY PERIOD ON REACTION TIMES AND P3 LATENCIES IN NEWLY LEARNED AND OVERLEARNED S-R INCOMPATIBILITY TASKS C. A. Christensen* and K. J. Drake. Dept. of Psychology, Vassar College, Poughkeepsie, NY 12601.

Pfefferbaum, Christensen, Ford & Kopell (1986) found that P3 latency was prolonged on incompatible trials in a S-R incompatibility task, suggesting that P3 latency may reflect aspects of response processing in that task. This conflicts with the widely held hypothesis that P3 latency reflects the duration of stimulus evaluation alone. The P3 prolongation was observed only when compatible (C) and incompatible (I) trials were tested in separate blocks, however. They speculated that the more rapid responding observed during blocked trials may reflect concurrent stimulus evaluation, response selection and execution, conditions which may predispose to prolonged P3 latencies on I trials. In a study of 16 Ss we evaluated this possibility by testing C and I trials together but cued the trial type either 1 or 2 sec in advance of the stimulus words RIGHT or LEFT. We predicted that P3 latency would not be prolonged on I trials with the 1 sec preparatory interval (a finding obtained by McCarthy & Donchin, 1981) but would be observed with a longer period of preparation. The longer preparatory interval produced only marginal effects on RT and P3 latency. As expected, reaction times (RTs) were prolonged on I trials with both intervals (~70 msec), but contrary to the prediction, P3 latency was prolonged at both intervals as well (~30 msec). A second set of 16 Ss was tested in the same task but using two nonsense words instead of the overlearned stimuli LEFT and RIGHT. The findings of the two studies were not significantly different suggesting that the incompatibility effects on RT and P3 latency are robust.

145.8

ERP CORRELATES OF WITHIN SESSION VARIATIONS IN DISCRIMINATION PERFORMANCE. D. Ryan-Jones, G. Lewis, & D. Wetzel*. Navy Personnel Research and Development Center, San Diego, CA 92152-6800.

Large individual differences in choice reaction time (RT), and response accuracy (HITS) are often seen on relatively simple psychophysiological tasks under conditions in which only minor differences would be expected. Many subjects may exhibit significant performance variability over trial blocks. These changes in performance have been attributed to mechanisms such as learning, adaptation, habituation, fatigue, and changes in cognitive strategies. Although average RT, and HITS may be significantly correlated, it is not clear how ERP amplitude, and latency measures covary with changes in performance over trials for these two behavioral measures. The purpose of this study was to investigate the ERP correlates to changes in performance. ERP activity was recorded from site Pz for 119 male subjects during a 400 trial, 2-letter visual discrimination task which lasted 24 minutes. Behavioral and ERP data were analyzed with respect to eight 3-minute blocks of 50 trials, as well as P3 amplitude and latency at the single epoch level. Variables of interest included absolute performance (HITS and RT), block-to-block performance variability (HSE and RTSE), and rate-of-change in performance over blocks (HSLOPE and RTSLOPE). For the grand averages, HITS and HSE were correlated with P1, N1, P2 and P3 amplitudes, and HSLOPE was correlated with early SW amplitude in grand average ERPs. In contrast, RTTOTAL, and RTSE were correlated with P2, P3, and early SW amplitudes amplitude. For the single-epoch P3 measures, the P3 amplitude was correlated with HITS and RT, and P3 latency was correlated with HITS, HSE, RT, and RTSE. The data suggest that RT and HIT measures are not equivalent measures of performance, and that the two measures may be mediated by different cognitive mechanisms. This abstract does not necessarily reflect the views of the Navy Department.

145.9

BRAIN VARIABILITY, INDIVIDUAL DIFFERENCES, AND TASK PERFORMANCE OVER TIME. G. W. Lewis* & D. L. Ryan-Jones. Navy Personnel Research and Development Center, San Diego, CA 92152-6800.

Brain variability has been shown to be inversely related to short-term task and job performance in both neuroelectric (ERP) and neuromagnetic data. This research addresses relationships between brain variabilty and performance on a 400 trial, 2 letter visual discrimination task which lasted about 24 minutes. ERP data were analyzed from site PZ for 69 male subjects (23 +/- 3 yrs). Data were recorded from 200 ms prestimulus to 1000 ms poststimulus. ERPs were separated into 25 windows, and trials were separated into 8 blocks of 50 epochs each. The first 10 epochs in each block were used to obtain the ERPs. Trial-to-trial variability (TTV) was the summed variance of each of the 154 points in the 10 epoch average of each block. Two groups [high decrement (HD), N=34, low decrement (LD), N=35] were determined from the regression line slope of correct hits. TTV was significantly greater for the HD than the LD from the start of the task, and increased throughout the testing session. The ERP prestimulus TTV showed large statistically significant group differences. The large, and increasing poststimulus TTV of the HD over the LD suggested that the HD may have less adaptation to the task than the LD. This difference in ERP variability probably was not due to attentional factors as the N1 amplitudes were not statistically significant over time. The large group differences in TTV were maximal during prestimulus, early (50-200 ms), and late (850-1000 ms) poststimulus, but were not statistically significant in the 200-850 ms interval. These data suggested that the HD group may be showing a decreased ability to suppress irrelevant background information which would result in a decreased stimulus sensitivity over time. However, the significantly greater TTV in the HD group in the prestimulus records also suggested that the HD individuals may be showing inherently greater processing variability. This abstract does not necessarily reflect the views of the Navy Department.

145.10

THETA-ACTIVITY RELATED TO PERFORMANCE S.J.Laukka*, J.Lindqvist, T.Järvilehto, Yu.I.Alexandrov. Laboratory of Developmental Neuropsychology, University of Oulu, Kasarmintie 4, SF 90100 Oulu, Finland.

Frontal midline theta-activity reported to appear during mental task (Ishihara et al., 1972) was investigated during goal-directed behavior. In a simulated traffic situation subject had to find the right way for driving a'car' through a set of roads in a computer game. During driving two interdependent decisions had to be made at two crossroads. Feedback about quality of performance was given after each trial. Duration of trial was 10 s. EEG was recorded from Fz.

Theta-activity during consecutive phases of behavior was analysed. Theta periods were time-locked with the instants of decisions.

Correct driving at crossroads was associated with significantly more theta than the wrong one. The results demonstrate a relation between the appearance of theta and quality of performance in dependence on the phase of behavior.

145.11

ATTENTIONAL DEVELOPMENT DURING MATURATION AS ASSESSED WITH SCALP ENDOGENOUS POTENTIALS AND RELATED MEASURES. S.F. Gilson*, J.W. Rohrbaugh, J.M. Stapleton, K.Y. Sirocco, G.L. Brown, J.L. Varner, M.J. Eckardt. National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20892; National Institute on Drug Abuse, Addiction Research Center, Baltimore, MD 21224; Dept. of Psychiatry, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The maturation of attention was studied using event-related potential (ERP) and choice reaction time (RT) measures, obtained from 24 children without significant medical diagnosis and without psychiatric diagnosis. Subjects were grouped according to age (8.0 to 13.0, and 13.1 to 18.0 years) and sex. Dichotic listening tasks were designed to elicit several ERP components related to different attentional and cognitive processes; these included N2, P3, Processing Negativity (PRN), and Mismatch Negativity (MMN). ERPs were recorded from midline and lateral central scalp sites.

RT and ERP measures showed pronounced developmental changes. Young children had slower RTs and made more errors. Although P3 was not affected by age, the younger children produced larger N2, PRN and MMN components. This effect occurred in the absence of any discernible N1 component, attesting to the independence of the attention-related components from the exogenous N1. Performance and P3 amplitude diminished rapidly over time when the task was difficult, at the same rate for younger and older children. The N2 component also diminished but at a faster rate in younger children. No decrement over time was observed for PRN or MMN. These results provide evidence regarding the maturational course of attention, and suggest that the course may not be a linear progression from simple to complex processing.

145.12

MATURATIONAL CONSTRAINTS ON CEREBRAL SPECIALIZATIONS FOR LANGUAGE PROCESSING: ERP AND BEHAVIORAL EVIDENCE IN BILINGUAL SPEAKERS. C.M. Weber-Fox*, H.J. Neville. The Salk Institute, LaJolla, CA 92037.

Recent evidence from event-related brain potentials (ERPs) suggests that cerebral organization is highly specialized for processing functionally distinct aspects of language. ERPs elicited by words that convey the meaning of a sentence (semantics) are distinct in timing and distribution from the ERPs elicited by words that specify the relationship between words in a sentence (syntax). Further, unique patterns of ERP responses were found to distinguish between three types of syntactic processing. Our goal was to investigate the hypothesis that different maturational constraints may affect distinct functional specializations of language systems within and between the hemispheres. Subjects were 61 adult Chinese/English bilinguals who were exposed to English at different points in development: 1-3, 4-6, 7-10, 11-13, and after 16 years of age. The language stimuli were 240 sentences which included semantic anomalies, three types of syntactic violations (phrase structure, specificity and subjacency constraints), and their controls. ERPs were recorded as the randomized sentence stimuli were presented on a monitor one word/540ms. Subjects judged whether or not each sentence was a "good English sentence." Our findings indicate that age of exposure to a language significantly determines functionally distinct aspects of language processing, and that different aspects of language processing are not uniformly affected by delays in exposure. Behavioral and ERP (N400) responses to semantic stimuli were found to be least vulnerable to delays in exposure (>11 yrs). The ERP and behavioral responses to each of the three types of syntactic stimuli showed earlier (<11 yrs), but not uniform, sensitivities to delays. Our findings are consistent with the view that maturational changes significantly determine the capabilities of humans to acquire language and constrain the development of relevant functional cerebral specializations for language. (NIH DC00054, NIH DC00128)

145.13

CONFIGURAL AND FEATURAL PROCESSING OF FACES: ERP AND BEHAVIORAL EVIDENCE T.D. Sarfaty*, D. Mills, P. Knaudt, and H. J. Neville. UCSD Dept. of Neuroscience, La Jolla, Ca 92093, and The Salk Institute, San Diego, Ca. 92037

Previous research has shown that inverted faces are more difficult to recognize than are upright faces. This 'inversion effect' is disproportionately greater for faces than for other types of stimuli. These findings have been interpreted to indicate that adults employ configural analysis for the recognition of upright faces and featural analysis for the recognition of inverted faces. Clinical and behavioral research have also suggested that the right hemisphere is superior for configural analysis in general, and for the recognition of upright faces in particular.

We sought physiological evidence concerning these hypotheses and recorded event related potentials (ERPs) from midline and lateral sites in 20 normal adults. Pairs of faces (upright or inverted) were sequentially presented, and subjects were required to make an identity discrimination.

Subjects were faster and more accurate in their responses to the upright faces. Difference ERPs formed by subtracting the ERPs to matched from ERPs to mismatched stimuli revealed that the identity mismatch effect for upright faces was characterized by an asymmetrical negativity (larger over the right hemisphere) beginning around 250 msec. with an antero-central distribution. By contrast, the effect for inverted faces was a symmetrical positivity that onset around 500 msec., with a posterior distribution.

ERPs recorded in response to upright and inverted faces thus differed in latency, amplitude, polarity, and distribution. These data support the hypotheses that a) the right hemisphere is preferentially involved in the recognition of upright faces and b) the recognition of upright and inverted faces is mediated by different neural systems that may be organized for configural and featural processes, respectively. (NIH DC00481)

145.14

SIGNALING IN DISTRIBUTED NEURAL SYSTEMS. V. Menon*. EEG Systems Lab., 51 Federal St., San Francisco, CA,94107

A theoretical study of nonlinear interaction between neuronal populations was undertaken to gain insight into the functioning of distributed neural systems. Temporal characteristics of the interaction are studied using, in addition to numerical simulations, simplified relations between the frequency, amplitude, and phase of the response.

Signaling between populations is shown to give rise to synchronization, desynchronization, and resynchronization (with a large jump in frequency and spatial phase difference) of the oscillatory activity as the latency of the reentrant signal is varied. During the desynchronized phase, the temporal response is chaotic and the two-population correlation is reduced substantially. This phenomenon represents a rapid (correlation-dependent) switching mechanism. The study further reveals topological effects - (1) spatial amplitude modulation and (2) existence of stabilizing (closed loop) and destabilizing (open loop) circuits.

These spatio-temporal features are likely to be important in cognitive processing.

145.15

EVENT-RELATED POTENTIALS CORRELATE OF IMPLICIT AND EXPLICIT MEMORY OF FACES. S. Hertz, P. Porjesz, H. Begleiter* and D. Chorlian, Neurodynamics Lab., SUNY HSC @ Brooklyn, Brookyln, NY 11203

We have conducted an electrophysiologic experiment to assess the effects of priming in both implicit and explicit face matching paradigms. Subjects were shown a series of pictures of faces interspersed with scrambled pictures of faces of equal size, position and luminance; intermittantly, the same face or scramble would be repeated. During the implicit task, subjects were asked to differentially press buttons to the face and scramble. Subjects were then asked to identify faces from hand held pictures mixed with faces not previously presented to test recognition and ensure that the implicit task was truly implicit. During the explicit task, subjects were presented with the same series of faces and scrambles but this time instructed to press one button to immediately repeated faces or scrambles and another button to different faces and scrambles. For both tasks, recordings were made from 32 EEG electrodes placed according to the 10/20 system, along with an electrode placed over an eye to monitor eye movements. Correct trials were sorted and averaged according to the combination of stimulus and preceeding stimulus: face/same face, face/different face, face/scramble, scramble/same scramble, scramble/different scramble and scramble/face. Reaction times were recorded. We observed a series of evoked potentials consisting predominantly of a P160, N180 and P240 with a posterior, bitemporal distribution and an amplitude maximum over the right posterior temporal region. Implicit and explicit priming effects were present predominantly over bilateral, anterior, mid and posterior temporal regions and reflected by reduction of the P240 amplitude. This P240 amplitude effect was more pronounced for the explicit task. These data reflect a novel set of neurophysiological observations on implicit and explicit memory for faces.

Supported in part by the New York State Office of Mental Retardation and Developmental Disabilities, CMEDD Fellowship

145.16

NON-LINEAR CORTICAL DYNAMICS ("CHAOS") AND MENTAL COMPLEXITY. N. Birbaumer*, W. Lutzenberger & T. Elbert. Inst. of Medical Psychology, Univ. of Tübingen, Tübingen, Germany, 74.

EEG-recordings from 16 cortical sites during mental tasks with different complexity were recorded in 52 healthy subjects with above average (IQ>100) and below average (IQ<100) intellectual ability. EEGs were analyzed with conventional power-spectra and (non-linear) dimensional analysis using the algorithm of Farmer et al (83) to determine phase space dimensions of extended time series such as the EEG.

Tasks were of sensory (tactile and visual discrimination) and verbal (alliteration) nature, all tasks were performed "in-vivo" and in imagination. Several imagery tasks were used. Results demonstrate higher dimensionality ("fractal complexity") during non-task rest conditions in intelligent subjects and increased frontal complexity during imagery compared to "in-vivo" tasks. Conventional EEG analysis did not show meaningful differences.

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145.17

EVENT-RELATED POTENTIALS ASSOCIATED WITH SHIFTING VISUAL ATTENTION IN NORMAL CHILDREN AND CHILDREN WITH CEREBELLAR LESIONS. N. A. Akshoomoff*, and E. Courchesne. Depts. of Psychology and Neurosciences, UCSD, La Jolla, CA 92093, and Neuropsychology Research, Children's Hosp. Res. Ctr., San Diego, CA 92123.

Event-related potentials (ERPs) were recorded while 8 normal children (ages 7-11) and 5 children with cerebellar lesions as a result of removal of a cerebellar astrocytoma (ages 8-11) were presented with randomized sequences of centrally located form and color stimuli on a computer screen. In the shift attention experiment, subjects responded to the target stimulus in one category (form or color), shifted their attention to the other stimulus category, and responded to the first target stimulus in that category. The target thus served as a cue to shift attention. In the focus attention experiment, subjects maintained their attention and responded to the target stimulus in one category. The frontal Nc component was found to be more negative when normal children maintained their attention than when they shifted their attention. The posterior P3b component was similar in amplitude across both experiments in normal children. Difference waves between focus and shift attention revealed a posterior positive component longer in latency than the P3b (labeled the Shift Difference or Sd wave). The present data demonstrate that children have ERPs that are similar to adults when they rapidly shift their attention. Compared to normal children, the patients with cerebellar lesions had abnormally small P3b and Sd responses to the signal to shift attention and were significantly impaired in their ability to rapidly shift attention. Both the reduced cognitive ERPs and reduced capacity to rapidly shift attention in these patients have important implications for the role of the cerebellum in cognitive operations, and suggest that cerebellar damage affects operations that may be primarily mediated by the cerebral cortex.

145.18

INTRACEREBRAL MEASURES OF LATERALIZED PROCESSING IN HUMANS: EFFECTS OF VISUAL FIELD, RESPONSE HAND AND PROCESSING STAGE. [1]J.M. Clarke*, [1]P. Chauvel, [2]J.-M. Scarabin, [1]E. Halgren. [1]Laboratoire de Neurosciences, INSERM CJF90-12 & [2]INSERM U335, Univ. Rennes I, 35033 Rennes, France.

Intracerebral recordings were acquired from 17 epileptic patients while they performed a lateralized visual discrimination task. Across these neurosurgical candidates, a total of 115 'depth' electrodes were implanted for seizure localization, permitting recordings from over 1,000 cortical and limbic sites. While maintaining central fixation, subjects responded to rarely occurring targets (e.g. a 'o'), but not to frequent nontargets (e.g. a '+'), presented to the four corners of a CRT screen.

A series of visual components (N170-P200-N260) were recorded in occipital and posterior inferotemporal cortices, which were larger (by 20-30 μV) and usually earlier (by about 20 ms) for stimuli presented to the visual field (VF) contralateral to the side of the electrode. An unmistakable visual quadrant effect (260 ms peak latency) was apparent in one patient in putative visual area 19. 'Cognitive' P3-like components to rare stimuli (300 to 500 ms peak latency) were reliably recorded in limbic and orbitofrontal locations, and sometimes in parietal sites. When present, the P3s were elicited by either VF with equal amplitudes. Hippocampal P3s showed VF latency effects, being typically 20 ms earlier for contralateral presentations. Finally, a late negative-going component (400 to 600 ms onset) was common for medial and lateral peri-rolandic sites, and was usually undifferentiated by VF or response hand. Clearly lateralized somatosensory and motor responses (500-650 ms peak latency) were recorded in one electrode that coursed through the somatomotor strip.

For this task, it appears that only early visual input and final motor output stages are lateralized. Both cerebral hemispheres appear to be equally engaged for intermediate cognitive stages even though this type of task probably can be performed by a single hemisphere.

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146.1

CONDITIONING STIMULUS PREEXPOSURE MODIFIES PRE-TRAINING HIPPOCAMPAL ACTIVITY. S.D. Berry*, R.L. Borgnis, and L.J. Dreshfield. Psych.Dept., Miami Univ., Oxford, OH 45056.

To clarify the role of the hippocampus in classical conditioning, we examined the effects of prior exposure to conditioning stimuli on patterns of hippocampal activity related to acquisition rates in rabbits. New Zealand White rabbits were implanted with stainless steel extracellular microelectrodes under anesthesia (ketamine 50mg/kg, xylazine 10mg/kg). Group P was exposed to the stimuli prior to the onset of classical jaw movement conditioning. Group C was not pre-treated. One minute samples of unit and slow wave activity from CA1 were recorded before and after each session. Slow wave frequency analysis showed that there were no differences between groups prior to adaptation to the apparatus (F (1,9)=0.67, $p>.44$). After the preexposure phase but before the beginning of conditioning, the groups had significantly different frequency distributions (F (1, 9)=11.22, $p<.006$). Such variations have been shown to precede differences in the rate of behavioral learning and in the development of conditioned unit activity (Berger, Berry, & Thompson, 1986). This result suggests that the effects of stimulus preexposure alter the behavioral state of the animal prior to (as well as in response to) the occurrence of the conditioning stimuli, and that the hippocampus participates in the hypothesized reduction in stimulus salience.

146.2

LONG-TERM DEPRESSION AND CLASSICAL CONDITIONING OF THE RABBIT NICTITATING MEMBRANE RESPONSE: AN ASSESSMENT USING THE RABBIT CEREBELLAR SLICE. B. G. Schreurs* and D. L. Alkon. Neural Systems Section, NINDS, NIH, Bethesda, MD 20892.

Long-term depression (LTD) has been proposed as a mechanism underlying classical conditioning of the rabbit nictitating membrane/eyelid response (NMR). However, LTD in cerebellar slices has only been obtained in the presence of GABA inhibitors which abolish disynaptic ipsps, and even then the temporal sequence of events (climbing fiber - parallel fiber) has been the reverse of that used in classical conditioning (CS-US) .

Based on intradendritic Purkinje-cell recordings obtained from rabbit cerebellar slices, we report that stimulation (80 μs, 4 Hz, 30 s) of parallel fibers in the absence of GABA inhibitors produced a small, brief depression of parallel fiber epsp amplitude (-10%, 4 min, n=17) that then became a longer lasting potentiation (20%, 12 min). However, if parallel fibers were stimulated during depolarization-induced local dendritic calcium spikes, significant, prolonged depression emerged (-35%, 17 min, n=19, $p<.001$). Presentation of climbing fiber and parallel fiber stimulation in either an LTD or classical conditioning-like order (80 μs, 4 Hz, 30 s, 50 ms interstimulus interval) without GABA inhibitors produced brief, weak depression (-20%, n=10; -15%, n=13, respectively). Presumeably, the depolarization and consequent calcium influx into the Purkinje-cell dendrite produced by climbing fiber synaptic potentials were less effective than the prolonged, direct depolarization induced by current injection through the recording electrode. Finally, stimulation of parallel fibers at frequencies used in in vivo conditioning experiments (100 Hz) produced profound, long-lasting epsp depression. The depression occurred in the absence of climbing fiber stimulation and was reminiscent of transmitter rundown.

146.3

Changes in Conditioning-Related Neuronal Activity in Rabbit Lobule HVI of Cerebellar Cortex During Backward, Unpaired and Forward Conditioning. T. J. Gould* & J. E. Steinmetz. Dept of Psych., Prog. in Neural Science. Indiana University, Bloomington, IN 47405

At least two areas of the cerebellum are believed to be involved in conditioned stimulus (CS)-unconditioned stimulus (US) association during classical conditioning; lobule HVI and the interpositus nucleus. The goals of the present study were to examine multiple unit HVI activity during two scenarios: 1.) Backward conditioning (US-CS paired presentation) followed by forward conditioning (CS-US paired presentation), and 2.) Unpaired training and subsequent forward conditioning. Twelve animals received 5 consecutive days of backward conditioning followed by forward conditioning until the animal reached a conditioning criterion for two days. Tone CSs and airpuff USs were used. Four additional animal received 5 days of explicitly unpaired training followed by forward conditioning until reaching criterion for two days. In some HVI recording sites, a significant increase in US-related activity was seen over the five days of backward training and these sites all showed well-sculpted amplitude-time course models of the learned response during forward training. In other sites, a significant decrease in US-related activity was seen over the five days of backward training and these sites did not show amplitude-time course models of the learned response during forward training. Both of these patterns were seen to a lesser extent in animals receiving explicitly unpaired training. During both backward and unpaired training, activity was largely concentrated early in the trial but across forward training large amounts of activity in both the early CS period and US period decreased as activity in the late CS period increased. These data demonstrate that several patterns of excitatory, multiple-unit, conditioning-related activity in cerebellar cortex can be observed as training constraints change.

146.4

A POSSIBLE MECHANISM FOR ASSOCIATIVELY DECREMENTING US PROCESSING IN RABBIT EYEBLINK CONDITIONING T. Canli*, J. Anthony, and N.H. Donegan. Dept. of Psychology, Yale Univ., New Haven, CT. 06520.

The cerebellar interpositus nucleus (IP) and red nucleus (RN) are activated by a conditioned stimulus in eyeblink conditioning, and are thought to be involved in decrementing US processing and contribute to the Kamin blocking effect and conditioned diminution of the UR (Donegan et al, 1989, *Psy. Learn. & Motiv.* 1989, *23*, 109). Support comes from the finding that electrical stimulation of the IP or the RN shortly before a periorbital shock-US decrements the unconditioned eyeblink response in rabbits (Canli, Whitney, & Donegan, *Neurosci. Abstr.*,1991). This decrement was observed both ipsi- and contralateral to the stimulation site. The present experiment was designed to assess the extent to which the decremental effect of IP stimulation is relayed through the RN. We combined electrical stimulation of the IP with chemical inactivation of the RN, using the excitatory amino acid antagonist glutamyl-glycine (0.5 μl, 50 nmol). Fourteen New Zealand male rabbits were implanted with a pair of unipolar stimulating electrodes in the region of the left IP, and a guide/infusion cannula targeted at the right RN. The effect of IP stimulation was tested for 22 trials (10 US-alone, 10 Stim ->US, 2 Stim-alone trials; parameters as in Canli et al., 1991). Subjects then received infusions of glu-gly (day 1) or aCSF (day 2) into the RN. Testing was then resumed for another 78 trials (35 US-alone, 35 Stim->US, 8 Stim-alone trials). Infusion of glu-gly into the RN significantly attenuated IP stimulation-induced UR diminution, regardless of whether diminution was observed ipsi- or contralateral to the IP stimulation site. Thus, the decremental effect of IP stimulation on eyeblink UR amplitude appears to be relayed through the RN. The same subjects were then tested for the effect of chemical stimulation of the RN in response to repeated facial shock-USs, using the excitatory amino acid kainate (0.2 μl, 235 pmol). Chemical stimulation of the RN bilaterally decremented the average eyeblink amplitude, suggesting that activation of the RN, not fibers of passage, affected US processing; possibly directly, via crossed rubrospinal projections, and indirectly, via uncrossed rubrobulbar projections .

146.5

SINGLE-UNIT ACTIVITY IN VENTROLATERAL PONS DURING CONDITIONING OF THE RABBIT NICTITATING MEMBRANE RESPONSE. M. J. Hirl* and J.W. Moore. Dept of Psychol, Univ of Mass, Amherst, MA 01003.

This study examined the activity of single-units in the ventrolateral pons during differential classical conditioning of the rabbit nictitating membrane (NM) response. We were particularly interested in the response of units in the pontine reticular formation (RF) and dorsolateral pontine nuclei (DLPN). Moore et al., (*Biol. Cybern.*, 62:17, 1989) present a brain implementation of a model of NM conditioning which purports parallel learning in cerebellar cortex and brainstem with RF as a possible brainstem site. For RF to be a site of parallel learning, it must receive US information, and thus should contain units which respond to US. The model assumes that DLPN propagates CS information to cerebellar cortex. CR-related activity in DLPN is not predicted by the model's implementation, given the motor program originates efferent to DLPN.

McCormick et al., (*Brain Research*, 271:73, 1983) investigated multi-unit recordings from RF and DLPN. Both showed activity closely coupled to the conditioned stimulus (CS) and CR. They did not observe activity to the unconditioned stimulus (US).

We trained rabbits to differentially respond to a reinforced CS+ and non-reinforced CS- (tones of 1200 and 600 Hz, 80 dB, 350 ms). The US was a single pulse of DC current applied to the periorbital region of the right eye. Under anesthesia rabbits were prepared for subsequent recording by exposing the dura and cementing a recording chamber in place. RF units were responsive to events during conditioning, 39% were coupled to CSs, 33% to US, and 28% to CRs. Thus far, we have found no units in DLPN with activity related to CRs. Our results support the the model's implementation.

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146.6

CONDITIONED POTENTIATION OF THE RAT EYEBLINK REFLEX. N. H. Donegan*[1] and T. H. Brown[1,2]. Depts. of Psychology[1] and Cellular and Molecular Physiology[2], Yale University, New Haven, CT. 06520.

The eyeblink reflex has proven useful for neurobiological studies of Pavlovian conditioning (Donegan et al, *Psychol. of Learn. and Motiv. 23*, 109, 1989). Although most of the work has been done on restrained rabbits, recent studies have begun to explore eyelid conditioning in unrestrained rats (Skelton, *Behav. Neurosci. 102*, 586, 1988). The use of rats may be superior for studies that require the same species to be used for both *in vivo* and *in vitro* analysis.

The lid closure during a reflex blink is generated by a passive downward force plus active contraction of the orbicularis occuli (oo) muscle. Reflex eyeblinks were elicited by electrically stimulating the fifth cranial nerve. Stimulation was delivered through a bipolar electrode that was uninsulated on the inner surface of a cuff that surrounded the nerve. The EMG response was measured with a monopolar electrode that was implanted in the oo muscle. The amplified EMG was filtered at 0.3 kHz and 5 kHz, digitized at 5 kHz, and rectified. The EMG response was roughly 100 times greater than the recording noise (about 20 μV).

Stimulation of the fifth nerve produced two distinguishable EMG responses. The R1 response had a short latency (6 - 6.5 ms) that was relatively invariant over trials. The R2 response had a lower threshold and longer latency (15 - 20 ms) that was quite variable. In response to 1 Hz stimulation, the R2 amplitude diminished whereas the R1 amplitude remained invariant. We are currently examining the modulatory effects of fear conditioning on the R1 and R2 amplitudes. Of particular interest is the possible role of the amygdala in conditioned potentiation of the eyeblink reflex (cf. Harden et al, *Soc. Neurosci. Abstr. 16*, 269, 1990). *Supported by NIH and the Whitehall Foundation*

146.7

RETENTION OF NM VISUAL CONDITIONING AFTER VISUAL DISCONNECTION OF THE CEREBELLUM. J. Steele Russell*, Anatomy, Texas A&M University, College Station, TX. 77843.

A mid-sagittal section of the optic chiasma in the rabbit will leave intact only the ipsilateral retinal ganglion cell fibers. All retinal NOT projections are destroyed.

Rabbits were trained on both brightness and pattern discriminations in the 2-choice situation, and then to both a light and a tone CS using NM response. The optic chiasma was cut, and they were tested for retention in both situations. All animals showed rapid re-learning of both visual tasks, clearly establishing the point that all animals could see.

Without exception all animals with complete section of the optic chiasma showed no retention of the Pavlovian visual conditioning. They were also unable to relearn it. In contrast rapid and acquisition of NM responses was obtained to the tone CS, showing that the failure of visual conditioning was not due to a motor impairment. With incomplete chiasma section there was a graded relationship between the amount of crossed fibre sparing and the amount of OKN and NM conditioning. When the chiasma-section was complete there was neither any OKN nor any NM conditioned responding. All animals conditioned to the auditory CS, independent of the amount of chiasma fibre sparing.

This argues that the same oculomotor circuitry that is controlling the OKN and VOR is also involved in visual conditioning of the NM response. Thus it is possible that lesions of the cerebellum affect Pavlovian conditioning either by disrupting the oculomotor control system that mediates the expression of the learning, or because it destroys the neural site of memory storage. Where only one measure is taken of conditioning, which involves an oculomotor response system, these two theoretical alternatives are inseparable.

146.8

LESIONS OF THE MEDIAL PREFRONTAL CORTEX IMPAIR REVERSAL OF CLASSICALLY CONDITIONED EYEBLINK RESPONSES IN RABBITS. M.E. Chachich* and D.A. Powell. VA Medical Center, Columbia, SC 29201 and Department of Psychology, University of South Carolina, Columbia, SC 29208.

The medial prefrontal cortex (mPfc) is important for flexible response strategies, such as those involved in the Wisconsin Card Sorting Task. This study extends the findings that the mPfc is involved in non-perserverative responding to classical eyeblink (EB) conditioning.

The mPfc of rabbits was bilaterally aspirated from bregma to the olfactory bulbs. In sham operated animals a skull flap was removed, but no lesion was made. Both groups received differential EB conditioning (304 & 1216 Hz tones were CSs and paraorbital shock was the US), until responding to the CS+ was 30% greater than to the CS- over two consecutive days. Reversal training then began, in which the reinforced CS+ and nonreinforced CS- were switched. Again reversal was terminated when the rabbits responded to the CS+ at a 30% higher frequency than to the CS- over two consecutive days.

mPfc lesions had no effect on the original discrimination, but when compared to the sham operated control animals, lesioned animals showed an impairment in reversal learning ($p<.01$). These results thus suggest that the mPfc is necessary for conditioned reversal of the EB response.

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146.9

HIPPOCAMPAL LESIONS DISRUPT LATENT INHIBITION. B.A Christiansen and N. A. Schmajuk*. Department of Psychology, Northwestern University, Evanston, IL 60208.

The effect of hippocampal lesions on latent inhibition of the classically conditioned eyeblink response in rats was examined with a procedure described by Schmajuk and Christiansen (1990). Male, Long-Evans hooded rats received either sham (S), cortical control (CC) or hippocampal (HL) lesions. Animals were assigned to either the preexposed (PRE) or the non-preexposed condition (SIT). PRE animals received 100 CS-alone presentations per day for four consecutive days following habituation. SIT animals remained in the experimental chamber without stimulus presentations during the preexposure phase. On the fifth day animals received 50 CS-alone presentations (or 25 minutes in the SIT condition), followed by 50 CS-US pairings. All groups received four additional days of conditioning (100 trials per day). The conditioned stimulus (CS) was a 85 dB, 500 ms, 1,200 Hz tone; the unconditioned stimulus (US) was a 5psi, 150 ms air puff; the interstimulus interval was 350 ms; and the intertrial interval was 30 s.

Both CC and S groups, but not the HL group showed latent inhibition. On the final day of training, PRE control animals exhibited significantly fewer CRs than SIT control animals ($F [1, 17] = 5.76, p < .05$). Conversely, PRE HL and SIT HL animals did not differ in the number of CRs generated ($F [1, 17] = 1.64, p > .05$). Furthermore, a comparison between HL and control animals revealed a significant interaction between lesion type and treatment ($F [1, 17] = 4.80, p < .05$). (Supported by ONR Grant N00014-91-J-1764.)

146.10

EXCITABILITY TO INTRACELLARLY INJECTED DEPOLARIZING CURRENT OF CORTICAL NEURONS SHOWING PRE-MOVEMENT SPIKE ACTIVITY IN CONSCIOUS RATS TRAINED TO LICK TO A LIGHT CUE STIMULUS. C.D. Woody* and E. Gruen. UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024.

Rats were trained to lick water after the onset of a light cue stimulus. Extracellular and intracellular recordings of unit activity were made from the rostral cortex during performance of the behavior. Averages of activity were made a) synchronized to the light onset and b) synchronized to the first lick after light onset (by back averaging). Neural excitability was measured by the number of spikes elicted by intracellularly applied, 80 ms, 1 nA, depolaring pulses. Mean (n = 74 units) increases in activity > 3Z above background activity were found to be temporally correlated with production of the licking rather than responding to the visual cue. No differences in excitability were found between 28 cells showing increased pre-lick activity (mean 4.14 spikes/pulse) and 14 other cells that did not (4.28 spikes). The results favor the hypothesis that a mechanism other than the tonic excitability changes found to support Pavlovian conditioning supports the increased lick activity in these cells and the large, previously reported EPSPs that precede licking, or that the adaptations occur in cells prior to these along the involved sensorimotor pathways. (Supp. HD05958. We thank M.P. Kristensen for his contributions to this study and G. Cheng and R. Folia for technical assistance.)

146.11

MODULATION OF ACTIVITY IN THE MEDIAL GENICULATE NUCLEUS DURING AUDITORY TRACE CONDITIONING. K.N. O'Connor and J.W. Moore*. Dept of Psychol, Univ of Mass, Amherst, MA 01003.

In classical trace conditioning the acquisition of a CR is dependent on neural coding of the CS following its termination. There is evidence that the medial or magnocellular region of the medial genicualate nucleus (mMGN) is one area where this coding may occur; it is essential to the acquisition and maintenance of successful discrimination of auditory stimuli in the conditioning of bradycardia in rabbits (Jarrel, et al., *Brain Res*, 382:199, 1986), and multi-unit reponses to reinforced tones in this region increase during conditioning in comparison to non-reinforced tones (Disterhoft & Olds, *J. Neurophys*, 35:665, 1972).

Single cell activity was recorded from the MGN during differential trace conditioning of the rabbit nictitating membrane response (NMR). Rabbits were first trained to discriminate between a reinforced CS+ and nonreinforced CS- (tones of 600 or 1200 Hz) 150 ms in duration followed by a 200 ms trace interval. The US was electrostimulation to the periorbital region of the eye. The rabbits were then prepared for subsequent recording; under anesthesia the dura was exposed, and a recording chamber cemented in place.

Responses of cells in the mMGN tend to be transient and related to the type of CS, primarily showing greater modulation in firing rate to the CS+. Presentation of probe CSs of varying duration suggest a relationship between these responses and NMR timing and topography.

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146.12

SIMULTANEOUS SINGLE UNIT RECORDING IN THE ACOUSTIC THALAMUS AND THE AMYGDALA THROUGHOUT HABITUATION, DIFFERENTIAL ACQUISITION, AND EXTINCTION OF THE RABBIT'S CONDITIONED HEART RATE. M.D. McEchron, P.M. McCabe*, E.J. Green, and N. Schneiderman. Department of Psychology and Neurosciences Program, University of Miami, Coral Gables, FL 33124.

Previous research has shown that the posterior acoustic thalamus and the amygdala are critical regions for the acquisition and expression of classically conditioned heart rate (HR) using acoustic conditioned stimuli (CS). It has been demonstrated that single units in the medial subnucleus of the medial geniculate nucleus (mMG) and the amygdaloid central nucleus (ACe) exhibit associative changes in response to acoustic CSs. The purpose of the present study was to examine simultaneous single unit responses in the acoustic thalamus and the amygdala during different phases of conditioning. New Zealand albino rabbits were implanted with stereotrode electrodes in the acoustic thalamus and in the amygdala. Following recovery, each animal received one conditioning session that included habituation, differential acquisition, and extinction trials. During differential HR conditioning two tonal CSs (2 s; 560 or 1350 Hz) were presented, one of which was paired with a corneal airpuff unconditioned stimulus (US; 0.5 s; 15 N/cm^2). The results focus on two areas of the posterior acoustic thalamus, the mMG and the posterior intralaminar nucleus (PIN). Within the amygdala, most recorded cells were in ACe. The areas mMG, PIN, and ACe were found to contain cells which show short latency (<100 ms) responses to the initial presentation of both tones. During differential conditioning, units in these regions exhibited differential responding to the tones. Differential responses developed gradually, and in some cases were correlated with the magnitude of the HR response on an individual trial basis. Extinction was associated with a decrease in differential responding in the mMG, PIN, and, ACe. Cells in these areas also exhibited short latency responses to the US alone. These findings provide evidence that associative changes in unit responding occur in the posterior acoustic thalamus and the amygdala throughout differential HR conditioning and extinction. Supported by NS24874 and HL07426.

146.13

DISCRIMINATION TRAINING INDUCES SELECTIVE RECEPTIVE FIELD (RF) PLASTICITY IN THE AUDITORY CORTEX DURING BOTH EASY AND DIFFICULT TASK. Jean-Marc Edeline* & Norman Weinberger. Center Neurobiology of Learning and Memory and Dept Psychobiology, UC IRVINE CA 92717.

CS frequency-specific RF plasticity develops in the auditory cortex (ACx) during classical conditioning to a single tone (Br. Res. 372: 357, 1986; Br. Res. 536:271, 1990). We report here the effects of discrimination training at two levels of task difficulty. RF (single unit n=9, cluster n=32) were determined before and after a single session of tone shock training. The CS+ and the CS- (6 s in duration) were pure tone frequencies selected to be in the cells' RF without being the pre-training best frequency (BF). A clear behavioral discrimination (conditioned bradycardia to the CS+ only) was observed after completion of training in the easy task, while the same animals failed to discriminate (they showed similar bradycardia to the CS+ and the CS-) when the 2 frequencies were closer during the difficult task. However, at both levels of task difficulty, selective RF plasticity was observed in the ACx. Responses to the CS+ increased while responses to the pre-training BF and to the CS- decreased . These changes were large enough to shift the BF to the CS+ frequency in 49% cases (20/41). The shifts to the CS+ frequency were maintained and even stronger 1hr. post training. These results suggest that selective RF plasticity is not tightly linked to behavioral performance but rather reflects highly selective acquisition of information.

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146.14

LONG-LASTING CS-SPECIFIC RECEPTIVE FIELD PLASTICITY IN AUDITORY CORTEX IS EXPRESSED UNDER ANESTHESIA. Roxanna Javid, Branko Lepan, Gabriel K Hui and Norman M Weinberger* Cntr. Neurobiol. Learn. Mem. & Dept. Psychobio. Univ. California, Irvine, Ca., 92717.

Receptive field plasticity in sensory neocortex during development and after peripheral denervation has been observed in anesthetized subjects. Brief learning experience (classical conditioning) induces frequency specific receptive field (RF) plasticity in the auditory cortex (AC) of the cat (Diamond and Weinberger, *Br. Res.* 372:357, 1986) and guinea pig (Bakin and Weinberger, *Br. Res.* 536:271, 1990) in the waking state. This plasticity is characterized as increased response to the CS frequency and decreased responses to other frequencies, including the pretraining best frequency (BF). This experiment determined if such RF plasticity established in the waking state can be expressed under general anesthesia. We obtained frequency RFs in guinea pigs under Nembutal (Nem) or Ketamine/Xylazine (K/X) anesthesia, before and repeatedly after (1 hr. – 8 wks.) a 20-30 trial session of non-BF tone-shock pairings. CS-specific RF plasticity was found in 6/7 (86%) Nem and 6/8 (75%) K/X subjects. In several cases, this plasticity lasted 8 wks. after training. Therefore, learning-induced CS-specific RF plasticity transcends the state under which it was established and can be expressed under anesthesia for very long retention periods. The extent to which RF plasticity in development and following injury involves learning remains to be determined. Supported by ONR # N0014-91-J-1193.

146.15

SINGLE UNIT CS-SPECIFIC RECEPTIVE FIELD PLASTICITY IN THE AUDITORY CORTEX IN AVOIDANCE TRAINING. D. A. South*, J. S. Bakin and N. M. Weinberger. Cntr. Neurobiol. Learn. &

Neurophysiological studies of learning in behaving subjects have benefitted from cluster recordings (CC). For example, classical and instrumental conditioning produce CS-specific receptive field (RF) plasticity in guinea pig primary auditory cortex (AC) (Bakin et al, *Neurosci. Abst.*, 1988, 14, 862; Bakin & Weinberger, *Br. Res.*, 1990, *536*, 271). However, as CL consist of contributions from an indeterminate number of single unit waveforms (SU), the extent to which highly specific plasticity develops within SU in the primary AC and the degree to which CL consists of heterogenous SU plasticity are unknown. To resolve these issues, and also to determine if RF plasticity at the level of SU develops for instrumental conditioning, we used waveform sorting algorithms to obtain SU and CL simultaneously from the primary AC of guinea pigs trained to avoid shock in a running wheel (CS = 10 sec tone, 30-60 trials in one session to a criterion of consistent avoidance). RF were obtained before and after training. We found highly specific RF plasticity within SU, e.g., increased response to CS frequency and decreased response to pretraining best frequency. Also, different patterns of plasticity were observed for SU within cluster recordings. Therefore, highly specific RF plasticity is a property of single cortical cells and cells within the same locale (which yield CL) can exhibit different dynamics due to learning. Supported by ONR N00014-91-J-1193.

146.16

THE DIRECTION OF THE CONDITIONED NATURAL KILLER CELL RESPONSE CAN BE RE-DIRECTED WITH INDOMETHACIN AND/OR HANDLING. C. Rogers*, V. Ghanta, C-M. Hsueh, N. Hiramoto, and R. Hiramoto. Departments of Biology and Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294.

We have used the pairing of camphor odor conditioned stimulus (CS) and injection of poly I:C unconditioned stimulus (US) in a short 3 day single trial conditioning paradigm. Conditioning was done by exposing mice to the CS/US combination on day 0 and reexposing the conditioned animals to the CS on day 2. This results in a conditioned augmentation of the natural killer (NK) cell response. Indomethacin treatment and/or handling stress induced by simply measuring rectal temperature was found to dramatically alter the direction of the conditioned NK cell response. Conditioning of indomethacin treated mice produced a conditioned suppression of the NK cell response mimicking a conditioned tolerance response. If handling stress was superimposed on day 2 the conditioned suppression response was replaced by a conditioned augmentation of the NK cell response. Even with one trial conditioning, drugs and handling stress can serve as additional cues to alter the direction of the conditioned response. The studies also show that the conditioning of the fever response is independent of conditioning of the NK cell response.

This work supported by grants NIH CA37570 and ACS IM509.

LEARNING AND MEMORY: PHARMACOLOGY—ACETYLCHOLINE

147.1

GONADAL STEROIDS AND CHOLINE INTERACT DURING DEVELOPMENT TO IMPROVE RADIAL-ARM MAZE PERFORMANCE AND INCREASE HIPPOCAMPAL NGF IN ADULT RATS. E. Gorry[1], R. Loy[2], J.K. Blusztajn[3]*, W.H. Meck[4], & C.L. Williams[5]. College of Physicians and Surgeons[1] and Depts. of Psychology, Columbia Univ.[4] and Barnard College[5], New York, NY 10027; Canandaigua VAMC and Dept. of Neurology, Univ. Rochester[2], Rochester, NY 14620; Dept. of Pathology, Boston Univ. School of Medicine[3], Boston, MA 02118.

Recent work has shown that choline chloride administered during ED 12-17 and PD 16-30 produces a long-term enhancement of visuospatial memory in male rats. In female rats choline is less effective in producing a memory improvement. To determine whether sensitivity to choline is influenced by endogenous perinatal gonadal steroids in male rats, choline-treated (ED 12 - PD 30) and control rats were either castrated or given sham surgery within 24 hrs of birth. At 50 days the sham surgery rats were castrated. Training on a 12-arm radial maze with 8 baited and 4 unbaited arms revealed an enhancement of overall choice performance by neonatally intact choline-treated male rats compared to the other 3 groups. Following behavioral training, 4 subjects from each of the groups were sacrificed, hippocampal regions dissected, frozen, homogenized, and assayed by ELISA for NGF. Hippocampal NGF was significantly elevated in neonatally intact choline-treated rats. Neonatally castrated rats did not show the choline-induced increase in NGF. These data suggest that organizational effects of perinatal choline on spatial memory may be dependent upon exposure to neonatal steroids. Both factors may play a role in regulating NGF levels in the developing hippocampus. (Supported by PO1 AG09523)

147.2

SELECTIVE DEVELOPMENTAL ACCELERATION OF RELATIONAL CUE NAVIGATION AND REDUCTION OF AGE-RELATED IMPAIRMENTS IN SPATIAL PROCESSING BY PRENATAL SUPPLEMENTATION WITH CHOLINE. N. L. Dallal[1]*, W. H. Meck[1] & C. L. Williams[2], Departments of Psychology, Columbia University[1] and Barnard College[2], New York, NY 10027.

Choline supplementation during prenatal development has been shown to enhance rats' spatial memory as adults. Because prior studies have focused on adult behavior as a consequence of these organizational changes occurring during infancy, the impetus for the current study was to look at these effects as they appear developmentally and to examine the longevity of memory enhancement into old age. A comparison of water maze performance for prenatal choline-treated (embryonic days 12 to birth) versus untreated Sprague-Dawley male and female rats was made at three time-frames spanning postnatal days 18-28. The results indicated that the development of relational, but not proximal, cue use is advanced by as much as three days for choline-treated rats. Due to the involvement of the hippocampus in relational cue use, these results support previous findings that this late developing structure is sensitive to prenatal choline supplementation. Additionally, repeated-acquisition water maze testing with these same groups of rats at 21 months showed that the level of age-related impairments in spatial working-memory function was significantly reduced for both male and female rats treated prenatally with choline.These data support the proposal that prenatal choline supplementation offers advantages for hippocampal-dependent memory function across the entire lifespan. (Supported by PO1 AG09523)

147.3

CHOLINE SUPPLEMENTATION DURING PRENATAL DEVELOPMENT INOCULATES AGAINST THE AGE-RELATED DECLINE IN SPATIAL MEMORY OF 24 - 26 MONTH OLD RATS. W.H. Meck & C.L. Williams*. Depts. of Psychol., Columbia Univ. & Barnard College, New York, NY 10027.

Male Sprague-Dawley rats were supplemented with choline during embryonic days 12-17 by adding choline chloride to the drinking water of their dams. Dams consumed 1 mmol/day. Controls received choline-free water. When these rats were trained on a 12-arm radial maze (with 8 baited and 4 unbaited arms) at 90 days of age, prenatal choline-treated rats made fewer working and reference memory errors than controls throughout training (Days 1-30). During reacquisition of this maze task at 24 months of age, prenatal choline-treated rats were more accurate in their choice performance than age-matched controls. Following this retraining phase, rats (now 26 months old) were tested on a retention-interval task where a delay (1.25, 2.5, 5, or 10 hrs) was inserted between the 4th and 5th correct choice. After waiting in their home cage, the rats were placed back on the maze to complete the trial. Performance during delay trials was evaluated relative to performance during normal training sessions which were interspersed with delay trials. All rats showed a reliable increase in both working and reference memory errors as a function of the length of the retention interval. Choline-treated rats showed a significantly lower rate of forgetting than control rats as indexed by working memory errors and made reliably fewer reference memory errors at all delays. These data demonstrate that prenatal choline supplementation causes permanent organizational changes in brain and behavior that appear to reduce or delay the normal age-related impairment in spatial memory. (Supported by PO1 AG09525).

147.4

SUPPLEMENTATION WITH CHOLINE *IN UTERO* REDUCES BRAIN CHOLINE ACETYLTRANSFERASE (CAT) ACTIVITY DURING POSTNATAL DEVELOPMENT IN THE RAT. D.A. Jackson[1,*], W.H. Meck[2], C.L. Williams[3] & J.K. Blusztajn[1]. [1]Dept. of Pathology, Boston Univ. Sch. of Med., Boston, MA 02118; Depts. of Psychology, [2]Columbia Univ. & [3]Barnard College, New York, NY 10027.

Supplementation of rats with choline during embryonic days (ED) 12-17 has been shown to facilitate spatial memory processes in adulthood. Therefore we hypothesized that supplemental choline may alter the development of the brain cholinergic system. Sprague-Dawley rats consumed 1 mmol/day of choline chloride in their drinking water during days 12-17 of pregnancy in addition to the choline in their chow. Controls received choline-free water. All of the pups were cross-fostered to control females. CAT activity, determined at postnatal day (PD) 35, was significantly reduced in rats supplemented with choline, by 14.1% and by 8.8% in the striatum and in the hippocampus, respectively. Cortical CAT activity tended also to be reduced. At PD90, the average CAT activity was lower in rats supplemented with choline *in utero* than in controls, although this effect was not significant in any of the above brain regions. The overall reduction of CAT activity in ED12-17 choline supplemented animals was statistically significant ($p<0.01$) by three-way ANOVA using choline treatment, age, and brain region as the independent variables. The results confirm our previous observations obtained with rats treated with choline during the ED12-PD30 period and indicate that the reduced CAT activity in the developing brain, in rats supplemented with choline perinatally, provides a biochemical correlate of the behavioral effects of this treatment. (Supported by P01 AG09525.)

147.5

NEUROCHEMICAL SUBSTRATES OF MEMORY: IMPLICATIONS OF CHRONIC PHYSOSTIGMINE INFUSION. R.A. Booth, D.J. Jenden*, R.W. Russell, K.M. Rice, M. Roch and A.S. Chang, Dept of Pharmacology and Brain Research Institute, UCLA, Los Angeles CA 90024.

Osmotic minipumps were implanted subcutaneously in rats to deliver physostigmine (PHY) (1.5 $mg.kg^{-1}.day^{-1}$) for 49 days. Neurochemical, physiological and behavioral variables were measured. Using a modified radiometric method, brain and blood ChE activity were ~20% of control and did not change significantly during the infusion. Acetylcholine (ACh) and choline (Ch) levels and ACh turnover were estimated in cerebral cortex (CTX), hippocampus (HIP) and striatum (STR) by measuring $[^2H_0]$- and $[^2H_4]$-Ch and ACh 1 min after IV $[^2H_4]$-Ch (20 $\mu mol.kg^{-1}$). Levels of total ACh in CTX, HIP and STR rose significantly by 20, 13 and 7% ($p \leq 2.7 \times 10^{-2}$) and levels of total Ch fell significantly by 13, 8 and 10% ($p \leq 3.7 \times 10^{-2}$). $[^2H_4]$-Ch uptake and $[^2H_4]$-ACh synthesis were affected differentially. The greatest decrease in ACh synthesis was seen in HIP (-14%; $p = 1.0 \times 10^{-3}$); the mole ratio of $[^2H_4]$-ACh was reduced by 25% ($p < 10^{-4}$) in HIP and by 12% ($p = 1.6 \times 10^{-3}$) in CTX. Ch uptake was less only in the CTX (-14%: $p = 1.2 \times 10^{-2}$). Core body temperature and weight fell after day 1 of infusion but showed tolerance, returning to control levels by 14 days. Rates of habituation in rearing and locomotion were significantly reduced at 13 days ($p < 10^{-4}$). Retention in the conditioned avoidance paradigm was impaired after 14 days of PHY infusion when the infusion began 1 day after training. At 14 days, controls showed positive transfer of memory ($p = 4.0 \times 10^{-4}$) and made fewer errors than the experimental animals ($p = 7.4 \times 10^{-7}$). Overall, the neurochemical changes were greatest in CTX and HIP, regions associated with higher cognitive function. Since these neurochemical variables did not change during treatment, physiological and behavioral tolerance are presumably the result of other processes, perhaps receptor down-regulation. Supported by DAMD-88-C-8045 and MH 17691.

147.6

E2030, A NOVEL ACETYLCHOLINESTERASE INHIBITOR (1): NEUROCHEMICAL STUDIES ON THE CENTRAL NERVOUS SYSTEM. Y. Yamanishi*, T. Kosasa and T. Kaneko. Tsukuba Res. Lab., Eisai Co. Ltd., 5-1-3 Tokodai Tsukuba-shi, Ibaraki 300-26, Japan.

E2030, 3-[2-[1-(1,3-dioxolan-2-ylmethyl)-4-piperidyl] ethyl]-2H-3,4-dihydro-6-methoxy-1,3-benzoxazin-2,4-dione hydrochloride, is a novel acetylcholinesterase (AChE) inhibitor. The *in vitro* IC_{50} of E2030 for AChE was 155.4 nM. E2030 inhibited AChE 2700-fold more selectively than butyrylcholinesterase. The mode of inhibition was non-competitive and the inhibition was reversible. In an *ex vivo* study, E2030 (80 mg/kg, p.o.) showed slight but significant inhibition of brain AChE in rats. The compound increased the rat brain acetylcholine (ACh) content and counteracted the decrease in ACh content induced by scopolamine or a lesion of the nucleus basalis magnocellularis, dose-dependently, at doses of 5-20 mg/kg, p.o. The microdialysis studies revealed that E2030 increased the extracellular ACh, norepinephrine and dopamine metabolites (DOPAC and HVA) in the brain of rats. These results indicate that E2030 increases ACh content not only in the intact but also in the cholinergic hypofunction models of rats, and furthermore, that E2030 may stimulate monoamine metabolism. E2030 may serve as a new type of acetylcholinesterase inhibitor for Alzheimer therapy.

147.7

E2030, A NOVEL ACETYLCHOLINESTERASE INHIBITOR (2): BEHAVIORAL STUDIES ON THE CENTRAL CHOLINERGIC SYSTEM. H. Ogura, K. Uchikoshi, Y. Furuya, T. Kosasa, Y. Yamanishi and T. Kaneko*. Tsukuba Research Laboratories, Eisai Co. Ltd., Tsukuba-shi, Ibaraki 300-26, Japan.

These studies, using behavioral tests in rats, were undertaken to explore the effects of E2030, a newly synthesized acetylcholinesterase inhibitor, on the central cholinergic system. E2030 and tetrahydroaminoacridine (THA) elicited yawning significantly at more than 4 mg/kg, p.o. E2030 caused only mild fasciculation, a peripheral cholinergic sign, at the highest dose, 16 mg/kg, p.o., whereas THA produced the symptom at more than 4 mg/kg, p.o. Scopolamine, but not methylscopolamine, inhibited E2030-induced yawning and haloperidol partially blocked it, suggesting that central cholinergic and, in part, dopaminergic mechanisms may be involved in E2030-induced yawning. Secondly, we examined the effects of E2030 on scopolamine-induced impairment in two different learning tasks, the 8-arm radial maze and the 3-panel runway. Scopolamine (0.5 mg/kg, i.p.) disrupted both performances, i.e. increased errors. Pretreatment with E2030 significantly counteracted scopolamine's effect at 1 mg/kg, p.o., in the two tasks. THA antagonized the deficit at 2 mg/kg, p.o. in the radial maze, but was not effective in the 3-panel runway at the range of 1-3 mg/kg. These results indicate that E2030 has a potent cholinergic activity and is more centrally selective than THA.

147.8

GLYCERYLPHOSPHORYLCHOLINE PREVENTS SCOPOLAMINE-INDUCED AMNESIA AND ENHANCES HIPPOCAMPAL CHOLINERGIC TRANSMISSION IN THE RAT. C.Missale*, S.Sigala, P.Rizzonelli, E.Zanelli, and P.F.Spano. Inst. of Pharmacology, Univ. of Brescia Sch. of Med., Brescia, Italy.

The effects of L-α-glycerylphosphorylcholine (GPC) on scopolamine-induced memory impairment and on hippocampal acetylcholine (ACh) release were investigated in rats. Oral administration of GPC 3h before aquisition of a passive avoidance response prevented the learning impairment induced by scopolamine (1 mg/kg). Similarly, the retrograde amnesia induced by scopolamine was also completely blocked by the drug. These effects were dose-dependent (50-600 mg/kg) with a maximum at 300 mg/kg. The effects of oral GPC administration on [3H]ACh release in rat hippocampal slices were then studied. K^+-stimulated [3H]ACh release was highly potentiated by the drug. The dose-response and time curves of this effect were superimposable on those found in the behavioural test with a maximum with the drug given at 300 mg/kg 3h before killing. The data suggest that the behavioural effects of GPC may be related to its property to increase hippocampal ACh release.

147.9

TIME-GRADIENT OF POST-TEST VULNERABILITY TO SCOPOLAMINE-INDUCED AMNESIA IN MICE : CORRELATION WITH THE TIME-COURSE OF MEMORY TEST-INDUCED ACTIVATION OF ASCENDING CHOLINERGIC PATHWAYS. T.P.Durkin* and A. Toumane. Lab. Neurosciences Comportementales et Cognitives, CNRS URA n° 339, Univ. Bordeaux I, Av. des Facultés, 33405 Talence, France.

Our previous studies showed that the initial session of acquisition of a concurrent spatial discrimination reference memory task in mice induces a long-lasting (up to 3 hrs) post-test activation of the cholinergic neurones of the ascending septo-hippocampal and nBM-cortical cholinergic pathways (Toumane et al., 1988, Behav. Brain Res. 30, 225-234). In order to reply to the question: is this testing-induced activation of cholinergic neurones a critical step for long-term retention of information?, we have conducted a study examining the effect of blockade of central muscarinic receptors at various times post-test. In comparison to groups injected with either scopolamine-MBr or saline, the injection of scopolamine-HCl (1 mg/Kg i.p.) during the period 30sec-3hrs post-test produced significant deficits in 24 hr. retention performance (amnesia). The severity of this amnesia decreased progressively as a function of the time of treatment following learning. No such amnesia was observed if the scopolamine injection was delayed from between 3-6 hrs post-test, attesting to the absence of any significant long-term pro-active effects of scopolamine on the ability to perform the retention test. These results show that **there exists a time window of up to three hours post-learning for vulnerability to the amnesiant effects of scopolamine.** This time window corresponds to **the time course of the testing-induced activation of the ascending cholinergic pathways** observed previously, and more particularly, to that of **the nBM-cortical cholinergic pathway.**

147.10

EFFECTS OF BICUCULINE ON SCOPOLAMINE-INDUCED AMNESIA. S. E. Cruz-Morales* and R. A. Prado-Alcala. Behav. Pharmacol. Program, ENEP-Iztacala and Physiol. Dept., Med. Sch., Natl. Univ. of Mexico, P.O.B. 314, Tlalnepantla, Edo. de Mexico, 54030, México.

The aim of this experiment was to study the interaction of GABAergic and cholinergic systems in memory processes. Male Wistar rats (250-350 g) were trained with 2.5 mA, on a passive avoidance task and tested 24 hr later. The rats were assigned to one of 17 groups: two groups were injected 5 min post-training (ip) with scopolamine (SCP) (4 or 8 mg/kg); six groups with bicuculline (BI) (0.25, 0.5, 1.0, 2.0, 4.0 or 6.0 mg/kg) six groups received 8 mg/kg of SCP in combination with BI (0.25, 0.5, 1.0,2.0, 4,0 or 6.0 mg/kg); and three control groups, one injected with isotonic saline, an intact group and a group with no shock. SCP induced amnesia in a dose dependent way; BI induced amnesia with low doses. Except the group with 1.0 of BI, all combinations of BI and SCP reversed the SCP-induced amnesia.
(Supported by CONACYT P228CC0X891608)

147.11

POST-TRAINING INSULIN ATTENUATES SCOPOLAMINE-INDUCED AMNESIA. C. MESSIER*, J. SIF, and C. DESTRADE. Lab. de Neurosci. Cognitives et Comport., URA. CNRS n° 339, Université de Bordeaux I, FRANCE and * School of Psychology, U. of Ottawa, (ONT) CANADA K1N 6N5.

Scopolamine is a well-known amnestic drug. Most of the agents that attenuate scopolamine amnesia can also improve memory when injected alone. We report here that insulin can abolish scopolamine-induced amnesia even though insulin per se has little memory-improving action. Male Balb/c mice were trained in an appetitive bar-pressing task. Immediately after the mice had completed 15 trials, scopolamine hydrochloride was injected (0.1, 0.2, 0.3, 0.6, 1.0 mg/kg i.p.) either alone or in conjunction with insulin (0.8 I.U./kg, s.c.). The retention of the animals for the training was tested the next day. The 0.6 and 1.0 mg/kg doses of scopolamine (but not the lower doses) produced an amnesia for the bar-pressing task training. This amnesia was significantly attenuated by the insulin injection. However, the injection of insulin alone had no effect on memory processes. These results show that insulin can protect from scopolamine amnesia. It is not clear by which mechanisms insulin has this action since insulin has been shown to reduce acetylcholine (ACH) turnover. This reduction should have accentuated the effect of scopolamine which effectively depletes acetylcholine stores. However insulin may also modulate scopolamine amnesia through its CNS receptors.

147.12

GALANIN MIMICS SCOPOLAMINE IN DISRUPTING DELAYED NON-MATCHING TO SAMPLE (DNMTS) IN RATS J.K. Robinson* and J.N. Crawley. NIMH, Building 10, Room 4N214, Bethesda, MD 20892.

The neuropeptide galanin coexists with acetylcholine in the rat medial septal region and inhibits cholinergic functions in vitro and in vivo (Fisone, et al, PNAS, 1987). The water maze, T-maze, and operant DNMTS are memory paradigms sensitive to lesions of the septo-hippocampal pathway and to cholinergic antagonists, especially scopolamine. In the present study, the effects of galanin (.5, 2, and 5 ug, i.c.v.) and scopolamine (.06, .12, .25, and 1.0 mg/kg, i.p.) on spatial DNMTS were compared. Both compounds impaired choice accuracy in a dose-dependent but delay-independent manner, but did not alter the frequency of sample-phase discrimination errors. Both compounds decreased trial completion rate and moderately decreased retention-interval VI lever pressing at the largest dose. The effects of intraventricularly administered galanin and intraperitoneally administered scopolamine therefore appear to be qualitatively analogous in this spatial memory paradigm.

147.13

DIFFERENTIAL INVOLVEMENT OF STRIATAL REGIONS IN MEMORY CONSOLIDATION: EFFECTS OF CHOLINERGIC BLOCKADE. G. Quirarte, M.A. Guevara, and R.A. Prado-Alcalá* Psychol. Sch., Univ. of Guadalajara, Physiol. Dept., Med. Sch., and Psych. Sch., Natl. Univ. of México, México, D.F. 04510.

Cholinergic blockade of the general area of the anterior caudate-putamen (CPU) produces amnesia. We now report the effects of atropine injections into each of four discrete regions of the anterior CPU (dorsomedial, dorsolateral, ventromedial, and ventrolateral), and into three regions of the posterior CPU (dorsomedial, dorsolateral, and ventral). Rats were trained in a passive avoidance task and 2 min. later injected, unilaterally, with atropine (60 µg/µl) into one of the seven regions. Appropriate control groups were also studied. A test of retention was given 24 hours later. A strong amnesic effect was found in the ventral regions, a milder amnesia in the dorsal-anterior regions, and no effect in the postero-dorsal CPU.
(Supported by DGAPA IN202791 and CGEP).

147.14

EFFECT OF FR121196, A NOVEL COGNITIVE ENHANCER, ON THE MEMORY IMPAIRMENT OF RATS IN PASSIVE AVOIDANCE, WATER MAZE AND RADIAL ARM MAZE TASKS. N. Matsuoka*, N. Maeda, M. Yamazaki, Y. Ohkubo and I. Yamaguchi. Basic Research Group, Tsukuba Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 5-2-3 Tokodai, Tsukuba, Ibaraki 300-26, JAPAN

We investigated effects of FR121196 in various animal models of dementia, and the results were compared with those of methamphetamine and physostigmine. FR121196 (N-(4-acetyl-1-piperazinyl)-4-fluorobenzenesulfonamide), a newly introduced cognitive enhancer, ameliorated the failure of memory retention in passive avoidance task seen in young male rats subjected to scopolamine treatment, nucleus basalis magnocellularis (NBM) lesion or in aged rats (24-26 months old) with bell-shaped dose-response curves in doses ranging from 0.1 to 10 mg/kg. Similar dose-response curves were obtained with methamphetamine (0.1-10 mg/kg). On the other hand, physostigmine (0.01-1.0 mg/kg) attenuated the amnesia in scopolamine-treated rats but hardly affected that in NBM-lesioned or aged rats. In the water maze task, FR121196 significantly restored the acquisition deficits of spatial memory observed in young animals with NBM lesion or in aged rats. In the radial arm maze task, behavioral indices of spatial memory, such as first correct choices and numbers of errors, were impaired following an injection of scopolamine or lesions of medial septum (MS) or fimbria-fornix (FF). FR121196 ameliorated the scopolamine-induced spatial memory deficit with a bell-shaped dose response curve, while methamphetamine rather tended to aggravate the deficit. These two drugs had little effect on the memory deficits in MS- or FF-lesioned rats. On the other hand, physostigmine ameliorated the memory deficits in scopolamine-treated and MS-lesioned rats but not in FF-lesioned rats. These results suggest that intact septo-hippocampal cholinergic neurons are necessary for FR121196 to ameliorate the memory deficits.

147.15

AF64A IMPAIRS TASTE AVERSION AND SPATIAL LEARNING IN A MORRIS WATER MAZE TASK. K. Opello *, S. Ackerman, R.W. Stackman, T.J. Walsh. Dept. of Psychology, Rutgers Univ. New Brunswick, NJ 08903.

AF64A (ethylcholine aziridinium ion) produces a long-term hypofunction of the cholinergic septohippocampal pathway and spatial working memory deficits. However, the effects of AF64A on non-spatial memory and reference memory have not been well-characterized. The following studies examined whether AF64A would differentially affect the acquisition or extinction of a non-spatial task; conditioned taste aversion (CTA), or the performance of spatial or non-spatial reference memory tasks in the Morris water maze. Male Sprague Dawley rats were injected (icv) with either 2 ul artificial CSF or 2.5 nmol/side AF64A. During CTA water-deprived rats were injected (i.p.) with either 0.15 M LiCl or the NaCl vehicle, either immediately (0 delay) or 4 hrs (4 hr delay) after exposure to a preferred 0.2 % saccharin solution. At the 0 delay condition the AF64A-treated group exhibited a comparable aversion to the saccharin solution on the first day of testing but an accelerated extinction of the CTA. In contrast, with the 4 hr delay the AF64A group exhibited a significantly attenuated CTA but a rate of extinction that was comparable to the controls. Cognitive impairments in these animals were further assessed using both a standard spatial Morris water maze task and a non-spatial cued-version of the task. AF64A significantly impaired acquisition of the spatial task as evidenced by longer escape latencies and pathlengths and less time spent in the target quadrant during a one minute probe trial. However, AF64A had no effect on the acquisition or performance of the cued version of the task. These cognitive deficits were associated with a significant, 42% decrease in high affinity choline uptake in the hippocampus and with no alterations of uptake in the striatum. These data demonstrate that AF64A produces memory impairments that depend upon the temporal and spatial demands of the task.

147.16

A POTENTIAL PRIMATE MODEL OF DISRUPTIVE BEHAVIOR OF DEMENTIA. C.K. Fleming, R.F. Schlemmer, Jr., J.E. Young, N.L. Katz, and J.M. Davis*. Dept. of Pharmacodynamics, Univ. of Illinois at Chicago and Research Dept., Illinois State Psychiatric Institute, Chicago, IL 60612.

The centrally active antimuscarinic scopolamine (SCOP) has been used to model dementia because it induces a disruption of short-term memory in humans and animals. In our laboratory SCOP, 0.01-0.03 mg/kg, disrupts responding in the delayed match-to-sample paradigm in monkeys. This study was designed to examine the behavioral effects of SCOP in free-ranging aged vs. non-aged adult monkeys. Parallel experiments were conducted in two stumptail macaque (Macaca arctoides) social colonies of 4 females and 1 male each. The females in one group were aged (≥ 20 yr) and, in the other, the females were younger adults (< 20 yr). Following baseline observation, the females from each colony received 7 acute doses of SCOP HBr, 0.001-0.05 mg/kg, in a cross-over design. Each dose was given intramuscularly 15 min prior to a 1 hr behavioral observation session. A blind observer scored the behavior of each monkey using a checklist of more than 40 social and solitary behaviors. SCOP induced dose-dependent increases in locomotion and checking (visual scanning) in both age groups; aged monkeys exhibited greater sensitivity to the drug. Social interaction, particularly social grooming initiated by treated monkeys, was disrupted. SCOP induced an increase in distancing of aged monkeys from other colony members suggestive of social withdrawal. There was no significant change in aggression. These results suggest that increased locomotion and hypervigilance may exemplify a state of agitation in monkeys; whereas the disruption of social grooming represents a deficit in social interaction. Hence, this paradigm may serve as an animal model for the study of disruptive behavior and social deficits of dementia.

147.17

ESTABLISHMENT AND IMPLEMENTATION OF AN AUTOMATED SYSTEM FOR EVALUATING A DELAYED MATCH-TO-SAMPLE TASK IN STUMPTAIL MACAQUE MONKEYS. N. L. Katz*, D. C. Jolly, R. F. Schlemmer, Jr., H. J. Haigler and J. M. Davis. Dept. of Pharmacodynamics, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612 and Research Dept., Illinois State Psychiatric Institute, Chicago, IL 60612.

We will describe a computer controlled system used to train and test stumptail macaque (Macaca arctoides) monkeys on a delayed match-to-sample (DMTS) task and present preliminary data collected thus far. The DMTS is an operant behavioral procedure which requires monkeys to discriminate a particular stimulus from several choices at varying time intervals after its initial presentation. The data from a DMTS allow quantitative assessment of attention to environmental stimuli, reaction time, and recent memory function. In 2 young adult female monkeys trained to criterion performance, the percentage of correct matches (i.e., success ratio) declined only slightly as a function of delay length, indicating involvement of memory in performing the task. Furthermore, in these animals, 0.015 mg/kg im of the acetylcholine antagonist scopolamine (SCO) did not affect DMTS performance at delay intervals less than 5 sec. Higher doses of SCO consistently reduced the success ratio at 2, 5, and 10 but not 0 sec delay. The amnestic effect of SCO was attenuated by the cholinergic agonist physostigmine (PHY). This computer controlled implementation of the DMTS shows promise as a means for studying neuropharmacologic modulation of memory and attention, and for the pre-clinical screening of drugs for the treatment of dementia and other age-, disease-, or trauma-related cognitive dysfunctions. Also, the stumptail macaque is a highly social species. Extensive systematic studies of the effects of drugs on the social behavior of the stumptail macaque are routinely conducted in this laboratory. The DMTS paradigm should complement studies of drug-induced changes in free ranging social and solitary behavior.

147.18

EFFECTS OF AN M_1 MUSCARINIC AGONIST, YM796, ON DISTURBANCE OF LEARNING BEHAVIOR IN CEREBRAL ISCHEMIC RODENTS. Koichiro Takahashi*, Mitsuko Oovama, Takashi Yamaguchi, Sachiko Saito, Minoru Yamamoto. Applied Pharmacology & Development Laboratories, Yamanouchi Pharmaceutical Co., Ltd., Itabashi-ku, Tokyo 174, Japan.

An M_1 muscarinic agonist, YM796((-)-S-2,8-dimethyl-3-methylene-1-oxa-8-azaspiro[4.5]decane L-tartarate monohydrate)[1], ameliorates the disturbance of learning behavior in nucleus basalis magnocellularis-lesioned and scopolamine-treated rats [2]. The present study examined the effects of YM796 on passive avoidance response in gerbils subjected to 3 repeated 2-min occlusions of the bilateral carotid arteries, and middle cerebral artery-occluded rats (MCA rats). Latency of step-through in the passive avoidance behavior was shortened in both cerebral ischemic rodents. Neuronal degeneration of the CA1 region of the hippocampus in ischemic gerbils, and that of cerebral cortex and striatum was observed in MCA rats. YM796 in a dose of 0.1 mg/kg (p.o.) or doses of 0.1-1 mg/kg (p.o.) ameliorated the shortened latency in gerbils and MCA rats, respectively, without affecting spontaneous movement and pain response. Neuronal degeneration was not changed by administration of YM796. These results suggest that YM796 improves the disturbance of learning behavior presumably due to activation of M_1 receptors in the brain including the cerebral cortices and hippocampus of the cerebral ischemic rodents.

[1]Wei, H. et al., Life Sci. 50, 355-363, 1992
[2]Wanibuchi, F. et al., Soc. Neurosci. Abstr. 17, 488.5, 1991

147.19

PERIPHERAL ADMINISTRATION OF MUSCARINIC RECEPTOR ANTAGONISTS MODULATE HIPPOCAMPAL ACETYLCHOLINE RELEASE: POSSIBLE RELATION TO MEMORY PROCESSES. A. Wilson*, J. Richard, R. Quirion, & N.M. White. Douglas Hospital Research Centre and Department of Psychology, McGill University, 1205 Dr. Penfield Avenue, Montreal, Quebec, Canada H3A 1B1.

Previous evidence indicates that peripheral post-training injections of a selective cholinergic M2 receptor antagonist, AFDX 384, enhances retention on a task sensitive to lesions of the hippocampal system (win-shift). The extent to which subcutaneous injections of AFDX 384 modulates the release of acetylcholine in the CA1 region of the hippocampus was examined using the in vivo microdialysis technique. Male Long-Evans rats were dialyzed using Ungerstedt ringer buffer containing physostigmine (75 uM) and ACh levels were determined using gas chromatography-mass spectometry. Doses of AFDX 384 which improved performance in the win-shift task (1.0 mg/kg) did not significantly alter measurable hippocampal ACh release; higher doses of the drug increased ACh release to a minor extent (125% and 150% over basal levels, 10.0 and 2.0 mg/kg respectively). For comparison, we observed that at 5.0 mg/kg atropine sulfate increased ACh release 500% over basal levels. These results indicate that peripheral injections of rather selective M2 receptor antagonists such as AFDX 384 are able to modulate ACh release in the CA1 region of the hippocampus, but that the effective doses mediating the memory modulating effect and ACh release in hippocampus do not coincide. This may be due to the sensitivity of the methods used here which only monitor ACh overflow. While preliminary evidence indicates that intrahippocampal injections of AFDX 384 also enhance retention on the win-shift task we cannot rule out the possibility that the memory modulating effect of peripheral injections of the drug are mediated by other mechanisms.

147.20

INTERACTION BETWEEN THE MUSCARINIC CHOLINERGIC AND THE D2-DOPAMINERGIC SYSTEMS ON THE MODULATION OF MEMORY STORAGE I.B. Introini-Collison*, A. Gasbarri, M. Packard and J.L. McGaugh. Center for Neurobio. of Learning & Memory and Dept. of Psychobio., U. of Calif., Irvine, CA 92717.

Evidence exists indicating that both the muscarinic cholinergic and the dopaminergic systems are involved in the modulation of memory storage. Posttraining administration of muscarinic cholinergic receptor agonists facilitate memory while antagonists induce amnesia. The D2 antagonist sulpiride appears to impair memory. The present experiments examined the interaction between the two systems on the modulation of memory storage. Male CD1 mice (60 days old) were trained in an inhibitory avoidance and a Y-maze visual discrimination task. Retention was evaluated 48 hrs after training. Sulpiride (10.0, 30.0 or 100.0 mg/kg; ip) impaired retention in both tasks in a dose-dependent manner. In the inhibitory avoidance task, the muscarinic agonist oxotremorine (35.0, 50.0 or 70.0 µg/kg; ip) significantly prevented sulpiride-induced impairment of memory. In the Y-maze visual discrimination task, sulpiride (10.0, 30.0 or 100.0 mg/kg; ip) prevented oxotremorine (35.0, 50.0 or 70.0 µg/kg; ip)-induced memory facilitation. Although both the muscarinic cholinergic and the D2 systems modulate retention of both tasks, the two systems appear to interact differently in influencing retention in the two learning tasks.

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147.21

NB LESION-INDUCED PA DEFICIT MAY BE MEDIATED VIA AMYGDALA. P. Riekkinen Jr*, J. Sirviö, M.Riekkinen and P. Riekkinen. Department of Neurology, University of Kuopio, 70211 Kuopio, Finland.

The present study was designed to elucidate the role of cholinergic innervation of amygdala (AG) in inhibitory avoidance (passive avoidance, PA) and spatial reference memory (water maze, WM) performance. Nucleus basalis (NB) quisqualic acid lesioning decreased ChAT activity in the AG and frontal cortex, and impaired PA retention and reversal WM performance. AG lesioning impaired PA retention but did not affect PA or WM performance in NB-lesioned rats. Scopolamine (a muscarinic antagonist) and mecamylamine (a nicotinic antagonist) dose dependently impaired PA and WM performance in intact rats. The cholinergic antagonist effects on PA performance were blocked (low antagonist doses) or decreased (high antagonist doses) by lesions of the AG. AG lesions did not modulate the performance impairing effect of cholinergic antagonist on WM performance. The present results suggest that the NB cholinergic projection may modulate the functioning of amygdala via muscarinic and nicotinic receptors.

147.22

MECAMYLAMINE DELAYS ACQUISITION OF THE CLASSICALLY CONDITIONED NM RESPONSE BUT DOES NOT AFFECT NICOTINIC RECEPTOR BINDING IN RABBIT BRAIN. Y.-T.Li[1]*,D.S.Woodruff-Pak[1], A. Kazmi[2], & W.R.Kem[2]. Dept. of Psych.[1], Temple Univ., Philadelphia, PA 19122 & Dept. of Pharmacol. & Therapeutics[2], Univ.of Florida, Gainesville, FL 32610.

The septo-hippocampal acetylcholinergic system plays an important modulatory role in classical conditioning of the nictitating membrane (NM) response in rabbit. The effects of low doses (0.5mg/kg, sc) of the nicotinic receptor antagonist, mecamylamine, were compared to vehicle, in 18 young rabbits (7-8 mo.) classically conditioned for 10 sessions in the delay NM conditioning paradigm with a 750 msec interval between the tone CS and corneal airpuff US that were paired or explicitly unpaired. Nicotinic receptor densities were measured by vacuum filtration after incubating the synaptosomal membranes with [^{3}H]methylcarbamylcholine for one hour at 0°C. Mecamylamine prolonged acquisition of conditioned responses (CRs) so that young rabbits learned like older rabbits. Mecamylamine-treated young rabbits took a mean of 780 trails to learning criterion, whereas the saline-treated ones attained criterion in 422 trails ($T=3.7427;p<.05$). The percentage of conditioned responses (CRs) were also strikingly different ($F_{[2,15]}=9.31;p<.003$). Motor unconditioned responses were not affected by the compound ($F_{[2,15]}=1.29;p>.3$). Nicotinic receptor concentrations in cerebral cortex, cerebellum, and remaining brain tissues were 40.2, 7.4, and 64.6 fmol/mg membrane protein, respectively for saline-treated rabbits. For mecamylamine-treated rabbits the corresponding B_{max} estimates were 40.5, 7.5, and 57.3 fmol/mg protein. The mean estimates of these two groups were not significantly different. Thus, the deleterious effect of mecamylamine upon NM classical conditioning was not accompanied by a measurable change in nicotinic receptor concentration, at least for these three brain regions.

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147.23

CHRONIC NICOTINE-MECAMYLAMINE INTERACTIONS AND RADIAL-ARM MAZE PERFORMANCE IN RATS. E.D. Levin*, S.J. Briggs, N.C. Christopher and J.E. Rose. Nicotine Research Laboratory, Department of Psychiatry, Duke University and VA Medical Centers, Durham, NC 27705

Acute or chronic nicotine administration has been found to improve working memory performance, whereas acute doses of the nicotinic antagonist mecamylamine have been found to impair it. In the current study, the effects of chronic nicotine and mecamylamine on working memory in the radial-arm maze were examined in adult female Sprague-Dawley rats implanted subcutaneously with osmotic minipumps delivering saline (N=12), 12 mg/kg/day of nicotine (N=12), 3 mg/kg/day of mecamylamine (N=11) or these doses of nicotine and mecamylamine (N=12). Replicating our earlier work, nicotine was found to improve working memory performance ($p<0.05$) Concurrent administration of mecamylamine with nicotine eliminated the facilitation. Surprisingly, unlike acute mecamylamine, chronic mecamylamine did not cause a deficit in memory performance and actually improved memory performance during the first week of administration ($p<0.001$). This effect subsequently dissipated. These results demonstrate that chronic nicotine-induced facilitation of working memory performance is a robust effect which appropriately is blocked by concurrent administration of a nicotinic antagonist. The facilitation of working memory performance by chronic mecamylamine is an interesting effect which deserves further investigation. (Supported by funding from Abbott Labs.)

147.24

LEARNING AND MEMORY EFFECTS OF LOBELINE, A NICOTINIC AGONIST, IN RATS. M.W. Decker*, M.J. Majchrzak, and S.P. Arneric. Neuroscience Research, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064.

Lobeline crosses the blood brain barrier readily and binds to nicotinic sites, but does not produce the nicotine cue (Reavill et al., 1990, Neuropharmacol., 29: 619), This suggests that the behavioral effects of lobeline and nicotine differ. However, these differences have not been well characterized. Since we have found that nicotine enhances retention of inhibitory avoidance (IA) training in rats (Majchrzak and Decker, 1991, Neurosci. Abstr., 17: 1236) and improves the performance of rats with septal lesions in a spatial discrimination Morris water maze (Decker et al., 1992, Br. Res., 572: 281), we were interested in determining if lobeline had similar effects.

Male, Long-Evans rats were used in these experiments. For the IA experiment, lobeline (0, .62, 1.9, 6.2, and 19 µmol/kg IP, n=15/group) was administered immediately after training, and retention was assessed 24 h later. Lobeline-treated rats (19 µmol/kg) took significantly ($p<.05$) longer to enter the shock compartment on the test day than saline-treated rats. In the water maze experiment, rats with radiofrequency and sham lesions of the septal area learned to use spatial cues to discriminate an escape platform from a similar appearing false platform. Lobeline (0, 1.9, and 19 µmol/kg IP) was administered 15 min. before training on each of 4 consecutive days (n=4-5/group). Lobeline significantly improved performance of rats with septal lesions (lobeline by lesion interaction, $p<.025$), particularly at the lower dose. This finding was confirmed when rats were retrained using new spatial locations and saline and low dose treatments were reversed in a crossover design.

Thus, lobeline's effects on learning and memory may be similar to nicotine's. Coupled with previous reports that lobeline does not produce the nicotine cue, our results suggest that nicotinic receptors involved in the modulation of memory processes may be distinct from those involved in producing the nicotine cue.

NEURAL PLASTICITY I

148.1

TEMPERATURE-DEPENDENT BLOCK OF LTP BY THE NO SYNTHASE INHIBITOR L-NARG. Y.-G. Li, M.L. Errington, J.H. Williams and T.V.P. Bliss*. Nat. Inst. Med. Res., London, NW7, UK.

We have previously failed to detect an effect of the Nitric Oxide (NO) synthase inhibitor Nʷ-Nitro-L-arginine (L-NARG) on the induction of LTP in area CA1 of rat hippocampal slices maintained at 29°C (Abstr. Soc. Neurosci. 17, 951,1991). We have now confirmed recent reports that L-NARG blocks LTP in CA1 at room temperature (24°C). CA1 minislices were prepared from male Sprague-Dawley rats (70-100g), and maintained in an interface chamber. At 29°C L-NARG (100µM, Sigma) had no effect on the magnitude of tetanically-induced LTP; the normalized change in population EPSP slope 60 min post-tetanus relative to pre-tetanus values was 47.8 ± 6.7% in control medium (mean ± sem, n=8), and 53.3 ± 7.8% (n=8) in slices exposed to L-NARG for 2-4 hours pre-tetanus. In contrast, at 24°C L-NARG blocked LTP (% change 60 min post-tetanus was -14.9 ± 4.9% (n=8) compared to 29.2± 1.7% in control medium (n=8)). D-NARG (100µM) was ineffective. The block by L-NARG at 24°C was reversed by 1mM arginine. L-NARG did not reduce LTP in dentate minislices at either temperature; this may be related to the presence of bicuculline (100µM) and/or higher concentrations of Mg and Ca in the medium. Haemoglobin (20µM, Sigma) significantly reduced the magnitude of LTP at 24°C but not at 29°C in CA1 minislices and in 5/5 experiments blocked the induction of LTP in the perforant path in vivo. L-NARG (100µM (n=3) or 500µM (n=2)) failed to block LTP in vivo when perfused into the dentate gyrus for 2h pre-tetanus. These data raise doubts about the role of NO in LTP at physiological temperatures.

148.2

EFFECTS OF GLUCOCORTICOID TYPE I AND TYPE II AGONISTS ON HIPPOCAMPAL LONG-TERM POTENTIATION. C. Pavlides*, Y. Watanabe A.M. Magarinos and B.S. McEwen. The Rockefeller University, New York, NY.

A number of studies have demonstrated that high levels of glucocorticoids (GC), correlate negatively with hippocampal long-term potentiation (LTP). Similar deficits have recently been described with subbasal levels of GC. Thus, optimal plasticity can be obtained at an intermediate level of GC (Diamond et al. 1991). In the present experiments we investigated the effects of specific low affinity (Type I) and high affinity (Type II) GC receptor agonists on LTP in the dentate gyrus granule cell layer (DG). Male, Sprague Dawley rats were ADX 3 days prior to LTP testing. Approx. 2 h before recording, rats were administered (s.c.,10 µg/100 g/body weight) with either RU-28362 (Type II agonist), aldosterone (ALDO; Type I agonist), or the vehicle (propylene glycol). The animals were then implanted, under Chloropent anesthesia, with a stimulating electrode in the perforant path and a recording electrode in the DG. Following baseline recording, high frequency stimulation was applied (10 pulses, 200 Hz, 6X, @ 0.1 Hz) followed by recording for 30-40 min.

RU-28362 produced an extremely potent suppression in LTP induction as measured by bot'. the pop spike and the EPSP slope. ADX itself also produced a suppressing effect on LTP, in comparison to nonADX controls. ALDO admin. reversed the ADX induced suppression and produced even higher LTP than non operated controls. These results confirm previous observations indicating an opposing modulatory effect of GC, mediated by Type I and Type II receptors. Supported by a Whitehall Foundation grant to CP.

148.3

RATS HOUSED IN A COMPLEX ENVIRONMENT EXHIBIT GREATER HILAR LTP THAN INDIVIDUALLY HOUSED LITTERMATES. E.L. Hargreaves*, F. Boon, and D.P. Cain. Dept. Psych., Univ. Western Ontario, London, Ont. CANADA, N6A 5C2.

Male rats (n=18) approximately 50 days of age were housed either together in a meter^3 cage filled with ramps, boxes, and toys that were re-arranged on a daily basis, or individually in suspended cages located in the same colony room. After a month of such housing an overall behavioral assessment was undertaken. Some of the findings from this assessment have been reported in brief, elsewhere (rats housed in the complex environment were stronger, swam faster, balanced better, weighed less, drank less, were less neophobic to novel tastes, less aggressive to strange conspecifics, and faster at acquiring the Morris Water-Maze task than their individually housed littermates; however, they were behaviorally similar to their individually housed littermates in their open-field activity levels, food consumption, and retention of the Morris Water-Maze).

Subsequent to the behavioral assessment, rats were surgically implanted, with stimulating/recording electrodes in the perforant-path/dentate-gyrus of the hippocampus for the recording of hilar evoked potentials. After 10 days recovery, the complex housed rats were reintroduced to the environment. Full I/O curves were recorded a month later using behavioral clamping and compared on identical intensities. No differences between the groups on pop-spike amplitude were found. After a further three weeks of housing, rats were matched at approximately 60% of the maximum I/O pop-spike amplitude and electrically potentiated using standard lab parameters. Potentiation was assessed at 0, 1, 3, 24, 72, and 168 Hrs after LTP induction. Results indicate that rats housed in the complex environment (n=6) sustained, and maintained over the 7 day period, higher levels of LTP than their individually housed littermates (n=8). Supported by a grant from NSERC to DPC.

148.4

REM SLEEP DEPRIVATION EFFECTS LTP IN THE RAT HIPPOCAMPAL SLICE. R.N. Morrissette*,R.A. Hicks, S.A. Veregge. San Jose State University, San Jose, CA. 95192.

REM sleep (RS) consolidation theory postulates that RS may serve to consolidate learning. Current evidence suggests that LTP occurs when animals learn. It is hypothsized that RS deprived (RSd) rats will show enhanced LTP characteristics. RSd was initiated using the platform technique and LTP was elicited and monitored in hippocampal slices of control and 4-day RSd rats. Four characteristics of the field potential response were measured, as well as, the frequency of occurrence and duration of LTP. Results show a significant increase in population spike amplitudes for the RSd rats as compared to controls. Although these results offer support for the hypothesis, non-significant differences using the other measures weaken this support.

148.5

ASSOCIATIVE LONG-TERM POTENTIATION/DEPRESSION OF SYNAPTIC TRANSMISSION IN CINGULATE CORTEX SLICE. T.G. Hedberg*, G.V. Simpson and P.K. Stanton. Department of Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

A persistent increase in synaptic efficacy (LTP) has been demonstrated in hippocampus and neocortex following brief high-frequency stimulation. LTP is associative in nature: synchronous (in phase) activation of convergent inputs to the same neuron can enhance synaptic strength. Alternately, out of phase coactivation of separate afferents can elicit enduring synaptic depression (LTD). We used high and θ frequency stimulation of callosal (CAL) and subicular (SCT) afferents to characterize synaptic plasticity in superficial (II/III-IV) vs deep (V-VI) laminae of rat cingulate cortex slices. SCT/CAL stimulation allowed both synchronous and asynchronous activation of pyramidal apical and basal dendritic arbors. Trains of 100Hz bursts applied to the CAL yielded persistent (>15-60 min post-stim) decreases in latency (0.6-2.3 msec) and increases in amplitude of population potentials in both laminae. In contrast, 100Hz bursts via SCT yielded LTP in deep laminae only (ΔEPSP +51% ± 7%, n=8). When 5Hz SCT pulse trains were given in or out of phase with 100Hz bursts to CAL, no associative changes in synaptic strength were elicited. However, 5Hz CAL pulse trains elicited associative LTP (ΔEPSP +24% -3%; n=5) when in phase and LTD (ΔEPSP -13% +7% n=9) when out of phase with 100Hz bursts via SCT. Thus, CAL afferents show enhanced capacity for associative plasticity. *(Supported by NIMH Grant #45752 and the Office of Naval Research)*

148.6

INCREASED EXPRESSION OF GAP-43 , SOMATOSTATIN AND NEUROPETIDE Y mRNA IN HIPPOCAMPUS DURING DEVELOPMENT OF HIPPOCAMPAL KINDLING IN RATS. C. Bendotti*, G. Tarizzo, A. Vezzani and R. Samanin. Istituto di Ricerche Farmacologiche "Mario Negri", 20157, Milano, Italy.

It has been suggested that during the development of kindling, an animal model of temporal lobe epilepsy and neuronal plasticity, a synaptic reorganization occurs in certain neuronal populations of the hippocampus. Recently, we have shown a specific distribution of GAP-43 mRNA, a putative marker of neuritic growth, within the CA3 pyramidal neurons and the polymorph hilar neurons of dentate gyrus of the hippocampus from adult rat (Kruger et al. Mol. Brain Res.,13,267,1992) . In this study, using in situ hybridization technique and computer assisted grain counting at cellular level, we investigated whether changes in the distribution and expression of GAP-43 mRNA occured in the hippocampus during the development of electrical hippocampal kindling. A significant increase of grains number was found in the CA3 pyramidal neurons and hilar polymorph neurons of the dentate gyrus, 48 hours after stage 2 (preconvulsive stage) but not stage 5 (full seizure expression) kindling in the stimulated hippocampus. No modifications in the distribution of GAP-43 mRNA were observed in the hippocampus of kindled rat as compared to sham stimulated animals. We also found a marked increase of somatostatin(SOM) and neuropetideY(NPY) mRNA in the neurons of the hilus of the dentate gyrus at both stages of kindling,2 days but not 30 days after last simulation. This study suggests that GAP-43 may play a role in the synaptic remodeling occuring during kindling and an increased synthesis of SOM and NPY may have a role in epileptogenesis.

148.7

CLASSICAL CONDITIONING OF EYEBLINK RESPONSE IN TRACE PARADIGM MODIFIES AMPA AND CNQX BINDING IN THE RABBIT HIPPOCAMPUS. A.J. Annala*, G. Tocco, M. Baudry & R.F. Thompson. Neuroscience Program, University of Southern California, Los Angeles, CA 90089-2520.

Trace classical conditioning of the rabbit eyeblink response requires an intact hippocampus. Because we have observed increased AMPA binding in the hippocampus following classical conditioning of the rabbit eyeblink response in the delay paradigm we were interested in determining whether a similar effect would be observed after trace conditioning.

New Zealand White rabbits were divided into three groups: Paired, Unpaired, and Naive. Rabbits in the paired group received paired presentations of CS (1 KHz 85db 250ms tone) and US (3 psi 100 ms air puff) in a classical conditioning paradigm with a 500 ms trace interval. Rabbits in the unpaired group received explicitly unpaired CS and US presentations. Paired rabbits exhibited significantly increased ^{3}H-AMPA binding in hippocampal CA1 stratum oriens and radiatum/lacunosum, CA3 stratum radiatum/lacunosum, and molecular layer of dentate gyrus. Paired rabbits also exhibited significantly decreased ^{3}H-CNQX binding in hippocampal CA1 stratum oriens and radiatum/lacunosum, and molecular layer of dentate gyrus.

The results suggest that classical conditioning is accompanied by a change in affinity of the AMPA receptors, and that this cellular modification might play a critical part in learning-induced synaptic plasticity.

Research supported by NSF BNS-8718300, ONR N0001488K0112 and McKnight Foundation grants to R.F. Thompson.

148.8

ASSOCIATING CHOLINERGIC AND SCHAFFER COLLATERAL STIMULATION INITIATES PROTEIN SYNTHESIS IN CA1 PYRAMIDAL CELL DENDRITES. P. Lipton* & S. Feig. Depts. Physiol. Anat. U. Wisconsin Med. Sch. Madison, WI 53706.

A large body of evidence suggests that protein synthesis in the hippocampus around the time of learning is essential for memory consolidation. One way in which such synthesis might be involved is if associated synaptic inputs activate protein synthesis in the ribosomes which are located in subsynaptic regions of dendrites. Such a process could have great specificity.

Schaffer collaterals in the guinea-pig hippocampal slice were stimulated intermittently at 10 Hz over 20'. The cholinergic agonist carbachol (50 μM) was added to the bath to mimic the effect of cholinergic stimulation. Protein synthesis in pyramidal cell layer dendrites, and somata, was measured autoradiographically, following 3' exposure to (3H)-leucine (to prevent dendritic flow) at the end of the 20'period.

Cycloheximide-dependent (3H) incorporation (protein synthesis) was negligible in the resting state and was not activated by Schaffer collateral stimulation, or carbachol, when administered separately. However, associating the two stimuli increased incorporation in dendrites 3-fold, without increasing uptake of free (3H)-leucine. The increase was cycloheximide-dependent (protein synthesis). There was no effect in cell somata. The increased synthesis in dendrites was completely blocked by the muscarinic antagonist, atropine, and by the NMDA-antagonist, D-APV.

Thus, associating inputs which may occur together during learning initiates protein synthesis in target dendrites of CA1 pyramidal cells. This is dependent on activation of NMDA receptors.

148.9

PROGRESSIVE ENTORHINAL LESIONS ACCELERATE HIPPOCAMPAL SPROUTING IN RATS. K.R. Bulsara, J.F. Manibo and J.J. Ramirez*. Neuroscience Program and Department of Psychology, Davidson College, Davidson, NC 28036.

Previously, we have shown that two-stage (progressive) lesions of the entorhinal cortex (EC) restricted to one hemisphere result in a 300% acceleration in: A) sprouting by the crossed temporodentate pathway (CTD) and B) recovery from spatial alternation deficits following unilateral EC lesions. The purpose of this study was to determine electrophysiologically whether progressive lesions accelerate the rate at which the CTD forms functional synapses.

Animals were assigned to one of four groups: progressive lesion, control, priming lesion, or one-stage lesion. Electrophysiological recordings were made in the dentate gyrus contralateral to the lesion site either 6 or 12 days postlesion.

CTD stimulation in the progressive lesion group resulted in a 250% increase in the evoked population EPSPs of the dentate granule cells when compared to the one-stage group. Thus, the synaptic drive of the CTD in the progressive lesion cases was significantly greater than that of the one-stage group.

Based on our anatomical, behavioral, and electrophysiological data, we propose that progressive EC lesions accelerate the formation of functional CTD synapses that are behaviorally meaningful.

Supported by grants from the National Science Foundation (grant no. BNS 9020151) and The Pew Charitable Trusts to J.J.R.

148.10

GLUTATHIONE REDUCES SPATIAL LEARNING DEFICITS IN THE MORRIS WATER TASK AFTER MEDIAL FRONTAL CORTEX LESIONS IN THE RAT. M. S. Weaver*, T. A. Jones, M. L. Swoboda, T. Schallert, and S. W. Leslie. Institute for Neuroscience, University of Texas, Austin, TX 78713.

The endogenous tripeptide glutathione (GSH; γ-glutamyl-cysteinlglycine) interacts specifically with the NMDA recognition site (Leslie, et al., 1992). This peptide also acts as a free radical scavenger (Halliwell & Gutteridge, 1989). Since both of these processes are linked with attenuation of excitotoxicity, the present study was conducted to evaluate the neuroprotective effects of GSH after brain injury. The influence on spatial learning deficits after medial frontal cortex (MFC) lesions was assessed. Following 5 days of baseline training on Whishaw's learning-set version of the water task, rats sustained bilateral MFC lesions or sham operations, and cannulae were implanted chronically in either the left or right lateral ventricle. Infusions of GSH (10 µl of 20 mM; i.c.v.) or vehicle (a-CSF) were given at 1, 12, and 24 hrs after surgery and then once daily for 7 days. Training was resumed 4 days after surgery and continued for 12 days. As expected (Weaver et al., 1991), bilateral MFC lesions significantly impaired spatial learning ability in the water task. GSH-treated rats were significantly less impaired and had faster rates of recovery than vehicle-treated lesioned animals. Preliminary anatomical results indicate that GSH may protect against lesion-associated shrinkage of the CA1 region. (Funded by NS-23964 and AA-07471).

148.11

RECEPTIVE FIELD PLASTICITY IN THE ADULT RAT BARREL CORTEX. M.E. Diamond*, M.A. Armstrong-James, and F.F. Ebner, Institute for Developmental Neuroscience, Vanderbilt University, Nashville, TN 37203.

Experiments were carried out to determine if the receptive fields (RFs) of neurons in layer IV barrels are shaped by changes in sensory experience. All whiskers on one side of the face -- except D2 and one neighbor (D1 or D3) -- were clipped to a length of 3 mm and trimmed daily. After 3-30 days, the responses of single units in barrel D2 of anesthetized rats to stimulation of whisker D2 (the center RF), and 4 whiskers in the surround RF (SRF), D1, D3, C2, and E2, were measured. In "control" rats, cells in barrel D2 responded maximally to whisker D2 at latencies < 10 ms; whiskers in the SRF evoked a less powerful response at longer latencies. The response to whiskers D1 and D3 was symmetric. Strong asymmetry in the RFs of D2 barrel cells developed as early as 3 days after whisker trimming. Responses of D2 cells to D2's "paired" neighbor (D1 or D3) became more powerful and the response to the trimmed neighbor became less powerful: the ratio of response magnitudes for paired/trimmed whisker (D1/D3 or D3/D1) was 2.0. The latency to the paired whisker decreased and to the trimmed whisker increased. The response to whiskers C2 and E2 decreased. The changes in response to SRF whiskers occurred mainly in the 10-20 ms and 20-50 ms poststimulus epochs, but not in the 0-10 ms epoch, indicating that thalamocortical drive was not significantly affected. The results suggest that behavioral pairing of whiskers rapidly potentiates the linkage of the co-active barrels; whereas un-pairing weakens the linkage. (Supported by NS-25907, P30-HD15052, and the Wellcome Trust).

148.12

THE CORTICAL METABOLIC EFFECTS OF TRAINING A SPARED RAT VIBRISSA IN THE ADULT C.L. Hand*, R.L. Craik, K.M. Gallo, & P.J. Hand, Idaho State U., Pocatello, ID 83209; Beaver College, Glenside, PA 19038; and U. of Pennsylvania, Phila., PA 19104.

We are exploring treatment interventions that **maximize functionally-appropriate brain alterations**. This study combined a bilateral spared vibrissa preparation with unilateral associatively-paired (AP) or disassociatively-paired (DAP) training in 19 **adult** rats. Subtotal vibrissa deafferentation involved bilateral sparing of a single whisker (SC3) on postnatal day 45. AP (pairing vibrissa stroking with a 20% honey water positive reinforcer) or DAP (unpaired vibrissa stroking and honey water administration) training on SC3 was initiated on PND 50 for 5 min/day for 30 days. Prior work in **neonatal** rat indicates a robust response of the damaged SC3 system to AP training, but not to DAP training. For neonates, the quantitative [^{14}C]-2DG technique revealed a 65±38% **increase** in the area of metabolic labeling in the **AP** trained SC3 cortical representation and a 31 ±10% **decrease** in the **DAP** trained animals. In the **adult**, there was a 40 ± 12% **increase** in the **AP** trained SC3 representation and a 2 ± 9% **decrease** in the **DAP** trained SC3 cortical representation. Results demonstrate that the **adult** cortical metabolic representation of vibrissae is also dynamically maintained and responds differentially to peripheral interventions. NS22283

148.13

ENHANCED DENDRITIC ARBORIZATION AND PRUNING AFTER SENSORIMOTOR CORTEX DAMAGE IN ADULT RATS: ROLE OF THE NMDA RECEPTOR. D.A. Kozlowski*, T.A. Jones, & T. Schallert, Neuroscience Institute & Department of Psychology, University of Texas at Austin, Austin, TX 78712.

Unilateral damage to the forelimb-representation area of the sensorimotor cortex in adult rats causes a time dependent proliferation of dendritic processes followed by a partial pruning of layer V pyramidal neurons of the homotopic contralateral cortex (Jones & Schallert, 1992). Dendritic growth appears to be linked to a hyper-reliance on the unimpaired forelimb, especially for postural-motor behaviors, whereas dendritic pruning is associated with a return to symmetrical limb use.

Unilaterally lesioned rats were administered an NMDA receptor antagonist, MK801 (1mg/kg, i.p. once every four days), or saline control, during the dendritic pruning phase. MK801 reinstated severe and chronic unilateral postural-motor asymmetries. These behavioral events may be associated with anatomical changes. Analysis of Golgi-Cox stained tissue in the homotopic cortex of the undamaged hemisphere indicated that MK801 prevented the pruning (that is, enhanced dendritic arborization was maintained). Pruning of adult dendrites may depend on glutamatergic activation of the NMDA receptor and may be important for restoration of function after brain damage.

148.14

ICMS INDUCED EMERGENCE OF NEW SKIN FIELD REPRESENTATIONS IN RAT SOMATOSENSORY CORTEX. F. Spengler* and H.R. Dinse.
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Intracortical microstimulation (ICMS) induces short time plasticity in somatosensory cortex of adult rats and monkeys[1]. ICMS alters not only cortical representations and receptive fields, but also neuronal sensitivity functions and transfer properties [2]. The present studies demonstrates that ICMS in addition induces receptive fields beyond the representational borders. Neural activity in deep layer III and IV within the hindpaw representation were recorded in adult Urethan anesthetized rats using 1 or 2 microelectrodes. Low-threshold cutaneous receptive fields were defined by handplotting and quantitative averaging techniques before and after ICMS. First, the rostromedial and caudal borders of the hindpaw representation were mapped in detail. Recordings made outside this region revealed neuronal activity that was neither drivable by cutaneous stimulation nor by stimulation of joint or muscle receptors. ICMS was then applied at a location close to the representational border for several hours. After ICMS, this border region was remapped. The borders of the skin field representations were shifted rostro-medially or caudally resp., up to 1mm into zones that were under control not sensitive to tactile stimuli. These results demonstrate that cortical networks are dynamically maintained not only within, but also beyond the representational borders. Further studies are required to clarify the nature of thalamic projection within the border region.

1:Recanzone GH, Merzenich MM, Dinse HR (in press) Cerebral Cortex
2:Dinse HR (1991) in: Synapse-Transmission-Modulation, Thieme, pp 532

148.15

COMPARATIVE PLASTICITY OF SOMATOSENSORY AND AUDITORY CORTEX IN RAT P.H.Bedenbaugh*, P.E.Maldonado and G.L.Gerstein Dept. of Physiology, Univ. of Pennsylvania, Philadelphia PA 19104

We have studied plasticity induced by electric intra cortical microstimulation (ICMS) in primary somatosensory and auditory cortex of the rat using essentially identical experimental, recording and analytic procedures. Multi-unit, separable recordings were obtained with various arrays of electrodes, and processed with appropriate spike shape sorting devices. Recordings and characterization of unit and assembly properties were made before and after a period of supragranular or granular ICMS. This consisted of spaced trains of biphasic 5μA pulses applied through one of the recording electrodes.

1. After a period of ICMS the responses in both cortices to appropriate sensory stimulus of cells recorded near but not at the stimulating electrode became more similar to those of cells at the stimulating electrode. These changes grew and subsequently seemed to be reversible with a similar time course of several hours.
2. Irregular somewhat bursting firing (< 10 Hz) , approximately synchronized across electrodes was observed in both cortices under several anesthetic conditions. This pattern of activity was enhanced by ICMS. Sensory stimulus entrained this bursting behavior for two or three cycles, particularly after the ICMS period.
3. Cross-correlograms among all simultaneously recorded cells showed that many cell pairs increase their correlation of firing after the ICMS period, while a few decrease correlation. This is expressed as changes in peak areas and shapes.
4. Joint peri-stimulus histograms (JPST) for cell pairs in auditory cortex showed that much of the ICMS related increases in correlated firing occurred during the interstimulus time; less increase occurred during the sensory stimulus response.
5. Our interpretation of these observations is that (a) ICMS reveals the plastic nature of the sensory map structure, and (b) that ICMS forces an increase of intracortical neuronal coupling. The detailed mechanisms remain to be investigated.

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148.16

CORTICAL PLASTICITY AND NEURONAL ASSEMBLIES IN RAT AUDITORY CORTEX P.E.Maldonado* and G.L.Gerstein Dept. of Physiology, Univ. of Pennsylvania, Philadelphia PA 19104

Neuronal assemblies as defined in terms of cooperativity of neuron firing were studied during cortical reorganization induced by electrical microstimulation. Multiple single-unit recordings from rat primary auditory cortex (supragranular layers) were obtained with a linear array of four parlyne-insulated tungsten microelectrodes in ketamine-xylazine and nembutal anesthetized animals. Receptive fields (RF) and after-discharge responses were characterized before and after cortical microstimulation (ICMS) through one of the recording electrodes. Auditory stimuli were free field 50 msec tone burst over the range of 4-40 Khz, 20-30 dB over threshold, randomly presented through a tweeter located 60 degrees at the contralateral side of the site of recording. For RF determination, burst tones ocurred every 600 msec; for after-discharges burst tones were given every 2 sec. ICMS were trains of 13 pulses of 5 uA spaced 5 ms, trains repeated every 0.5 sec. We found changes in RFs of cells recorded from the electrodes adjacent to the stimulating electrode which consisted mainly in new sensitivity to the best frequency of cells recorded from the stimulating electrode. In most cases the cells remained with sensitivity to the former best frequency. RF of cells recorded from stimulating electrodes did not change their best frequency. Cross-correlation analysis showed that ICMS changes the distribution of cooperativity of neuron firing between cells located at different recording electrodes. Spontaneous activity shows a rhythmic synchronized pattern of firing between most cells in cortex in the range of 4-8 Hz. Auditory stimulus response entrained this oscillation; JPST analysis shows significant correlation between cells during interstimulus time, but not during stimulus response time. ICMS increases particularly the correlation of firing during interstimulus interval. Differences between Ketamine and Nembutal anesthesia relate mainly to spontaneous activity, strength of coherent firing and shapes of RFs. Plasticity occurs with both anesthetics.

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148.17

THICKNESS OF THE MOTOR CORTEX INCREASES FOLLOWING 28 DAYS OF EXERCISE OR MOTOR SKILL TRAINING. **B.J. Anderson*, P.B. Eckburg, K.I. Relucio* & W.T. Greenough. Dept. of Psych., Neurosci. Prog., Cell Struct. Bio., Univ. of Ill., Urbana, IL 61801, *Rush Med. College, Chicago,IL 60612.**

Voluntary exercise in an exercise wheel in 10 mo. old female rats was previously shown to increase the density of capillaries in the paramedian lobule of the cerebellar cortex, whereas training on an obstacle course caused an increase in both the volume of the molecular layer and number of synapses per neuron (Black, et al., 1990). We investigated the effects of these motor tasks on the thickness of the motor cortex. Six mo. old female littermates were balanced across three treatment conditions each lasting 28 days. The exercise group had free access to a running wheel (running a mean of 150 Km). The acrobatic group traversed an increasingly difficult obstacle course requiring balance and coordination five times a day (travelling a mean of 125 m). Control rats were raised in standard cages and handled daily. Rats were perfused, brains were embedded in celloidin, sectioned at 40 μm and stained with Methylene Blue/Azure II. Motor cortical thickness was measured in four coronal planes between -2.33, & -.3 mm posterior to bregma (Paxinos and Watson, 1982). Thickness was measured at 13 medial-lateral positions starting from the midline. At the plane -2.33 mm from bregma the acrobatic group had significantly thicker cortices at four positions. The acrobatic and exercising rats had significantly thicker cortices in the other three planes than the control rats. Although the pattern of differences in thickness are somewhat dissimilar between the acrobatic and exercise groups, both treatments increased cortical thickness. The discrepancy between the exercise effects in this study and those in the cerebellum may reflect the greater amount of exercise performed by the rats in this study.

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148.18

ROLE OF NMDA RECEPTOR IN THE BEHAVIORAL EFFECTS OF ENVIRONMENTAL COMPLEXITY. A. Mohammed*, R. Liljequist, B. Henriksson, N. Latif, T. Pham and B. Winblad. Dept. of Geriatric Medicine, Karolinska Institute, S-141 86 Huddinge, Sweden.

This study examined the involvement of NMDA receptor in environmentally-induced cognitive changes. Juvenile male Sprague-Dawley rats were housed in group cages. They were treated with the noncompetitive NMDA receptor blocker MK-801 (0.16 mg/kg/day, i.p.) or saline and exposed for two hours to a complex environment (EC). Other animals remained in the standard (social) condition (SC) after drug or saline treatment. Drug treatment and exposure to EC were given on alternate days for 4 weeks. All the animals remained in home cages in groups of 4 per cage for 3 months and then tested for motor activity and spatial learning. During the exposure to EC, observation of the animals using a behavioral checklist was carried out at 10 min intervals. Drug treatment caused ataxia, increased locomotion, and decreased rearing with resultant diminished interaction with objects in the complex environment. For saline treated rats EC resulted in higher rearing scores and rapid habituation, as well as superior spatial learning when compared to SC. This EC-SC difference was absent in drug treated rats where the performance of rats from SC was at the level of those from EC. MK-801 treatment modified the effects of both EC and SC. Radioligand binding studies showed that drug treatment decreased [^{3}H]-MK-801 binding sites in cortex and hippocampus. These observations demonstrate that the NMDA receptor is critically involved in mediating environmentally dependent cognitive changes, and are in line with the findings implicating NMDA receptors in neuronal plasticity underlying learning and memory.

NEUROETHOLOGY: INVERTEBRATES, ELECTRIC FISH

149.1

NEURON SOMA EXCITABILITY: A COMPARATIVE APPROACH BY ANALYSIS OF Na^+ CURRENT IN MOLLUSCAN NEURONS. Rhanor Gillette* and William F. Gilly, Dept. Physiol., Univ. Illinois, Urbana, IL and Hopkins Marine Station, Stanford Univ., Pacific Grove, CA.

The excitability of neuron somata of opisthobranch and pulmonate snails may be exceptional, since the somata of cephalopods and some other molluscs are non-spiking, like those of arthropods and annelids. In most cases, neither the significance nor biophysical basis of these conditions is known. We measured I_{Na} with whole cell patch clamp of acutely dissociated neurons (0-1 day culture, < 100 microns dia.). I_{Na} is not prominent in larger somata of stellate ganglion neurons of *Loligo* (squid) nor *Octopus* until several days in culture. Properties of I_{Na} in these cells are similar to squid giant axon. In contrast, small stellate neurons of these species as well as CNS neurons of opisthobranchs *Pleurobranchaea* and *Doriopsella* show I_{Na} of relatively high density. I_{Na} kinetics in these differ from squid giant axon: 1. Steady-state curves for activation and inactivation are displaced to positive voltages; 2. I_{Na} kinetics of activation, inactivation, and deactivation in the operating (intermediate) voltage range are much slower than those of giant axon cells, but are similar at extreme ends of the range. Such findings may relate to the electrical relationship of the soma to the synaptic integrating region of the neuron.

149.2

CHEMICALLY MEDIATED DEFENSIVE INKING IN *APLYSIA CALIFORNICA*. C. Kicklighter, P.M. Johnson and T.G. Nolen*, Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, FL 33124.

Ink release by *Aplysia* in response to noxious stimulation may function as an anti-predator, chemical defense. While this adaptive hypothesis has yet to be tested directly, considerable indirect evidence supports it: Inking involves an elaborate neural releasing mechanism [Carew & Kandel, 1977]; ink release is directed to the site of attack [Walters & Erickson, 1986]; the toxic secondary plant compounds concentrated by the animal in its ink can act as an effective deterrent [Ambrose et al, 1979]; and finally, ink itself elicits escape behaviors in neighboring juvenile *Aplysia* [Johnson & Nolen, 1991]. Inking is thought to be triggered only by high threshold, noxious stimulation. But we have found that relatively mild handling can elicit inking and that much of this effect appears to be due to chemical components found on the experimenter's skin.

Forty-five 100 gm *Aplysia* were tested twice, on successive days, for inking in response to gentle exposure of the length of the foot to BARE skin or GLOVED skin. Comparisons were made between BARE *vs* GLOVED, BARE *vs* BARE, GLOVED *vs* BARE, GLOVED *vs* GLOVED, and for prior handling effects. BARE skin elicited inking in 43 to 93% of the trials compared to GLOVED skin, *ca* 20 to 23% ($p<0.05$). The probability of BARE skin triggering inking increased following handling with either BARE or GLOVED skin ($p<<0.05$ respectively) whereas the probability of GLOVED skin eliciting inking did not change ($p>0.05$). When mechanical handling effects are accounted for, chemical factors appear to be correlated with increased ink release. Chemical sensitivity to predators is common in molluscs: Saponins and related naturally occurring surfactants in the skin of predatory starfish elicit escape behavior in many other gastropods. Currently we are examining the behavioral and neural mechanisms of chemically mediated inking — in response to a variety of natural, sympatric starfish predators — in several species of *Aplysia*.

149.3

ESTIMATION OF THE SIZE OF A FUNCTIONAL GROUP OF INTERNEURONS CONTROLLING ABDOMINAL POSITIONING IN THE CRAYFISH. L.D. Brewer, and J.L. Larimer*. Dept. of Zoology, Univ. of Texas at Austin, Austin, TX 78712.

The command system controlling abdominal positioning (flexion and extension) in the crayfish *Procambarus clarkii* has been extensively studied (Larimer 1988 TINS: 11). Several studies indicate that the command elements underlying this behavior are organized into parallel and serial functional groups through extensive synaptic connections (Jellies and Larimer 1985, J.Comp. Physiol., 156; Murphy *et al.* 1989, J. Comp. Physiol. A, 165). We devised a method to estimate the number of interneurons which comprise a functional group controlling abdominal positioning. Fictive abdominal positioning was observed by recording from motor roots. Single command elements were stimulated intracellularly in isolated, perfused nerve cords. Recordings were also made from rostral and caudal hemiconnectives (1-2 and 5-6) that were divided into bundles. When these command elements were stimulated they activated a number of previously silent interneurons and increased or inhibited the spontaneous activity of others. We then compared spontaneous with evoked activity. The number of recruited interneurons ranged from 4 to 19 (average = 10; n = 10). Vigorous motor output was usually correlated with a greater recruitment of interneurons. Sometimes a stronger motor output was generated either rostrally or caudally; and interneuronal activity was also directionally biased. Occasionally, evoked activity was of such high frequency that individual spikes could not be identified. Therefore, the actual number of recruited cells is probably higher than reported. Finally, since the output of these command elements are usually symmetrical, the interneurons underlying the behavior may be distributed equally in each half of the nerve cord. Since our numbers are based on counts from one hemiconnective the total number of interneurons in a functional group is probably twice that given above. These data support the functional group hypothesis. (Supported by NIH Grant NS 05423, to J.L.L.)

149.4

TIMING OF THE COCKROACH ESCAPE RESPONSE AND THE INFLUENCE OF CHOLINOACTIVE DRUGS. S. Ye*and C.M. Comer. Neuroscience Group, Dept. Biol. Sci., Univ. of Illinois, Chicago, IL 60680

The wind-evoked escape response of the cockroach occurs at short latencies and is mediated by giant interneurons in the ventral nerve cord which are putatively cholinergic. A preliminary report (Krähling, Neurosci. Lett., 62: S514, 1985) claimed that the frequency distribution of latencies for wind elicited escape had several peaks, and that cholinergic drugs could selectively shift some of the peaks. We were interested in verifying that the escape response has different latency classes, and also in assessing the sensitivity of escape latency to cholinoactive substances.

We observed the escape responses of adult male *Periplaneta americana* with a high speed video system (240 pictures/sec). Wind puffs were delivered to the animals under free-ranging conditions. The frequency distribution of latencies observed in normal animals showed two peaks: an early peak (latencies < 44 ms from time of triggering wind machine) and a later peak (latencies > 44 ms). The relative positioning of these two peaks depended, in part, on wind intensity.

Injecting animals with atropine (200 ug/animal), a cholinergic antagonist, diminished the early peak and slightly shifted the later peak to the right. Injecting animals with carbamylcholine (20 ug to 100 ug/animal), a cholinergic agonist, shifted latencies in the opposite direction -- it increased the number of trials comprising the early peak and slightly shifted both peaks to the left. These results partially confirm the observation that there exist more than one class of latencies for the wind-evoked escape. In addition, cholinergic agents affect these classes differently.

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149.5

NEURAL ELEMENTS AND MOTOR PATTERNS UNDERLYING EGG PROGRESSION IN CRICKETS.
T.N. Ricciardi and G.A. Wyse*, Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Sugawara and Loher (1986, J. Insect Physiol. 32:179) described the sequence of events underlying egg movements and oviposition in the reproductive system of female crickets. In an attempt to characterize the motor patterns to musculature involved in egg progression, we recorded the EMG activity of the genital chamber (GC) and muscle 2 (M2) in dissected preparations of adult *Acheta domesticus*. GC and M2 show ongoing rhythmic bursting that appears to represent motor patterns underlying egg movements during oviposition. The anatomy of motor neurons supplying these muscles was determined by cobalt backfills of the 7th and 8th segment ventral nerves, and by intracellular dye fills of single motor neurons. These experiments showed that the 7th and 8th segments of the terminal abdominal ganglion (TAG) contain bilaterally paired clusters of motor neurons, as well as cells similar in morphology to dorsal unpaired median (DUM) cells. *In situ* intracellular recordings of GC motor neurons (GCMN) show rhythmic bursting that is coincident with ipsilateral GC contraction. Intracellular spikes of GCMN cause time-locked excitatory junctional currents in the GC muscle. Some recordings of single GCMN showed various sizes and shapes of spikes, suggestive of multiple sites of spike initiation. It has been hypothesized that chemical mating factors passed via the spermatophore can activate egg progression and oviposition by turning on pattern-generating circuitry in the TAG. Our system is well suited to test such a hypothesis.

149.6

FUNCTIONAL REPRESENTATION OF WIND DIRECTION IN THE CRICKET CERCAL SENSORY SYSTEM. Jacob Levin and Gwen Jacobs* Dept. of Molecular and Cell Biology, UC Berkeley, Berkeley, CA. 94720

We are interested in how a topographically mapped sensory system represents the functional characteristics of sensory stimuli anatomically in the nervous system. In the cricket cercal sensory system, mechanoreceptors on the cerci of the animal are sensitive to both the direction and velocity of wind stimuli. They have broad overlapping tuning curves which encode wind stimulus orientation with respect to the animal's body. We have made a 3-D computer reconstruction of the topographic map formed by the sensory receptors in the CNS by staining and reconstructing single sensory afferents encoding all wind directions. These reconstructions preserve the precise spatial location and the density distribution of terminals within each afferent arbor. Our results show that overlap between the arbors of different afferent types decreases exponentially with separation between tuning curves. Thus, wind direction may be represented in a continuous fashion within the map. To examine this observation in detail, a vector field map consisting of density-weighted average sensitivity vectors at each point in the map was generated, enabling us to visualize how the representation of wind direction changed with position in the map. We have found that the map is composed of two large regions within which the direction of peak sensitivity changes in a continuous fashion. There are, however, abrupt changes in the vector field between these two regions. A similar reconstruction technique was performed on the sensory interneurons. By convolving the IN dendritic arbor with the afferent vector field we were able to illustrate how interneurons construct their receptive fields by sampling different regions of the topographic map.

149.7

PREDICTING INTERNEURON TUNING CURVES FOR DIFFF .ENT WIND VELOCITIES IN THE CRICKET CERCAL SENSORY SYSTEM Michael Landolfa*,Frederic Theunissen and Gwen Jacobs Molecular and Cell Biology, and Graduate Group in Biopysics, UC Berkeley, Berkeley, CA.

The cricket cercal sensory system encodes the direction of wind stimuli over a large range of wind velocities. Both the primary sensory interneurons (INs) and the sensory mechanoreceptors exhibit changes in the variance and shape of their directional tuning curves as a function of changes in wind velocity. To determine the extent to which the IN response properties depend upon the characteristics of the afferent input, we predicted the tuning curves of INs using a simple model and compared those results to data obtained from physiological experiments. First, a computer reconstruction of the topographic map of the sensory afferent arbors was created based on their directional sensitivity to wind and receptor density on the cercus. Hypothetical IN tuning curves were then computed based on (1) the position of the IN dendritic arborizations within this map, (2) the shape of the afferent tuning curves and (3) the electoanatomy of the IN. Hypothetical IN tuning curves were computed using afferent tuning curves measured at several different wind velocities. The computed IN tuning curves were very similar to tuning curves measured physiologically at matched wind velocities.

In previous studies, we demonstrated that the position of peak response of the IN depended on features of the dendritic arborization pattern. We have tested these predictions by blocking inputs to specific dendrites through immobilizing mechanoreceptors on one cercus. The observed shifts in directional tuning curves will be discussed in light of the results predicted by the model.

149.8

WIND VELOCITY ENCODING BY SPIKES IN INTERNEURONS OF THE CRICKET CERCAL SENSORY SYSTEM: QUANTIFICATION BY THE REVERSE RECONSTRUCTION TECHNIQUE. F.E. Theunissen and J.P Miller*. Mol. & Cell Biology Dept., University of California, Berkeley 94720.

The activity patterns of primary sensory interneurons in the cricket cercal sensory system encode information about the direction and velocity of air current stimuli in the animal's immediate environment. In previous work the statistical principles of information theory were used to calculate the maximum *directional accuracy* attainable from the probabilistic response ensemble of four low velocity interneurons from this system. Here we quantified the accuracy with which the *velocity aspect* of wind stimuli are encoded in this class of interneurons.

The dimension of the velocity space was too large to allow estimation the conditional probability of the spike train given a particular wind waveform. Instead, the "reverse reconstruction" technique (Bialek et al. Science 1991) was used to estimate the equivalent measure of signal to noise. For our case, we calculated the filter which yielded the best estimate the air current stimulus velocity from the recorded interneuronal spike trains. The effective noise was defined as the difference between the reconstructed stimulus waveform and the actual stimulus waveform. From this signal to noise ratio, the quality of the velocity encoding was characterized by calculating the information theoretic measure of transinformation. We found that the shape of the filter derived for the interneuron, as well as the quality of the associated stimulus reconstruction, were very different from those of the primary sensory afferents in our system (obtained in an earlier study.) This reflects aspects of neural processing carried out between these layers.

149.9

THE SUBLEMNISCAL PREPACEMAKER NUCLEUS (SPPN) MODULATES THE FREQUENCY OF THE MEDULLARY PACEMAKER NUCLEUS IN THE GYMNOTIFORM FISH, *EIGENMANNIA*.

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Eigenmannia is able to raise or to lower the frequency of its medullary pacemaker to shift its own electric organ discharge (EOD) frequency away from similar interfering frequencies of its neighbors (= jamming avoidance response, JAR). These frequency shifts are generated by the diencephalic prepacemaker nucleus (PPn-G/CP) and by the sublemniscal prepacemaker nucleus (SPPn), respectively. Activation of the PPn-G/CP raises the pacemaker frequency through AMPA-mediated excitation of cells in the pacemaker nucleus. The SPPn, on the other hand, appears to maintain a stable pacemaker frequency through tonic, NMDA - mediated excitation of pacemaker cells. As a consequence, a GABA-mediated inhibition of the SPPn causes a drop in the pacemaker frequency. Therefore, the motor side of the JAR appears to be controlled according to a push-and-pull principle.

149.10

CENTRAL PROJECTIONS OF THE LATERAL LINE NERVES IN GYMNARCHUS NILOTICUS (GN) IDENTIFIED BY HRP AXONAL TRANSPORT.

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As other weak electric fish GN possesses a posterior (ELL) and an anterior (NM) lateral line lobe (Bass & Hopkins, 1982), the presumed main central projection areas of the lateral line nerves.HRP labeling of the posterior (PLLn) and anterior (ALLn) lateral line nerves demonstrates that indeed both nerves project to the ELL and NM as well as to the eminentia granularis and corpus cerebelli. In the ELL, the ALLn projects to its medial, the PLLn to its lateral half, and both projection fields extend from the ELL's posterior pole to its anterior border. Each nerve projects to three distinct ELL cortical regions: to the medial (MZ), dorsal (DZ) and ventral (VZ) cortical zones. Thick fibers of each nerve end in the inner cell layer (IC) of MZ, whereas medium sized fibers project to the inner cell layer of DZ and VZ. According to the fiber diametre spectrum, the MZ, DZ and VZ cortical areas appear to be the projection areas of the three GN electroreceptor types, Gymnarchomast I, II and ampullary receptor (Szabo, 1965), respectively. In addition, fine fibers terminate on the giant cells of the ELL deep fiber layer, which seems to be a particularity of GN.

149.11

POSSIBLE IONIC MECHANISMS UNDERLYING THE CONTROL OF PACEMAKING IN THE WEAKLY ELECTRIC FISH, *HYPOPOMUS*.

J. E. Spiro* and W. Heiligenberg. Departments of Biology and Neuroscience, UCSD, La Jolla, CA 92093.

Hypopomus discharges an electric organ in its tail which is driven by a signal from an endogenous oscillator, the medullary pacemaker nucleus. In different behavioral contexts, the otherwise regular discharge is modulated to produce a number of distinct output patterns. We are investigating the ionic mechanisms underlying these modulations in an intact preparation as well as in a newly developed whole-brain *in vitro* preparation by recording intracellulary from the pacemaker cells while inducing changes of the rhythm by stimulating previously characterized presynaptic regions in the diencephalon. Prior investigations have shown that glutamate serves as the transmitter and that the various modulations result from a specific placement of the subtypes of the glutamate receptor complex on the two cell types that make up the pacemaker nucleus.

We have found that the pacemaker is especially sensitive to cesium, which has been shown in other preparations to block an inward current that is activated by hyperpolarization, and is implicated in the control of pacemaking (termed I_f or I_h). Cesium at 1-5mM slows the pacemaker rhythm without affecting spike shape and may impede slow modulations. We suggest that one mechanism for the control of frequency of the pacemaker may involve modulation of this current, which sets the slope of the pacemaking potential, and therefore increases or decreases the interpulse interval.

149.12

THE EMERGENCE OF ELECTROTONIC CONNECTIONS IN THE DEVELOPING MEDULLARY PACEMAKER NUCLEUS OF APTERONOTID ELECTRIC FISH.

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The medullary pacemaker nucleus of gymnotiform electric fish consists of electrotonically coupled pacemaker cells, which generate the oscillation, and relay cells, which transmit the pacemaker signal to the spinal motor neurons of the electric organ. Pacemaker cells form chemical synapses on relay cells in species such as Hypopomus, which discharge their electric organ at relatively low rates (in the range of tens of Hz) and are considered 'ancestral' in this regard. The most modern gymnotiform fish appear to be those of the apteronotid family, which discharge at the highest known frequencies (in the range of 1000 Hz), and have converted the ancestral myogenic electric organ to a faster, neurogenic organ. This evolution is repeated in the juvenile development of these fish (Kirschbaum F, Naturwissenschaften 70: 205 (1983)). In addition, adult apteronotid fish have established purely electrotonic junctions between pacemaker cells and relay cells (see reviews in Bullock and Heiligenberg: Electroreception, Wiley (1986)). These connections, however, are 'still' chemical in juvenile apternotids and are then replaced by electrotonic junctions as the fish's initially low pacemaker frequency approaches adult levels.

149.13

LOCAL CIRCUITRY WITHIN THE ELECTROSENSORY LATERAL LINE LOBE (ELLL) OF STERNOPYGUS: ROLE OF POLYMORPHIC NEURONS

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High frequency weakly electric fish, Apteronotus and Eigenmannia, exhibit a well known behavioral reflex, the jamming avoidance response (JAR). This behavior apparently allows these species to electrolocate in the presence of noise generated by the electric organ discharges of neighboring conspecifics. Neuronal processing underlying the JAR relies heavily on the amplitude and phase coding pathways. A closely related low frequency species, Sternopygus, does not exhibit JAR behavior. Furthermore, earlier anatomical studies showed that the ELLL, a major electro-sensory nucleus receiving both primary afferent and descending inputs, differs markedly between the low and high frequency species, suggesting that microcircuits in the ELLL of Sternopygus may be related to behaviors other than the JAR.

In this study, we used intracellular micropipettes to iontophorese lucifer yellow into cells in lightly fixed slices to examine the somatodendritic morphology of selected neurons in the ELLL of Sternopygus. A confocal laser scanning microscope (BioRad MRC 600) was used to collect serial optical sections which were then projected onto a single plane to render a photographic equivalent of a camera lucida image of each neuron. This technique allowed us to study a large number of immunohistochemically identified neurons, in combination with retrograde labeling or plant lectin staining, in order to identify cell types and their local connectivity within the ELLL. Our survey revealed a number of new cell types, most of which appear to be modified polymorphic neurons, were far more prevalent in the tuberous subdivisions of Sternopygus when compared to the high frequency species. The dendritic field dimensions of polymorphic, basilar and non-basilar cells near the M/CM, CM/CL and CL/L borders were measured. Dendritic asymmetries were present mostly at the CM/M border. This work funded by NSERC 5-80310 (JM).

149.14

FAST NMDA TRANSMISSION IN A SENSORY FEEDBACK PATHWAY.

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Within the electrosensory system of weakly electric fish, feedback projections constitute a significant yet poorly understood functional component. Two sensory feedback pathways (one direct and one indirect) project back to the first order electrosensory processing nucleus the *Electrosensory Lateral Line Lobe* (ELL), with spatially discrete zones of synaptic termination in the ventral (VML) and dorsal (DML) molecular layers respectively. While the response characteristics of the projection neurons of the direct *tractus stratum fibrosum* (Tsf) feedback projection have been investigated *Invivo* (Bratton and Bastian, 1990), the physiological and pharmacological characteristics of their synaptic terminations in the VML have yet to be fully elucidated. Since these synapses are excitatory in appearance, and NMDA and Non-NMDA receptors have been demonstrated in this region, we investigated the effects of various EAA receptor antagonists on synaptic transmission in the VML *Invitro* . Under control conditions, electrical stimulation of the Tsf evoked a characteristic extracellular field potential in the VML which (1) consisted of both an ***early*** (3-10 msec) and late (>10 msec) phase, (2) was restricted to the zone of Tsf synaptic termination, (3) was blocked by Mn2+, and (4) was reversibly blocked by focal pressure ejection of kynurenic acid (2mM) at the recording site in the VML. Pressure ejection of the NMDA receptor antagonist CPP (1mM) reduced both the *early* and late phase of the VML response, and while both phases of the VML response were enhanced under 0-Mg+, this effect was completely blocked by CPP. Under control conditions, CNQX (1mM) completely blocked the VML response, however, under 0-Mg+ a ***fast*** residual component of the response was seen which was subsequently blocked by CPP. Theses results confirm the existence of ***fast NMDA*** receptor mediated synaptic transmission .

149.15

INFORMATION CODING BY PRIMARY ELECTROSENSORY AFFERENTS IN *APTERONOTUS*. M.E. Nelson*, J.R. Payne, Z. Xu. Dept. of Physiology and Biophysics, Beckman Institute, Univ. of Illinois, Urbana, IL 61801.

The first stage of information processing in the electrosensory system is the encoding of electrical stimuli by primary electrosensory afferents. Natural stimuli give rise to perturbations in the fish's electric field which typically influence a large number of electroreceptors on the body surface. Individual receptors within the region of influence can have varied properties in terms of threshold, gain, dynamic range and temporal filtering properties. In order to understand how sensory information is encoded at the periphery, it is necessary to characterize the distributions of response properties within the primary afferent population. To address this issue, we characterize response properties in probability coding (P-type) afferents in the brown ghost knife fish, Apteronotus leptorhynchus. We quantify the response properties of individual afferents by fitting a time-domain model of primary afferent dynamics to experimentally measured responses to step and sinusoidal amplitude modulations. We use a linear-nonlinear cascade model, consisting of a linear dynamic block followed by a static nonlinearity. The linear part of the model consists of a spontaneous rate term, a tonic gain term, and two phasic components (fast and slow). The output of the linear model feeds into a sigmoidal-type nonlinearity which accounts for the effects of rectification and saturation. The model provides a basis for quantifying how information about the electrosensory world is represented at the electrosensory periphery.

149.16

SEXUAL DIMORPHISM IN CHIRPING BEHAVIOR INDUCED BY ELECTRICAL STIMULATION OF THE WEAKLY ELECTRIC TELEOST, *APTERONOTUS LEPTORHYNCHUS*. G.K.H. Zupanc[1,2]* and L. Maler[2]. [1]Abteilung Physikalische Biologie, Max-Planck-Institut für Entwicklungsbiologie, W-7400 Tübingen, F.R.G., and [2]Dept. Anatomy, University of Ottawa, Canada K1H 8M5.

The brown ghost, *Apteronotus leptorhynchus*, produces quasi-sinusoidal electric organ discharges (EODs) of highly constant frequency and waveform. In the context of aggression, courtship, and mating short-term modulations of the EOD may occur. One common form are "chirps", brief and sharp rises in EOD frequency (T.H. Bullock, Brain Behav. Evol. *2:* 85-118, 1969; M. Hagedorn and W. Heiligenberg, Anim. Behav. *33:* 254-265, 1985). By stimulating a fish electrically with a sine wave of slightly different frequency than its own EOD, chirps can be elicited under controlled experimental conditions (J. Dye, J. Comp. Physiol. A *161:* 175-185, 1987; L. Maler and W.G. Ellis, Behav. Brain Res. *25:* 75-81, 1987). In the present study, we used such a stimulation regime consisting of four 30 s trials to examine some of the parameters that may influence the propensity to chirp.

While total length, body weight, relative gonadal weight, and EOD frequency were only weakly correlated with the rate of chirping, the sex of the fish turned out to be a decisive factor. Females, on the average, chirped only 0.5 times per 30 s, and 7 out of 9 female fish tested did not chirp at all during the 30 s stimulation periods. Males, in contrast, generated on the average 21.9 chirps per 30 s, and even the weakest male produced a total of 6 chirps during the four 30 s trials. The difference between males and females is highly significant when comparing the mean chirp rates of individuals ($p = 0.0001$, Mann-Whitney U Test, 2-tailed, $n = 9$ females and 21 males). We hypothesize that this sexual dimorphism may be caused by differences between males and females in the structure of the prepacemaker nucleus, a diencephalic cluster of neurons controlling chirp-like modulations of the EOD (M. Kawasaki *et al.*, J. Comp. Neurol. *276:* 113-131, 1988).

149.17

MOVING OBJECT RESPONSES OF PERIPHERAL AND TECTAL MECHANOSENSORY, AND BIMODAL ELECTRO/ MECHANOSENSORY LATERAL LINE UNITS IN WEAKLY ELECTRIC FISH. Randy Zelick* and Horst Bleckmann. Biology Dept., Portland State University, Portland, OR 97207 and Fak. für Biologie II, Universität Bielefeld, W-4800 Bielefeld 1, FRG

In weakly electric fish *Eigenmannia virescens* primary lateral line units were very sensitive to small vibrations from a moving object (2.0x 1.5 x 0.6 cm) for object speeds ≥ 2.5 cm/s. Peak spike rate and total number of spikes elicited increased with increasing object speed. In the velocity range tested (2.3 - 20 cm/s) saturation of neural responses did not always occur. Compared to tuberous electroreceptors, object detectability over distance declined more slowly in the mechanosensory lateral line. Opposite directions of object movement often caused an inversion of the main features of the response histogram. Increases in spike frequency became decreases and vice versa. But in terms of peak spike rate or total number of spikes elicited, primary lateral line afferents were not directionally sensitive.

Midbrain lateral line units of *Apteronotus leptorhynchus* had low spontaneous activity and a weak,jittery response to object motion. If the object was set into vibration while moving past the fish, however, the total number of spikes elicited increased and masked object position. In contrast to the peripheral lateral line, many higher order lateral line neurons were direction-sensitive. Highly directional ampullary and tuberous midbrain units were also recorded. The receptive fields of single units demonstrated a somatotopic organization of lateral line input: anterior body areas project to rostral midbrain, posterior body areas project to caudal midbrain. Bimodal lateral line-tuberous or lateral line-ampullary units recorded were OR units, i.e., the units were reliably driven by a unimodal stimulus of either modality.

Thus object motion is coded by mutlimodal mechano- and electrosensory input. Tuberous electrosensory input provides good low-speed information over a shorter range, while the mechanosensory system preserves higher-frequency motion and is sensitive over a longer range.

149.18

TRANSNEURONAL BIOCYTIN OUTLINES THE VOCAL MOTOR CIRCUIT IN A TELEOST FISH. A. Bass*, M. Marchaterre, B. Horvath and R. Baker. Neurobiology and Behavior, Cornell Univ., Ithaca, N.Y. 14853; Bodega Marine Laboratory, Bodega Bay, CA. 94923; Physiology & Biophysics, NYU Medical Center, New York, N.Y. 10016.

The plainfin midshipman *Porichthys notatus* vocalizes by the simultaneous contraction of sonic muscles attached to the lateral walls of the swimbladder. Intracellular staining and recording has demonstrated that each muscle is innervated ipsilaterally by large motoneurons (lm) located in caudal brainstem sonic motor nuclei (SMN). Certain pacemaker neurons (pm) lie ventrolateral along the entire extent of the SMN and innervate it bilaterally, consistent with the hypothesis that they synchronize motoneuron activation. Biocytin was used as a transneuronal tracer to more completely identify the extent and position of pacemaker and command neurons. Following ipsilateral injections into the sonic muscle or nerve, 4 groups of biocytin-labelled neurons with a Golgi-like filling of their processes and somata were observed: lm ipsilateral, pm bilateral, 'small' motoneurons (sm) bilateral but restricted to the rostral SMN, and ventrolateral reticular formation neurons (vrf) bilateral but rostral to the SMN. Sm, unlike lm, had extensive dendritic branching lateral to the SMN and possible axonal branching to both sonic muscles. Axons of the vrf ascended and terminated at the level of brainstem auditory and vestibular nuclei. The vrf may be a part of the pacemaker circuitry and include command neurons providing temporal information to sensory-recipient nuclei. Support by NSF, NIH and New York State Hatch Act.

149.19

SOUND PRODUCTION EVOKED BY ELECTRICAL STIMULATION OF THE OYSTER TOADFISH FOREBRAIN. M.A. Perini and M.L. Fine*. Dept. of Biology, VA. Commonwealth Univ., Richmond, VA 23284.

Although sonic pathways in vertebrates are considered homologous, forebrain involvement in sound production has not been investigated in fishes. We targeted potential forebrain structures in and close to the preoptic area based on their role in fish courtship, presence of steroid concentrating neurons and involvement in frog mating call production. The agonistic grunt and courtship boatwhistle call were evoked in the nucleus preopticus parvocellularis anterior (PPa) and the supracommissural nucleus of the ventral telencephalon (Vs), an amygdala homologue. Evoked sounds formed a continuum from knock to burst grunts to transition and complete boatwhistles, and sound pressure level (SPL), fundamental frequency and duration increased within the continuum. For all sound types, SPL exhibited smallest, fundamental frequency intermediate and duration widest variation. Boatwhistles exhibited the smallest variation suggesting maximal output of the CNS and sonic muscles, whereas grunt SPLs varied by as much as 18 dB, suggesting recruitment of variable numbers of motor units despite electrical coupling within the sonic motor nucleus.

149.20

STUDIES OF THE STARTLE REFLEX IN FATHEAD MINNOWS: METHODS AND INITIAL DATA. J.M. Wakeman, M.Q. McHenry, I.D. Kendrick and R.L. Seaman*. Depts. of Biological Sciences and Biomedical Engineering, Louisiana Tech Univ., Ruston, La 71272.

Startle reflexes in fathead minnows (Pimephales promelas) were evoked using brief (50-100 ms) sinusoidal sound stimuli at frequencies from 50 to 1000 Hz. The fish were held within a cylinder along which they could swim freely but could turn only with difficulty. An infrared beam at one end of the cylinder was directed towards an infrared detector at the other end and responses were digitized at 2000 samples/sec and stored on disk.

Initiation of the startle response occurred within 8 - 14 ms (mean = 10 ms) following onset of the stimulus. At frequencies below 700 Hz, startle responses were elicited in 71% of the trials. However, the response rapidly weakened at higher frequencies and no startle response could be evoked at 1000 Hz.

The techniques and initial data developed in this study will be applied in subsequent studies on modification of fathead minnow startle responses by prepulse stimuli and chemical exposure.

Supported by a Louisiana Tech Faculty Development Grant.

150.1

CHRONIC VARIABLE STRESS ENHANCES THE STIMULATORY ACTION OF LOW DOSES OF MORPHINE: REVERSAL BY DESIPRAMINE. V.A. Molina*, C.J. Heyser, and L.P. Spear. Center for Developmental Psychobiology and Dept. of Psychology, SUNY, Binghamton, NY 13902-6000.

The effect of a low dose of morphine on locomotor activity was investigated in rats previously exposed to a chronic variable stress treatment (CVS) with or without concurrent daily administration of desipramine (DMI). Animals given CVS were submitted daily to a different stressor following an injection of either saline or DMI (5 mg/kg, i.p.), whereas control animals were unmanipulated except for the injection process. Following 2 weeks of CVS treatment, control and stressed animals were administered saline or morphine (1.5 mg/kg, i.p.) and their locomotion assessed for 90 min. A substantially greater increase in locomotor activity following morphine administration was observed in chronically stressed rats as compared to control rats. This potentiated locomotor response to morphine in CVS rats was attenuated by DMI pretreatment prior to the daily stressors. These data support the suggestion that a CVS regimen results in sensitization to the low dose stimulant effects of opioid stimulation, and that pretreatment with the tricyclic antidepressant DMI prior to the daily stressor blocks the development of this sensitization.

150.2

VISCERAL AND BEHAVIORAL HYPERSENSITIVITY CONSEQUENT TO REPEATED STRESSOR EXPOSURES: FURTHER EVIDENCE TOWARD AN ANIMAL MODEL OF PTSD. **R.J. Servatius*, J.E. Ottenweller, S. Soldan, J.L. Gross, B.H. Natelson. V.A. Medical Center & New Jersey Medical School, East Orange, N.J. 07019.**

Human PTSD, a stress-related mental illness, is characterized by persistent post-stressor abnormalities including visceral hyperresponsiveness and exaggerated startle responding. We previously reported an animal model of PTSD exhibited elevated basal corticosterone (CORT) and altered behavior for up to 3 days post-stressor. To extend our analogy to PTSD we examined hypersensitivity in our model. In Experiment 1, rats were exposed to 3 or 1 day(s) of 40, 2-mA tailshocks. Rats were then given a single tailshock probe 10 days post-stressor. For both repeatedly stressed groups the CORT response to the probe at 15-min and 60-min post-stressor was elevated compared to nonstressed probed rats. In Experiment 2, rats exposed to 3 days of tailshock stress were tested for their startle response to high and low auditory stimuli. Repeatedly stressed rats exhibited an exaggerated startle response to the low stimulus 4 days post-stressor, but not at either 1 day or 10 days post-stressor. There were no difference in startle responding to the high stimulus. The hyperresponsiveness in our model, both visceral and behavioral, makes it a prime choice for the study of the etiology and treatment of PTSD. **Supported by V.A. Medical Research funds.**

150.3

CHRONIC STRESS DOES NOT AFFECT ARTERIAL BLOOD PRESSURE IN THE RAT. **Y. Tizabi*, V.J. Massari and P.J. Gatti. Dept. of Pharmacol., Howard Univ., Washington, D.C. 20059.**

In humans, stress or psychological stimulation may be an important factor in the etiology of hypertension. Several studies in the rat have provided evidence in support of this hypothesis. However, these studies have utilized the tail-cuff method for measurement of blood pressure (BP). Since a variety of factors can influence the outcome of measurement by this method, rigorous control of the environment is required. In addition modest changes in BP may be difficult to detect with this technique. This study was designed to correlate the changes in BP, measured by direct intraarterial cannulation, with various durations of stress. Male Sprague-Dawley rats were immobilized for 2.5 hr daily, 5 days a week for 2, 4 or 6 weeks. Twenty four hours after the last stress, rats were anesthetized with urethane (1.5g/kg, i.p.) and the carotid artery was cannulated for BP measurement. The anesthetic urethane was shown not to affect BP. The study verified that the weight gain in stressed animals was considerably lower than in controls. However, no significant difference in BP or heart rate between any stressed group or controls was detected. The mean systolic and diastolic pressures were approximately 92 and 52 mmHg respectively. The mean heart rate was approximately 300 beats/min. Thus, the question of whether chronic stress does indeed result in hypertension in rats requires further clarification. **(Supported by American Heart Association/ NCA)**

150.4

THE EFFECTS OF CONTINUOUS AND INTERMITTENT (SART) COLD EXPOSURE ON HYPOTHALAMIC-PITUITARY-THYROID AXIS IN RATS. K. Fukuhara[1,3], G. Cizza*[2], R. Kvetnansky[1], V.K. Weise[1], H. Ohara[3], K. Go[3] and I.J. Kopin[1]. [1]NINDS and [2]NIMH, NIH, Bethesda, MD 20892, [3]Inst. of Bio-Active Science, Nippon Zoki, Hyogo, Japan.

Immobilization stress produces an activation of the sympathoadrenal and hypothalamic-pituitary-adrenocortical systems but inhibits the hypothalamic-pituitary-thyroid axis activity in rats. Recently, we reported that SART (Specific Alternation of Rhythm in Temperature) stress, i.e. continuous cold exposure interrupted by four hourly intervals of room temperature daily, activates mainly the sympathoneural system in rats. In the present study, we examined the effects of long-term SART and continuous cold on hypothalamic-pituitary-thyroid axis, which is functionally related to the sympathoadrenal system. Male Wistar rats (280-350 g) were exposed to -3 °C for 5 days, either continuously, either with four hourly intervals of room temperature. Plasma TSH and free T3 levels were significantly increased by both SART and cold stress, whereas plasma free T4 levels were not changed. KCl-stimulated TRH *in vitro* secretion from hypothalamic explants was four times higher in both SART and cold stressed-rats compared to controls (room temperature), whereas basal TRH secretion was not modified by cold exposure. Plasma ACTH and corticosterone levels were not significantly changed in rats exposed to either SART or continuous cold stress for 5 days. These results suggest that the hypothalamic-pituitary-thyroid axis is highly activated by continuous or intermittent (SART) cold exposure. It appears that hypothalamic-pituitary-adrenocortical system, which is highly activated by some stressors (e.g. immobilization), is not significantly affected by long-term cold exposure.

150.5

CHRONIC SOCIAL STRESS IN RATS: EFFECTS ON GLUCOCORTICOID SECRETION AND CORTICOSTEROID BINDING GLOBULIN R.R. Sakai*, S.M. Weiss, D.C. Blanchard, R.J. Blanchard, R.L. Spencer, A.H. Miller and B.S. McEwen. The Rockefeller University 1230 York Ave., NY., NY 10021, Mount. Sinai Medical Center. NY, NY 10029 and The University of Hawaii, 2430 Campus Rd. Hono., HI 96822.

The use of a visible burrow system in the analysis of social interactions in rat colonies has been shown to provide an effective model for the examination of behavioral and neuroendocrine effects of prolonged social stress. In a previous study we showed that dominant male rats within each colony had lower basal plasma corticosterone than subordinate rats after two weeks in the colony situation.

We have extended these studies to show that, in addition to an elevated basal level of corticosterone, the response of subordinate rats after 1 hour of a novel restraint stress was blunted as compared to the typical elevation in corticosterone seen in control animals.

Levels of serum corticosteroid binding globulin (CBG) were also lowest in subordinates, higher in dominants and highest in singly-housed controls. Low CBG in subordinates may lead to higher concentrations of free corticosterone in these animals and may explain the increase in receptor activation (decrease in Type II glucocorticoid receptor binding) found in spleen tissue of subordinate rats compared to dominant and control animals.

These data provide further support that subordinate and dominant male rats in a burrow system habitat provide a useful model for the analysis of neuroendocrine effects of prolonged social stress. (Supported by AA0622, MH41256, MH42803, MH47674)

150.6

NIMODIPINE COUNTERACTS BEHAVIORAL EFFECTS OF LONG TERM CORTICOSTERONE TREATMENT

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Corticosterone (COR) slow release pellets were implanted subcutaneously in young Fischer rats, in an attempt to produce an animal model for the central effects of stress. COR treated rats exhibited cognitive impairments during the initial acquisition stage of the radial arm maze (RAM), and morphological specific damages in their pyramidal hippocampal cells, mainly at CA1 and CA4 regions. In a continuation study, COR and placebo groups were divided into two sub-groups, fed by either regular food or food containing 860 ppm of nimodipine. During the second week of this treatment, their activity in a new environment was measured. Placebo-treated rats, explored the new environment and showed a characteristic habituation, leading, two days later, to a low activity in the same maze, while COR treated group regarded it as completely new. COR rats treated with nimodipine behaved during this test as controls. The protection afforded by nimodipine against morphological and cognitive impairments induced by long term COR treatment will be substantiated using the RAM and histological evaluation (Supported by Bayer AG).

150.7

CHRONIC COCAINE CHANGES CHRONIC STRESS-INDUCED ALTERATIONS IN DOPAMINE (DA) AND 5-HT NEUROCHEMISTRY. David J. Mokler*, Mark Dixon and Denise Gay Dept. Pharmacol., Univ. New England Col. Osteopathic Med., Biddeford, ME 04005.

The purpose of the present experiment was to examine the effects of chronic cocaine administration on the stress-induced alterations in DA and 5-HT neurochemistry. Male C57Bl/6J mice were exposed to an acoustic stressor (100 dB, 8 kh tone for 1 sec on a variable 90 sec interval) during 1 hr sessions for 14 d. Half of each stress group was administered either saline or 25 mg/kg cocaine HCl 3 hours after the end of the session. Five min after the last stress session, animals were decapitated. The CN, mPFC and NA were dissected from frozen slices and analyzed by HPLC. Cocaine and stress produce significant alterations in the levels of DA and metabolites, DOPAC and HVA, as well as, 5-HT and 5-HIAA in the mPFC. Turnover rates were increased for DA by cocaine and cocaine and stress; while 5-HT turnover was increased by cocaine and stress but not the combination.. In the CN, stress decreased DA, HVA, 5-HT and 5-HIAA. Cocaine did not alter the effects of stress. Turnover of DA and 5-HT were not altered by stress or cocaine. Thus chronic exposure to cocaine produces alterations in the response to chronic stress.

150.8

ESCAPABLE AND INESCAPABLE SHOCK DISRUPT DELAYED ALTERNATION IN RATS R. Bauman* and G. Kant. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307.

In our rodent model of sustained stress, a 12 hour light/dark cycle is maintained in individual living cages where a rat must feed itself by pressing a lever once for each food pellet. A rat in group **ES** is trained to pull a ceiling chain to avoid/escape signaled footshocks that are scheduled 24 hours/day. In group **IS**, a rat can not avoid/escape shock; shock delivery is yoked to the delivery of shocks in group **ES**. In an effort to evaluate the effects of sustained stress on a simple cognitive performance, pellets were delivered only if a rat alternated presses on two levers **and** a signaled minimum delay of 16 sec elapsed between presses.

Compared to rats who were not shocked, mean percent correct alternation (accuracy) of groups **ES** and **IS** was reduced during the dark but not the light period of the first day of shock. The mean accuracy of group **IS** was most reduced and compared to the accuracy of group **ES** recovered much more slowly during seven days of sustained shock. These data suggest that this model is sensitive to the differential impairment of cognitive performance by controllable and uncontrollable stress.

150.9

FOOTSHOCK-INDUCED EXACERBATION OF AMPHETAMINE CTA. W.J. Bowers, M.A. Gingras & Z. Amit*, Center for Studies in Behavioral Neurobiology, Dept. Psychology, Concordia University, Montreal, Canada, H3G 1M8.

Stressors have been reported to block the development of CTA to lithium but not morphine. However, it is unclear if this effect generalizes to other self-administered drugs. Since cross sensitization exists between amphetamine and stressors, we expected that footshock would augment amphetamine-induced CTA. In experiment 1 rats were placed on a restricted water schedule. On pairing days animals were given .1% saccharin instead of water and were exposed to either footshock or no shock. Half the animals in each group were then injected with either saline or 2mg/kg amphetamine. A second pairing was conducted 2 days later followed by 4 extinction trials on alternate days and access to water on intervening days. Experiment 2 was identical except footshock was given 2 and 4 days preceding the amphetamine-saccharin pairing. On pairing days, half the animals in each shock group received either saline or amphetamine. A second pairing was conducted 2 days later followed by 4 extinction trials as in experiment 1. For both experiments, a CTA was evident on the second pairing day and continued until the third extinction trial. Footshock alone had no effect on saccharin consumption but it did exacerbate the amphetamine-induced CTA evident on the second pairing day.

150.10

CENTRAL ALPHA$_2$ ADRENOCEPTORS AND FOOTSHOCK STRESS. G.B. Kovachich*, R.E. Poland, A. Frazer & C.E. Aronson, Vet. Affairs Med. Ctr., Phila. PA*, Harbor-UCLA Med. Ctr., Torrance, CA, Univ. of Pa. School of Med., Phila. PA.

Stress activates the hypothalamic-pituitary-adrenal (HPA) axis leading to augmented release of ACTH and corticosterone (CS), and repeated exposure to the same stressor attenuates this response. Alpha$_2$ adrenoceptors appear to play a key role in regulation of ACTH and CS release, since administration of clonidine alters their release under either basal or stress conditions. We examined the effect of daily exposure of rats to 30 min sessions of intermittent footshock (0.15mA shocks calibrated across 53K Ohms; 0.5sec duration with 0.5sec intervals delivered 15 times every 5 min) for 1, 6 or 10 days on central alpha$_2$ adrenoceptors, measured by quantitative autoradiography using ^{3}H-Idazoxan (3nM). Baseline CS increased slightly, but not significantly, in rats given footshock for 6 days. However, a single initial footshock session increased plasma CS by 209 ± 28% but the same footshock session only increased plasma CS by 47 ± 23% in rats given five prior footshock sessions ($p<0.001$). This suggests adaptation of the stress response. Central alpha$_2$ adrenoceptors showed no change in the paraventricular nucleus of the hypothalamus (where CRF producing neurons originate) or in noradrenergic cell body areas A1, A2, or A6, either after 1, 6 or 10 days of footshock. By contrast, chronic but not acute exposure of rats to footshock significantly elevated alpha$_2$ adrenoceptors in several areas of the dorsal hippocampus. The results support recent results indicating the importance of the hippocampus in the adaptive response to chronic stress. (Supported by Research Funds from the Veterans Administration).

150.11

CHRONIC ABERRANT BEHAVIOR IN THE MENTALLY RETARDED (MR): ANIMAL MODELS, RISK FACTORS AND REPEATED STRESS. C. J. Stodgell*, P. S. Loupe, & R. E. Tessel Dept. of Pharmacol. & Toxicol. Univ. of Kansas, Lawrence, KS 66045

Certain risk factors appear to contribute to the likelihood that the MR will chronically manifest aberrant behaviors (CAB). Previously our laboratory demonstrated that acute stress (foot shock) can elicit CAB-like behaviors in rats modeling these risk factors. In the present study we examined the behavioral effects of chronic (repeated) stress in these risk-factor models. Spontaneously hypertensive (SHR), Sprague-Dawley (SD), methylazoxymethanol-induced microencephalic (MAM), and 6-Hydroxydopamine (6-HD) neonatal catecholamine depleted rats were group housed (GH) or socially isolated (SI) for 3 months post weaning. Then, every 3-4 days rats received foot shock (6/2s every 10s/1h X 7 sessions.), The sessions were video taped and behaviors scored (e.g., self injurious behavior, aggression, stereotypy, and hyperactivity). Differences in groups and housing condition were as follows (">" = $p< 0.05$; lack of housing designation indicates no sig. housing effect). **CAGE BITING (aggression);** Session 1: SHR-SI>all other group. Session 7: 6-HD-SI>all other groups. **MAINTAINED REARING (stereotypy);** Sessions 1 and 7: MAM>all other groups. Session 1 v. 7: Maintained rearing is increased in the MAMS. **LOCOMOTOR ACTIVITY DURING SHOCK (hyperactivity);** Session 1: MAM>(SD-SI>SD-GH)=(SHR-SI>SHR-GH)>MAM. Session 1 v 7: Locomotor Activity is decreased in all of the groups. **FREEZING (LEARNED HELPLESSNESS?);** SESSION 1: SHR>SD=6-HD>MAM. Session 7: SHR=SD=6-HD>MAM. Session 1 v 7: There is an increase in Freezing behavior in all groups except MAMs. These data support the hypothesis that stress can contribute to both the chronicity and topography of CAB displayed by the MR. (Supported by NICHHD grant 1 PO 1 HD26927)

150.12

FEAR-POTENTIATED STARTLE IN POST-TRAUMATIC STRESS DISORDER C.A. Morgan III M.D., C.Grillon Ph.D.*, M.Davis Ph.D., D.S.Charney M.D. Yale University, West Haven VA Medical Center,West Haven, CT. 06516.

Objective studies have given conflicting results as to whether or not startle is increased in Post Traumatic Stress Disorder (PTSD). Normal, increased and reduced startle have all been reported. Increased startle can be considered as part of a conditioned emotional response to stimuli reminiscent of trauma. A reasonable model for the conditioned emotional response in PTSD is the fear-potentiated startle paradigm in which a central state of fear/anxiety increases the magnitude of the startle reflex. This study was designed to explore both the magnitude and timing of the effect of anticipatory anxiety on startle in combat veterans with PTSD. The eyeblink component of the startle reflex was measured at various times preceding and following the anticipation of electric shock in 9 PTSD subjects and 10 age-matched controls. Results indicate that startle was greater throughout the experiment in the PTSD subjects, compared to the controls. In addition, the magnitude of startle potentiation at the time of shock expectation was larger in the PTSD, compared to the control group. Because data from our laboratory indicate normal baseline startle in the same cohort of PTSD subjects when no aversive stimulation is anticipated, these data suggest that exaggerated startle is contextual in PTSD.

150.13

STRESSOR CONTROLLABILITY AND PLASMA CHOLESTEROL. Francis X. Brennan, Jr.*, Linda R. Watkins, & Steven F. Maier. Psychology Department, University of Colorado, Boulder, CO, 80309, USA.

Elevations in the level of cholesterol in plasma are a risk factor for the development of Coronary Heart Disease (CHD). We have recently reported (Brennan, Job, Watkins, & Maier, 1992) that three sessions of inescapable tailshock, one session per day for three consecutive days, is sufficient to increase the total plasma cholesterol levels of rats versus nonshocked controls. Uncontrollable stress produces a myriad of behavioral and physiological changes that do not occur, or occur with a marked reduction in severity, if the physically identical stressor is presented in a controllable manner. Berger, Starzec, Mason, and DeVito (1980) demonstrated that total plasma cholesterol was sensitive to stressor controllability using a 30 day leverpress avoidance paradigm. The purpose of the present study was to explore whether various cholesterol parameters were differentially sensitive to the controllability of our relatively brief stress procedure.

Male Sprague-Dawley rats were exposed to 3 sessions of either escapable shock, inescapable shock, or no shock. Escape subjects could terminate a 1.0 mA shock pulse, delivered to the tail via an attached electrode, by performing a wheel turn response. Each escape subject had a yoked partner that received the identical pattern of shock as his escape partner. Control animals were left undisturbed in their home cages. Escape and yoke subjects received one 100-shock session per day, for three consecutive days. Blood samples were taken prior to stress, and four hours after the conclusion of the third stress session.

Results indicated that stressed groups showed elevated levels of both total cholesterol, as well as LDL and VLDL cholesterol, relative to nonshocked controls. No differences were observed between escape and yoke animals. The fact that cholesterol is sensitive to controllability in more chronic stress regimens (Berger et al., 1980), while the controllability of the stressor is not a relevant variable in our brief stress paradigm, may have implications for the pathogenesis of CHD.

150.14

LINKAGE OF STRESS-INDUCED HYPOCALCEMIA, GASTRIC ULCER AND BEHAVIORAL DESPAIR IN WISTAR KYOTO RATS. S. AOU, J. MA and T. HORI, Dept. of Physiology, Fac. of Med., Kyushu Univ., Fukuoka 812, Japan.

Recently the stomach has been suggested as a critical organ for etiology of immobilization-induced hypocalcemia (Aou et al., 1991). Changes in calcium metabolism can affect many physiological functions, however, pathophysiological implication of stress-induced hypocalcemia is unknown. In the present study, stress-induced hypocalcemia was investigated in relation to gastric ulcer and emotional behavior in Wistar Kyoto (WKY) rats which have been known to be vulnerable to behavioral despair and gastric ulcer (Pare, 1989). Wistar rats were used as a control. In forced-swimming test, WKY rats showed significantly longer duration of immobility and shorter duration of struggling than Wistar rats as previously reported. In immobilization stress under water immersion, the WKY rats showed significantly severer gastric ulcer and hypocalcemia than the Wistar rats. The degrees of hypocalcemia and total ulcer length were positively correlated in both strains. The severity of both ulcer and hypocalcemia were positively correlated with the immobility time in the WKY rats, while those were negatively correlated with the struggling time in the Wistar rats. It is concluded that vulnerability to stress-induced hypocalcemia are closely related to gastric ulcer and behavioral responsiveness to the stress condition. Those factors link each other in strain dependent manner. The stress-induced hypocalcemia seems to be an emotionally induced pathological phenomenon associated with formation of gastric ulcer.

150.15

HUMAN RESPONSE TO HIGH DOSE NALOXONE FOLLOWING EXPOSURE TO CONTROLLABLE AND UNCONTROLLABLE STRESS J. Fertig*, R. Peters, J. Leu, G. Meuller, G. Kamimori. Walter Reed Army Institute of Research, Washington, D.C. 20307

In an attempt to characterize the biochemical and physiological response to naloxone administration following exposure to stress, forty healthy male subjects were exposed to bursts of 95 dB noise while attempting to solve a visual-spatial task under either controllable stress (CS) or uncontrollable stress (UCS) conditions. The stress induction procedure was followed by a double blind infusion of the opiate antagonist naloxone (1.5 mg/kg) or placebo. Physiologic reactivity, biochemical response, mood and physical symptoms were monitored throughout the stress induction and drug administration procedures. Naloxone had a highly significant effect on heart rate ($p<.0001$), plasma cortisol ($p<.0001$), and immunoreactive beta-endorphin ($p<.0001$). Diastolic blood pressure, systolic blood pressure, and plasma norepinephrine which had been differentially elevated by stress were unaffected by the administration of naloxone. Subjects who received naloxone also reported an increased number of physical symptoms as well as disturbances of mood. Results suggest that the opiate antagonist naloxone has a selective effect on the biochemical and physiological responses to stress.

150.16

NEUROENDOCRINE STRESS RESPONSE IN ADOLESCENT PREGNANCY. A. Chicz-DeMet*, B. Simon, E.M. DeMet, C.K. Cho and F.M. Crinella. Dept. Psychiatry & Human Behavior, UC Irvine, CA 92717 and State Dev. Res. Inst., Costa Mesa, CA 92626.

Three biochemical stress indices, plasma β-endorphin (βE), ACTH, and cortisol, were studied in 26 adolescent and 59 adult mothers during the third trimester of pregnancy. A multivariate comparison failed to reveal any significant group difference ($F=1.11$; $df=3, 81$) although βE levels were marginally elevated (11.6%) in the adolescent group. Plasma ACTH and cortisol levels were significantly correlated in adults ($r=0.31$; $p<0.02$), but not adolescents ($r=0.10$) although the group difference was also not significant. In contrast, adolescent βE levels were significantly correlated with cortisol ($r=0.52$; $p<0.007$) whereas adult levels were not ($r=0.01$), and this group difference was significant ($F=5.8$; $p<0.005$). Plasma βE and ACTH levels were significantly correlated in both groups although the regression lines significantly differed ($F=6.6$; $p<0.003$). Adjustment of both variables for the influence of feedback regulation on the HPA axis (partialling of cortisol values) significantly improved the model fit in adults ($F=6.5$; $p<0.02$) but not adolescents ($F=0.13$, $df=1, 23$). The results suggest that stress induced changes in plasma cortisol may be selectively linked to βE increases in adolescent as opposed to adult mothers.

MONOAMINES AND BEHAVIOR: SEROTONIN

151.1

$5HT_2$ RECEPTOR CONTRIBUTION TO THE LOCOMOTOR ACTIVATION PRODUCED BY THE AMPHETAMINE ANALOGUE, 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA). J.H. KEHNE*, H.J. KETTELER, R.A. PADICH, T.C. MCCLOSKEY, C.K. SULLIVAN, AND C.J. SCHMIDT. Marion Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati, OH 45215.

Understanding the mechanisms of amphetamine and related compounds is critical for their utilization in models of CNS dysfunction. Given data that amphetamines acutely release both 5HT and DA, the present study evaluated the relative roles of these monoamines in the locomotor stimulating effects of the ring-substituted analogue MDMA.

MDMA (0.25-20 mg/kg) dose-dependently stimulated locomotion in rats as measured with photocell activity cages. MDMA stimulation (20 mg/kg; 0-30 min post-injection) was significantly reduced (but not abolished) in rats with severe central 5HT depletions achieved with i.c.v. 5,7-DHT. A similar magnitude attenuation was produced by the highly selective $5HT_2$ antagonist MDL 100,907 (R(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluoro-phenylethyl)]-4-piperidinemethanol), or by the DA antagonist haloperidol. In contrast, low dose (2 mg/kg) MDMA locomotor stimulation was reduced by haloperidol but not MDL 100,907.

The data suggest that $5HT_2$ and DA receptor stimulation both contribute to the early locomotor activating effects of high doses of MDMA.

151.2

EFFECTS OF SEROTONERGIC DRUGS ON A 5,7-DIHYDROXYTRYPTAMINE-ASSOCIATED BEHAVIORAL DEFICIT IN RATS PERFORMING ON A DIFFERENTIAL REINFORCEMENT OF LOW RATE-72 SECOND OPERANT SCHEDULE OF WATER REINFORCEMENT. D. C. Jolly*, J. B. Richards, and L. S. Seiden, Dept. of Pharmacol. and Physiol., University of Chicago, Chicago, Illinois. 60637.

The Differential Reinforcement of Low Rate-72 second (DRL-72s) operant schedule of water reinforcement in rats is a behavioral screening procedure for antidepressant drugs. Chemical lesion of the brain serotonin (5-HT) system disrupts DRL-72s behavior. This experiment was an attempt to discern the 5-HT receptor subtypes involved in the performance of DRL-72s behavior. Trained rats were given bilateral, intracerebroventricular (icv) infusions of 100 µg of 5,7-dihydroxytryptamine (5,7-DHT). A control group received icv infusions of vehicle. After the rats recovered from surgery, DRL-72s performance was continually assessed for a period of 17 weeks. During drug free (baseline) sessions, all measures of the behavior of the control group remained unchanged, but the 5,7-DHT rats showed a behavioral deficit. This behavioral deficit was characterized by increased response rate, decreased reinforcement rate, and disruption of the temporal distribution of DRL-72s responding relative to pre-lesion baseline behavior. The results of 5-hydroxytryptophan administration to the 5,7-DHT treated rats suggested partial amelioration of the DRL-72s behavioral deficit. The 5-HT1A agonist drugs 8-hydroxy-2-di-N-propylaminotetralin (8-OH-DPAT), or 5-Methoxy-dimethyltryptamine (5-MeODMT) reversed the behavioral deficit observed in the 5,7-DHT treated rats. The neurochemical assay results indicated specific depletion of 5-HT in the 5,7-DHT treated rats, in all brain regions examined. These results suggest that 5-HT1A receptors play a significant role in the serotonergic mediation of DRL-72s response timing.

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151.3

DOPAMINE AND LEARNED HELPLESSNESS: *IN VIVO* MEDIATION BY SEROTONIN IN FRONTAL CORTEX. F. Petty*, L. Kramer, L. Wilson, M. Waddill and Y.L. Chae. VA Medical Center and the University of Texas Southwestern Medical School, Dallas, TX 75216.

Learned helplessness (LH) is a maladaptive behavioral depression following inescapable stress (IS) which may be a model of human depression. LH can be induced neurochemically in naive mice by injection of the dopamine (DA) receptor blocker haloperidol (HDL). We have replicated this in the rat, with doses of 10 and 20 mg/kg (but not 5 mg/kg) inducing LH. These doses all produced increased release of DA (measured by *in vivo* microdialysis), but only 10 mg/kg elevated basal 5-HT release. When HDL was perfused in the microdialysis probe, increased release of both DA and 5-HT was produced by 100 μM HDL. Also, perfusion of DA itself through the probe led to increased release of 5-HT measured in perfusate. Of note is the fact that even at the highest dose intraperitoneal, HDL only produced behavioral depression in 67% of rats (similar to IS). Therefore increased frontal cortex (FC) DA release, known to be a common and non-specific stress effect, may play a necessary but not sufficient role in LH development. Along with previous research, increased FC 5-HT release and subsequent 5-HT depletion cause the subsequent LH.

151.4

ANXIETY, 5-HT RELEASE AND THE EFFECT OF DRUGS IN THE GUINEA-PIG. A. REX, C. A. MARSDEN AND H. FINK[1] (SPON: Brain Research Association) Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, UK, and [1]Institute of Pharmacology and Toxicology, Humboldt University, 1040 Berlin.

In the present microdialysis study exposure of the guinea-pig to the elevated plus maze increased extracellular 5-HT in the frontal cortex; an effect similar to that seen in the rat. Diazepam and the 5-HT_{1A} agonist 8-OH-DPAT decreased not only basal extracellular 5-HT but also reduced the increase in 5-HT observed on exposure to the x-maze and produced 'anxiolytic' behaviour. Pretreatment with flumazenil (10.0 mg/kg) and methiothepin (10.0 mg/kg) antagonised the neurochemical and behavioural effects of diazepam and 8-OH-DPAT, respectively. Flumazenil alone also decreased basal extracellular 5-HT and prevented the increase seen when the guinea-pigs were on the x-maze, but had no effect on behaviour. 5-CT, a 5-$HT_{1D/1A}$ agonist prevented the increase in extracellular 5-HT but had no anxiolytic profile. The results show that an increase in extracellular 5-HT occurs in the guinea-pig exposed to aversive conditions. While the anxiolytic effects of diazepam in the guinea-pig were associated with decreased extracellular 5-HT, there was no relationship between the decrease in extracellular 5-HT after either flumazenil or 5-CT and behaviour.

151.5

DIFFERENTIAL EFFECT OF TWO 5HT AGONISTS ON REM SLEEP INITIATION AND PGO GENERATION. R.J. Ross, L.D. Sanford, A.E. Seggos, A.R. Morrison, W.A. Ball*, and G.L. Mann. Depts. of Anim. Biol., and Psychiatry, Univ. of Penn. and VAMC., Phila., PA 19104.

Ponto-geniculo-occipital waves (PGO), identifying markers of REM, appear to be generated by "burst" neurons in mesopontine cholinergic nuclei. Serotonin (5HT) may modulate REM sleep and inhibit PGO generation. We unilaterally infused the relatively specific $5HT_{1A}$ agonist 8-hydroxy-2-(n-dipropylamino)tetralin (DPAT; 0.0, 0.002, 0.01, and 0.08 μg/0.5μl) and the less specific 5HT agonist 1(3-chlorophenyl)piperazine (mCPP; 0.0, 0.02, 0.2, 2.0, and 20.0 μg/0.5μl) into the mesopontine peribrachial region (PB) of cats to examine the role of 5HT receptor mechanisms in sleep/wake patterns and PGO generation. Six-hour sleep studies separated by a minimum of 7 days were conducted for each dose. The number of REM episodes ($\geq$ 1 min) was significantly decreased by DPAT (0.01 μg). REM% was decreased 71% by this same dose; however, there was no significant effect on REM episode duration once a cat actually entered REM. A higher dose (0.08 μg) did not significantly decrease the number of REM episodes relative to saline, perhaps due to the activation of additional 5HT receptor populations. All doses of DPAT increased aborted REM episodes (<1 min) and periods of increased PGO rate in non-REM (NREM) that did not lead to REM. DPAT did not appear to affect PGO generation (ipsilateral to injection site) independently of its effect on REM; PGO rate/min in REM episodes ($\geq$1 min) was not significantly different from the saline control. REM% and total number of REM episodes ($\geq$ 1 min) were only marginally decreased by mCPP (0.02 and 0.2 μg; $p < .08$) relative to saline. At higher dosages of mCPP (2.0 and 20.0 μg) sleep architecture was not discernible from that during saline control, and increases in aborted episodes of REM were not noticed. $5HT_{1A}$ receptor mechanisms have been suggested to regulate REM and waking. We propose that actual REM initiation is regulated by $5HT_{1A}$ receptor mechanisms located in PB. Supported by MH-42903, MH-18825 and the D.V.A. Med. Res. Serv.

151.6

ANTAGONISTS AT THE 5-HT_3 RECEPTOR CAN REDUCE THE RAT POTENTIATED STARTLE RESPONSE: BUT IS THIS SUPPORT FOR ANXIOLYTIC ACTIVITY? E. W. Anthony* and M. E. Nevins. Neurological Diseases Research, SEARLE, Skokie, IL 60077.

The ability of drugs to reduce the "conditioned fear" leading to the potentiated startle response in rats is believed to predict anxiolytic efficacy in humans. Clinically-effective anxiolytics, such as the benzodiazepines and buspirone, reduce the potentiated startle response. Antagonists at the 5-HT_3 receptor are currently under clinical investigation for anxiolytic utility: and one report [Glenn & Green, Behav. Pharmacol. 1: 91-94, 1989] found ondansetron to block potentiated startle. The current study was done to determine the effects of three 5-HT_3 antagonists on the potentiated startle response. Two conditioning protocols were used: a) 10 conditioning trials on each of two days using 0.5 mA footshock (i.e., the "standard" conditioning protocol), and b) 15 conditioning trials in 1 day using 0.25 mA footshock (which presumably produces a less intense conditioned fear). Ondansetron, (+)-zacopride, granisetron, diazepam and buspirone were evaluated for their ability to reduce the potentiated startle produced by both protocols. While diazepam and buspirone effectively reduced the potentiated startle produced by both protocols, the 5-HT_3 antagonists were potently effective only in the modified protocol. Ondansetron and granisetron were ineffective, at doses up to 1 mg/kg, and (+)-zacopride was effective only at the highest dose (1 mg/kg) when the "standard" conditioning protocol was used. These results suggest that the 5-HT_3 antagonists may not have the broad spectrum of clinical efficacy characteristic of the benzodiazepines and buspirone.

151.7

COMPULSIVE ORAL MOTOR BEHAVIOR INDUCED BY SEROTONERGIC STIMULATION OF THE VENTROLATERAL STRIATUM. S.K. Yeghiayan* and A.E. Kelley. Department of Psychology, Northeastern University, Boston, MA 02115.

Both anatomical and behavioral studies have suggested that the striatum can be divided into various functional subregions. One area, the ventrolateral striatum (VLS) is thought to play a critical role in oral motor behavior. The striatum is heavily innervated by both dopaminergic and serotonergic neurons arising from the midbrain. The current experiments were aimed at exploring the role of the serotonergic terminals in this region. In Experiment I, 5-hydroxytryptamine hydrochloride (5-HT) was microinjected into the VLS in pargyline (25 mg/kg i.p.) -pretreated rats and various behaviors were observed using a time-sampling procedure. 5-HT (0, 1, 10, 20, and 30 μg/0.5 μl) induced strong head-down sniffing, biting and licking of cage bars, self-gnawing and mouth movements. In Experiment II, oral stereotypy induced by 5-HT (30 μg/1.0μl) was significantly reduced by intra-VLS microinjection of the nonselective 5-HT antagonist methysergide (15, 30 μg/1.0μl). Furthermore, the serotonin-mediated oral behavior was also blocked by coadministration of SCH23390 (0.1 mg/kg i.p.), a D1 receptor antagonist, and raclopride (1.0 mg/kg i.p.), a D2 receptor antagonist. These data provide evidence for a role of serotonin within the ventrolateral striatum in repetitive oral motor behavior, and suggest that dopamine may be involved in this effect.

151.8

SEROTONIN RECEPTOR SUBTYPES IN NIPPLE ATTACHMENT IN INFANT RATS. S. A. Rabchenuk* and C. P. Cramer. Dept. of Psychology, Dartmouth College, Hanover, N.H. 03755

One possible mechanism underlying weaning in rat pups is the maturation of the serotonin system. Serotonin antagonists, such as methysergide, enhance suckling in nondeprived rat pups after 15 days of age (Williams, Rosenblatt, & Hall, 1979); but at 3 days, antagonists inhibit suckling (Spear & Ristine, 1982). Conversely, the serotonin agonist quipazine inhibits suckling in deprived rats beginning at 10 days of age. One possibility for these ontogenetic changes may lie in the time of development of different serotonin receptor subtypes. It is not yet known which receptor subtype(s) mediate serotonergic actions on suckling, because previously tested drugs were relatively nonselective. This experiment investigated the effects of selectively-acting serotonin agents in rat pups at different ages.

Nondeprived, Long-Evans rats were tested at different ages before weaning. Eight litters were used in each condition; 4 pups within a litter each received a different dose: Ketanserin, a 5-HT2 receptor antagonist (1.0, 2.5, 5.0 mg/kg); and DOI, a 5-HT2/5-HT1c agonist (1.0, 2.0, 3.0 mg/kg) (IP). Latency to attach to a nipple and total time attached were measured in the following 45-min. observation period. To determine the receptor specificity of the agonist effect, 20-day-olds were subsequently pretreated with ketanserin (5.0mg/kg) 25 min. prior to the administration of DOI (2.0mg/kg).

Ketanserin significantly inhibited nipple attachment in 10-day-old pups, at all doses, but had no effect on either measures in 20-day-olds. DOI likewise significantly inhibited nipple attachment in 10-day-old pups. Conversely, DOI had no significant effect on latency to attach in 20-day-olds, but significantly reduced their total time attached. Pretreatment with ketanserin blocked the inhibition of nipple attachment by DOI in 20-day-olds. The inhibition of nipple attachment with DOI thus appears to be via the 5HT2 receptor. The facilitation with nonselective agents in older pups appears to act through a different subtype or combination of subtypes.

151.9

A MORE PRONOUNCED INCREASE IN BRAIN TRYPTOPHAN AND 5HIAA IN A TCDD-SUSCEPTIBLE THAN A TCDD-RESISTANT RAT STRAIN BY TCDD.
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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent anorexigen in rats. However, there is an over 300-fold difference in the LD_{50}-values for TCDD between two rat strains, Long-Evans (L-E) and Han/Wistar (H/W). This difference is reflected in the degree of hypofagia after TCDD exposure. As central monoamines are putative regulators of feed intake, we compared the effects of TCDD on brain neurochemistry between the rat strains. Rats of both strains were given a single ip dose of TCDD (50 μg/kg; LD_{100} for L-E, nonlethal to H/W) or vehicle. Four or 10 days after TCDD exposure, half the rats were injected with α-methyl-p-tyrosine (AMPT), an inhibitor of catecholamine synthesis, or vehicle. Three hr later, the rats were decapitated. Noradrenalin, dopamine and indoleamines (5HT, 5HIAA, tryptophan; from those not treated with AMPT) were then determined by HPLC-EC in 9 brain regions. L-E rats decreased dramatically their daily food intake and body weight after TCDD exposure while H/W rats responded only marginally to TCDD administration. Four days after exposure, L-E rats exhibited 36 and 43% elevated levels of the 5HIAA and tryptophan, respectively. These increases were still present 10 days after exposure. In contrast, the resistant H/W rats exhibited only slightly elevated tryptophan and 5HIAA four days after TCDD, and these changes had levelled off by 10 days after TCDD administration. L-E rats pair-fed for 4 days to their TCDD-treated counterparts exhibited a slight tendency toward increased 5HIAA and tryptophan levels in the brain areas measured. However, this change was clearly less pronounced than that in TCDD-treated L-E rats. TCDD had only a negligible effect on noradrenaline or dopamine metabolism. These findings imply that increased serotonergic activity may be, indirectly or directly, associated with TCDD lethality.

151.10

AUGMENTATION OF THE EFFECTS OF FLUVOXAMINE BY FLESINOXAN IN RATS?: ADDITION IN 2 MODELS OF ANXIETY, ANTAGONISM ON PARAMETERS OF THE 5-HT SYNDROME. A.M. Van der Poel, B.Olivier*, J.Mos and H.E.Molewijk. CNS Pharmacology, Solvay Duphar B.V., P.O.Box 900, 1380 DA Weesp, The Netherlands.

Recent clinical findings indicate that buspirone adjuvant therapy may be beneficial to OCD and depressed patients treated with 5-HT uptake blockers (e.g. Pigott et al., J.Clin.Psychopharmacol. 12:11-18, 1992). However, supporting animal data are lacking. We therefore studied the effects of combined treatment with the $5\text{-}HT_{1A}$ receptor agonist flesinoxan and the 5-HT uptake blocker fluvoxamine on two measures of anxiety (isolation-induced ultrasonic distress calls in pups and shock-induced ultrasonic vocalizations in adults) and a number of parameters of the 5-HT syndrome in rats. Both flesinoxan and fluvoxamine suppress ultrasonic distress calls dose-dependently in pups as well as in adult rats. Combined treatment with marginally effective doses of flesinoxan (pups and adult rats: 0.03 mg/kg IP) and fluvoxamine (pups: 0.3 mg/kg IP; adults 1 mg/kg IP) produced a significant suppression of calling. However, doubling the dose of flesinoxan as well as doubling the dose of fluvoxamine produced similar reductions of calling. Similar additive effects were found with flat body posture, part of the 5-HT syndrome produced by both flesinoxan and fluvoxamine. High doses of flesinoxan (3 mg/kg IP and higher) produce diarrhoea and piloerection. These effects are effectively antagonized by fluvoxamine. On the other hand, high doses of fluvoxamine (15 mg/kg IP and higher) produce immobility and reduce rearing, which effects are effectively antagonized by flesinoxan. If these animal data can be taken as predictive for the clinical situation, it can be concluded that combined treatment with a $5\text{-}HT_{1A}$ agonist and a 5-HT uptake blocker yields little advantage over monotherapy with either flesinoxan or fluvoxamine.

151.11

SEEKING RELATIONSHIPS BETWEEN BLOOD CHOLESTEROL AND CSF 5-HIAA IN A MIXED PSYCHIATRIC SAMPLE
D. L. Ringo, K. F. Faull, S. E. Lindley, W. O. Faustman*, Stanford/VA Mental Health Clinical Research Center, VA Medical Center, 3801 Miranda Ave., Palo Alto, CA 94304, USA.

Meta-analysis of treatment studies to reduce blood cholesterol suggests that decreased cardiac mortality is balanced by increased non-medical mortality (e.g., suicide,violence). A small literature notes an association between low cholesterol and violent/aggressive behavior in humans and animals. Many works have linked diminished serotonergic measures (e.g., CSF 5-hydroxyindoleacetic acid, 5-HIAA) and violent/impulsive acts in humans. These parallel findings have recently led to speculation about lipid/neurotransmitter interactions. We performed two retrospective analyses of 5-HIAA and cholesterol relationships in partially overlapping samples of male psychiatric inpatients (predominantly schizophrenia and depression). CSF was obtained in patients, free of psychiatric treatment medications, studied over the past 15 years. The initial analysis correlated the closest available routine clinical value of plasma cholesterol with CSF 5-HIAA. No overall correlation was obtained between 5-HIAA and cholesterol ($r = 0.13$, $N = 84$). However, an analysis of those patients who had clinical cholesterol samples within 7 days of the CSF collection suggested a trend towards a relationship with 5-HIAA ($r = 0.33$, $P = .08$, $N = 31$). In a second analysis we assayed blood plasma obtained at the time of the CSF collection, stored frozen at -70°, from patients studied over the past 5 years. Measures were obtained for total cholesterol, HDL, LDL, and triglycerides. In this partially overlapping sample we were not able to find a significant relationship between 5-HIAA and lipid measures (total cholesterol $r = 0.15$, HDL $r = 0.18$, LDL $r = 0.07$, triglyceride $r = -0.01$, $N = 70$). The results do not offer strong support for cholesterol/CSF 5-HIAA relationships. Such relationships may be subtle, and therefore easily obscured by methodological issues (e.g., small sample size, stored samples). Prospective studies may be needed to clarify a possible serotonin/cholesterol interaction. Supported by MH-30854.

151.12

EXTREME AGGRESSION AND SOCIAL WITHDRAWAL ARE RELATED TO REDUCED CENTRAL SEROTONERGIC ACTIVITY IN MALE CYNOMOLGUS MACAQUES. M.B. Botchin*, J.R. Kaplan, S.B. Manuck, and J.J. Mann. Dept. of Comparative Med., Bowman Gray Sch. of Med., Winston-Salem, N.C. 27157 and Depts. of Psychology and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We examined behavioral differences between monkeys characterized as having low vs high serotonergic responsivity. The subjects were 75 male cynomolgus macaques (*Macaca fascicularis*), housed in five-membered social groups for 28 months. Behavioral assessment consisted of *ad libitum* sampling of specific behaviors for 30 minutes, twice a week, for each social group. Extreme aggression was calculated as the ratio of severe forms (bite, grab, chase) to total aggression (including facial threat and displacement). Sociability was assessed by %time spent alone and %time in body contact. The mean of these behaviors over the entire experiment was used in the statistical analyses. Central serotonergic function was assessed by serum prolactin response to fenfluramine HCl injection. The challenge was performed once during the final six months of the study. Mean prolactin response for the low responders was 14.3 ng/ml (SE±.43) compared to 44.5 ng/ml (SE±1.1) for the high responders. It was observed that the low responders exhibited significantly higher levels of extreme aggression ($p < 0.05$), less time in body contact with others ($p < 0.05$), and a trend toward more time alone ($p = 0.06$). These data support the hypothesis that aggressivity is related to reduced central serotonergic activity. Further, the tendency toward less positive social interaction is also related to reduced central serotonergic activity.

151.13

AGGRESSIVE BEHAVIOR AND BRAIN SEROTONERGIC RESPONSIVITY IN MALE CYNOMOLGUS MACAQUES. R.C. Kyes*, M.B. Botchin, J.R. Kaplan, S.B. Manuck, and J.J. Mann. Dept. of Comparative Med., Bowman Gray Sch. of Med., Winston-Salem, NC 27157 and Depts. of Psychology and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We examined the relationship between behavioral responsivity and an index of CNS serotonergic activity (serum prolactin response to fenfluramine injection) in adult male cynomolgus macaques (*Macaca fascicularis*). Prolactin response to fenfluramine was distributed bimodally. Fifteen low and 15 high prolactin responders were compared. Mean prolactin response was 13.2 ng/ml (SE ±0.3) for low responders and 44.3 ng/ml (SE ±1.9) for high responders ($p < .001$). Behavioral responsivity was assessed by placing the monkeys individually in an open-field enclosure and presenting a series of photographic slides depicting both threatening and nonthreatening stimuli. Monkeys that were low prolactin responders displayed significantly more aggressive gestures in response to the threatening slides than did the high prolactin responders ($p < .05$). No other behaviors differentiated these groups with respect to the threatening slides, nor did animals differ in any behavioral responses to the nonthreatening slides. These data support the hypothesis that high aggressivity is associated with low CNS serotonergic activity, as indicated by a blunted serum prolactin response to fenfluramine.

151.14

INDOLEAMINE METABOLISM IS AFFECTED BY THE BEHAVIORAL MUTANT JERKY IN XENOPUS LAEVIS. R. Tompkins*, T. Le, B. F. Ferrer, D. C. Reinschmidt. Cell and Molecular Biology Department, Tulane University, New Orleans, LA 70118.

The recessive mutant gene, jerky, of Xenopus laevis causes in the larval stages of homozygous animals a transient abnormal behavioral syndrome characterized by uncoordinated swimming movements which grade toward immobility and paralysis. Onset of the syndrome is first apparent about stage 48 and continues until metamorphosis. Environmental factors including photoperiod, light intensity, kind of food, and amount of food have been shown to affect the severity of the syndrome. In addition, lithium has been found to have a prophylactic effect on the expression of the motor disturbances. These results point to a centrally acting lesion. Levels of catecholamines and indoleamines in the brains of mutant and normal animals were compared using HPLC with electrochemical detection. Mutant tadpoles consistently had more serotonin (5-HT) in their forebrains than did normal tadpoles with a concurrent rise in the levels of 5-hydroxyindoleacetic acid (5-HIAA), a major metabolite. Differences were more pronounced in tadpoles kept in the light than in those kept in the dark.

151.15

INCREASE IN SEROTONIN LEVELS IN THE DOG ILEUM AND BLOOD BY CISPLATIN AS MEASURED BY MICRODIALYSIS. M. Yamamoto*, H. Fukui, T. Kondo, S. Sato and T. Ando. Drug Safety Research Labs., Takeda Chemical Ind., Ltd., Osaka, 569 Japan.

Serotonin (5-HT), especially in the intestine, is known to be involved in cisplatin-induced emesis. In the present study, we investigated the effects of cisplatin on 5-HT release in the ileum and 5-HT concentrations in blood using a microdialysis technique. The dialysis probe was inserted into the wall of the ileum in anesthetized dogs or into the cephalic vein in conscious dogs. The probes were perfused with Ringer solution at a rate of 2 µl/min, and the perfusate was collected at 20-min intervals. In anesthetized dogs, 5-HT levels in the ileum increased gradually beginning immediately after cisplatin administration (3 mg/kg, i.v.) and reached a peak 2-3 hrs later, the period when emesis occurs most frequently. 5-HT levels then decreased and had returned to the baseline levels 5 hrs after dosing, the time when emetic episodes stop. The increase in 5-HT levels was confirmed in conscious dogs. 5-HT concentrations in blood increased synchronously with the occurrence of emesis. Furthermore, an immunohistochemical study revealed that the number of 5-HT-immunoreactive cells in the ileum was increased by cisplatin. These results strongly suggest that increases in the synthesis and release of 5-HT in the enterochromaffin cells are intimately involved in cisplatin-induced emesis.

151.16

EFFECTS OF RAPHE LESIONS AND SEROTONIN DEPLETION ON PASSIVE AVOIDANCE. D. Wirtshafter*, Dept. Psychol., Univ. Ill. at Chicago, Box 4348, Chicago, IL, 60680.

Many studies have shown that electrolytic lesions of the median raphe nucleus (MR) can produce dramatic disturbances in performance in a variety of learning paradigms. We report here that electrolytic MR lesions produce a marked impairment in the acquisition of several different step-through passive avoidance tasks in which rats were shocked when they crossed from a large, brightly illuminated, to a small dark compartment. A similar impairment was seen whether or not escape from the shocked compartment was allowed and whether an intertrial interval of 30 sec or 24 hr was employed. After criterion was reached, rats were directly placed in the dark compartment of the box, with the shock turned off, and allowed to cross over into the "safe" compartment. Under these conditions, MR lesioned animals showed significantly longer "escape" latencies than did controls. In another study MR lesions failed to alter the acquisition of a one way avoidance task in which rats had to cross from the dark to the light compartment to avoid shock. When tested on passive avoidance of the dark compartment, however, these lesioned animals showed no transfer from the active to the passive avoidance task.

Electrolytic lesions of the dorsal raphe failed to reproduce the effects of MR lesions, as did specific depletions of serotonin produced by systemic treatment with p-chloroamphetamine or intra-MR injections of 5,7-DHT. These findings suggest that the effects of raphe lesions on passive avoidance may result from damage to nonserotonergic cells within the MR.

151.17

LOCOMOTOR EFFECTS OF MICROINJECTIONS OF KAINIC ACID INTO THE MEDIAN RAPHE (MR), DORSAL RAPHE (DR), VENTRAL TEGMENTAL AREA (VTA), AND THE PONTINE RAPHE NUCLEUS (NRP). M.R. Pitzer* and D. Wirtshafter. Dept. Psychology, Univ. of Il. at Chicago, Box 4348, Chicago, Il.

We have previously reported dose dependent decreases in feeding, drinking, and both spontaneous and methylphenidate-induced locomotion subsequent to intra-median raphe (MR) injections of the glutamate analogue kainic acid (KA). The current series of experiments was designed to investigate the anatomical specificity of KA-induced suppression of activity within the midbrain. Microinjections of KA (0,5,10,20ng) were made into the MR, DR, VTA, and the NRP of animals previously injected with d-amphetamine. The data revealed a pronounced suppression of activity following MR injections and to a lesser extent, following DR injections. Much smaller effects were observed from injections at the remaining sites. These current findings suggest that the MR is the most sensitive site in the midbrain tegmentum for the suppression of locomotion by kainate receptor stimulation. Preliminary work has suggested these effects may be partially mediated by serotonin.

151.18

EFFECTS OF DESIPRAMINE ON THE DRL ANTIDEPRESSANT SCREEN DEPEND UPON HOW LONG THE RATS ARE REQUIRED TO WAIT. J.B. Richards*, K.E. Sabol, M.J. Baggott, L.S. Seiden. University of Chicago, Department of Pharm/Phys Sci., Chicago, IL 60637.

The differential-reinforcement-of-low-rate-72-s (DRL 72-s) schedule requires animals to wait 72 seconds between lever press responses in order to gain access to a reinforcer (.05 ml water). When required to wait 72-s between responses for reinforcement normal rats typically produce peak (modal) interresponse time (IRT) durations which are less than the 72-s criterion for reinforcement. For rats trained on a DRL-72s scheule, a variety of antidepressant compounds (tricyclics, monoamine oxidase inhibitors, and atypical antidepressants) shift the peak IRT duration toward longer durations resulting in an increase in the number or reinforcers. Three groups of rats were trained on DRL schedules of reinforcement with criterion values of 18-s, 36-s, and 72-s. After training, the peak IRT duration for the DRL 18-s group was greater than the criterion (18.3 s). For the DRL 36-s and 72-s groups however, the peak values were less than the DRL criterion values (34.6 s and 58.7 s respectively). The effect of the antidepressant desipramine (DMI) (2.5, 5.0, 10.0 and 20.0 mg/kg) on peak IRT duration was determined. DMI shifted the peak of the IRT distribution to the right - toward longer IRT durations - at all three DRL criterion values. However, the shift to the right was disproportionately larger at the longer DRL criterion values. At the 10.0 mg/kg dose of DMI the peak IRT durations were 19.8, 39.3, and 69.8 s respectively for the DRL 18, 36, and 72-s schedules. DMI increased the number of reinforcers earned at all three DRL criterion values. DMI did not increase dispersion of the IRT distributions indicating that its effects were not due to a nonspecific disruption of responding. The results are not explained well by the hypothesis that DMI changed the rats' perception of time. The results are more consistent with the hypothesis that DMI enhanced the rats' ability to wait between bar press responses. (Supported by: MH-11191; RSA-10562, L. Seiden)

151.19

DISCRIMINATIVE STIMULUS PROPERTIES OF ELTOPRAZINE AND FLESINOXAN IN RATS AND PIGEONS. A.van Hest*, B.Olivier, J.Mos, A.M. Van der Poel, C.E.Ybema and J.L.Slangen. CNS Pharmacology, Solvay Duphar B.V., P.O.Box 900, 1380 DA Weesp, The Netherlands.

The 5-HT_{1A} receptor agonist flesinoxan and the 5-$HT_{1A,1B}$ receptor agonist eltoprazine, have clear stimulus properties in rats and pigeons and can be easily trained to discriminate either compound from vehicle. The flesinoxan cue in both species (0.5 mg/kg i.p. in rats; 0.25 mg/kg p.o. in pigeons) seems exclusively mediated by activation of the 5-HT_{1A} receptor. In rats ipsapirone, 8-OH-DPAT, gepirone and yohimbine generalize to the flesinoxan cue, whereas buspirone and idazoxan partly substitute for flesinoxan. The 5-HT_{1A} activity of the α_2-adrenergic antagonists yohimbine and idazoxan could explain their generalization towards flesinoxan. The flesinoxan cue could be antagonized by (±)pindolol, (±)propranolol and (partly) by NAN-190, all presumed 5-HT_{1A} receptor antagonists. In pigeons, buspirone, eltoprazine and ipsapirone completely substitute for flesinoxan.

The eltoprazine cue in rats (0.5 mg/kg i.p.) fully generalized to 8-OH-DPAT, TFMPP, RU 24969, (±)pindolol, (±)propranolol and partly to buspirone, flesinoxan, mCPP, mesulergine, idazoxan and fluvoxamine. No antagonism of the eltoprazine-cue was found with any 5-$HT_{1,2}$ or $_3$ receptor antagonist. The stimulus properties of eltoprazine in rats seem to comprise at least a 5-HT_{1A} and 5-HT_{1B} receptor agonistic component. In pigeons, which do not posses 5-HT_{1B} receptors, eltoprazine (5.0 mg/kg, p.o.) generalized to flesinoxan, partly to buspirone (50%) and not to ipsapirone. 5-HT_{1A} receptor activation clearly plays a role in the eltoprazine stimulus properties in pigeons, but other mechanisms (5-HT_{1C}, 5-HT_{1D}) may also be involved.

151.20

SEASONAL AND STATE-DEPENDENT CHANGES IN REGIONAL BRAIN CONTENT OF MONOAMINES IN THE HIBERNATING GROUND SQUIRREL. A.C. Russo†, A. Izquierdo, R.C. Lowry, C. Kay-Nishiyama and T.L. Stanton*. Dept. of Physical Therapy† & Dept. of Physiology, California State University, Long Beach, CA 90840

The levels of 5-HT, NE, and their metabolites were measured by HPLC in regionally dissected brains of summer euthermic (SE), winter euthermic (WE), interbout (I), and hibernating (H) Citellus lateralis. Dramatic state-dependent increases (H>I) were observed in 5-HIAA/5-HT ratios in all 10 brain regions. The concentration of 5-HIAA was also significantly increased in 5 regions of H animals, most notably in the hypothalamus. No seasonal (SE vs WE) differences were noted in the ratio values, but 5-HIAA levels were significantly increased in 8 regions of SE animals. In the NE system, significant seasonal (SE>WE) differences in MHPG and MHPG/NE ratios were noted in 7 regions. Small but significant state-dependent differences (H>I) in the MHPG/NE ratios were also seen in 5 of these regions, all located in the brainstem. MHPG levels did not vary significantly across state in any region. These results demonstrate dynamic seasonal and state-dependent changes in 5-HT and NE neural systems in Citellus lateralis. (Supported by NIH Grant # GM08238)

152.1

DIFFERENTIAL ABILITY OF NK-1 AGONISTS TO PRODUCE SCRATCHING AND GROOMING BEHAVIOURS IN MICE. S. Ravard, J. Betschart, V. Fardin, A. Doble* and J.C. Blanchard. Rhône-Poulenc Rorer, CRVA, 13 Quai J. Guesde, 94403 Vitry, France.

We have compared the effects of different NK-1 agonists to produce reciprocal hind-limb scratches and grooming in mice. Selective NK-1 agonists used were [Apa^{9,10}]SP (0.1-10 μg), [Pro^{9}]SP, [Sar^{9},Met(O$_2$)^{11}]SP and septide (0.0125-1 μg) We have also tested a mixed NK-1/NK-2 agonist, [Lys^{5},Tyr^{7},Pro^{8}]NKA(4-10) (0.1-10 μg). Drugs were administered icv to CD1 mice in a volume of 5 μl/mouse. Consecutive to the icv injection, the number of scratches and time spent grooming were measured in a 10-min test session. Induction of scratches and intense grooming, behaviours not seen in vehicle-treated mice, was observed with [Pro^{9}]SP, [Sar^{9},Met(O$_2$)^{11}]SP and septide in a dose-dependent manner. The rank order of efficacy was septide > [Sar^{9},Met(O$_2$)^{11}]SP > [Pro^{9}]SP. [Apa^{9,10}]SP produced scratches and grooming only at a dose of 10 μg. The mixed NK-1/NK-2 agonist [Lys^{5},Tyr^{7},Pro^{8}]NKA(4-10) had no effect in the dose range tested. In contrast to [Pro^{9}]SP and [Sar^{9},Met(O$_2$)^{11}]SP, results from binding experiments performed on brain preparations have shown that septide exhibits a weak affinity for NK-1 sites. Since septide was more potent than [Pro^{9}]SP and [Sar^{9},Met(O$_2$)^{11}]SP in our experiments, a mechanism other than NK-1 receptors may account for the present results. It cannot, however, be excluded that differential degradation of these agonists may affect the behaviours observed.

152.2

SUBSTANCE P PATHWAY FROM THE MEDIAL AMYGDALA TO MEDIAL HYPOTHALAMIC DEFENSIVE RAGE SITES. M.B. Shaikh, A. Steinberg, and A. Siegel*. Department. of Neurosciences, New Jersey Medical School, Newark, N. J. 07103.

We have recently shown that the medial amygdala (ME) powerfully facilitates defensive rage behavior (DR) in the cat. This study tested the hypothesis that the underlying neurochemical substrate includes a substance P (SP) pathway from the ME to the medial hypothalamus (MH) from which DR is elicited. Cannula-electrodes were implanted into DR sites in the MH and microinjections of the retrograde tracer, Fluoro-Gold (8% in 0.5 μl), were placed into these sites. Following survival periods of 6-8 days, animals were sacrificed and the tissue was processed for immunoreactivity for SP-positive cells and fibers. Large numbers of retrogradely labelled cells were identified in the ME. Likewise, high concentrations of SP-positive cells were observed in ME. Of particular significance was the presence of large numbers of cells in the ME labelled for both Fluoro-Gold and SP. Such labelled cells were most highly concentrated in the dorsal aspect of ME -- sites which facilitate DR. In addition, dense quantities of SP-positive axons and preterminals were noted in the region of the MH from which DR is elicited. These findings provide further evidence that the pathway from ME to MH, which facilitates DR, utilizes SP as a neurotransmitter. [Supported by NIH Grant NS 07941-22].

152.3

THE ROLE OF SUBSTANCE P IN MEDIAL AMYGDALOID FACILITATION OF DEFENSIVE RAGE BEHAVIOR IN THE CAT. A. Steinberg, M.B. Shaikh* and A. Siegel. Department of Neurosciences, N. J. Medical School, Newark, N. J. 07103.

The present study tested the hypotheses that: (1) the medial amygdala (ME) facilitates feline defensive rage behavior (DR) and; and (2) substance P (SP) is utilized as a neurotransmitter in the facilitatory pathway from the amygdala. In phase I of the experiment, stimulating electrodes were implanted into the ME and cannula-electrodes were implanted into the medial hypothalamus (MH) from which DR could be elicited by electrical stimulation. Response latencies for DR were significantly ($p < .01$) lowered following dual stimulation of the ME and MH relative to single stimulation of the MH alone. In phase II, following the I.P. administration of the NK1 antagonist, CP 96,345, in doses of 0.05, 2.0, and 4.0 mg/kg, dose and time dependent decreases in ME-induced facilitation (by as much as 30% at the highest dose level) were observed. In phase III of the study, CP 96,345 was microinjected directly into DR sites within the MH in doses of 0.05, 0.50, 2.5 and 5.0 nmoles. Again, dose and time dependent decreases in ME-induced facilitation of DR (by as much as 50% for the highest dose level) were noted. The results suggest that ME facilitates DR by acting through an SP mechanism at the level of the MH. [Supported by NIH Grant NS 07941-22].

152.4

LEARNING ENHANCED BY SUBSTANCE P IN RATS WITH HIPPOCAMPAL LESIONS AND GRAFTS OF FETAL NERVOUS TISSUE.

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Grafting of fetal nervous tissue into the CNS may improve performance of brain damaged animals. The present study was undertaken to examine the interaction of the neurokinin Substance P (SP) with the effects of fetal grafts on the consequences of bilateral hippocampal lesions. It is known that SP has memory enhancing, rewarding, and neurotrophic effects.

One week after bilateral kainic acid injections into the hippocampus rats received suspension grafts of fetal tissue at the sites of their lesions. Spatial learning was tested weekly in a Morris water-maze. SP (at 5 and 50 μg/kg) was injected peripherally, daily 5 hours after the learning trial over a 9 weeks period. Animals with grafts performed superior to non-grafted animals. SP injections (50 μg/kg) facilitated performance in both grafted and non-grafted animals.

SP also inhibited seizure activity in the rats with hippocampal lesions over the whole period of nine weeks.

152.5

APOMORPHINE- AND OXYTOCIN-INDUCED PENILE ERECTION AND YAWNING: ROLE OF SEXUAL STEROIDS. M.R. Melis, A. Mauri & A. Argiolas*. B.B. Brodie Dept. Neurosci., Cagliari Univ., 09124 Cagliari, Italy.

In intact male rats, apomorphine (APO) (80 μg/kg s.c.) and oxytocin (OXY) (30 ng i.c.v.), induce penile erection (PE) and yawning (Y). Castration prevented both APO and OXY responses. In castrated rats, testosterone (T) (100 μg/kg s.c. once a day for 3 days) restored PE while estradiol (E) (10 μg/kg s.c. once a day for 3 days), restored Y induced by APO or by OXY. 5-dihydrotestosterone (5DHT) and progesterone (P) (100 μg/kg s.c. once a day for 3 days) were ineffective. E + 5DHT partially restored APO-induced Y and PE, while E + P restored only Y. In intact rats, P increased, and E decreased, respectively, APO-induced Y. Both steroids were ineffective on APO-induced PE. T and 5DHT were ineffective on both APO responses. E effect was prevented by the antiestrogen tamoxiphen (1 mg/kg s.c. once a day for 3 days), which per se failed to modify APO responses, by T or by P. Similar results were found with OXY-induced Y and PE, except that Y was prevented by E much less than that induced by APO. The results suggest that sexual steroids modulate PE and Y induced by APO and by OXY in a way that depends on the endocrine status of the animals.

152.6

APOMORPHINE-, OXYTOCIN- AND ACTH-INDUCED PENILE ERECTION AND YAWNING: ROLE OF EXCITATORY AMINO ACIDS (EAAs). A. Argiolas, R. Stancampiano & M.R. Melis*. B. B. Brodie Dept. Neurosci., Cagliari Univ., 09124 Cagliari, Italy.

The effect of the EAA receptor antagonists MK-801, CPP, CNQX and AP-4 on penile erection (PE) and yawning (Y) induced by the dopaminergic agonist apomorphine (APO), oxytocin (OXY) and ACTH 1-24 was studied in male rats. Systemic (0.1-0.4 mg/kg i.p.) and i.c.v. (10-50 μg) MK-801 prevented dose-dependently PE AND Y induced by APO (80 μg/kg s.c.), by OXY (30 ng i.c.v.) or by ACTH 1-24 (10 μg i.c.v.). MK-801 prevention of PE and Y was parallel to the appearance of a complex behavioral syndrome characterized by head weaving, body rolling, hyperlocomotion and ataxia. A similar prevention of PE and Y, especially in the number of PE, was also found with high, but not low doses of CPP and CNQX, which impaired normal motor activity. AP-4 was ineffective at all doses tested. All the above compounds failed to modify PE and Y when injected into the paraventricular nucleus of the hypothalamus, the brain area most sensitive to APO and OXY for the induction of PE and Y. The results suggest that EAAs do not play an important role in the expression of PE and Y induced by the above compounds.

152.7

GONADAL STEROIDS ALTER VASOTOCIN RECEPTOR CONCENTRATIONS IN AMPHIBIAN BRAINS. S.K. Boyd.* Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556.

Since arginine vasotocin (AVT) levels are sexually dimorphic in the bullfrog (Rana catesbeiana) brain, we used in vitro autoradiography with ^{3}H-vasopressin to determine whether sexual differences also exist in concentrations of putative receptors for this peptide. Receptor concentrations were greater in female bullfrogs, compared to males, in the amygdala pars lateralis and hypothalamus. Levels were greater in male bullfrogs in the pretrigeminal nucleus and auditory dorsolateral nucleus. Next, bullfrogs of both sexes were gonadectomized and given implants of DHT or estradiol. In the amygdala, gonadectomy significantly reduced receptor concentrations in males and females while estradiol therapy restored concentrations to control levels. In the pretrigeminal nucleus, castration of males significantly decreased receptor number and these effects could be reversed by either DHT or estradiol treatment. Ovariectomy and steroid therapy had no effect on pretrigeminal receptors in female bullfrogs. Likewise, these treatments did not alter receptor levels in the hypothalamus or dorsolateral nucleus of either sex. These receptor populations may represent the sites where AVT interacts with gonadal steroids to alter sexually dimorphic behaviors in the bullfrog.

152.8

ESTRADIOL ALTERS THE BEHAVIORAL RESPONSE OF THE MPOA-AH TO ARGININE VASOPRESSIN (AVP) IN SYRIAN HAMSTERS. K.L. HUHMAN* & H.E. ALBERS. Lab. of Neuroendocrinology & Behavior, Depts of Biol. & Psychol., Georgia State Univ., Atlanta, GA 30303.

Vasopressin (AVP) in the medial preoptic-anterior hypothalamus (MPOA-AH) is known to be important in the control of flank marking, a form of scent marking seen in hamsters. In males, there is evidence that testosterone influences the frequency of flank marking by altering the response of the MPOA-AH to AVP. The purpose of this experiment was to determine if estradiol alters the response of the MPOA-AH to AVP in female hamsters. Hamsters (N = 26) were ovariectomized and implanted with blank Silastic capsules or with capsules containing estradiol benzoate (EB). Four weeks later, each hamster was implanted with a guide cannula into the MPOA-AH and was given microinjections of AVP (0.09, 0.9, 9.0, & 90.0 μM in 100 nl saline) over four testing days. AVP-induced flank marking was significantly higher in hamsters implanted with EB capsules than in hamsters receiving blank implants. These data support the hypothesis that gonadal hormones influence flank marking by altering the response of the MPOA-AH to AVP. (Supported by NSF BNS-8910863)

152.9

VASOPRESSINERGIC NEURAL CIRCUITS IN GOLDEN HAMSTERS. Y. Delville*, G.J. DeVries, and C.F. Ferris. Physiol. Dept., Univ. of Mass. Med. Ctr., Worcester, MA 01655, and Neuroscience and Behavior Program, Univ. of Mass., Amherst, MA 01003.

In golden hamsters, the anterior hypothalamus, the lateral septum and the midbrain central grey are target sites for vasopressin in the regulation of a stereotypic behavior: flank marking. As a first approach to study the underlying neural network controlling this behavior, *Phaseolus vulgaris* leucoagglutinin, an anterograde tracer, was injected within the anterior hypothalamus, which contains vasopressinergic neurons. Projections originating from this area were observed within the entire limbic system, particularly within the lateral septum, hippocampus, preoptic area, central amygdala, midbrain central grey, and the nucleus solitarius. Interestingly, these sites also contain high affinity vasopressin receptors and vasopressin-immunoreactive fiber terminals. These results suggest that the neural network controlling vasopressin-induced flank marking may originate from the anterior hypothalamus. This hypothesis is being tested through combined retrograde tracing and immunocytochemistry for vasopressin.

152.10

CHANGES IN VASOPRESSIN PATHWAYS OF PRAIRIE-VOLE MALES RELATED TO REPRODUCTION. M.Bamshad*, M.A.Novak, G.J. De Vries. Program of Neuroscience and Behavior and Dept. of Psychology, Univ of Massachusetts., Amherst, MA 01003.

Prairie-vole males display paternal behavior. We have previously found that, in comparison to virgin males, parental males have a lower density of the vasopressin immunoreactive (AVP-ir) fiber plexus in the lateral septum and lateral habenular nucleus, which presumably is derived from the bed nucleus of the stria terminalis (BNST) and medial amygdaloid nucleus (MA). It is not clear whether AVP-ir projections get less dense before or after the birth of pups.

We compared AVP-ir fiber density in the lateral septum and lateral habenular nucleus and the paraventricular nucleus of the thalamus and medial preoptic area among prairie-vole males and females that were either housed separately or housed together for 72 hrs, or until 10 or 18 days into the gestation period, or until six days after the birth of pups. Under all these conditions, AVP-ir fiber density in the lateral septum and lateral habenular nucleus was much denser in males than in females. In males, the lowest density was found at 72 hrs after being housed together. An intermediate density was found at 10 days in the gestational period and at 6 days after birth of pups. An equally high density was found in sexually inexperienced males and in males that were housed together for 18 days. In females, no changes in AVP-ir fiber density were found. In addition, in both sexes no differences were found in the AVP-ir fiber density in the paraventricular nucleus of the thalamus and the medial preoptic area. These data indicate that the AVP-ir projections from the BNST and MA of males become less dense after mating and once again after the pups are born. This suggests that these projections may not only play a role in becoming parental but may also underlie some of the behavioral changes seen in prairie voles after mating.

152.11

ARE SEX DIFFERENCES IN HAMSTER FLANK MARKING MEDIATED BY VASOPRESSIN (AVP) WITHIN THE MEDIAL PREOPTIC-ANTERIOR HYPOTHALAMUS (MPOA-AH)? A.C. HENNESSEY*, K.L. HUHMAN & H.E. ALBERS. Lab. of Neuroendocrinol. & Behavior, Depts. of Biol. & Psychol., Georgia State Univ., Atlanta, GA 30303.

Female hamsters flank mark more than males in response to the odors of conspecifics. Since AVP activity within the MPOA-AH is critical for the control of hamster flank marking, we investigated whether these sex differences may be mediated by differences in AVP activity within the MPOA-AH. In experiment 1, immunohistochemical analysis revealed no significant differences in AVP-IR cell counts within the BNST, MPOA-AH, SON, PVN and SCN in male (n = 6) and female (n = 6) hamsters. In experiment 2, males (n = 8) and females (n = 8) were implanted with guide cannulae aimed at the MPOA-AH and were microinjected with AVP (0.009, 0.09, 0.9 or 9.0 μM) on four consecutive days. AVP stimulated flank marking in a dose-dependent manner in both male and female hamsters ($p < 0.01$). No significant sex differences in AVP-stimulated marking were found. These data suggest that sex differences in odor-stimulated flank marking are not due to sex differences in AVP availability or release within the MPOA-AH. (NSF BNS-8910863).

152.12

EEFECT OF VASOPRESSIN ON CIRCADIAN ACTIVITY AND BODY TEMPERATURE IN FOOD-RESTRICTION STRESS. G. R. Nadzam, C. H. Wideman*, and H. M. Murphy*. John Carroll University, Cleveland, OH 44118.

Male, vasopressin-containing, Long-Evans (LE) and vasopressin-deficient, Brattleboro (DI) rats were maintained in individual cages while telemetered activity (Am) and core body temperature (Tb) data were collected. The rats were exposed to a 12:12 light-dark cycle with a room temperature of 23 ± 1°C and allowed to habituate for 12 to 14 days with ad-lib food and water. The habituation period was followed by an experimental period in which a stress of 23h of food restriction per day was introduced. A 1h feeding period was initiated 1h after the beginning of the light cycle. While circadian rhythms of Am and Tb were nearly identical between DI and LE rats during habituation, the experimental period produced marked differences. Food stress resulted in a marked increase in DI activity, but resulted in an overall decrease in LE activity. Initially, LE peak Tb per day was significantly elevated and was followed by a gradual daily decrease in trough Tb. In spite of the dramatic increase in DI Am, peak Tb per day was only slightly elevated from the habituation period, but trough Tb per day decreased more than 0.5°C per day until death.

152.13

EVIDENCE THAT CELLS IN THE LUMBAR REGION OF THE SPINAL CORD PROJECT TO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS. L.Y. Yang and L.G. Clemens* Neuroscience Program and Department of Zoology, 209 Biology Research Center, Michigan State University, East Lansing, MI 48824

We have demonstrated that neurons of the paraventricular nucleus of the hypothalamus (PVN) project to the sexually dimorphic, lower lumbar region of the spinal cord (Wagner and Clemens, Brain Research 539:254-262, 1991). The present study extended this analysis by examining whether neurons in the lumbar region of the spinal cord project to the PVN. Fluorogold (4%, 0.1uL) was stereotaxically injected into the PVN. Fluorogold-labelled cell bodies were found in the lumbar region of the spinal cord. When combined with previous studies, this experiment demonstrates that reciprocal connections exist between the PVN and the lumbar region of the spinal cord. (NSF Grant: BNS-9109292)

152.14

OXYTOCIN PROJECTIONS FROM THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS TO LOWER LUMBAR SPINAL CORD AND THE DISTRIBUTION OF OXYTOCIN FIBERS IN THE REGION OF THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS IN MALE AND FEMALE RATS. A.E. Ackerman* and L.G. Clemens, Department of Zoology and Neuroscience Program, Michigan State University, East Lansing, MI 48824.

Oxytocin-immunoreactive (OT-IR) neurons are found within the paraventricular nucleus (PVN) of the hypothalamus and project throughout the CNS, including the spinal cord, (Cechetto, D.F. and Saper, C.B., *Comp. Neurol.* 272: 579, 1988). We recently demonstrated loss of neurophysin-immunoreactive fibers in the sexually dimorphic lower lumbar cord (L_5-L_6) following PVN lesions in male rats (Wagner, C.K. and Clemens, L.G., *Unpublished observations*). In the present study, we extend this analysis of PVN projections to L_5-L_6 by comparing the distribution of PVN, OT-IR neurons in male and female rats. This comparison was made using PVN tissue from rats injected with 0.5µl of 4% Fluorogold into segments L_5-L_6. Following 1-2 weeks, animals were perfused and immunohistochemistry for OT was performed on 30µm sections through PVN. In addition to comparing the distribution of double labelled cells in PVN, other male and female rats were used to compare the density of OT-IR fibers within lower lumbar cord. Results demonstrate that OT-IR fibers, presumably originating in the PVN, project to the sexually dimorphic lower lumbar segments of the spinal cord in both male and female rats. (Supported by NSF BNS-9109292)

152.15

CHRONIC OXYTOCIN INFUSIONS AFFECT CNS OXYTOCIN RECEPTORS MEDIATING SOCIAL BEHAVIOR. Diane M. Witt*& Thomas R. Insel. Lab. of Neurophysiology, NIMH, Poolesville, MD 20837

The central release of oxytocin (OT) has been implicated in the neural mediation of both male and female sexual behavior. The current studies examined the effects of chronic central administration of OT on (1) male sexual and social behavior, and (2) brain OT receptors. Either artificial cerebrospinal fluid (CSF) or OT was centrally infused (via osmotic mini-pump) to gonadally-intact male rats. Behavioral observations were made on males paired with either ovariectomized or estrous females during a 6-hour time period. The durations of physical contact were doubled in pairs containing OT-infused males, even in the absence of sexual interactions. OT-infused males also showed significantly higher levels of anogenital sniffing of females and autogrooming, however sexual behavior *per se* was unaffected by chronic OT. Chronic OT had no effect on body temperature, analgesia or exploratory behavior in an open field. Subsequently, brains from these animals were processed for *in vitro* receptor autoradiography, using [^{125}I]-OTA. OT receptor binding was decreased in every target field by 50 %, even 24 hours after pump removal, suggesting an OT receptor down-regulation. These findings suggest that chronic OT in male rats has behavioral effects which enhance adult social (non-sexual) interactions possibly through alterations in olfactory and somatosensory information processing. Alterations in OT receptors, in response to prolonged OT stimulation, may provide insights into mechanisms by which central OT affects neurotransmission.

DRUGS OF ABUSE: BENZODIAZEPINES AND BARBITURATES

153.1

DIFFERENTIAL REGULATION OF THE BEHAVIORAL EFFECTS OF CHLORDIAZEPOXIDE. J.S. Shumsky* and I. Lucki, Departments of Pharmacology and Psychiatry, University of Pennsylvania, Philadelphia, PA 19104

Chronic administration of the benzodiazepine (BZ) receptor agonist, chlordiazepoxide (CDP), produced tolerance to its motor impairing effects but no tolerance to its anxiolytic or amnesic effects. Male Sprague-Dawley rats were pretreated for 14 days with 25 mg/kg CDP (IP, b.i.d.) or saline and chronic treatment was maintained throughout the experiments. Tolerance to the ataxic effects of CDP on the rotorod was observed as a 9-fold shift to the right in the cumulative dose-effect curve between chronic CDP- and saline-treated animals. Anxiolytic effects were examined using operant behavior reinforced under a multiple schedule with unpunished (RI 80-s) and punished (CRF with incremental shock intensities) components. Tolerance developed to CDP's effects on unpunished responding, as shown by a 7-fold shift to the right in the dose-effect curve. Conversely, tolerance did not develop to the effects of CDP on punished responding. In addition, baseline responding was elevated by chronic CDP treatment throughout the experiment, also indicating a lack of tolerance to CDP's anxiolytic effects. Furthermore, no tolerance developed to the amnesic effects of CDP in a model of memory impairment using the radial arm maze. These results are consistent with the effects of chronic BZ treatment in humans and suggest a homologous regulation of drug effects, potentially mediated by regional differences in BZ receptor subtype regulation or composition. Supported by USPHS grant DA 05186.

153.2

EVIDENCE FOR DISCRIMINATION OF BENZODIAZEPINE WITHDRAWAL BY RATS. D.A. Lytle and M.W. Emmett-Oglesby*, Dept. of Pharmacology, TCOM, Fort Worth, TX 76107

This experiment determined whether withdrawal from benzodiazepine dependence could be trained as a discriminative stimulus. Rats were chronically maintained on a nutritionally-complete liquid diet that contained 100mg/kg/day of chlordiazepoxide (CDP); diet was administered in two portions daily. Subjects were trained to discriminate a benzodiazepine antagonist, flumazenil (FLUM), 2.5mg/kg, from vehicle using a food-reinforced two-lever choice paradigm. Flumazenil substituted for FLUM over a broad generalization gradient (ED50 = 0.045mg/kg). The anxiogenic drug pentylenetetrazole (PTZ) substituted for FLUM, and pentobarbital (20mg/kg) shifted the dose-effect curve of FLUM to the right. Twenty-four hr. after termination of CDP-diet, vehicle substituted for FLUM. This substitution of vehicle for FLUM was dose-dependently blocked by CDP and abecarnil. At 14 days after termination of CDP-diet, both vehicle and FLUM failed to substitute for FLUM; however, PTZ fully substituted for FLUM at that time. These data show that the FLUM discriminative stimulus is dependent on the presence of chronic CDP, and the results support the hypothesis that FLUM produced a discriminative stimulus based upon precipitated withdrawal from CDP dependence. The substitution of vehicle for FLUM at 24-hr after termination of CDP is consistent with the occurrence of spontaneous withdrawal that substituted for a discriminative stimulus based upon precipitated withdrawal. Supported by grant DA RO1-3521.

153.3

EFFECTS OF DIAZEPAM ON SCHEDULE CONTROLLED BEHAVIOR IN A RADIAL MAZE MODEL OF FORAGING.

G.R. Sessions*, J.J. Pilcher, and E.L. Closser-Gomez. Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

The effects of diazepam on scheduled controlled behaviors and motor performance was studied in a novel radial-arm maze model of foraging. Rats were allowed one hour access to a radial maze where delivery of food was controlled by concurrent fixed interval (FI) schedules of reinforcement in each of 8 arms, ranging from 55 to 759 sec. Diazepam (0.625-10.0 mg/kg ip) produced dose-dependent decreases in measures of performance, but reference memory for the relative contingencies associated with the eight individual FI schedules was relatively unaffected. The patterning of responding on the individual schedules, however, was disrupted at doses lower that those which produced frank motor deficits. The observed effects on schedule controlled behaviors in this complex task may be attributable to diazepam-induced alterations in response timing, due to impairments in either working memory for time or internal clock mechanisms. The radial maze foraging model provides a useful paradigm for simultaneous drug testing for behavioral effects on motoric and complex learned behaviors.

153.4

EFFECTS OF DIAZEPAM ON PERFORMANCE AND MEMORY IN A RADIAL MAZE MODEL.

J.J. Pilcher*, G.R. Sessions, and E.L. Closser-Gomez. Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

The effects of diazepam on performance and memory were studied in an eight-arm radial maze paradigm designed to be sensitive to the disruption of working memory. Moderately food-deprived rats (n = 10) were trained to obtain a food reward at the end of each arm of the maze. Working memory errors were counted when a rat revisited an arm after obtaining the reward. Latency measures were used as a measure of overall task performance. On drug trials the rats were injected with 0, 1.25, 2.5, or 5 mg/kg diazepam (i.p., counterbalanced order, 2 replications for each dose) 30 min prior to the start of a trial. Diazepam produced a small but significant increase in working memory errors at 5 mg/kg. The drug had no significant effects on latency to complete the trial, average choice latency or in number of pellets obtained. This study shows that working memory deficits induced by diazepam are independent of generalized performance decrement or motoric impairments.

153.5

CHARACTERIZATION OF THE DISCRIMINATIVE STIMULUS (DS) EFFECTS OF MIDAZOLAM (MDZ) USING A 3-LEVER DRUG DISCRIMINATION PARADIGM IN RATS.

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Discrimination procedures are useful to characterize a drug's behavioral effects and to evaluate its mechanisms of action. In the present study, 12 rats were trained to discriminate 2 doses of the benzodiazepine (BZ) agonist MDZ (0.32 mg/kg & 3.2 mg/kg MDZ, s.c.) and no-drug (ND), in daily sessions consisting of multiple discrete 20 min trials: 15 min timeout (TO), 5 min FR 10 schedule for food. At the start of each TO, rats received either 0.32 MDZ mg/kg, 3.2 mg/kg MDZ or sham treatment. During tests, vehicle or a dose of test drug was given at the start of each TO; 10 responses on any lever produced food. MDZ produced dose-dependent increases in 0.32 and 3.2 mg/kg MDZ lever responding; this effect was dose-dependently antagonized by flumazenil. Diazepam and triazolam produced similar patterns of generalization. Chlordiazepoxide, lorazepam, and flurazepam did not produce 3.2 MDZ lever responding up to rate-decreasing doses. Clonazepam produced only ND lever responding in 4/10 rats. Other tests suggested that the DS effects of 3.2 MDZ are BZ-like, and not mediated by sedative or muscle-relaxant effects. The DS effects of 0.32 mg/kg MDZ in this context appeared to be less specific than 3.2 mg/kg MDZ and different than the profile seen in animals trained in 2-lever tasks.The DS effects produced by the 2 MDZ doses were qualitatively different and may not reflect simply magnitude differences.

153.6

HORMONE-RELATED DIFFERENCES IN NEURAL ADAPTATIONS TO CHRONIC BENZODIAZEPINE EXPOSURE.

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The gonadal hormone milieu modifies both the anticonvulsant tolerance and the GABA receptor changes associated with prolonged benzodiazepine (BZ) treatment in rats (Wilson & Biscardi, Eur.J.Pharm., in press). The current studies compare adaptations in physiological GABA/BZ responses induced by chronic BZ exposure in intact MALE and ovariectomized (OVX) rats. GABA sensitivity and BZ responses were assessed in single substantia nigra pars reticulata (SNr) neurons using electrophysiological techniques and in cortical synaptosomes with ^{36}chloride flux analysis.

Chronic diazepam exposure (DZ; 3 wk) reduced the subsequent effects of diazepam on SNr activity in MALE ($F=3.9$, $p<0.01$), but not OVX ($F=0.62$,NS), rats. Diazepam (0.25 mg/kg,iv) decreased SNr firing rates $24\pm9\%$ ($N=6$) below basal values in DZ-treated MALES, compared to a $57\pm9\%$ ($N=5$) decrease in MALE controls. In contrast, DZ (0.25mg/kg,iv) decreased SNr firing similarly in OVX control and DZ-treated groups ($41\pm15\%$ and $34\pm17\%$, $Ns=4$). In MALES, chronic DZ treatment did not alter basal SNr activity, but caused a compensatory increase in firing rate after RO15-1788 administration. In OVX DZ-treated rats, basal firing rates were nonsignificantly decreased compared to controls and no Ro15-1788-induced increase was observed. In both MALE and OVX rats, chronic DZ exposure did not affect iontophoretic GABA sensitivity and a slight decrease in responses to iontophoretic BZ was observed. Chronic DZ exposure also increased GABA-activation and reduced BZ modulation of cortical ^{36}chloride influx in OVX, but not MALE, rats. These results suggest that gonadal hormones modify the neural adaptations associated with chronic BZ exposure in a region-specific manner. (Supported by DA05932)

153.7

FULL AND PARTIAL BENZODIAZEPINE AGONISTS AND PRIMATE VOCAL COMMUNICATION IN AVERSIVE AND POSITIVE SOCIAL CONTEXTS.

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In socially relevant situations squirrel monkeys emit a rich repertoire of calls that range in frequency from 0.1 to 16 kHz. These calls consist of specific structural and functional components and may represent intense affective expressions within the social context. During brief periods of isolation from their mother or social group, juveniles (11-30 mo.) exhibit behavioral agitation and emit numerous calls or "peeps". These calls are slightly reduced by partial social contact and are markedly reduced by full physical contact with mother. The benzodiazepine anxiolytics, chlordiazepoxide (1-10 mg/kg,i.m.) and Ro16-6028 (0.1-30 mg/kg, i.m.), reduced peeps and explosive motor behaviors in a manner similar to full contact with mother. Interestingly, vocalizations associated with quiet affiliative behavior are also susceptible to benzodiazepine modulation. Adults with full visual, auditory and olfactory contact with the social group engage in vit, twitter and cackle calls when exposed to light stimuli signaling subsequent access to highly preferred or standard foods. Chlordiazepoxide reduced these types of calls at the same doses that reduced isolation peeps, but increased feeding. Vocal communications in squirrel monkeys may convey subtle and intense affect that are sensitive to anxiolytics.

153.8

DIFFERENTIAL EXPRESSION OF BENZODIAZEPINE ANTICONVULSANT CROSS-TOLERANCE ACCORDING TO TIME FOLLOWING FLURAZEPAM TREATMENT.

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Chronic benzodiazepine (BZ) treatment can cause tolerance, and cross-tolerance to other BZs. In studies of the anti-pentylenetetrazol (PTZ) effect of BZs, the demonstration of tolerance depended on the BZ given acutely to test for tolerance (Pharmacol. Biochem. Behav. 39:711, 1992) and the BZ used for chronic treatment (FASEB J. 5: A1211, 1991). In this study, the time course of tolerance reversal was studied in rats treated one week with flurazepam (FZP). Groups were tested 12 hr, 2 and 7 days after ending FZP treatment. BZ effect was measured by the increase in convulsive threshold during a constant i.v. PTZ infusion. In controls, the BZ doses used increased PTZ clonus threshold approximately 3-fold. 12 hr after 1-week FZP treatment, all BZs were significantly less effective, showing tolerance. 48 hr after the 1-week FZP treatment, tolerance to clonazepam was no longer present, but was still robust for both diazepam and FZP. Rats tested a week after the 1-week FZP treatment were no longer tolerant. The results suggest differences in the way these BZs interact with their receptors, allowing differential expression of tolerance between clonazepam vs FZP and diazepam. Moreover, the molecular basis for the differential interaction of the BZs with their receptors must have been altered by FZP treatment, then recovered between 12 and 48 hr after the end of treatment. Supported by DA02194.

153.9

CENTRAL, NOT PERIPHERAL, BENZODIAZEPINE RECEPTOR ANTAGONIST PRODUCES ANXIETY-LIKE STIMULUS IN DIAZEPAM-DEPENDENT RATS. C.M. Harris*, Dept. Pharmacol., NYCOM, Old Westbury, NY 11568.

The peripheral benzodiazepine receptor antagonist PK 11195 (PK) has been reported to precipitate overt signs of diazepam-withdrawal similar to those precipitated by the central-type receptor antagonist flumazenil (FL) (Martinez et al., Pharmacol., Biochem. Behav. 41:461, 1992). To determine whether PK also produces anxiety-like subjective effects, 13 rats were trained to discriminate the anxiogenic drug PTZ, 20 mg/kg, from saline in a 2-lever operant task with food reward, and then tested with PK. Prior to chronic diazepam, neither PK nor FL (up to 40 mg/kg each) substituted for PTZ. After 3-4 days of diazepam (240 mg/kg per day in the diet) PK, 10 and 15 mg/kg (doses up to 3 times those reported to precipitate withdrawal) still did not substitute for PTZ, while FL, 10 mg/kg did. These preliminary results suggest that the the anxiety symptom of benzodiazepine withdrawal may be mediated selectively by the central-type benzodiazepine receptor.

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153.10

CENTROMEDIAL AMYGDALOID LESIONS BLOCK THE ANXIOLYTIC EFFECT OF DIAZEPAM DURING RESTRAIN OR SWIM INDUCED STRESS. I. Zarco de Coronado*, M. de J. Vivar Estudillo, M. A. Barrios Figueroa, M. de L. Palacios Pérez. Biology School. U.A.P. and Physiology Department. Medical School. U.N.A.M. Apdo. Postal 70250. MEXICO.

Emotional stress induce anxiety. The centro medial amygdaloid nucleus (CMA) contains GABA A receptors and benzodiazepine (BDZ) component. To study the role of CMA in anxiolytic effects of diazepam, rats with electrolitically produced CMA lesions and sham operated controls were used. After five operative days (food and water intake ad libitum), ip diazepam (8mg/Kg B.W.) was injected two hours prior to swimming in water mantained at 31*C, or restrain stress.

The experimental animals showed more difficult, incoordinated, and shorter time of swimming behavior. There was lesser gastric ulcerations in the control animals. Beside, lesioned animals increase body weight more than controls. These data indicate that diazepam anxiolysis requires intact CMA to bind adequate GABA receptors.

153.11

DAILY FLUMAZENIL (FLU) DOSING DURING CHLORDIAZEPOXIDE (CDP) DEPENDENCE INDUCTION ATTENUATES FLU PRECIPITATED (PWD) BUT NOT SPONTANEOUS WITHDRAWAL (SWD). N.R. Boisse*, L.P. Martin, J.J. Guarino and G.M. Samoriski. Dept. of Pharm. Sci., Northeastern University, Boston, MA 02115.

Several labs reported periodic benzodiazepine (BZ) receptor antagonist FLU during chronic BZ dosing attenuates FLU PWD. Indeed, Gallager et al (Eur J Pharm 132: 31, '86) suggested intermittent FLU as a therapeutic approach to preventing BZ dependence & WD. However, none monitored SWD which is a more relevant criterion than PWD for BZ WD in man. To re-evaluate FLU in preventing BZ WD, rats were made dependent on CDP 75 mg/kg, po bid daily for 5 wks; chronic controls received water. Two hrs after AM CDP doses, FLU 25 mg/kg or vehicle were given po. After the last CDP dose (4-6 hrs), PWD was elicited by FLU 25 mg/kg, ip & monitored for 60 mins by treatment blind observers. SWD was monitored daily x 14 days. The WD score (sum of all grades for 20 WD signs) and AUC for PWD and SWD were compared. Co-treatment with FLU attenuated PWD by 35% while SWD was unchanged. Clearly, the PWD results are not predictive of SWD and imply differences in molecular mechanisms underlying BZ SWD vs PWD. These results don't support intermittent FLU as a tool to prevent BZ SWD in man. (BRSG SO7-RR-05830-03)

153.12

EFFECTS OF CHLORDIAZEPOXIDE ON SEPTAL SINGLE UNIT ACTIVITY FOLLOWING PAVLOVIAN CONDITIONING: A REGIONAL COMPARISON. C.E. Strickland, E. Thomas* and E. Yadin. Dept. of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010 and Dept. of Psychiatry. Medical College of Pennsylvania, Philadelphia, PA 19129.

Single unit activity was recorded from several regions of the septal area in male Sprague-Dawley rats using chronically implanted miniwires. Following Pavlovian conditioning there was a decrease in activity in the dorsolateral (LSD) and ventrolateral septum (LSV) when a stimulus signaling footshock (CS+) was presented. Few consistent changes were observed in the intermediate lateral septum (LSI) and medial septum (MS). Gross movement was also recorded using a video tracking system. Different behavioral patterns developed as a result of conditioning and depended on the type of CS presented. Chlordiazepoxide (CDP, 10mg/kg i.p.) produced an increase in baseline unit activity (as measured during the ten second period prior to CS presentation) in the LSD and LSV and a decrease in the LSI and MS areas. CDP also appreciably reduced the effects of CS+ on unit activity in LSD and LSV. These results are consistent with other results in our laboratory which suggest that activity in the lateral septum may mediate the action of anxiolytic drugs. The results also indicate that the lateral and medial septum may have reciprocal functions with regard to anxiety.

153.13

GENETIC SELECTION FOR REPLICATE LINES OF DIAZEPAM-SENSITIVE AND -RESISTANT MICE. E.J. Gallaher*, M.L. Helms, S.E. Gionet. VA Medical Center and Oregon Health Sciences University, Portland OR 97201.

We have previously reported the genetic selection of mice for diazepam sensitivity. In the earlier study we used a fixed-speed rotarod and a pass-fail test to monitor the duration of diazepam impairment. DS (sensitive) and DR (resistant) mice were shown to differ with respect to CNS sensitivity rather than the rate of diazepam elimination. However, this procedure includes the possibility that differences in innate rotarod performance or diazepam elimination contribute to the observed difference between DS and DR mice.

We have recently developed replicate lines using within-family selection. An accelerating rotarod was used and each mouse was pretested to determine its baseline rotarod ability. All mice were then injected with 40 mg/kg diazepam ip and tested for rotarod performance at 30 min. Selection for mating was based on the decrement from baseline. Since all mice are tested at the same time, this protocol eliminates the possible confounds of innate rotarod ability and diazepam elimination rate.

The sensitive and resistant lines were found to diverge significantly by the third generation, with additional divergence in subsequent generations. Correlated behaviors (seizure threshold, anxiolysis, sedation, muscle relaxation) will be examined during the course of selection to determine which of these benzodiazepine effects segregate concomitantly with rotarod impairment.

Supported by the VA Medical Research Service.

153.14

DIFFERENCES IN DURATION OF INHIBITION OF CORTICAL AND HIPPOCAMPAL PROTEIN SYNTHESIS FOLLOWING AN ACUTE ADMINISTRATION OF LORAZEPAM. V.Carson[1]*, M.Carson[1], and S.Tewari[2]. 1 Dept. of biology, Chapman Univ., Orange, CA 92666 and 2 Dept. of Psychiatry and Human Behavior, College of Med., UCI, Irvine, CA 92717.

Benzodiazepine derivatives such as Lorazepam (LRZ) interfere with memory consolidation as well as act as anxiolytics. We have found that the degree of inhibition of protein syntheses differs in cortices and hippocampi following an acute administration of LRZ. The time course of these effects was then investigated. An experimental group of Sprague-Dawley rats received 1 mg/kg LRZ via intra-gastric intubation and a control group of rats received 1 ml H_2O/kg. Two subgroups were trained in a water maze 50 or 70 min. after intubation and sacrificed 5 min. after training. A third subgroup was sacrificed 24 hours after intubation. Response latencies were significantly greater in LRZ rats at 70 but not 50 min. Free and membrane bound polysomal RNAs were isolated from the pooled cortices or hippocampi of these rats. The effects of LRZ treatment on the translational abilities of the polysomal RNAs were determined using a mRNA dependent rabbit reticulocyte lysate system containing ^{14}C leucine. Free polysomal RNAs from cortices of LRZ treated rats displayed impaired protein synthesis only 24 hrs. after treatment, while bound cortical polysomal RNAs displayed impaired synthesis at all three time points. In contrast, free polysomal RNAs from hippocampi displayed impaired protein synthesis at all 3 time points while bound polysomal RNAs from hippocampi displayed inhibition only 55 and 75 min. after treatment. Free polysomes are associated with the production of cytoplasmic proteins and bound polysomes are associated with the production of membrane proteins. These results imply that LRZ affects cortical and hippocampal protein synthesis differently.

153.15

HISTOLOGICAL ALTERATIONS OF THE CEREBELLAR CORTEX OF MOUSE FETUSES EXPOSED "IN UTERO" TO DIAZEPAM. A. Márquez-Orozco*, R. Andrade-Martínez, Ma.C. Márquez-Orozco and M.V. Gazca-Ramírez. Embryol. Dept. Sch of Med. Natl. Univ. of Mexico. Mexico 04510 D.F.

Diazepam (DZ) accumulates in the fetal cerebral and cerebellar cortices. We investigated DZ effects upon the development of the cerebellar cortex of mouse fetuses DZ-exposed during gestation. Single daily doses of DZ (2.7 mg/kg/b wt) were s.c. administered to one group (DZ), from the 6th to 17th day of pregnancy. An other group received 0.9% saline solution and a third was non-treated. At day 18th all females were killed with CO_2 atmosphere to remove the fetuses. Their cerebellums were fixed with 2.5% glutaraldehyde, post-fixed in OsO_4 and embedded in epoxic resine. The semifine sections were stained with toluidine blue and observed under the fotonic microscope. In the DZ fetuses the cerebellar cortex revealed a significant delay in the differentiation and neuroblastic migration it evidenced by their accumulation in the germinal external granular layer, the molecular layer, and the density of the internal granular layer ($p < 0.05$). The delay in neuroblastic diferentiation could explain the behavioral alterations observed in newborn mice exposed prenatally to DZ.

153.16

EFFECTS OF PRENATAL DIAZEPAM ON THE RUNNING BEHAVIOR IN ADULT MICE. Ma.C. Márquez-Orozco*, A. Márquez-Orozco and I. Zarco de Coronado. Embryol. and Physiol. Depts. Sch of Med. Natl. Univ. of Mexico. Mexico 04510 D.F.

Previous results in newborn mice indicated impaired motor activity after prenatal administration of diazepam. The underlaying mechanisms involved both peripheral and central alterations in motor regulation. This study analyzes the locomotor activity of 8-weeks old mice prenatally exposed to diazepam.

Diazepam (Valium), 2.7 mg/kg/b.w., was intraperitoneally injected to pregnant mice (days 6th to 17th). Control animals received 0.9% saline solution. The offsprings were maintained for 8-weeks (food and water intake ad libitum). Running activity in a cilinder was evaluated in both groups. Experimental animals showed significant decreased motor activity ($p < 0.05$). These results indicate that long lasting effects produced by prenatal diazepam include reduction in the recovery capacity after damage.

153.17

Sensitivity of substantia nigra reticulata (SNR) neurons to GABA and benzodiazepines (BZs) during chlordiazepoxide (CDP) withdrawal.

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BZs can cause dependence and withdrawal signs in lab animals and humans. We used single-unit extracellular recording to study SNR neuronal activity and responses to GABA and BZs during CDP withdrawal.

Rats (10/group) were given CDP (75 mg/kg/day p.o.) or vehicle for 5 weeks and tested 5 days after the last dose. Recordings were made in each rat to: (1) count spontaneously active SNR neurons in 6 electrode penetrations and measure firing rates, (2) measure the current-response to iontophoretically applied GABA (0.01M, pH 4), and to (3) GABA with concurrently applied flurazepam (0.1M, pH 4, 2-5 nA) and (4) determine the dose-response to i.v. diazepam.

There was no difference between the number or firing rate of SNR cells in CDP-treated rats (1.9 ± 0.1 cells/track, 20.9 ± 2.3 spikes/sec, N=9 rats) and vehicle-treated controls (2.3 ± 0.2 cells/track, 20.7 ± 1.6 spikes/sec, N=9). However, GABA sensitivity was significantly reduced over the range of ejection currents (2-20 nA) in CDP-treated rats. Currents to eject GABA for inhibiting firing by 50% (I50) were 13.7 nA for CDP-withdrawal rats and 7.6 nA for vehicle (N=11-22 cells in 9 rats/group). Nevertheless, neuronal sensitivity to BZs was unchanged. Co-application of flurazepam shifted the GABA I50 from 13.7 to 9.2 nA in CDP-withdrawn rats and from 7.6 to 3.1 nA in controls. Diazepam (0.25-1 mg/kg i.v.) reduced SNR firing by 82% in CDP-withdrawn rats (ED50 0.25 mg/kg, N=7) and by 83% in controls (ED50 0.23 mg/kg, N=7).

Our results demonstrate a modest, but significant reduction of GABA sensitivity for SNR neurons during withdrawal from chronic CDP.

153.18

CHANGES IN $GABA_A$ERGIC RECEPTOR FUNCTION IN PENTOBARBITAL-DEPENDENT RATS. A. Nabeshima, T. Saito*, H. Ozawa, N. Takahata. Dept. of Neuropsychiat. Sapporo Med. Col., Sapporo, JAPAN

Changes in $GABA_A$ergic receptor function were studied in pentobarbital(PB)-dependent rats. Physical dependence on PB in male Lewis rats was induced by using the drug-admixed food (DAF) method. The GABA dependent $^{36}Cl^-$ influx into cerebral cortical synaptosomes of rats was significantly decreased at the time of PB withdrawal (PB-dependent rats) and was significantly increased 20 hrs after PB withdrawal (PB-withdrawal rats). ^{3}H-Muscimol binding to low affinity sites of $GABA_A$ receptors was significantly decreased in the PB-dependent rats. Although ^{3}H-flunitrazepam (FZ) binding to the cortical membrane was not changed, the enhancement of GABA-dependent $^{36}Cl^-$ influx induced by the addition of FZ disappeared in the PB-dependent rats. Additions of picrotoxinin and bicuculline had no effect on the GABA-dependent $^{36}Cl^-$ influx in the PB-dependent rats. These results suggest that physical dependence on PB leads to change of the GABA binding site associated with changes in the GABA/benzodiazepine/chloride channel coupling.

153.19

TOLERANCE AND SENSITIZATION TO THE BEHAVIORAL EFFECTS OF TRIADIMEFON, A NOVEL STIMULANT. A.R. Allen* and R.C. MacPhail. Dept. of Psych., UNC-CH, Chapel Hill, NC 27514 and Neurotoxicology Div., US EPA, RTP, NC 2711.

Triadimefon (TDF) is a fungicide which has been shown to produce many of the behavioral effects associated with stimulant drugs. Previous work in our laboratory has shown that tolerance can develop to the effects of TDF on schedule-controlled behavior when chronically administered. The present experiment was conducted in an effort to determine whether tolerance would develop to the effects of TDF only when administered prior to the session, as is reported for stimulants. The acute effects of TDF (30 -170 mg/kg, 30 min presession, i.p.) were determined in 24 male Long-Evans rats performing under a multiple FR 10 FI 3-min schedule of milk reinforcement. Rats were then divided into three groups (N=8). A dose of TDF for chronic treatment was selected for each rat that was based on the acute effects of TDF. One group received this dose daily before each session; a second group received this dose after each session and the third group received vehicle before and after each session. Following 30 days of treatments, TDF dose-response functions were redetermined and compared to the acute TDF dose-response functions. Tolerance developed to the effects of TDF on FR overall response and run rates in rats that received TDF presession, but only at the dose that they had received daily. Rats that received TDF postsession were sensitive to the effects of several doses of TDF on performance in both schedule components. No overall change in the effectiveness of TDF on performance was seen in the rats receiving vehicle daily. These results indicate that repeated administration of TDF can result in either tolerance or sensitivity, depending upon the temporal relationship between treatment and the test session. It is possible that the tolerance in rats receiving presession TDF may have countered changes that otherwise would have resulted in sensitivity.

154.1

Chronic MDMA administration down-regulates CCK but not tyrosine hydroxylase mRNA in neurones of the rat substantia nigra.

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Abuse of the psychotropic drug, methylenedioxymethamphetamine (MDMA) is widespread. Chronic administration of MDMA causes selective neurodegeneration of serotonin neurones whilst levels of dopamine are relatively unaffected by MDMA.

We have measured the effect of MDMA on cholecystokinin and tyrosine hydroxylase messenger RNA expression which are co-expressed in a subgroup of substantia nigra neurones. Using 35S-labelled oligonucleotide probes and liquid emulsion techniques, we measured mean grain densities (grain area/cell area) over positive neurones from control animals and MDMA-treated animals. Twenty-four hours after the last of eight injections of MDMA (10 mg/kg, i.p.) administered over four days, CCK mRNA levels were reduced to 40% of control levels (MDMA treated level = 13.52 ± 0.68 (n=463), control level = 32.34 ± 1.00 (n=533)). In sections from the same animals, tyrosine hydroxylase mRNA levels were relatively unchanged (MDMA treated level = 32.87 ± 0.94 (n=407), control level = 29.16 ± 0.79 (n=396)). Two weeks after the last dose, CCK mRNA levels remained depressed (43% of control levels). The effect was dose-related since three injections of MDMA (10 mg/kg, i.p.) administered over two days produced a smaller reduction in CCK mRNA levels 24 hours after the last injection (75% of control levels) and, two weeks after the last dose CCK mRNA levels in substantia nigra had returned to normal (98% of control levels).

The study shows that chronic MDMA can produce long-term selective alterations in CCK mRNA levels in substantia nigra. The results may relate to the recent reports that MDMA abuse can lead to chronic atypical psychosis in man, since CCK or its antagonists can modulate these behaviours.

154.2

PHOSPHORYLATION OF NEUROMODULIN (GAP-43) AFTER AN AMPHETAMINE-SENSITIZING REGIMEN AND ACUTE AMPHETAMINE.

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An increase in stimulus-induced DA release and synaptosomal calmodulin (CaM) occur in rat striatum after withdrawal from a sensitizing regimen of amphetamine (A). The CaM-binding protein, neuromodulin (NM), promotes neurotransmitter release and dissociates CaM upon phosphorylation by protein kinase C (PKC). To assess whether these processes were altered in A-sensitization, the *in vivo* phosphorylated state of NM was examined by a back phosphorylation assay, SDS-PAGE and autoradiography. Purified striatal synaptic membranes were phosphorylated by PKC and [^{32}P]ATP. Female, Sprague-Dawley rats received a 4 wk-escalating dose regimen of A or saline (S); a challenge dose of 1 mg/kg A or S was given 4 weeks after the last dose of A to create 4 groups: S-S, S-A, A-S, A-A. EGTA in the homogenizing buffers reduced endogenous dephosphorylation. *In vitro* PKC-mediated back-phosphorylation of NM was significantly reduced in S-A, A-S and A-A groups compared to S-S controls. [^{32}P] content of the cut bands was: S-S, 293 ± 5; S-A, 231 ± 15, A-S, 201 ± 16, A-A, 199 ± 11, N = 6 (ANOVA, $p < 0.001$). Anti-NM immunoblots showed no change in NM levels in any group. These results suggest that acute A (as in the S-A group) increases NM phosphorylation and this change endures 4 wks after repeated A. We have found an effect of 10 μM A on NM phosphorylation in ^{32}Pi-incubated synaptosomes. Since sensitization can be induced with one dose of A, it is possible that phosphorylation of NM could contribute to events leading to an increase in CaM, release of DA and A-induced behavioral sensitization. Funded by NIDA grant, DA05066.

154.3

IDENTIFICATION OF AP-1 BINDING PROTEINS IN NORMAL AND METAMPHETAMINE-TREATED MOUSE BRAINS BY IN SITU DNA BINDING METHOD.

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We developed a novel method for detecting specific DNA-binding proteins in situ (In situ DNA binding method, ISDB). Using an oligo-DNA probe containing AP-1 consensus sequence, we examined the distribution of AP-1 binding proteins in mouse brains and its changes induced by metamphetamine. The specific bindings to AP-1 element showed regional distribution coincided with that of jun/fos in the brain and the binding pattern was clearly different from that of SP-1 whose site is recognized by the other transcription factors. Antibody against jun which recognizes a DNA-binding domain markedly reduced AP-1 binding but not SP-1 binding. Gel shift assay with the nuclear extracts from individual regions of brain paralleled with data of ISDB method. Distribution of AP-1 binding proteins was greatly different from that of control brain, when this method was applied for the mouse brains intoxicated with metamphetamine acutely or chronically. These results suggest that ISDB method is useful and convenient for visualizing distribution of a specific DNA binding protein in vivo.

154.4

C-*FOS* EXPRESSION IN MOUSE STRIATUM (STR) IS INDUCED BY AMPHETAMINE (AMPH).

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Previous studies (Karler et al., *Life Sci.* 45:599, 1989) have indicated that NMDA receptors may be involved in the behavioral sensitization associated with amphetamine-induced stereotypy. We have measured c-*fos* mRNA in total cellular RNA extracts of individual mouse STR (N=5) using a quantitative solution hybridization assay. One hr after D-AMPH, 16 mg/kg ip, c-*fos* mRNA levels were increased nearly 10 fold above controls (saline = 0.56 ± 0.7 pg/μg RNA, AMPH = 5.2 ± 0.7). Pretreatment with MK-801 (MK) or haloperidol (HAL) 0.5 mg/kg ip 30 min prior to AMPH significantly reduced the induction of c-*fos* mRNA (AMPH + MK = 2.5 ± 0.2, AMPH + HAL = 1.7 ± 0.2). The results provide quantitative information on the ability of a dose of AMPH that produces stereotypy to induce c-*fos* mRNA in mouse striatum, a structure implicated in the psychomotor actions of AMPH. The demonstration that a dopamine (HAL) or an NMDA antagonist (MK) can attenuate c-*fos* induction suggests that the measurement of c-*fos* mRNA, an unambiguous indicator of c-*fos* gene expression, may be useful in identifying brain regions and neuroeffector systems associated with AMPH's behavioral effects. Supp. in part by DA-00346, DA-01457, DA-07239.

154.5

TRANSCRIPTION FACTOR GENE EXPRESSION DURING RAT AMPHETAMINE WITHDRAWAL: CORRELATIONS WITH NOVELTY RESPONSES AND DOPAMINE LEVELS.

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Changes in transcription factor gene expression could participate in the behavioral and neurochemical alterations induced by chronic psychostimulant use. Rats injected with 7.5 mg/kg d-amphetamine sulfate bid for 14 days display enhanced expression of c-fos, fos-b, fra-1, jun-b, c-jun and zif 268 mRNAs that manifests tolerance during treatment and cross-tolerance with saline injection stress. Withdrawal decreases expression of each factor; this begins 12 hours after the last injection and largely recovers to control levels by 54 hours. These results show temporal correlations with the onset of decreased stereotypy time on a novelty test and with modest decreases in striatal dopamine levels; each begins 12 hours after the last injection. Interestingly, dopamine levels are also normal by 54 hours after withdrawal, while novelty response alterations last longer. Decreased novelty responses noted during amphetamine withdrawal are thus accompanied by decreased levels of dopamine and reduced expression of genes encoding several transcription factors. These effects each return to baseline values more rapidly than the abnormal behavioral responses, however.

154.6

MULTIPLE EXPOSURE TO D-AMPHETAMINE SENSITIZES C-FOS EXPRESSION IN RAT STRIATUM.

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Exposure of animals to psychomotor stimulants such as amphetamine or cocaine induces an increase in the behavioral effects of these drugs on subsequent exposure. D-amphetamine stimulates the transcription of the immediate early gene c-fos in rat striatum and, therefore, the fos protein can act as a neurochemical marker of postsynaptic responses to d-amphetamine. We investigated fos induction in rat striatum following multiple exposure to d-amphetamine. Male Sprague Dawley rats received an initial injection of either d-amphetamine (5 mg/kg i.p.)(n=7) or an equal volume of saline (n=8) and three days later all rats were administered a second injection of d-amphetamine (2.5 mg/kg i.p.). Three hours post-injection, rats were perfused and brain sections processed for immunocytochemistry for fos. In striatum from rats challenged twice with d-amphetamine there was a significant increase in the total number of fos immunoreactive nuclei and in the intensity of staining compared to that observed in striatum of rats receiving a single challenge of d-amphetamine. Thus, there was an apparent sensitization of the response of striatal cells with respect to fos induction following multiple exposure to d-amphetamine suggesting a possible postsynaptic component of the sensitization response. This change in gene expression may reflect a mechanism by which long term changes in sensitivity to psychomotor stimulants may be produced. (Supported by RO3 MH45253)

154.7

MULTIPLE EXPOSURES TO APOMORPHINE SENSITIZES C-FOS EXPRESSION IN 6-HYDROXYDOPAMINE LESIONED RAT STRIATUM. Wayne Ouellette*, Jennifer M. Klug, Heidi C. Landen, Herbert R. Thompson and Andrew B. Norman, Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

The sensitization to the behavioral effects of psychomotor stimulants has been suggested to be mediated via an increase in the release of dopamine in the basal ganglia. However, in dopamine deafferented rats there is a behavioral sensitization following multiple challenges with apomorphine implying that a postsynaptically mediated sensitization response also exists. Dopamine receptor mediated stimulation of c-fos induction can represent a marker of post-synaptic responses. We, therefore, investigated fos induction in rat striatum following multiple exposure to apomorphine. Male Sprague-Dawley rats received unilateral 6-hydroxydopamine (6-OHDA) lesions and were divided into two groups. The first group (n=7) received three injections of apomorphine (0.25 mg/kg sc) separated by three day intervals. The second group (n=6) received two saline injections followed by an injection of apomorphine (0.25 mg/kg sc). Three hours post-injection the rats were perfused and brain sections were processed for immunocytochemistry for fos. In 6-OHDA lesioned striatum from rats challenged three times with apomorphine, there was a significant increase in the total number of fos immunoreactive nuclei and in the intensity of staining as compared to that observed in rats receiving the single challenge of apomorphine. Thus, there was an apparent sensitization in the response of cells with respect to c-fos induction in 6-OHDA lesioned striatum following multiple exposure to apomorphine. These data indicate that the sensitization in lesioned striatum following multiple challenges with apomorphine can be independent of dopaminergic input and may be mediated by postsynaptic mechanisms. (Supported by RO3 MH45253)

154.8

PHARMACOKINETIC / PHARMACODYNAMIC ANALYSIS OF D-AMPHETAMINE AND D-METHAMPHETAMINE. W.P. Melega*, A.E. Williams, D. Schmitz, E.D. Stefano, A.K. Cho Division of Nuclear Medicine and Dept of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024

Plasma and brain profiles of the kinetics and metabolism of d-amphetamine (Ampt) and d-methamphetamine (MAmpt) were correlated with the magnitude of the drug-evoked dopamine release as measured with microdialysis.

Ampt or MAmpt (1.0, 2.5, 5, 10 mg/kg, iv), was administered to chloral hydrate anesthetized rats. Striatum, cortex, cerebellum and plasma levels were quantitated from 5 to 120 min; brain levels increased proportionally with increases in plasma levels for both compounds; regional differences among brain regions were not observed. For MAmpt, metabolites consisted of amphetamine and para-hydroxymethamphetamine, the latter being more concentrated in striatum relative to cortex or cerebellum.

Pharmacodynamic profiles were obtained in parallel studies with microdialysis in the anterior striatum. Extracellular dopamine (DA) was assayed between 5 and 90 min post-drug. At 2.5 mg/kg, the MAmpt-induced DA release was greater than that from equimolar Ampt; whereas at 5 mg/kg, equimolar doses of MAmpt and Ampt produced comparable effects. Maximal DA release (at 10-20 min) lagged behind peak brain levels (at 5-10 min) of either compound. DOPAC and HVA levels decreased (0-90 min) but at 5 min post-drug, a small increase in DOPAC was observed; HIAA was not affected.

154.9

CONDITIONED INCREASES IN LOCOMOTOR ACTIVITY IN MICE PRODUCED BY AMPHETAMINE OR COCAINE ARE ANTAGONIZED BY MK-801, BUT NOT BY SCH-23390 OR SPIPERONE. G.P. Vincent*, S. Williams, and J. Sepinwall, Neurobiology Research, Hoffmann-La Roche Inc., Nutley, NJ 07110.

Groups of mice (n=16/group) were tested in locomotor activity cages for 1h on each of two consecutive days. Drugs were given ip (mg/kg) as shown below.

	Day 1		Day 2
Gp.	15' Before Activity	1h Post	15' Before Activity
SSC	Saline	Saline	Cocaine (10)
SCC	Saline	Cocaine (40)	Cocaine (10)
CSC	Cocaine (40)	Saline	Cocaine (10)
SSA	Saline	Saline	Amphetamine (3)
SAA	Saline	Amphetamine (10)	Amphetamine (3)
ASA	Amphetamine (10)	Saline	Amphetamine (3)

Groups CSC and ASA on Day 2 exhibited increases in motor activity compared to the other four groups. This behavioral sensitization could be antagonized by the NMDA antagonist MK-801 (1mg/kg), but not by the dopamine D_1 antagonist SCH 23390 (0.04mg/kg) or the D_2 antagonist spiperone (0.04mg/kg), when given immediately after the Day 1 test. MK-801 was much less active when given 2h after the Day 1 session. Thus, NMDA receptor blockade, but not D_1 or D_2 blockade, may interfere with long term memory formation processes that underlie conditioned activity increases produced by amphetamine or cocaine.

154.10

METHAMPHETAMINE-INDUCED ALTERATIONS IN DOPAMINE AND SEROTONIN NEURONS IN FETAL RAPHE CULTURES.

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Methamphetamine (METH) and its structural analogues have been demonstrated to be neurotoxic to CNS dopamine (DA) and serotonin (5-HT) neurons both *in vivo* and *in vitro*. Our laboratory has been involved in an extensive characterization of mesencephalic cultures and the effects of exposure to METH as determined by both neurochemical and immunochemical indices. We have initiated studies of the midbrain raphe nuclei which contain 5-HT as well as DA neurons. The purpose of the present studies was to extend our previous findings and compare them with METH-induced alterations in cultured fetal raphe cells. Fetal rat brains (E19) were removed and the rhombencephalon obtained and dispersed into single cells. METH (10^{-4} M) was added to the cultures on day 2 and fresh daily thereafter. The effects of chronic METH exposure on [^{3}H]-DA and [^{3}H]-5-HT uptake was determined after 5 days of drug treatment. Additional cultures were fixed with 5% acrolein and the presence of TH-and 5-HT-containing cells was determined by immunocytochemistry. Results indicate that chronic METH exposure decreased DA and 5-HT uptake (approximately 40 and 30%, respectively). Preliminary immunocytochemical studies suggest that METH administration also results in a reduction in the number of TH and 5-HT immunopositive cells. These results, like those found with mesencephalic cultures, suggest that chronic METH exposure can induce alterations in raphe aminergic neurons. *Supported by DA-05073 (BAB) and DA-05381 (JMP).*

154.11

EFFECT OF d-AMPHETAMINE ON THE EXTRACELLULAR CONCENTRATIONS OF DOPAMINE AND GLUTAMATE IN IPRINDOLE TREATED RATS. J. F. Nash* and B. K. Yamamoto. Dept. of Psychiatry and Neuroscience, Case Western Reserve University, Cleveland, OH 44106-5000

The effect of d-amphetamine on the extracellular concentrations of dopamine (DA) and glutamate (GLU) was studied in iprindole treated rats using in vivo microdialysis. d-Amphetamine (18.4 mg/kg, ip) significantly increased the extracellular concentration of DA in the anteromedial striatum. Treatment with iprindole (10 mg/kg, ip) had no effect on the absolute increase in DA efflux but significantly prolonged the increase in the extracellular concentration of DA. d-Amphetamine administration increased the extracellular concentration of GLU only in iprindole treated rats. The increase in GLU efflux began 4-6 hr after d-amphetamine administration and the maximal increase was observed 8-12 hr after drug administration. Each rat was sacrificed 7 days after the dialysis study and the concentration of DA was determined in the contralateral striatum. Consistent with previous studies, d-amphetamine significantly decreased DA content in the contralateral striatum of iprindole treated rats without affecting the concentration of serotonin. Pretreatment with α-methylparatyrosine (150 mg/kg) significantly attenuated d-amphetamine-induced DA efflux and the long-term (7 day) depletion of DA in the striatum. Haloperidol (1 mg/kg) had no effect on d-amphetamine-induced DA efflux but completely blocked the depletion of DA observed 7 days later. The extracellular concentration of GLU was measured in both cases and will be presented. These data are suggestive that acute increases in the extracellular concentration of GLU plays a significant role in the depletion of DA produced by d-amphetamine in iprindole treated rats.

154.12

DOPAMINE RELEASE FROM A9 AND A10 NEURONS INDUCED BY ADMINISTRATION OF 4-METHYLAMINOREX AND AMPHETAMINE. W.F. Eberle, C.S. McNeish, C.R. Ashby, R.Y. Wang, and R.E. Strecker*.

The novel stimulant drug 4-methylaminorex (4-MAX) has properties similar to amphetamine in reports of its use in humans, and in drug discrimination paradigms with animals. The present study used microdialysis sample collection and quantitative neurotransmitter analysis by HPLC to quantify 4-MAX and amphetamine induced changes in dopamine (DA) release in the nucleus accumbens (NAS), frontal cortex (FC), and caudate-putamen (CP) of awake freely moving rats. Route of drug administration included both intraperitoneal (i.p.) injections and intra-ringer administration of d-amphetamine and the four isomers of 4-MAX. According to previous studies, the four isomers of 4-MAX differ in potency as follows: trans(4S,5S) > cis(4S,5R) = cis (4R,5S) > trans(4R,5R). Our results from the presentation of these compounds (at a concentration of 10^{-5}M) in the ringer solution into the NAS resulted in increases of DA release quite consistent with their expected potencies. Specifically, the trans 4S,5S and the cis 4R,5S isomer produced large increases (7.1 and 6.8 fold respectively), which were quantitatively similar to d-amphetamine, while the less active trans 4R,5R isomer produced only a 2.4 fold increase in DA release. Parallel electrophysiology studies by Ashby and Wang have suggested that the trans 4S,5S isomer of 4-MAX is more potent in suppressing cell firing in the NAS and FC (projection area of the A10 DA neurons) than in the CP (projection area of the A9 DA neurons). Following i.p. injections of the various 4-MAX isomers, a preliminary analysis indicated that there was no large difference between 4-MAX-induced increases in DA levels in the CP versus the NAS. Supported by PHS award DA07456.

154.13

AMPHETAMINE-INDUCED CHANGES IN MONOAMINE RELEASE IN AWAKE MONKEY (MACACA FASCICULARIS) AS ASSESSED BY IN VIVO MICRODIALYSIS. C.W. Berridge*, R. Kuczenski, D.S. Segal, S.L. Foote. Univ. Calif., San Diego, CA 92093.

Guide cannulae for microdialysis probes were permanently implanted over primary motor cortex (n=4), parietal cortex (n=4), and caudate nucleus (n=4) in an adult male monkey using MRI-determined stereotaxic coordinates. Following recovery, the monkey was habituated to daily chairing. Each dialysis experiment involved chairing the monkey 3-4 hr/day for 4 consecutive days. On day 1, dialysis probes were inserted into 2-3 sites and on days 2-4 samples were collected prior to and following amphetamine (AMPH; 0.25 mg/kg, sc) administration. Experiments were separated by 1-4 weeks. Dopamine (DA) was present in samples from caudate (60-150 fmol/sample) and motor cortex (2-7 fmol/sample) but not detectable (< 1.5 fmol) in parietal cortex; AMPH increased DA concentrations approximately 10-fold in caudate and motor cortex. Norepinephrine (NE) was detectable in parietal (2-4 fmol/sample) and motor cortices (2-5 fmol/sample); AMPH increased NE in samples from both regions approximately 10-fold. The DA catabolites HVA and DOPAC were present in all regions. Concentrations of HVA were approximately 10-fold greater than those of DOPAC. A delayed AMPH-induced decline in these catabolites was observed. These results demonstrate the feasibility of using microdialysis in cortical and subcortical structures in awake nonhuman primate.

154.14

IN VIVO MICRODIALYSIS AND CHRONOAMPEROMETRY: TIME-RELATED EFFECTS OF AMPHETAMINE ON RAT STRIATAL DOPAMINE EFFLUX AND LOCOMOTION. A.G. Phillips,* A. Coury, L.J. Atkinson and C.D. Blaha. Dept. Psych., Univ. British Columbia, Vancouver, BC, Canada V6T 1Z4.

Amphetamine (AMP, 2 mg/kg s.c.) effects on striatal dopamine (DA) efflux (CA: chronoamperometric 1 s pulse/30 s; MD: microdialysis-HPLC sample/900 s, 1.5 ul/min) and locomotor activity (photocell counts/10 min) were assessed 24 hr after bilateral implantation into rat striata of a CA electrode (CE: 150 um stearate+carbon paste surface) and MD probe (MP: 0.34x4 mm). AMP effects on DA for MD are given as a percentage of pre-AMP basal DA values (100%). In order to compare CA and MD measures of AMP effects on DA, a second study derived a mean value (100%) for basal DA oxidation current 24 hr after implantation into rat striatum of a parallel side-by-side CE|MP assembly. By virtue of MD drainage properties (Blaha et al., *Soc Neurosci* 17, 1991), perfusion (5 ul/min) of the MP maximally decreased baseline CA currents 9±0.4 nA (n=33). AMP effects on CA signals are thus given as a percentage of this mean basal DA current value. At the nadir of this decrease, the CE selectivity to DA was tested by serial perfusion of the MP with 10, 5, and 0.05 mM of ascorbate, DOPAC, and DA, respectively. Only DA reversed the MD-induced decrease in the CA signal. In the first study, MD and CA measures of AMP effects on DA efflux were similar in total duration (210 vs 230 min) and peak-time (30-45 vs 45-55 min) but differed in the magnitude of increase above baseline (475% vs 200%), respectively. These time course measurements of DA efflux correlated well with AMP action on locomotor activity (600% peak increase in 45 min with baseline recovery in 210 min) and demonstrate that both *in vivo* methods may be used to study the role of central DAergic systems in mediating specific behaviors. The disparity in magnitude of AMP effects may be due, in part, to the MP extraction process and its impact on steady-state concentrations of neurochemicals in the surrounding interstitial fluid.

154.15

REPEATED MICRODIALYSIS MEASUREMENTS OF CAUDATE EXTRACELLULAR DOPAMINE IN THE VERVET MONKEY: EFFECT OF AMPHETAMINE. C.W. Bradberry *, J. Nobelitti, R. Innis, R. Malison. Depts. of Psychiatry and Laboratory Medicine, Yale University School of Medicine, New Haven, CT and the VA Medical Center, West Haven, CT 06516.

We have performed repeated microdialysis experiments in the vervet monkey, examining the effect of intravenous amphetamine on extracellular DA in the caudate nucleus. MRI-directed stereotaxic placement of a guide cannula assembly was utilized in order to accurately position microdialysis probes. All experiments were performed by acute placement of a microdialysis probe in the isoflurane-anesthetized animal. Basal levels of extracellular DA were 18.8 ± 3.5 fmol/microliter (corrected for probe recovery), corresponding to an estimated extracellular concentration of 18.8 nM. Intravenous amphetamine sulfate resulted in a maximal 22-fold increase in extracellular dopamine, with an apparent ED_{50} of 0.3 mg/kg (salt weight). Careful use of sterile procedures completely avoided any infection as a result of either the initial surgery, or the microdialysis probe placement. For comparison, a series of experiments was performed in the chloral hydrate anesthetized rat. Probes were placed acutely into the lateral striatum. In the rat, intravenous amphetamine sulfate resulted in a maximal 40 fold increase in extracellular dopamine, with an apparent ED_{50} of 1.1 mg/kg. These results indicate an enhanced sensitivity of the primate to amphetamine in comparison to the rat, and establish the utility of the procedures for the study of psychostimulant action in the primate.

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154.16

THE EFFECTS OF ESCALATING DOSES OF d-AMPHETAMINE (AMPH) ON LATERAL HYPOTHALAMIC INTRACRANIAL SELF-STIMULATION (ICSS). E.M. Munn* and R.A. Wise, Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Canada, H3G 1M8.

It has been suggested that the transient behavioural depression observed following treatment with escalating, non-neurotoxic doses of AMPH may represent an animal model of the human withdrawal "distress syndrome" (Paulson et al., Psychopharmacology, 1991, **103**, 480-492). The effects of this injection paradigm were assessed in rats trained to lever press for lateral hypothalamic electrical stimulation. The animals were pretreated with either saline or AMPH twice a day 5 days a week for 6 weeks. Rate-frequency curves were collected weekly on the seventh day (>36 hrs after the last AMPH injection) during this period and for an additional 3 weeks following the termination of drug treatment. Animals receiving AMPH showed a progressive elevation of ICSS threshold whereas their asymptotic rate of responding decreased from baseline levels. These effects were transitory and 3 weeks later there were no significant differences in these measures. A subsequent AMPH challenge (1 mg/kg IP) reduced the ICSS threshold to the same degree in both groups. These observations suggest that the behavioural depression seen during AMPH withdrawal is accompanied by an attenuation of the rewarding effects of electrical stimulation. Unlike locomotion, however, AMPH pretreatment does not enhance the facilitating effects of subsequent AMPH on ICSS.

154.17

LESIONS OF THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS BLOCK DRUG-INDUCED REINFORCEMENT, BUT NOT AMPHETAMINE-INDUCED LOCOMOTION. M.C. Olmstead and K.B.J. Franklin*. Dept. Psychology, McGill University, Montreal, Canada, H3A 1B1

It has been proposed that the positive reinforcing and motor activating effects of drugs involve the activation of a single neural substrate. If this is true, manipulations that effect drug reinforcement should also effect the locomotor responses to a drug injection. To test this hypothesis, we examined the role of the pedunculopontine tegmental nucleus (PPTg) in drug reinforcement and stimulant induced locomotion.

In three separate experiments, rats were prepared with NMDA (.5 ul of .1M solution) or sham lesions of the PPTg. In two experiments, sham and lesioned animals were tested for the development of a conditioned place preference (CPP) to morphine (2 mg/kg X 3 pairings) or amphetamine (1.5 mg/kg X 3 pairings). Ten days later, spontaneous motor activity (SMA) was assessed following saline or amphetamine (1.5 mg/kg s.c.). The third group of drug-naive animals were tested for SMA only. Lesions of the PPTg blocked the development of a CPP to both morphine and amphetamine. In contrast, lesions had no effect on saline or amphetamine-stimulated SMA.

The PPTg appears to be involved in the reinforcing effects of amphetamine and morphine, but is not necessary for the expression of amphetamine-induced SMA.

154.18

CHOLINERGIC-DOPAMINERGIC INTERACTIONS IN THE SHELL AND CORE REGIONS OF NUCLEUS ACCUMBENS. R.M. Booze* and M.L. Smith. Department of Pharmacology, University of Kentucky Medical Center, Lexington, KY 40536-0084.

The mesocorticolimbic dopamine system has been recognized as an important neurobiological substrate for cocaine abuse. In these experiments, we have used (1) ChAT immunocytochemistry and 3-D computer reconstruction to localize cholinergic neurons within the nucleus accumbens and (2) quantitative receptor autoradiography/*in situ* hybridization to localize dopamine receptors in the shell and core regions of the nucleus accumbens.

Adult male Sprague-Dawley rats were perfused with 4% paraformaldehyde. The brains were sectioned (40 µm) and tissue sections collected through the nucleus accumbens. Serial sections were processed for ChAT-IR using standard ABC immunocytochemical detection techniques. Computerized 3-dimensional reconstructions were generated of the ChAT-IR interneurons in the dorsal striatum and nucleus accumbens. In a separate series of animals, cryostat-cut (16 µm) adjacent sections were collected through the rostral, mid, and caudal nucleus accumbens for D-1 and D-2 receptor autoradiography/*in situ* hybridization.

We found that ChAT-IR neurons were preferentially localized to the shell (dorso-medial) region of the nucleus accumbens. The shell region of nucleus accumbens also contained the highest levels of dopamine D-1 receptors and D-1 mRNA. In contrast, D-2 receptors and D-2 mRNA were predominant in the rostral nucleus accumbens and the core region. These findings suggest (1) that cholinergic interneurons serve to link the dopaminergic inputs from the VTA (A10) with GABAergic efferent neurons of the nucleus accumbens shell region and (2) the anatomical heterogeneity of the nucleus accumbens should be considered in determining the actions of cocaine. (Supported by DA 06638)

154.19

Amphetamine, cocaine and nicotine induce c-fos expression in different subnuclei of ventral tegmental area (VTA). Hideo Kiba, Ying Pang and A. Jayaraman* Dept. of Neurology, LSU Med. School., New Orleans, LA 70112

The euphoric and reinforcing properties of drugs of abuse have been speculated to be mediated by VTA dopaminergic neurons. Induction of c-fos is an immediate and early response in the cascade of molecular events that ultimately lead to long-term alterations in gene expression in neurons. To identify the precise subsets of VTA neurons that mediate the acute effects of some common drugs of abuse, the pattern of expression of c-fos gene was mapped in VTA with immunocytochemical methods. **Amphetamine** (5mg/kgi.p) resulted in Fos-Li in supramamillary area, parabrachialis pigmentosus, rostral linear nuclei, caudal VTA and retrorubral areas. Fos-Li was also prominent in the rostral and lateral areas of substantia nigra pars reticularis (SNpr). Neurons with Fos-Li were moderately labeled in nucleus paranigralis, but were absent in caudal linear nucleus and substantia nigra pars compacta (SNpc). **Cocaine** (7.5-30 mg/kg.i.p) resulted in prominent Fos-Li in paranigralis,caudal VTA, parabrachialis pigmentosus, and rostral linear nuclei. Only few cells of interfasicular and caudal linear nuclei contained Fos-Li. Many cells of rostromedial SNpc contained Fos-Li, but SNpr was free of labeled cells. **Nicotine** (0.3-1.4 mg/kg.,s.c.) resulted in prominent Fos-Li in interpeduncular nucleus, medial terminal nucleus of accessory optic tract and caudal linear nucleus of VTA. These **results** suggest that different drugs of abuse may mediate their acute (and possibly their reinforcing) properties via different subset of VTA nuclei. Supported by Smokeless Tobacco Research Council.

DRUGS OF ABUSE: COCAINE AND DEVELOPMENT

155.1

CHRONIC PRE AND POSTNATAL EXPOSURE TO COCAINE PRODUCES AN ALTERATION IN THE RELEASE OF DOPAMINE AND ACETYLCHOLINE IN THE DEVELOPING RAT PUP.

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and Department of Chemistry, University of Oklahoma, Norman, OK

Chronic exposure to cocaine produces a behavioral sensitization. The neurochemical basis for this behavioral sensitization has not been determined. In this study, pregnant rats were injected with cocaine (5 mg/kg s.c.) daily from day 10 (sperm + = day 0), through weaning (postnatal day 21). Dopamine and acetylcholine were measured simultaneously, using in vivo microdialysis coupled with HPLC-EC for DA and GC/MS for ACh. Experiments were performed in the rat pups at 10-12 ,21-22 and 34-36 days postnatal.

A challenge dose of cocaine (5 mg/kg) was given after a stable baseline was achieved. In pups chronically exposed to cocaine, basal release of DA was reduced 50%. However, the release of DA increased by approxiamately 400% after cocaine challenge.

In the 10-12 day pups the development of basal release of ACh was delayed. At 20-22 days, basal release of ACh was reduced by approxiamately 50% of control. This reduction in basal release was not altered after a cocaine challenge.

These finding provide a neurochemical basis for the behavioral sensitization seen after cocaine administration as well as the learning or motor deficits seen.

155.2

CHARACTERIZATION OF DOPAMINE AND SEROTONIN TRANSPORTERS IN AN ACUTE FETAL RAT STRIATAL PREPARATION. C.E. Hyde* and B.A. Bennett, Dept. Physiology and Pharmacology, Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27157.

Monoamine transporters have been implicated in the mechanisms underlying the behavioral effects of various abused drugs. Psychomotor stimulants decrease the uptake of amines by binding to and inhibiting transporter-mediated uptake. Studies have shown that the ability of cocaine to inhibit dopamine (DA) uptake correlates positively with its reinforcing effects. However, little is known about the role of serotonin (5-HT) transporters in the behavioral response to cocaine. In this study, we characterized the DA and 5-HT transporters in an acutely dispersed rat striatal preparation utilizing various neuronal uptake inhibitors. Brains from fetal rats (E19) were removed and the striatum was obtained and dispersed. This preparation is a crude homogenate, consisting primarily of cell bodies with both pre- and post-synaptic elements. DA and 5-HT uptake were performed using 15nM [^{3}H]-DA and 15nM [^{3}H]-5-HT respectively. Kinetic analysis of the data revealed high affinity uptake mechanisms. Relative inhibition potencies of the DA transporter was as follows: nomifensine>amfonelic acid> mazindol> CFT> dopamine> methamphetamine> cocaine>> bupropion> (-)norcocaine. Studies with the serotonin transporter revealed the following relative potencies: fluoxetine> CFT> mazindol> cocaine>> methamphetamine. Hill plot coefficients indicated one binding site for the dopamine transporter. The data indicate that the transporters in this preparation exhibit high affinity for inhibition, and their kinetics resemble those observed in synaptosomal or homogenate preparations. This novel preparation is currently being used to examine fetal and neonatal alterations in transporter function after *in utero* exposure to various abused drugs. *Supported by DA-05073 (BAB).*

155.3

PERINATAL EXPOSURE TO COCAINE OR FLUOXETINE INTERACTS WITH GENDER IN PREDICTING THE ACOUSTIC STARTLE RESPONSE FOLLOWING 8OHDPAT AND MCPP INJECTION IN ADULT RATS. H. E. Hughes*, E. A. Grose and D. L. Dow-Edwards. Laboratory of Cerebral Metabolism, Department of Pharmacology, SUNY Health Science Center, Brooklyn, N.Y., 11203.

It is likely that perinatal cocaine exposure impacts upon the development of multiple neurotransmitter systems. The acoustic startle response (ASR) in rats has been shown to be under dopaminergic and serotonergic control. Cocaine has been shown to block the presynaptic uptake of both amines and to alter ASR. This study compared the first ASR in response to 5-HT receptor agonists in adult rats that received cocaine or fluoxetine during a postnatal period of serotonergic development. Sprague-Dawley rats were mated in our animal facility. At birth, litters were culled to four males and four females. During postnatal days 11-20, each litter received daily sc injections of 25 mg/kg cocaine, 25 mg/kg fluoxetine or water. At 21 days, pups were weaned and housed with same sex littermates. At 76-80 days, rats were ASR tested for 30 min (121 trials) on two consecutive days. The startle stimulus was a 40 msec burst of 116 dB white noise. On Day 1 of testing, no test drug was used. On Day 2, subjects received a single sc injection of 0.5 mg/kg 8OHDPAT (5-HT1A agonist), 5.0 mg/kg MCPP (5-HT1B agonist) or saline ten min before testing. While postnatal treatment did not effect ASR on day 1 of testing, preliminary analysis of the data indicate that perinatal exposure to cocaine, but not fluoxetine, decreased first trial amplitude in response to MCPP in males. This finding suggests that the long-term effects of cocaine on ASR were not primarily due to cocaine's effects on 5-HT reuptake. Supported by ADAMHA grant #DA04118.

155.4

RELEASE OF ^{3}H-5HT FROM SYNAPTOSOMES BY COCAINE: A COMPARISON BETWEEN FETAL AND MATERNAL TISSUE.

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Our laboratories have previously reported that gestational cocaine (COC) administration significantly disrupts the serotonergic (5-HT) system. In adults, it is believed that these drugs exert their effects by inhibiting 5-HT re-uptake and/or promoting its release. However, these effects have never been clearly demonstrated in fetal rats making it difficult to determine how these drugs may be damaging to the fetal brain. This study investigated the ability of COC to cause release of ^{3}H-5HT from fetal synaptosomes, and then compares these results to release measured in the maternal brain.

Sprague-Dawley fetuses were removed by cesarian section on embryonic day 16 (E-16) and their brains removed. Fetal and maternal whole brain synaptosomes were prepared and tested simultaneously. Concentration curves were established for both ages to reveal the COC dosages that best induced release.

In vitro COC resulted in a bi-phasic release of ^{3}H-5HT in the fetal brain at 10^{-7}M (100.1% of total release) and a second at 10^{-4}M and 10^{-3}M (95.15% and 100% of total release). Conversely, significant release only occurred in the maternal brain at COC concentrations above 10^{-4}M (100% of total release).

These results indicate that COC may activate a high-affinity release response in the fetal brain. This site may be the product of an immature 5-HT transporter and only present during the early stages of ontogeny. We are currently investigating the effects of other drugs such as fenfluramine and 3,4-methylenedioxymeth-amphetamine (MDMA) on this system due to their ability to produce significant synaptosomal release of serotonin. (Supported by NICHD to P.M.W-A. and NIDA contract # 271-90-7403 to E.C.A.)

155.5

ACTIVITY AND RESPONSIVITY TO COCAINE FOLLOWING THIRD TRIMESTER COCAINE EXPOSURE IN RATS. S. Barron*, D.H. Kaiser, K.A. Spalmacin, L.S. Hansen. Psychology Department, University of Kentucky, Lexington, KY 40506.

This study was designed to look at the effects of third trimester cocaine exposure on activity and response to cocaine using a rodent model. Subjects were rat pups artificially-reared (AR) from postnatal (pn) days 4 - 10 with gastrostomy tubes. Treatment groups included two doses of cocaine (20 mg/kg/day and 40 mg/kg/day), an AR control and a suckled control. Subjects were tested at either PN 21 or PN 65. Subjects were placed in a running wheel for a 30 min baseline measure. This was followed by injections of saline on day 1 (ip) and 20 mg/kg cocaine hydrochloride on day 2. Activity was recorded for an additional 60 min. At PN 21, baseline hypoactivity was observed in both sexes at the higher cocaine dose and in females at the lower dose. There was no evidence of hypoactivity at PN 65. Neonatal treatment did not interact with cocaine-induced activation. However, there was a pronounced age effect since all PN 21 groups showed cocaine-induced increases in running wheel activity which contrasted with adults. These findings suggest a developmental difference in the responsivity to cocaine-induced activation in the running wheel. In addition, third trimester cocaine exposure resulted in changes in baseline activity which may be age-dependent and sex-dependent. However, there was no evidence of later sensitivity to the activating effects of cocaine. (Supported, in part, by NIDA DA06049).

155.6

Gonadal Hormones Modify the Effects of Acute and Chronic Cocaine Administration on Paced Responding F. van Haaren *, Univ. of Florida, Gainesville, FL 32611.

Intact and gonadectomized male and female Wistar rats were exposed to a two component multiple schedule to investigate whether or not the presence and absence of gonadal hormones would differentially affect the behavioral effects of acute and chronic cocaine administration. High response rates were maintained by a tandem (RI 30-s, DRH 0.5-s) schedule in one component, low rates by a tandem (RI 30-s, DRL 5-s) schedule in the other component. Acute cocaine administration (1-30 mg/kg, IP) decreased high and low rates at intermediate doses (5.6, 10.0 mg/kg). At 17.0 mg/kg responding was eliminated in intact and ovariectomized female rats and castrated male rats, but not in intact male rats. Chronic administration of a behaviorally active dose of cocaine produced sensitization to the rate-decreasing effects at doses exceeding the chronic dose of cocaine. High and low response rates were controlled by stringent schedule contingencies which may have prevented the observation of other differences.

155.7

POSTNATAL BIOCHEMICAL AND NEUROENDOCRINE DEFICITS IN SEROTONIN SYSTEMS FOLLOWING IN UTERO EXPOSURE TO COCAINE. T.M. Cabrera*, J. Yracheta, A.D. Levy, Q. Li, K. Kunimoto, H. Kusters, G. Kindel, I. Hanin, L.D. Van de Kar and G. Battaglia. Department of Pharmacology, Loyola University Chicago, Stritch School of Medicine, Maywood, IL 60153.

During fetal brain development, serotonin (5-HT) acts as a trophic factor regulating the development of 5-HT neurons and 5-HT target tissues. Since cocaine alters 5-HT neurotransmission, we hypothesized that exposure to cocaine during fetal development could result in long-term postnatal dysfunction of 5-HT systems. Pregnant rats, administered either saline or cocaine (15 mg/kg, s.c., b.i.d.) from gestational day 13 through 20, comprised 3 treatment groups (sal-inj/ad-lib fed; sal-inj/pair-fed; & cocaine-injected). All progeny were fostered to non-treated lactating dams. On postnatal day (PD) 30 (males & females) and PD 70 (males), rats received a single injection of either saline or the 5-HT releaser p-chloroamphetamine(PCA) to determine the functional integrity of presynaptic 5-HT neurons. HPLC data indicated no significant differences in cortical 5-HT or 5-HIAA content among the male progeny groups at either PD30 or PD70. Likewise, PD30 female cocaine progeny exhibited no differences in cortical 5-HT or 5-HIAA content from controls. However, deficits in the ability of PCA to deplete cortical 5-HT content were observed in male but not female cocaine progeny. Consistent with these biochemical data, radioimmunoassay of trunk blood revealed deficits in the ability of PCA to increase plasma adrenocorticotrophic hormone (-40%), plasma renin activity (-52%) & renin concentration (-60%) in male progeny. In contrast to these hormone data, but consistent with the HPLC data, no deficits in PCA-mediated increases in plasma renin were observed in female cocaine progeny. Both the biochemical and neuroendocrine data indicate in utero cocaine-induced long-term functional deficits in brain 5-HT systems in male progeny. These deficits appear to be, in part, due to dysfunction of the presynaptic 5-HT terminal, although the present data do not preclude the possibility of postsynaptic modifications as well. (Supported in part by Loyola University and by DA04865)

155.8

EFFECTS OF PRENATAL COCAINE EXPOSURE ON STRIATAL TYROSINE HYDROXYLASE IMMUNOREACTIVITY. D. Jackson*, M.S. Manley, L.D. Manley, S.J. Young, and P.M. Groves. Dept. of Psychiatry, Univ. of California at San Diego, La Jolla, CA, 92093-0603.

The effects of prenatal exposure to cocaine on CNS dopaminergic systems remains controversial in that evidence supports models for both dopamine (DA) repletion and depletion effects of cocaine. A variety of factors may contribute to altered DA transmission, including toxicity to monoaminergic systems or changes in DA synthesis, release, and receptors. We examined changes in TH labeling in the striatum of 20 day old Long-Evans rats following prenatal exposure to cocaine. Pregnant females were injected with cocaine hydrochloride (15 mg/kg b.i.d., s.c.) or saline during embryonic days 15-21. Animals were perfused and post-fixed with 4% paraformaldehyde. Brains were removed, cryoprotected, then cut with a freezing microtome. Tissue was processed for tyrosine hydroxylase (TH) immunoreactivity with a sheep anti-TH polyclonal followed by standard avidin-biotin staining methods. Brightfield images and density measurements were obtained from dorsal, mid-, and ventral striatum using an image analysis system. TH-positive patches in the most dorsal aspect of the striatum of offspring of saline control mothers were sparse (mean$\pm$SEM = 2.35$\pm$.53). Prenatal exposure to cocaine elevated the number of patches in both female (6.1$\pm$.61, n=4, p=.002) and male (5.6$\pm$.61 n= 4, p=.009) rats relative to saline controls. This finding may be related to observations that another monoaminergic drug, amphetamine, unmasks striatal patches in adult rats (Ryan et al., 1988). In contrast, density measurements of the TH labelling in striatal matrix were reduced by 33% in cocaine-exposed female rats relative to controls while males were unaffected. These results suggest that prenatal cocaine exposure produces different effects on patch and matrix regions. Prenatal treatment with cocaine produces a persistent enhancement of TH-positive patches. Loss of TH staining in cocaine-treated female rats may reflect sex-dependent terminal degeneration or decreased expression of TH in the matrix. Supported by grants NIDA DA 02854 and NSF BNS 9006155

155.9

INCREASED SEROTONIN IMMUNOREACTIVITY IN STRIATUM AND CORTEX OF ADULT RATS TREATED PRENATALLY WITH COCAINE. M.S. Manley*, D.Jackson, L.D.Manley, S.J.Young, and P.M.Groves. Dept. of Psychiatry, School of Medicine, University of Califomina, San Diego, La Jolla, CA 92093-0603

Previous studies involving administration of stimulant drugs to pregnant female rats have shown effects on monoaminergic systems in their offspring. In particular, administration of cocaine during the last week of gestation has been shown to cause a serotonin (5-HT) hyperinnervation in the striatum of immature offspring. We examined 5-HT innervation in the cortex and striatum of adult rats (>250g) prenatally treated with cocaine. Groups of pregnant Long-Evans females received either saline or cocaine hydrochloride (15 mg/kg qd, 15 mg/kg b.i.d. or 40 mg/kg qd s.c.) during the last week of gestation. Pups were weaned at 27 days, then were sacrificed as adults by transcardial perfusion with 4% paraformaldehyde (PF) under Nembutal anesthesia (100mg/kg). Brains were removed, postfixed 4 hrs in PF, cryoprotected, then sectioned. 5-HT was detected using a rabbit polyclonal antibody against 5-HT (Incstar) followed either by a standard avidin-biotin method, or fluorescein-conjugated secondary antibody. Measurements of 5-HT labelling in neostriatum and cortex were obtained using an image analysis system. Material was also examined with a confocal laser scanning microscope (Bio-Rad). Adult male rats treated prenatally with cocaine showed significantly increased 5-HT staining in neostriatum and cortex compared to saline controls. Preliminary results on adult females similarly treated with cocaine showed a trend toward increased 5-HT in the cortex, but not in the striatum. These results suggest that alterations in 5-HT innervation as a consequence of prenatal exposure to cocaine persist into adulthood. Data will also be presented on 5-HT fiber morphology and distribution. Supported by DA 02854 and PHS 5T32 MH18398.

155.10

PRENATAL COCAINE EXPOSURE HAS MINIMAL EFFECTS ON DEVELOPMENT AND ADULT BEHAVIOR IN RATS. L.W. Means*, E.W. Bass Jr., T.J. Fernandez and B.A. McMillen. Psych. & Pharm. Depts., East Carolina Univ., Greenville, NC 27858.

To examine the developmental and behavioral effects of prenatal exposure to cocaine, female Sprague-Dawley rats were given cocaine (15mg/kg s.c. b.i.d.) (group C) or an equivalent volume of saline (group S) throughout gestation. Half of the dams receiving saline were pair-fed with dams receiving cocaine (group SPF). At birth litters were culled to 5 male and 5 female pups, and half of the litters from each group were placed with untreated surrogate mothers. One male and one female from each litter was tested on cork gnawing (age 30 days), runway acquisition and response to a novel alley (60 days), CRF, FR, and DRL performance (90 days), and response to a plus maze (120 days). The C dams gained less weight during gestation than did the other dams ($p<.05$), and the C and SPF offspring had delayed eye opening compared to the S pups. In the plus maze, C offspring made more arm entries and spent more time in the open arms than did S offspring ($p<.05$). Also, due to two outliers, group C male pups had longer latencies during runway acquisition and exposure to the novel alley than pups in the other groups ($p<.05$). The groups did not differ on any other developmental or behavioral measure. Prenatal cocaine exposure produces minimal developmental and behavioral effects in rats. (DA 04895)

155.11

THE EFFECTS OF PRENATAL EXPOSURE TO COCAINE ON THE DISCRIMINATIVE PROPERTIES OF COCAINE IN ADULT RATS. C.J. Heyser*, L. Rajachandran, N.E. Spear, and L.P. Spear. Center for Developmental Psychobiology and Dept. of Psychology, SUNY, Binghamton, NY 13902-6000.

Adult male rats prenatally exposed to cocaine and control offspring were trained to discriminate 10 mg/kg cocaine hydrochloride (HCl) from saline in a two-lever discrimination procedure to examine sensitivity to the discriminative (interoceptive) properties of cocaine. Offspring were derived from Sprague Dawley dams that received daily subcutaneous injections of 40 mg/kg/3cc cocaine HCl (C40) from Gestational Day 8-20, pair-fed control dams receiving saline injections (PF), and nontreated control dams (LC). Following training, dose response curves were generated to a variety of substitute doses of cocaine HCl and the time course (to peak discriminability) of cocaine was assessed. No differences were observed among the prenatal treatment groups in bar press acquisition or the number of sessions to reach criterion. Cocaine was generalized to the cocaine training stimulus in a dose-dependent manner in all prenatal treatment groups. However, linear regression analysis of the dose response curves (Probit) indicated a significant shift to the right in the dose response curve for animals gestationally exposed to cocaine (ED50 = 2.70 mg/kg) when compared with LC (1.67 mg/kg) and PF (1.77 mg/kg) offspring. No differences were observed among the prenatal treatment groups in the temporal parameters of cocaine, with peak discrimination occurring 15 min following injection. Taken together these data suggest that offspring gestationally exposed to cocaine are less sensitive to the discriminative stimulus properties of cocaine.

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155.12

ONTOGENY OF NEURONS CONTAINING TYROSINE HYDROXYLASE (TH) IN FETAL MACAQUE BRAIN. O.K. Ronnekleiv*. Oregon Regional Primate Research Center, Beaverton, OR 97006, and Dept. of Physiology, OHSU, Portland, OR. 97201.

The development and distribution of neurons containing immunoreactive (IR)-TH (the rate-limiting enzyme involved in the synthesis of dopamine) have been examined in brain sections from days 38 to 60 fetal rhesus macaques. By day 38 of gestation, cells and fibers positive to TH were found in the mesencephalic flexure and the zona incerta. The IR-TH staining was greatly increased by days 40 and 41 of gestation. At this time increased number of IR-TH neurons were found in the mesencephalic flexure and the zona incerta; in addition, neurons were present in the preoptic area (POA) and the hypothalamus. A ventral and a dorsal fiber path could also be distinguished that projected towards the developing POA and nucleus accumbens (NAc), and the striatum, respectively. Large bundles of IR-TH extended in a dorso-ventral direction at the level of the optic chiasm in the day 41 fetal macaque. These bundles resembled those of the migrating GnRH neurons within the nasal septum. The number of IR-TH cells and fibers were further increased in the days 50 - 60 fetuses. We have initiated studies to explore the effects of maternal cocaine use (3mg/kg, four times a day) on the early development of dopamine neurons in fetal macaques. (Supported by PHS grant DA07165).

155.13

CONCENTRATIONS OF DOPAMINE D-1 AND D-2 RECEPTOR AND ENKEPHALIN mRNAs IN RAT CAUDATE AND NUCLEUS ACCUMBENS AFTER PRENATAL COCAINE TREATMENT. A. de Bartolomeis*, M.C. Austin, L.P. Spear§, D. Pickar, J.N. Crawley. Experimental Therapeutics Branch, NIMH, Bethesda, MD 20892 and §Department of Psychology, State University of New York Binghamton, NY 13901.

The effects of prenatal cocaine treatment on D-1, D-2 and enkephalin messenger RNA were investigated in rat forebrain. Pregnant Sprague-Dawley rats were administered one of the following treatments from gestational day 8 to gestational day 20: A) subcutaneous (sc) injection of cocaine hydrochloride 40 mg/kg /3cc; B) 0.9 % saline injection sc and pair fed to the cocaine group; C) no injection and ad libitum access to lab chow; D) saline injection and diet of 60% lab chow and 40% cellulose. Offspring were sacrificed at postnatal day 21 and the brains processed for in situ hybridization histochemistry. Coronal tissue sections were hybridized with oligonucleotides probes for D-1, D-2 and enkephalin genes, of the anterior to the posterior caudate nucleus and the nucleus accumbens. Quantitative analysis of the autoradiographic images demonstrated no significant differences in mRNA concentrations between experimental groups in any of the areas quantitated. These findings suggest that prenatal cocaine exposure does not change the transcription of D-1, D-2 or enkephalin genes in juvenile rats.

155.14

EFFECTS OF PRENATAL COCAINE TREATMENT ON POSTNATAL DEVELOPMENT OF NEOCORTEX IN WHITE MICE: IMMUNOCYTOCHEMISTRY OF CALBINDIN- AND PARVALBUMIN-POSITIVE POPULATIONS OF GABAERGIC NEURONS. E. Yablonsky-Alter*, I. I. Glezer C. Carter and M. Juvan. Dept. Pharmcol., Dept. Cell Biol. Anat. Sci., CUNY Med. Sch., Dept. Biology, CCNY of New York, New York, NY 10031.

GABAergic neurons-positive to calcium-binding proteins, calbindin D28-k (CB) and parvalbumin (PV) were studied immunocytochemically in the neocortex of developing white mice (line DC-1) prenatally treated with cocaine. Experimental group of ten pregnant white mice were daily injected intra-peritoneally with cocaine solution in dosage 10 mg per I kG. Control group of eight pregnanat mice was injected daily with saline. The injections in both groups were applied through the whole gestation period (21-22 days) and were stopped at birth of pups. The pups were sacrificed after intracardial perfusion with 0.5% of glutaraldehyde/ 2.5% formaldehyde in the following days after birth: 3, 7, 10-11, 15 and 20. In the first ten postnatal days the difference between control and experimental groups of animals was statistically insignificant. However, at day 15 numerical density of CB- and PV-positive neurons sharply deacreased in experimantal group and remained significantly ($p < 0.001$) lower than in control group of animals in later ontogenetic stages. Thus, treatment of pregnant mice with cocaine causes changes in quantitative and qualitative distribution of GABAergic cortical neurons in their offsprings. These effects became visible in critical period of postnatal development, when all important sensory systems (especially, visual and sensory-motor) became fully functional. Supported by grants PSC-CUNY RF- 662232, PSC- CUNY 662199 and by MBRS/CRS Program of CCNY.

155.15

EFFECTS OF CHRONIC PRENATAL COCAINE ON THE ONTOGENY OF THE DOPAMINE UPTAKE COMPLEX (DAUC). L.M. Collins* and J.S. Meyer. Dept. of Psychology, Neuroscience and Behavior Program, Univ. of Massachusetts, Amherst, MA 01003.

Cocaine has been hypothesized to block catecholamine uptake by binding to the transporter complex. Withdrawal from repeated cocaine exposure has been found to decrease [^{3}H]mazindol labeling of the dopamine transporter in adult rat nucleus accumbens (Sharpe et al., Eur. J. Pharmacol. 203:141, 1991), whereas chronic administration of L-deprenyl resulted in an upregulation of the dopamine transporter (Wiener et al., Eur. J. Pharmacol. 163:191, 1989). To assess the effects of in-utero cocaine exposure on the DAUC, pregnant Sprague-Dawley rats were subjected to either daily s.c. injections of cocaine HCl (40 mg/kg/day from gestational day 11 through 21) or to s.c. implantation of two capsules each containing 80 mg of cocaine base from gestational day 19 through 21. Quantitative autoradiography with [^{3}H]GBR 12935 was used to quantify DAUC density in the corpus striatum, nucleus accumbens, and other forebrain structures, as well as in the substantia nigra and ventral tegmental area. Data will be presented comparing cocaine-exposed and control offspring at postnatal days 1, 5, 10, 30 and 60. Supported by DA-06495.

155.16

NORMAL STRESS RESPONSE BUT DECREASED CELLULAR IMMUNE RESPONSE IN MALE RATS PRENATALLY EXPOSED TO COCAINE. **K. Bulloch*, D. Torres and R.F. McGivern. Univ. Cal. San Diego and San Diego State Univ., San Diego, CA 92120.**

Previous work from our laboratory has shown that exposure of fetal rats to cocaine during the last week of gestation significantly decreases thymus weight during development. In an initial evaluation of T cell function in these animals, we have examined the ability of thymocytes and splenocytes to respond to the T cell mitogen, concanavalin A (range 0.1 to 1.0 ug/well). Cells were removed from 30-32 day old male representatives born to 6 Sprague-Dawley dams administered cocaine (10 mg/kg, sc) twice daily from days 15 through 21 of gestation. Six control dams were injected with saline. Pups were weaned on day 24 and group housed by sex and treatment under a 12/12 hr light cycle. Thymus, but not spleen weights weighed significantly less in cocaine exposed males when indexed to body weight ($p < 0.01$). Con-A induced proliferation of thymocytes derived from cocaine exposed rats was 4-6 fold less than controls ($P < 0.01$). Proliferation in splenocytes was approximately half of that observed in controls ($p < 0.05$). Subsequent restraint stress studies in 35 days old littermates revealed normal basal and stress-induced levels of corticosterone. Overall, these results reveal marked reductions in Con-A stimulated cellular proliferation in animals prenatally exposed to cocaine which cannot be attributed to circulating glucocorticoids. Current studies are underway to examine potential cocaine induced alterations in the development of autonomic innervation of immune organs. (NIDA, DA04490 to RFM).

155.17

EVIDENCE FOR AN ENHANCED PSYCHOPHARMACOLOGICAL RESPONSE TO MU RECEPTOR STIMULATION IN ANIMALS PRENATALLY EXPOSED TO COCAINE. G. A. Goodwin*, F. Athalie, and L. P. Spear. Department of Psychology, Center for Developmental Psychobiology, State Univ. of NY at Binghamton, Binghamton, NY 13902-6000.

Prior work has revealed that weanling offspring of Sprague-Dawley dams given 40mg/kg cocaine during gestation exhibit an increase in [^{3}H]naloxone binding in autoradiograms of numerous brain regions (Hammer, et al., 1991). This increase in opiate binding was associated with an increase in responsiveness to the opiate agonist morphine when assessed in 10 day old (P10) offspring using morphine related suppression of isolation-induced ultrasounds as the response measure (Goodwin, Moody, & Spear, 1991). In the study reported here, P10 offspring gestationally exposed to cocaine and control offspring were injected i.c.v. with opiate agonists specific to the mu (0, .0005, .01 µg/5µl DAMGO) and delta (0, 0.3, 3.0 µg/5µl DPDPE) opiate receptors and tested for ultrasound production. Offspring exposed to cocaine were observed to be more sensitive to DAMGO than control animals. No differences were seen between prenatal treatment groups when animals were given DPDPE, although this may be due to a floor effect as the doses of DPDPE used strongly suppressed ultrasound production in all animals. These results suggest that the enhanced response to morphine seen in animals given prenatal exposure to cocaine is in part the consequence of increased activity at the mu receptor, whereas conclusions about responsiveness at the delta receptor are difficult to draw at this point. [Supported by NIDA Grants R01 DA04478 and K02 DA00140].

155.18

COCAINE TREATMENT ALTERS MATERNAL AND AGGRESSIVE BEHAVIOR. J.M. Johns*, L.I. Zimmerman, L.R. Noonan, L. Li and C.A. Pedersen. Dept. of Psychiatry, Univ. of North Carolina, Chapel Hill, N.C. 27599.

Cocaine abuse by pregnant women is well documented. We studied the effect of chronic and acute cocaine treatment on maternal behavior and aggression in rats. Gravid rats received one of three treatments; s.c. injections b.i.d. of saline or 15 mg/kg cocaine throughout gestation or one 15 mg/kg cocaine injection 30 min prior to testing sessions. Frequency, duration and latency of 11 maternal behaviors were recorded for a 30 min period beginning 30 mins following delivery of a dam's last pup. The same measures for 11 maternal aggressive behaviors were recorded on lactation day 6 for a ten min period during which a smaller male intruder was present in the cage. Fewer cocaine treated than saline dams had a crouch latency of <350 sec or a crouch duration of >500 sec. ($p<.005$, Fisher's Exact test). More chronic cocaine than saline dams displayed 8 or more bouts of aggressive posture and fight attack on the maternal aggression test ($p<.025$ Fisher's Exact test). Maternal behavior and aggression were altered by cocaine treatment. (HD 25255)

155.19

INTRAVENOUS COCAINE (C) ADMINISTRATION IN PREGNANT RATS: PROGENY DEVELOPMENT AND BEHAVIOR. P.M. Kunko*, D. Moyer and S.E. Robinson Dept. of Pharmacology and Toxicology, Med. Coll. of Virginia., Richmond, VA 23298-0613.

Use of cocaine (C) among pregnant women appears widespread. Developmental consequences for the offspring of these substance abusers are as yet unknown. The current study examined developmental and behavioral endpoints in the offspring of pregnant rats administered C intravenously (IV), which may more closely mimic the route of drug use in humans. Rats in the treatment group were injected IV with C (6 mg/kg) or saline on days 8 - 20 of gestation. An untreated control group was also included. This procedure produced no differences in weight gain or litter characteristics (litter size, resorptions) in the C group. Pups were sexed and weighed at birth, litter size reduced to 10, and fostered to untreated mothers. Pups were weighed daily and several developmental endpoints were assessed. On postnatal day 21 (P21) one male and one female from each litter were tested in an open field following an injection of C (10 mg/kg, IP), saline, or no treatment. Female pups from C treated dams weighed significantly less than controls, but this difference was resolved by P2. Both C and saline exposed pups gained weight less rapidly than pups from untreated dams. C exposed pups exhibited a trend for developmental delay in most assessments though significant differences were only found for negative geotaxic response and day of eye opening. On day P21, C exposed pups exhibited significantly more vertical movement following C challenge than did either control group. C exposed pups differed in the quality of their behavior , exhibiting more stereotypic or repetitive activity than the control groups. (NIDA Grant DA04746)

155.20

CHANGES IN BETA ADRENERGIC RECEPTORS FOLLOWING POSTNATAL COCAINE (COC) EXPOSURE. M.A. Kosek*, G.A. Ordway, R.M. Kliegman, Department of Pediatrics, Pharmacology and Psychiatry, Case Western Reserve University, Cleveland, Ohio 44106.

Because COC blocks neuronal catecholamine reuptake, we hypothesized that it may interfere with development of the brain monoamine system resulting in persistent changes in beta adrenergic receptor density. We measured central beta adrenoceptors in Sprague Dawley rat pups after chronic exposure to COC HCl (20mg/kg s.q. TID) or 0.9% saline in equivalent volume from birth to 15 days. Pups were sacrificed and the binding of ^{125}I-iodopindolol to brain beta-1 and beta-2 receptors were analyzed using quantitative autoradiography. At 15 days (after a one-day washout period) binding was significantly reduced in beta-1 receptors in the COC exposed group ranging from 18-40%. However, at 27 days (after a 12-day washout period) total beta receptor binding was moderately increased and statistically significantly in the COC group of 19%. In summary, there was a decrease in beta adrenoceptor density following chronic neonatal exposure that appears to show a compensatory rebound and elevation after withdrawal of COC. Whether these changes are sustained and effect behavior are subjects of future studies.

DRUGS OF ABUSE: OPIOIDS

156.1

CLONIDINE ATTENUATES CONDITIONED PLACE AVERSION TO NALOXONE-PRECIPITATED OPIATE WITHDRAWAL. T. A. Kosten*, PhD. Dept Psychiatry, Yale Univ School Medicine, New Haven, CT 06519.

Previous research shows that clonidine (CLON), an alpha-2 antagonist, attenuates the severity of opiate withdrawal signs in humans and in animals. Although CLON reduces some overt signs of withdrawal, its relief of subjective aspects of withdrawal has been questioned. We tested whether CLON affected conditioned aversion to opiate withdrawal in rats with conditioned place aversion (CPA) and conditioned taste aversion (CTA) paradigms. Rats were made morphine dependent by a subcutaneous morphine pellet implant. Withdrawal was precipitated 72 hr later by naloxone (NLX) injection (0.05 mg/kg, s.c.) which was paired with a saccharin solution (0.001 M) and with one side of the place apparatus. Vehicle injections were paired with an HCl solution (0.001 M) and the other side of the place apparatus. CTA was assessed with a 2 bottle preference test and CPA was assessed by measuring the time shift away from the NLX paired side compared to baseline preference. Six rats received CLON (0.4 mg/kg, i.p.) injections 5 min prior to NLX and 6 received vehicle CLON. The vehicle CLON group showed a significant shift away from the NLX paired side (-8.0 $\pm$ 0.8 min) compared to the CLON group (1.4 $\pm$ 1.9 min). Both groups showed a significant CTA to the NLX paired saccharin (57% and 34% intake) compared to controls (85% intake) and some mild opiate withdrawl signs. No CPA or CTA to NLX was shown in non-morphine dependent rats. Thus, conditioned aversion paradigms are sensitive measures of opiate withdrawal severity which is mediated, in part, by the alpha-2 noradrenergic system. Additional groups will be run to test the magnitude and specificity of the CLON effect on NLX CPA.

Supported by NIDA grant #P50-DA04060.

156.2

INCREASED EEG SPECTRAL POWER DURING ACTIVE PERIODS ACCOMPANIES MORPHINE WITHDRAWAL
K. Grasing* and Q.T. Lin, Departments of Medicine and Clinical Pharmacology, Robert Wood Johnson School of Medicine, New Brunswick, NJ 08903.

After nicotine withdrawal, EEG alpha frequency is diminished in human subjects with a heavy smoking history. We hypothesized that diminished arousal may also occur during opioid withdrawal. Three, adult male Sprague-Dawley rats were implanted with cortical EEG electrodes and intravenous catheters. Morphine and saline were administered as 12 hour infusions on alternate days. Each subject received two morphine infusions of 1.0, 3.0, and 10 mg/kg-hour. Cortical EEG was continuously digitized and analyzed by FFT. On days during which saline was infused, EEG spectral power was diminished during daytime inactive periods but increased during active nighttime hours. Theta activity did not differ significantly during withdrawal periods. Although opioid withdrawal has been associated with increased waking time and reduced EEG amplitude, opposing effects can be observed during active behavior.

156.3

EFFECT OF U-50,488H INDUCED TOLERANCE AND ABSTINENCE ON MET-ENKEPHALIN LEVELS IN BRAIN, SPINAL CORD PITUITARY AND PERIPHERAL TISSUES OF THE RAT. G.A. Tejwani, A.K. Rattan, K.L. Koo, S.A. Tjioe* and H.N. Bhargava, Dept. Pharmacology, Ohio State Univ., Columbus, OH 43210 and Dept. Pharmacodynamics, Univ. Ill. at Chicago, Chicago, IL 60612.

Male Sprague-Dawley rats were injected with U-50,488H (25 mg/kg, ip) or its vehicle twice a day for 4 days. Tolerance to the analgesic and hypothermic effect of U-50,488H developed as a result of this procedure. On day 5, rats from vehicle and U-50,488H treated groups were divided into 2 groups each. Rats from one group were sacrificed (abstinent rats) while n the other were injected with either vehicle or U-50,488H and sacrificed 1 hr later (tolerant rats). Brain, spinal cord, pituitary gland, plasma, adrenals, kidneys and heart were collected. Brain was dissected into 7 regions namely hypothalamus, cortex, hippocampus, pons and medulla, midbrain, amygdala and corpus striatum. Levels of met-enkephalin were determined by the RIA. Tolerance to U-50,488H was associated with increases in levels of met-enkephalin in the hippocampus but decreases in the adrenals. Changes were not seen in other brain regions, spinal cord and other peripheral tissues. On the other hand, abstinence from U-50,488H was associated with increases in the levels of met-enkephalin in the hypothalamus and heart. Thus tolerance-dependence and abstinence from U-50,488H alters met-enkephalin levels in the selected part of the CNS and periphery (Supported by a grant DA-02598 from the National Institute on Drug Abuse).

156.4

MODIFICATION OF DYNORPHIN (1-13) LEVELS IN CENTRAL AND PERIPHERAL TISSUES OF K-OPIATE TOLERANT AND ABSTINENT RATS. H.N. Bhargava, A.K. Rattan, K.L. Koo and G.A. Tejwani*, Dept. Pharmacol., Ohio State Univ., Columbus, OH 43210 and Dept. Pharmacodynamics, Univ. Ill. at Chicago, Chicago, IL 60612.

Male Sprague-Dawley rats were rendered tolerant to and physically dependent on U-50,488H, a k-opiate agonist, by injecting 25 mg/kg of the drug intraperitoneally twice a day for 4 days. Two sets of rats were used rats labeled as tolerant-dependent were injected with U-50,488H (25 mg/kg) on hour before sacrificing on day 5 whereas the abstinent rats were sacrificed on day 5 without the injection of U-50,488H. Of all the tissues examined, pituitary gland had the highest level of dynorphin (1-13), whereas heart had the lowest level. The levels of dynorphin (1-13) increased in hypothalamus hippocampus and pons and medulla of U-50,488H tolerant-dependent rats whereas in abstinent rats, the levels of dynorphin (1-13) were elevated only in the midbrain. The levels of dynorphin (1-13) in the pituitary gland of U-50,488H tolerant-dependent or abstinent rats were unchanged. In peripheral tissues, the levels of dynorphin (1-13) in the heart of U-50,488H tolerant-dependent rats were increased. In the abstinent rats they were elevated in the adrenals, spleen, and the heart but were decreased in the kidneys. Compared to morphine tolerant-dependent and abstinent rats were observed and may explain many pharmacological differences in the μ- and k- opiate induced tolerance-dependence and abstinence processes (Supported by a grant DA-02598 from the National Institute on Drug Abuse).

156.5

EFFECT OF STRESS ON MORPHINE AND FENTANYL SELF-ADMINISTRATION IN TWO BEHAVIORAL PARADIGMS IN RATS. Y. Shaham, and N.E. Grunberg*. Medical Psychology Dept. USUHS, Bethesda, MD 20814.

Clinical and epidemiological studies suggest that stress increases opioid use and relapse. Unfortunately, these human studies are correlational and do not establish a causal link. Recently (Shaham *et al.* 1992), we reported that immobilization (IM) stress increases oral opioid self-administration (SA) in a home cage setting by male rats during initiation and maintenance phases. The present experiments were designed to extend these findings: 1) to the relapse phase of the addiction process, and 2) to electric shock stressor and operant lever pressing for the drug. Experiment 1 examined the effect of IM stress (15 min/day) on oral opioid (morphine [0.5 mg/ml] or fentanyl [25 ug/ml]) SA after 2 months of opioid SA followed by 3 weeks of a drug-free period. Stressed rats increased their opioid preference and manifested a more severe withdrawal syndrome as compared to control rats not exposed to stress. Experiment 2 examined the effect of electric footshock (10 min/day) on fentanyl (75 ug/ml) SA in operant conditioning chambers. After one month of initiation of fentanyl SA, the animals were tested for lever pressing for fentanyl under fixed-ratio-4 and progressive ratio schedules of reinforcement for 30 min/day. Shock stress caused an increase in drug SA as compared to a non-stress condition. These results indicate that the effect of stress to increase opioid SA can be generalized to the relapse phase of the addiction process and can be detected by an operant conditioning paradigm. Therefore, rat SA paradigms can be used to examine mechanisms underlying the effect of stress on opioid use.

156.6

THE EVOLUTION OVER TIME OF OPIATE DEPENDENCE AND ABSTINENCE IN RATS. L.H. Gold*, L. Stinus and G.F. Koob. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA and Psychobiologie des Comportements Adaptatifs, INSERM Unité 259, Bordeaux, France.

Opiate withdrawal is a common occurrence in human opiate addicts that is not life threatening but is considered to be a significant factor which may prompt drug taking behavior in these opiate dependent individuals. Animal models can be used to study the time courses for the evolution of dependence and the development of the abstinence profile. In the following studies rats were made dependent by either implantation of 2 morphine pellets sc (75 mg morphine base) or 2 daily injections of morphine sulfate (5-10 mg/kg escalating dose/injection, ip) for 10 days. Withdrawal was evaluated by either cessation of morphine injection or an injection of the opiate antagonist, naloxone (1 mg/kg, sc). Morphine implanted rats exhibited analgesia as measured in a tail-dip assay, from 2-36 hrs post-implant, while hyperalgesia was measured in rats undergoing spontaneous withdrawal from 12-78 hrs following the last morphine injection. Rating of the abstinence syndrome following naloxone injection revealed significant withdrawal signs by 3 hrs post-implant which became increasingly intense up to 24 hrs post-implant. Withdrawal could be precipitated for at least 12 days post-implant, while by 18 days post-implant only minimal signs were observed. These results suggest that opiate abstinence develops gradually and follows the time course of the development of tolerance.

156.7

MK-801 INHIBITS THE DEVELOPMENT OF MORPHINE TOLERANCE IN THE RAT AT SPINAL SITES. HB Gutstein,* KA Trujillo, and H Akil. Dept. of Anes. and MHRI, Univ. of Michigan, Ann Arbor, MI

The development of morphine tolerance is a significant clinical problem in anesthesiology and other fields. Recent studies have shown that the non-competitive NMDA receptor antagonist MK-801 inhibits the development of morphine tolerance in rats (Science, 251:85-87, 1991, Brain Research, 547:77-81, 1991). Understanding the basic mechanisms underlying this inhibition could have important theoretical and clinical implications. The present study was undertaken to determine whether this effect could be mediated at spinal sites.

Sixteen male Sprague-Dawley rats underwent a T-8 level spinal transection under chloral hydrate anesthesia. Animals were allowed to recover for one week following surgery, and baseline tail flick measurements were obtained. Animals were then implanted with one 75 mg morphine pellet and an osmotic minipump infusing either MK-801 (0.1 mg/kg/day) or saline. On day 3, 3 additional morphine pellets were implanted in each animal. Tail flick latencies were measured 4 hours after pellet implantations and daily thereafter.

The data showed that MK-801 significantly inhibited the development of morphine tolerance in spinal transected rats. Also, one half of the rats in the MK-801 group died after receiving 3 morphine pellets, and the surviving rats appeared sedate. These changes were not observed in the saline-treated rats.

These data suggest that MK-801 inhibits the development of morphine tolerance at spinal sites. However, the data do not exclude the possibility that supraspinal sites may contribute to this inhibition in the intact animal. The data also suggest that the enhancement of morphine-induced lethality by MK-801 observed in previous studies (Pharmacology, Biochemistry and Behavior, 38:673-675, 1991) also occurs in spinalized animals. The mechanism of this synergism is unclear, and is currently under investigation. These results may have important implications both for understanding mechanisms of tolerance development and for the clinical management of patients requiring long term narcotic therapy.

156.8

INHIBITION OF MORPHINE TOLERANCE BY PHENCYCLIDINE. K.A. Trujillo* and H. Akil, Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan, 48109-0720.

We have previously reported that the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 inhibits the development of tolerance to the analgesic actions of morphine without affecting pain responsiveness on its own, and without altering acute morphine analgesia (these findings have now been replicated by at least two other groups). In addition to tolerance, we observed that this drug interferes with the development of physical dependence on morphine. These findings led us to suggest that NMDA receptors are involved in the behavioral and neural plasticity associated with long term opiate administration. However, although MK-801 is a potent and selective NMDA receptor antagonist, in order to definitively conclude that NMDA receptors are involved in opiate tolerance and dependence it must be demonstrated that other NMDA receptor antagonists produce similar effects. In the present experiments we examined the ability of phencyclidine (PCP), another potent non-competitive NMDA receptor antagonist, to inhibit tolerance to the analgesic actions of morphine in rats. PCP (1.0 mg/kg i.p.) or saline was administered 30 minutes prior to morphine (10 mg/kg s.c.) or saline, once each day for 9 consecutive days. The tail-flick test was used to assess analgesia, 60 minutes following the second injection, on odd-numbered days. PCP, in the absence of morphine, was found to have no effects on pain responsiveness. However, this drug significantly attenuated the development of tolerance to the analgesic actions of morphine. The effects of 1.0 mg/kg of PCP were very similar to those of 0.1 mg/kg of MK-801, matching very closely the relative affinity of these drugs for the NMDA receptor. These results support our suggestion that NMDA receptors are important in the development of opiate tolerance. Further studies are underway examining the effects of other NMDA receptor antagonists in opiate tolerance and dependence.

This work was supported by NIDA Grant DA02265 and NIMH Grant MH422251.

156.9

NALOXONE INDUCED A RELEASE OF NPFF FROM SPINAL CORD OF MORPHINE TREATED RATS. J.-M. Zhu*, and H.-Y.T. Yang. Lab. of Biochem. Genetics, NIMH, Neuroscience Center at St. Elizabeths, Washington, D.C. 20032.

Neuropeptide FF (FLFQPQRF-NH_2, NPFF), an endogenous peptide with morphine modulating activity, is highly localized in superficial laminae of dorsal spinal cord. Previously, using an in vitro superfusion of isolated rat spinal cords, we have demonstrated that NPFF can be released from the spinal cord in response to stimulation with 56 mM KCl or substance P. Recently there are studies suggesting the involvement of NPFF in both morphine tolerance and dependence Thus in this study, the effect of morphine on the release of NPFF was investigated using an in vitro superfusion of isolated rat spinal cord. We found that Naloxone caused a significant increase of NPFF efflux from the spinal cord of morphine treated rat. In contrast naloxone exerted only a very slight or no effect on the NPFF efflux from the spinal cord of control rat. Furthermore, amounts of NPFF release induced by naloxone seem to correlate with durations of morphine treatments. The results of this study strongly suggest an important role for NPFF in morphine dependence.

156.10

EFFECT OF MORPHINE ON DEVELOPMENT IN XENOPUS EMBRYOS. Q. Xu, S. Spector*. Dept. of Psychiatry, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232.

The incidence of fetal birth defects is greater in these mothers who abuse opiates. This also can be demonstrated in pups from rats treated with morphine during gestation. Our studies used Xenopus embryos as a model to understand the mechanism for the defects. Embryos were incubated with varying concentrations of morphine 10^{-4} to 10^{-10} M. Concentrations of 10^{-4} M proved to be toxic and lower concentrations can cause morbidity and deformed tadpoles also. To investigate the neurological effects we used the Homeobox gene XIHbox6 as the marker for the neuro-system differentiation and development. We measured mRNA amount using northern blot to evaluate the expression of the gene. The results show morphine at concentrations from 10^{-4} to 10^{-8} M increased the mRNA. It suggests that at the molecular level morphine may influence the differentiation and development of the neuro-system by an action on the Homeobox gene XIHbox6.

156.11

MORPHINE INDUCED TOLERANCE AND DEPENDENCE ARE CONTEMPORARY TO LARGE VARIATIONS OF F8Fa LEVELS , AN ANTI-OPIATE NEUROPEPTIDE. Simonnet G., Allard M., Gold** L.H and Stinus* L. INSERM U.176 and *U.259, Rue C. St Saëns Bordeaux 33076 France. **Dept of Neuropharmacology, The Scripps Research Institute, La Jolla, CA.

Tolerance may be an adaptative process which limits excessive effects of morphine and leads to dependency. Since no clear opiate receptor reduction may underlie the hyposensitivity to chronic morphine, an alternative hypothesis postulates a role of "antiopiate" peptides in the development of tolerance. F8Fa is a FMRF-NH_2 like peptide which may counteract the effects of opiates via F8Fa specific receptors (Allard et al., 1989). The administration of morphine could stimulate antiopiate systems such as F8Fa, as part of a homeostatic mechanism. To test this hypothesis, tail-flick latency, physical dependence and CNS F8Fa levels (RIA) were measured at different times after implantation of morphine pellets (2x75mg; NIDA). Three hours after morphine pellet treatment, the analgesic effect was maximum and then decreased rapidly during the following 12 hours. As early as three hours post-implant, rats displayed naloxone-precipitated withdrawal. F8Fa levels were measured in spinal cord, brainstem and hypothalamus. We observed a slight decrease (-25% to -45% according to structures) one hour after morphine pellet implantation followed by a drastic increase of F8Fa levels (+60 to +140%) between 3 and 6 hours. F8Fa levels rapidly return to baseline after 24-36 hours, this evolution parallels the development of morphine tolerance and dependence. These data suggest that the activation of F8Fa anti-opiate neurons following chronic exposure to morphine may explain, at least in part, morphine dependency.

156.12

ENHANCED ANTIOPIATE ACTIVITY IN CYCLOPROPLOGS OF FMRFamide. L.S. Corriere, T.M. Benson, J.R. Lake, D.A. Smith, R.K. Baugher, K-K. Ho[1], K. Burgess[1], K. Payza[2] and D.H. Malin*. Univ. of Houston-Clear Lake, Houston, TX 77058, [1]Rice Univ., Houston, TX 77251 and [2]NIMH, St. Elizabeths, Washington, D.C., 20032.

FMRFa is a molluscan peptide which has antiopiate activity in a number of test systems. Two conformationally constrained peptidomimetics of FMRFa containing stereoisomers of 2,3-methanomethionine were synthesized. This study determined the antiopiate potency of FMRFa and its two cycloproplogs in precipitating opiate abstinence syndrome in rats infused s.c. for 7 days with 0.27 mg/kg/hr morphine sulfate via one Alzet osmotic minipump. Each rat received a third ventricle injection of one of five doses (0.25-25.0µg) of FMRFa or one of its cycloproplogs. The rat was then observed for 20 mins. on a standard checklist of opiate abstinence signs (teeth chatter, writhes, wet shakes, etc.). Both of the cycloproplogs were approximately 50 times more potent than FMRFa itself. ANOVA of abstinence scores revealed a significant effect of conformation, $p<.01$, and a significant effect of dose, $p<.05$. The interaction effect (conformation x dose) was not significant. The mechanism of this heightened potency requires further investigation, since one of the cycloproplogs binds with less affinity than FMRFa to FMRFa receptors and mammalian neuropeptide FF receptors. (Supported by NIDA DA06554 and Texas Advanced Technology Program).

156.13

ANALOG OF NEUROPEPTIDE FF ATTENUATES MORPHINE TOLERANCE J.R. Lake*, K.M. Hebert, K.D. Deshotel, D.D. Hausam, W. Witherspoon, K.R. Arcangeli, K. Payza[1] and D.H. Malin. Univ. of Houston-Clear Lake, Houston, TX 77058 and [1]NIMH Neuroscience Ctr. at St. Elizabeths, Washington, D.C. 20032.

Neuropeptide FF (NPFF, F8Fa) appears to play a role in opiate tolerance since antibody against NPFF attenuates tolerance. Desamino-YFLFQPQRa (daY8Ra) was synthesized as a possible NPFF antagonist. It reverses behavioral effects of NPFF injection and attenuates morphine dependence. The present study assessed whether daY8Ra has a similar effect on opiate tolerance. Twelve rats were rendered tolerant by 7 days s.c. infusion of 0.53mg/kg/hr morphine via 1 Alzet osmotic minipump. They were then tested for pain sensitivity (tail flick, 15s cut-off) and injected i.c.v. with 600ng daY8Ra in saline or with saline alone. Thirty-eight mins. later they were injected with 6µg morphine i.c.v. and retested at 5, 12, 20 and 28 mins. after morphine. Morphine analgesia scores were computed as % of maximal effect. For purposes of comparison, 6 non-tolerant rats were challenged with 6µg morphine. Tolerant rats pretreated with saline never reached more then 6% of maximal morphine analgesia, while tolerant rats pretreated with daY8Ra ultimately reached 90%. The two groups differed significantly, $p<.01$, at each time interval. Morphine analgesia was generally comparable in non-tolerant rats and tolerant rats pretreated with daY8Ra. In contrast, the same pretreatment with daY8Ra did not affect analgesic response to i.c.v. morphine in 12 opiate-naive rats. (Supported by NIDA DA06554 & Tx Adv. Tech. Prog)

156.14

OPIATE WITHDRAWAL *IN VIVO* INDUCES LONG-TERM GLUTAMATE DESENSITIZATION IN LOCUS COERULEUS NEURONS: *EX VIVO* RECORDINGS IN RAT BRAIN SLICES

J.H. Kogan* and G.K. Aghajanian. Depts. of Cellular & Molecular Physiology, Psychiatry, & Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06508.

An increase in excitatory amino acid (EAA) activation contributes to the high firing rates of locus coeruleus (LC) neurons observed *in vivo* during naltrexone-precipitated withdrawal (Rasmussen and Aghajanian, 1989). The initially elevated firing rates rapidly decline within the first 2-4 hours of precipitated withdrawal (Rasmussen et al., 1991). In the present study we investigated whether EAA responses were altered in LC brain slices prepared from rats chronically treated with morphine and withdrawn with naltrexone *in vivo* for 1 hour prior to slice preparation. The responses to glutamate, but not carbachol, were substantially reduced in cells from opiate-dependent, withdrawn, animals. In contrast, responses were not reduced in cells from opiate-dependent, non-withdrawn animals. The glutamate desensitization in the cells from the withdrawn animals was most pronounced in the first hour after slice preparation and gradually recovered over several hours. A similar long-term glutamate desensitization (LTGD) phenomenon could also be induced *in vitro* by prolonged applications (5-30 min) of glutamate (0.5-1 mM) which recovered within several hours. There was cross-desensitization between LTGD induced *in vivo* and *in vitro* indicating a common mechanism. Induction of LTGD *in vitro* was blocked by the non-selective glutamate antagonist kynurenic acid (10 µM) but not by the selective NMDA antagonist AP-5 (100 µM). These results suggest that LTGD in LC neurons results from increased glutamate release and may contribute to the early rapid decline in the elevated firing rates during naltrexone-precipitated opiate withdrawal.

156.15

NEUROCHEMICAL CORRELATE OF SHORT-TERM TOLERANCE TO MORPHINE AND POSSIBLE MODULATION BY IBOGAINE. D.W. Johnson and S.D. Glick* Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y. 12208.

Two regimens of morphine pretreatment were compared in terms of the extent to which tolerance occurred to the locomotor depressant effect of morphine. Rats pretreated with the first regimen (30 mg/kg, 1x/day for 4 days, i.p.) did not differ in their response to the locomotor depressant effect of a single i.p. dose of 30 mg/kg morphine on day 5, compared to saline pretreated control animals. Rats pretreated with the second regimen (30 mg/kg 2x/day for 3 days, then 1x on day 4, i.p) showed tolerance (i.e., higher activity counts) after the day 5 morphine injection compared to saline pretreated controls. Using in vivo microdialysis, neurochemical correlates of tolerance were sought: the morphine-induced increase in extracellular DOPAC levels in the striata of rats pretreated with the tolerance-producing regimen of morphine administration was markedly reduced ($p < .005$) compared to saline-pretreated controls; no such change occurred in the nucleus accumbens. These data suggest that tolerance to morphine-induced locomotor depression is preferentially due to an effect in the striatum. The effect of ibogaine, a putative anti-addictive agent, on morphine tolerance is being investigated.

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156.16

LOW OPIATE CONCENTRATIONS CAN AUGMENT SYNAPTIC TRANSMISSION IN NUCLEUS ACCUMBENS VIA DISINHIBITION. S.G. Madamba*, Z. Nie and G.R. Siggins, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The nucleus accumbens (NAcc) is thought to be involved in the reinforcing properties of opiates. Our previous intracellular studies of a slice preparation of NAcc (Yuan et al., Neurosci. Lett. 134: 223, 1992) showed that 0.1-1 μM concentrations of opiates specific for μ, δ and κ receptors all reduced the size of both excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) in NAcc neurons, with no effect on resting membrane properties. The μ receptor agonist [D-Ala^2, NMe-Phe^4 Glyol]-enkephalin (DAGO) was the most potent in reducing synaptic potentials. In the present study, we tested the effects of superfusion of lower concentrations of DAGO (30-50 nM) on the size of EPSPs in neurons of the NAcc slice, using intracellular recording. These concentrations again had no effect on membrane potentials or input slope resistance. In contrast to the effects of higher concentrations, 50 nM DAGO increased the size of EPSPs in 7 of 8 NAcc neurons tested (to 112-146% of control), with recovery on washout; EPSP size was decreased in the other neuron. With 30 nM DAGO, EPSP amplitudes were increased in 2 cells, decreased in 2, and unchanged in another. Because higher opiate concentrations were previously seen to reduce IPSPs as well as EPSPs, we tested the possibility that the EPSP increases at low DAGO concentrations might be due to an indirect action via covert IPSP reduction. In slices continually superfused with 30 μM bicuculline to block $GABA_A$ receptors, DAGO 50 nM decreased EPSP size in all 5 cells studied (to 34-81% of control); in 4 cells the effect was reversed by 0.5 μM naloxone. These data suggest that μ receptor agonists at low concentrations can augment EPSPs in the NAcc, probably by diminishing the IPSPs more effectively than EPSPs, thus removing the opposing or shunting effects of the IPSPs.

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156.17

SELECTIVE BREEDING FOR OPIOID SELF-ADMINISTRATION IN RATS. K.R. Carlson*, M.S. Moolten and C.M. Saulnier. Pharmacology Dept., U. Massachusetts Medical Center, Worcester, MA 01655.

We are breeding lines which show a preference (P) or aversion (A) for drinking the potent opioid etonitazene (ETZ), and a randomly-bred (R) control line. The rats were offered only 2.5 μg/ml ETZ for 4 days; all lines drank equivalent amounts (21-23 ml), and exhibited stereotyped gnawing indicating that they were receiving behaviorally effective doses. They were then given a water-ETZ (2.5 μg/ml) choice for 7 days. A custom fluid-delivery apparatus negated the influence of position preferences; these rats drank equally from the two spouts when both contained water ($F<1.0$). The rats showing the most extreme choice behavior appropriate to their lines (P or A) were bred in brother-sister pairs, and breeders were chosen randomly from the R line.

By the second selected generation (S2) the lines had diverged significantly in the percent of total fluid consumed containing ETZ (P=37.7%, A=23.8%, R=31.0%; $F=11.1$, $p<0.0001$). This trend was enhanced in the S3 generation (P=42.9%, A=18.9%, R=30.8%; $F=14.7$, $p<0.00001$).

S2 rats were also given a choice between water and increasing concentrations of ethanol (3-25%). There were no differences between the lines ($F<1.0$) in percent of total fluid drunk containing ethanol, and an inverted-U relationship was found between concentration and percent ($F=43.5$), indicating that ethanol was a reinforcer for all the lines. These data suggest that we are breeding selectively for ETZ preference and not for a generalized tendency to drink psychoactive drugs such as ethanol.

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156.18

ACUTE EFFECTS OF BUPRENORPHINE ON CEREBRAL GLUCOSE UTILIZATION IN HUMAN SUBSTANCE ABUSERS. S.L. Walsh*, J.M. Stapleton, K.L. Preston, J.T. Sullivan, R.F. Dannals, R. Grayson, R.L. Phillips, G.E. Bigelow, D.R. Jasinski and E.D. London, Johns Hopkins School of Medicine and NIDA Addiction Research Center, Baltimore, MD 21224.

Buprenorphine (BUP), an opioid with mixed agonist/antagonist properties, is currently being investigated as a potential treatment for opiate addiction. We used the PET [18F]fluorodeoxyglucose (FDG) method to characterize the biochemical and neuroanatomical effects of BUP in brain, in a double-blind, placebo-controlled, crossover study. Volunteers were 6 healthy opioid-experienced males, ages 27-36 years, who were not physically dependent on opiates at the time of study. Subjects participated as inpatients in two PET scans following the administration of placebo and BUP (1.0 mg, i.m.), 15 minutes before FDG. BUP reduced glucose metabolism (range 13-27%) in many brain regions. Significant decrements occurred in cortical areas and limbic structures, including hippocampus and amygdala; more caudal brain structures also showed decreases in metabolism. BUP produced typical opiate effects, including pupillary constriction and subjective reports of "liking" and "good effects". BUP produced significant changes in arterial concentrations of oxygen (↓ 21%) and carbon dioxide (↑ 17%), which may have contributed to the observed decrease in glucose metabolism. Several drugs with known abuse potential, including alcohol, cocaine and morphine, have been shown to produce global decreases in glucose utilization when given acutely. The present data demonstrate that BUP produces global reductions in cerebral glucose utilization, consistent with a commonality in the effects of euphoriants on cerebral metabolism.

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156.19

OPIOID CONTROL OF CCK RELEASE FROM THE SPINAL CORD OF MORPHINE-TOLERANT AND NON-TOLERANT RATS. J.J. Benoliel, S. Bourgoin, A. Mauborgne, M. Pohl, L. Lantumey*, M. Hamon and F. Cesselin, INSERM U 288, CHU Pitié Salpêtrière, 91 Boulevard de l'Hôpital, 75013 Paris, France.

Behavioral studies suggest that activation of central CCKergic systems could be involved in opioid tolerance. This led us to examine the possible influence of opioids on the spinal release of CCK like-material (CCKLM) in naive or morphine-tolerant rats. The Ca^{2+}-dependent K^+-induced overflow of CCKLM from slices of the dorsal part of the lumbar enlargement from naive rats was reduced by 0.1 μM-10 μM DAGO (μ opioid agonist) or 0.01-3 μM DTLET (∂ agonist), unaffected by 1 μM U 50488 H (κ agonist), and enhanced by 10 μM DTLET. Naloxone (1 μM) and U 50488 H (1 μM) blocked the effects of 10 μM DAGO but not those of DTLET (3 μM, 10 μM). ICI 154129 (50 μM, ∂ antagonist) inhibited both the inhibitory and excitatory effects of DTLET but not those of DAGO. This suggests the involvement of two subtypes of ∂ receptors, and the existence of κ/μ, but not κ/∂ interactions. In the opioid control of spinal CCKLM release. Low concentrations of morphine (0.01-0.1 μM) significantly reduced CCKLM release. In contrast, 10 μM morphine enhanced CCKLM release. Complementary experiments, where the effects of 10 μM morphine were investigated in the presence of 1 μM naloxone, 50 μM ICI 154129 and/or 1 μM norbinaltorphimine (κ antagonist), indicated that the reduction in CCKLM release due to μ receptor stimulation by 10 μM morphine was prevented by κ receptor activation, leading to a net increase in CCKLM release due to the stimulation of ∂ receptors. In morphine-tolerant rats the inhibitory effects of 10 μM DAGO and 3 μM DTLET on spinal CCKLM release disappeared. In contrast, the stimulatory effect of 10 μM DTLET on the peptide release persisted. These data strongly support the idea that spinal CCKergic systems are activated in morphine-tolerant rats.

156.20

SYSTEMIC ADMINISTRATION OF THE SUBSTANCE P ANALOGUE, DiMe-C7, POTENTIATES THE DEVELOPMENT OF SENSITIZATION OF THE BEHAVIORAL ACTIVATING EFFECTS OF MORPHINE. M.L. Forgie*, C. Archambault, D. Rodaros and J. Stewart, Center for Studies in Behavioral Neurobiology, Psychology Dept., Concordia University, Montréal, Québec, Canada, H3G 1M8.

Repeated administrations of morphine (MOR) produce long-lasting sensitization of the behavioral activating effects of the drug, thought to be mediated by long-lasting changes in the mesolimbic dopamine (DA) system. Substance P (SP) neurons provide positive excitatory feedback on DA neurons and could thereby contribute to sensitization. Further, SP has been shown to be reinforcing in several learning paradigms. We investigated the interaction of SP with MOR in producing behavioral sensitization. Animals received either MOR (10 mg/kg, i.p.) or its vehicle (1 ml/kg) in conjunction with either the SP-analogue DiMe-C7 (32.6 μg/kg, i.p.) or its vehicle (5 ml/kg). Locomotor activity was measured for 2 h, every 2 days on 5 occasions. In tests for sensitization, all animals received MOR (5.0 mg/kg) or DiMe-C7 (32.6 μg/kg). In a second parallel experiment, DiMe-C7 (3 μg/side) or its vehicle (0.5 μl/side) were administered bilaterally into the ventral tegmental area (VTA). When both injections were systemic, animals that had previously been exposed to both DiMe-C7 and MOR showed greater sensitization to a challenge injection of MOR than did animals that had been exposed to MOR alone. This potentiation was not observed when DiMe-C7 was applied directly to the VTA at this dose. These data suggest that SP can potentiate sensitization to MOR, but the VTA may not be the site of this effect.

157.1

INTERACTIONS BETWEEN CANNABINOIDS AND STEROIDS IN THE RAT HIPPOCAMPUS. J.C. Eldridge*[1], Hong-Yu Hu[1], P.J. Extrom[1], and P.W. Landfield[2]. *Department of Physiology & Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157-1083[1] and Department of Pharmacology, University of Kentucky Chandler Medical Center, Lexington, KY 40536-0084[2]*

In previously published reports (Eldridge, *et al., Brain Research 534*:135, 1990; *Steroids 56*:226, 1991), we demonstrated that cannabinoids (THC) could interact with glucocorticoid (GC) binding to GC receptors in the rat hippocampus. In the present studies, cytosols were incubated with [^{3}H]-Δ^9-THC and a small but significant competition was exerted by excess unlabeled THC or CORT, but not by other steroids. A large amount of the total binding was not saturable and appeared to be non-specific. Sucrose gradients of cytosols incubated with [^{3}H]-THC demonstrated a slight but displaceable association in the 8S region. Additional displaceable binding, plus a large amount of non-saturable binding, was located in the 4-5 S region. In a separate study, analyses were conducted of mRNA for glial fibrillary acidic protein (GFAP), which has been shown to be specifically suppressed in hippocampus after GC administration (Nichols, *et al., Mol. Brain Res. 7*:1, 1990). Tissue RNA extracts were hybridized to [^{32}P]-labeled antisense probe prepared after amplification of a clone furnished to us by the authors. Dot blot radioactivities were quantified with a Betagen® scanner. Treatment of adrenalectomized rats with 5 mg THC suppressed hippocampal GFAP mRNA within 24 hr. Continued daily injections maintained suppression, although an accommodation was evident by 4 days. These results support our earlier proposal (Landfield, *et al., Brain Research 443*:47, 1988) that cannabinoids may exert glucocorticoid agonist effects on rat hippocampus.

(Supported by NIDA grants DA-06218 and DA-03637)

157.2

GLUCOCORTICOID AGONIST-LIKE EFFECT OF DELTA-9-THC ON IMMUNOREACTIVE CALBINDIN UPREGULATION IN CA1 HIPPOCAMPAL NEURONS. J.L. Geddes, J.C. Eldridge and P.W. Landfield*. Dept. of Pharmacology, Univ. Kentucky Col. of Med., Lexington, KY 40536 and Dept. of Physiology and Pharmacology, Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, N.C. 27157

Glucocorticoids have been shown to increase rat hippocampal neuronal loss during brain aging, possibly by elevating inward calcium currents (cf. review in Landfield and Eldridge, Acta Endocrinol., 1991, 125: 54-64). Because the molecular structure of delta-9-tetrahydrocannabinol (THC) strongly resembles that of glucocorticoids, the effects of THC on hippocampal cell loss and on hippocampal glucocorticoid receptors were studied. THC was found to increase neuronal loss in young rats, and to downregulate the hippocampal type II glucocorticoid receptor (GR) (cf. review in Eldridge et al, Steroids, 1991, 56: 226-231).

The present study was undertaken to determine whether THC interacts with the GR as an agonist or an antagonist. We have investigated the effects of corticosterone (CORT) and THC on immunoreactivity of the calcium binding protein, Calbindin-28K (CaBP). In the hippocampus, the upregulation of CaBP is CORT-dependent. Young (3-5 mo-old), adrenalectomized rats were dosed with THC, CORT or vehicle. CORT induced a consistent increase in the CaBP staining intensity in CA1 and CA2 pyramidal neurons, but not in CA3 and CA4, as measured by average optical density values using a computer-assisted digital image analyzer ($p < 0.04$). This indicates that CORT upregulates CaBP through the type II GR, which is found in high density in CA1 and CA2.

In addition, 1-2 weeks of THC treatment induced an increase in CaBP immunoreactivity in CA1, indicating a partial type II GR agonist-like effect of THC in the hippocampus. This effect may account for aspects of chronic THC-mediated neurotoxicity. (Supported by DA-03637, DA-06218).

157.3

SYNTHETIC CANNABINOID ANALOGS ENHANCE A FAST POTASSIUM CONDUCTANCE IN CULTURED HIPPOCAMPAL NEURONS.

S.A. Deadwyler*, T.A. Edwards, J. Mu, B.A. Bennett and R.E. Hampson. Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27157.

The recent discovery of the cannabinoid receptor in brain has provoked intense speculation regarding its major physiological action on CNS neurons. Herkenham *et al.* (J. Neurosci. 11:563-583, 1991) have provided autoradiographic evidence for the possible existence of presynaptically located cannabinoid receptors in both the substantia nigra (pars reticulata) and in the hippocampus (outer molecular layer of the dentate gyrus). The studies to be described investigate the physiological action of cannabinoids in cultured (18-20 day embryonic) hippocampal neurons. Whole cell patch clamp recordings revealed a profound modulation of fast-inactivating, voltage-sensitive "A" current in hippocampal neurons by both the Pfizer (CP-55,940, CP-55,244, levonantradol) and Sterling Drug Co. (WIN-55,212-2) synthetic cannabinoid receptor agonists. Potassium currents provoked by two different voltage-step protocols examined activation and inactivation of "A" currents before and during local pipette perfusion of the cannabinoids in TTX treated cultures. "A" current activation was enhanced while inactivation was decreased in cells exposed to the analogs. Moreover, the potency for producing these effects matched the receptor binding and subsequent biochemical potencies of these compounds reported by others (Howlett *et al.*TINS 13:420-423 1991; Pacheco *et al.* JPET 257:170-183, 1991). Comparisons to a similar action of baclofen to produce these same effects on "A" current via the GABA-B receptor (Gage, TINS 15:46-51, 1992) showed the synthetic cannabinoids to be 100 fold more potent than baclofen.

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157.4

CANNABINOID INDUCED ALTERATIONS IN REGIONAL CEREBRAL BLOOD FLOW IN THE RAT. A. S. Bloom*, S. Tershner, S. A. Fuller, E. A. Stein. Departments of Pharmacology and Psychiatry, Medical College of Wisconsin, Milwaukee, WI 53226.

The cannabinoid receptor has a wide but heterogeneous distribution in the mammalian brain. However, the functional role of these receptors is not yet well understood. We have therefore examined the effects of Δ^9-tetrahydrocannabinol (THC) and its active metabolite 11-OH-THC on regional cerebral blood flow (rCBF) in the rat to determine their functional sites of action. Conscious rats were injected iv with either THC (0.5, 1, 4, 16 mg/kg) or 11-OH-THC (4 mg/kg) 30 min prior to sacrifice. rCBF was determined using [^{14}C]Iodoantipyrine. Changes were observed in 14 of 33 areas measured. Significant dose-related decreases in rCBF were produced by THC in 10 areas including the CA1 and CA3 regions of the hippocampus, frontal and medial prefrontal cortex and the nucleus accumbens. Thresholds for these effects ranged from 0.5 to 4 mg/kg. 11-OH-THC also decreased rCBF in these areas. In contrast, a significant biphasic increase (4 mg/kg peak effect) in rCBF was seen in the arcuate nucleus and posterior hypothalamus. Other areas such as the medial septum, ventral tegmental area, caudate, temporal, parietal and occipital cortex and cerebellum were unaffected. Monitoring of cardiovascular and blood gas parameters revealed significant decreases in heart rate and increases in pCO_2. The decrease in rCBF produced by the cannabinoids in the presence of increased pCO_2 suggests a direct drug effect on the brain. It is significant that the areas where rCBF is altered are involved in major behavioral and physiological effects of the cannabinoids such as impairment of short-term memory and endocrine function. (Supported in part by NIDA grants DA03725 & DA05012)

157.5

CHRONIC CANNABINOID ADMINISTRATION ALTERS CANNABINOID RECEPTOR BINDING IN RAT BRAIN: AN AUTORADIOGRAPHIC STUDY

Angelica Oviedo†, John Glowa and Miles Herkenham*. Section on Functional Neuroanatomy, NIMH, Bethesda, MD 20892. †Howard Hughes Medical Institute-NIH Research Scholar

The active ingredient of marijuana is (-)-Δ^9-tetrahydrocannabinol (Δ^9-THC). Δ^9-THC and other natural and synthetic cannabinoids such as CP-55,940 produce catalepsy in animals in a receptor-mediated fashion. To test the hypothesis that tolerance to repeated administration might be mediated by changes in receptor binding characteristics, we used quantitative *in vitro* autoradiography of [^{3}H]CP-55,940 binding to striatal brain sections of rats treated either chronically or acutely with Δ^9-THC, CP-55,940, or cannabidiol, an inactive cannabinoid. In the chronic condition, rats were given daily i.p. injections of Δ^9-THC (10 mg/kg), cannabidiol (10 mg/kg), or CP-55,940 (1, 3, or 10 mg/kg) for two weeks and sacrificed one-half hour after the last injection. In the acute condition, animals received a single dose (10 mg/kg) just prior to sacrifice. Rats showed rapid tolerance to the cataleptic effects of Δ^9-THC and CP-55,940, assayed in an open field test on days 1, 7, and 14. Densitometry of [^{3}H]CP-55,940 binding showed that Δ^9-THC and CP-55,940 treated animals had decreased binding in all structures measured at a selected striatal level. Displacement curves using varying competing concentrations of CP-55,940 permitted analysis of binding kinetics. Alterations seen in the acute condition were attributed to changes in affinity (Kd), whereas the major changes in the chronic condition were attributed to a lowering of capacity (Bmax). These results suggest that tolerance to cannabinoids results at least in part from agonist-induced receptor down-regulation.

157.6

AGE DEPENDENT CHANGES IN HUMAN BRAIN CANNABINOID RECEPTOR DISTRIBUTION I. Kerman, O. Bar-Peled and A. Biegon*, Weizmann Inst. Sci., Rehovot, Israel and Lawrence Berkeley Lab., UC Berkeley, CA 94720

Lifespan changes in cannabinoid receptors and the presence of these recepotrs in the human fetal brain may explain age-dependent changes in drug seeking behavior and the selective effects of marijuana exposure during pregnancy. Cannabinoid recepotrs were examined by quantitative autoradiography of sections of humn brains obtained postmortem, using [3H]CP55,940 (N.E.N, specific activity 104 Ci/mmole) as a ligand. Normal fetal brains (N=5, gestational age 18, 20, 22 and 24 weeks) were obtained from voluntary abortions. Normal adult human brains (N=16, age range 18 to 78) were obtained from the medical examiner's office in NYC and Jaffa. The radioligand (5nM) was incubated with the sections for 3 hours at room temperature followed by 2X90 min wash at 4°C. Non specific binding was determined in the presence of 10μM unlabeled THC. Dried sections were exposed to tritium sensitive film for 1 to 4 weeks. Autoradiograms and tritium standards were quantified using a computerized image analysis system and the NIH Image software. Cannabinoid receptors exhibit a widespread but heterogeneous distribution in the human brain. The highest levels are found in the substantia nigra. High densities are also found in globus pallidus, caudate, putamen, cerebellum, hippocampus and cortex. A significant negative correlation ($R=0.69$, $p<0.003$) between binding and age was found in the cingulate cortex. Fetal brains contained moderate densities of receptors in cortex and hippocampus and very low levels in the basal ganglia, suggesting age dependent changes in human brain cannabinoid receptor distribution.

157.7

APPROACHES TO THE DETERMINATION OF THE CENTRAL SITES OF ACTION OF CANNABINOIDS. E.S. Onaivi*, P.J. Little and B.R. Martin. Dept. of Pharmacology, MCV-VCU, Richmond, VA 23298 and *Stanford University, CA, U.S.A.

The administration of Δ^9-THC to rodents alters some of the behaviorial measures in a manner indicative of psychoactivity. The aim of this study was directed towards the localization of the central sites of action of cannabinoids. Male ICR mice were subjected to standard stereotaxic surgery for the implantation of guide cannulae to allow microinjection of cannabinoids or its vehicle bilaterally into the ventricles or other selected brain structures. The ICV injection of Δ^9-THC or the analog (-)- ACD reduced locomotor activity (LA) and rectal temperature (RT) and enhanced the tail flick (TF) and immobility responses: the (-)-ACD compound being more potent. The ICV injection of lower doses of 5' TMA Δ^8-THC (3-9 µg) was effective only in the production of analgesia and catalepsy but did not alter the LA and RT. There was no significant changes in RT and TF tests following the injection of similar doses of Δ^9-THC (50-150 µg) into the center of the nucleus accumbens (ACB) except for the induction of catalepsy and a slight decrease in LA. In contrast, the injection of similar doses of Δ^9-THC into the central nucleus of amygdala (ACE) failed to alter mouse LA, RT, TF and catalepsy tests. Therefore, the mesolimbic ACB but not the ACE may be an important site contributing to the modification of motor activity and induction of catalepsy by some cannabinoids. The importance of other brain sites in these behaviors and other cannabinoid subjective effects remains to be determined. Supported by NIDA grant #DA-03672.

157.8

EFFECT OF ß-FNA I.C.V. ON HEROIN SELF-ADMINISTRATION IN NON-DEPENDENT RATS AND *IN SITU* OPIOID BINDING. T.J. MARTIN, S. L. DWORKIN and J. E. SMITH*. Center for Neurobiology of Drug Abuse. Dept. Physiology and Pharmacology. The Bowman Gray Sch. of Med./Wake Forest Univ. Winston-Salem, NC 27157.

ß-FNA has been shown to be an irreversible antagonist of µ opiate receptors both *in vitro* and *in vivo*. The effects of centrally administered ß-FNA on the reinforcing properties of opiates was investigated and compared to effects on opiate binding sites. Male Fisher 344 rats (250-300g) were implanted with intravenous jugular catheters and guide cannulae into each lateral ventricle. The animals were then trained to self-administer heroin (18 µg/kg/inf) on a fixed-ratio 10 schedule during 4 hr sessions. Saline was substituted for heroin twice prior to administration of 40 nmol of ß-FNA. Prior to administration of ß-FNA the animals self-administered 33.8 (2.0) mean (s.d.) infusions/session. Saline substitution resulted in 9.3 (0.3) infusions/session. The number of infusions/session 1, 2, 3, 4, 7 and 14 days after ß-FNA administration was 12 (11), 7 (1), 8.5 (4.5), 10.5 (3.5), 20.5 (2.5) and 39 (0) infusions/session, respectively. Binding to µ opiate receptors was assessed in 20 µm brain slices using [3H]DAMGO. Administration of 40 nmol of ß-FNA i.c.v. was found to decrease the Bmax by 38.2% 24 hr later while having no effect on the Kd. The Bmax was found to be 46.2 and 97.3% of control 7 and 14 days after ß-FNA treatment, respectively. Therefore, ß-FNA produces a temporary loss in µ binding sites correlated with attenuation of heroin reinforcement. Supported by NIDA grants DA-01999 and P50-DA06634.

157.9

HALOPERIDOL BLOCKS THE RESPONSE-REINSTATING EFFECTS OF HEROIN REINFORCEMENT IN AN OPERANT RUNWAY PARADIGM. A.Ettenberg*, L. MacConell and T.D. Geist. Behavioral Pharmacology Laboratory, Dept of Psychology, Univ. California, Santa Barbara, CA 93106

The motivating actions of drug reinforcers have been demonstrated in studies where previously extinguished operant behaviors are reinstated following a single "prime" with the original reinforcer. In the present study, male albino rats were trained to traverse a straight-alley for a reinforcer consisting of five iv injections of 0.06 mg/kg heroin. Once the alley-running had been established, the heroin reinforcer was removed and the operant behavior permitted to extinguish. On treatment day, animals were injected 45 min prior to testing with 0.0, 0.75 or 0.15 mg/kg of the dopamine receptor antagonist, haloperidol. A single trial was then conducted during which some animals continued to experience extinction conditions while others were injected with the heroin reinforcer. The effects of these manipulations were determined during an additional single trial conducted 24 hrs later when the subjects were no longer drugged. While heroin did lead to a reinstatement in operant responding, this effect was dose-dependently prevented by pretreatment with haloperidol. These data suggest that the motivating properties of opiate agonist drugs can be attenuated during dopamine receptor blockade.

157.10

MICRODIALYSIS ASSESSMENT OF NUCLEUS ACCUMBENS DOPAMINE AND METABOLITES DURING IV HEROIN SELF-ADMINISTRATION AND EXTINCTION. P. Leone*, R. Rivest and R.A. Wise. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

Opiates are known to increase dopaminergic impulse flow and to elevate dopamine turnover in nucleus accumbens; the aim of the present study was to determine whether such effects occur with self-selected doses of intravenous (IV) heroin. Rats with nucleus accumbens guide cannulae were trained to self-administer heroin hydrochloride (0.2 or 0.3 mg/kg/inj) on a FR-1 schedule of reinforcement. Extracellular dopamine and metabolites were sampled at 20-min intervals by micro-dialysis and measured using high performance liquid chromatography (HPLC) with electrochemical detection. Dopamine and DOPAC levels increased approximately 300% during 3-h self-administration sessions; dopamine levels fell quickly, and DOPAC levels more slowly, during response "extinction" when heroin injections were terminated at the end of the sessions. The fall in dopamine levels during extinction occurred at a time of accelerated lever-pressing ("extinction burst") thus indicating that the elevated dopamine levels were not a simple consequence of behavioral arousal or the initiation of goal-directed behavior. These data offer clear evidence that rewarding doses of heroin are sufficient to disinhibit the mesolimbic dopamine system and produce elevated dopamine release similar to that seen during amphetamine or cocaine self-administration. Inasmuch as dopaminergic actions in nucleus accumbens are rewarding in their own right, dopaminergic actions appear to make an important contribution to the abuse liability of IV heroin.

157.11

PROPERTIES OF NEURONS RECORDED IN THE RODENT NUCLEUS ACCUMBENS, IN VIVO: RELATIONSHIP TO BEHAVIORAL STATE AND HEROIN SELF-ADMINISTRATION Steven J. Henriksen*, Clifton W. Callaway, S. Stevens Negus, George F. Koob, Dawn E. Miller, Greta I. Berg, Linda R. Friedman and Catherine C. Engberg. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California 92037.

The nucleus accumbens septi (NAcc) is a complex brain structure increasingly implicated in the acquisition and expression of reinforced behaviors. There is strong evidence suggesting that the NAcc, including some of its efferent and afferent connections, are critical for the brain processes leading to self-administration of both psychostimulant and narcotic analgesic drugs in rats. In order to investigate the cellular substrate of these behavioral events we have recorded from identified NAcc neurons in unanesthetized, freely-moving rats and have attempted to correlate their discharge with on-going behaviors, state changes and episodes of heroin self-administration. Single accumbens neurons were recorded with either tungsten microelectrodes or nichrome micro-wires assembled in a moveable microdrive. With this configuration we have been able to record the firing patterns of 80 NAcc neurons for tens of minutes up to several hours. The patterns of responsivity of these neurons to afferent stimulation appeared similar to what we have previously observed in anesthetized preparations. In contrast to the anesthetized rat NAcc neurons in the awake, freely moving rat demonstrated considerable spontaneous activity (4.08 ± 3.86 Hz) with both positive and negative correlations with behaviorally associated theta activity recorded from cortical EEG electrodes. However, there was no correlation of NAcc discharge rate and locomotor behavior, per sé. The rate of firing of these neurons declined during transition to slow-wave sleep (1.77 ± 1.68 Hz) and had their highest relative discharge rates in REM sleep (4.22 ± 3.86 Hz), another theta-related state. In one animal lever trained to self-administer heroin (0.02 mg/injection through an indwelling catheter, FR-1 schedule) we observed the discharge characteristics of three NAcc neurons to rewarded bar-presses. Video analysis of bar press events indicated that NAcc neuronal activity dramatically decreased for up to one minute following rewarded bar presses compared to non-rewarded similar rearing behaviors. This preliminary analysis suggests that NAcc neurons have state-dependencies and are influenced by complex, reward-related behaviors. (Supported by DA-03665, DA-(KO2)-00131 and AA07365 to S.J.H.)

157.12

LOCALLY-INDUCED OPIATE WITHDRAWAL MODESTLY ACTIVATES NORADRENERGIC LOCUS COERULEUS (LC) NEURONS *IN VIVO*. H. Hirata*, H. Akaoka[1] and G. Aston-Jones. Dept. Mental Health Sci., Hahnemann Univ., Philadelphia, PA 19102; [1]INSERM U171, St. Genis-Laval, France.

Hyperactivity of LC neurons during opiate withdrawal (OW) is thought to contribute to opiate abuse. Recently, we and others showed that the bulk of LC activation during OW resulted from increased excitatory amino acid (EAA) input to the LC from the ventrolateral medulla. It has also been reported that LC neurons in slices from morphine treated rats exhibited modest OW-induced hyperactivity. Here, we tested whether OW induced locally in the LC activated these neurons *in vivo*.

Adult Sprague-Dawley rats were continuously administered morphine via an osmotic minipump for 6 days (approx. 45 mg/kg/day). Under halothane anesthesia impulse activity of individual LC neurons was recorded using double barrel glass micropipettes. A solution of methyl naloxone (MeNLX) was microinfused (60-140 nl) from one barrel to induce local OW in the LC while recording LC discharge. None of 7 neurons tested were activated by local injection of 10 mM MeNLX. However, the discharge rate of neurons recorded with such MeNLX pipettes was significantly higher than that of neurons recorded in opiate-treated animals without this drug in the pipette (2.1±0.3 vs. 0.8±0.1 spikes/sec). To ascertain if leakage of MeNLX had elevated LC activity and prevented further activation with microinfusion of MeNLX, we used pipettes containing a much lower concentration of NLX (1 µM). Each of 10 neurons tested with 1 µM NLX were modestly activated (from 0.8±0.1 to 1.7±0.2 spikes/sec). However, some of this increase could result from antagonism of morphine circulating in the animal. Additional calculations revealed that the discharge of LC neurons recorded after microinfusion of 10 mM MeNLX was higher than that of morphine-naive rats (2.1±0.3 vs. 1.3± 0.2 spikes/sec, n=7 and 19, respectively). Our results indicate that 1/3 or less of this increase results from local OW mechanisms within the LC area, while the remaining 2/3 or more derives from OW in brain areas outside of the LC (largely or entirely EAA inputs from the ventrolateral medulla). *Supported by PHS grants DA 06214 and NS 24698.*

157.13

EXPRESSION OF FOS PROTEIN IN CENTRAL CATECHOLAMINE NEURONS DURING OPIATE WITHDRAWAL. H. Akaoka*[1], Y. Zhu, M.T. Shipley[2] and G. Aston-Jones. Hahnemann Univ., Philadelphia, PA 19102; [1]INSERM U171, St. Genis-Laval, France; [2]U. Cincinnati, Cincinnati, OH 45267

Previous studies have strongly implicated central catecholamine (CA) neurons in drug abuse and withdrawal mechanisms. We studied the distribution of CA neurons stimulated during opiate withdrawal using double immunocytochemistry for Fos protein, a metabolic marker, and tyrosine hydroxylase (TH). Adult male Sprague-Dawley rats were injected with morphine twice daily for 4 days in escalating doses of 5, 10, 20 and 30 mg/kg per injection. Opiate withdrawal was precipitated on day 5 with naltrexone (1 mg/kg). Control groups included: chronic morphine - saline; chronic saline - saline; and chronic saline - naltrexone. Two hrs after the last injection animals were processed for Fos and TH immunoreactivity (ir). Opiate withdrawal induced Fos-ir in several regions of the brain and spinal cord; here, we analyzed only neurons in the major CA nuclei. In the brain stem, doubly labeled cells were consistently found in the ventrolateral medulla (A1 and C1 neurons), nucleus tractus solitarius (NTS; most prominently in mid-NTS), and locus coeruleus (mostly in rostral and dorsal regions). Numerous non-TH-ir neurons in the NTS also expressed Fos protein after withdrawal. Fewer, or no, doubly labeled cells were found in the A5 or A7 cell groups or in rostral parts of the dorsal medulla. In control groups, there were few or no Fos-positive cells in any of these areas. From the midbrain to the olfactory bulbs, no TH-ir cells contained a detectable level of Fos protein regardless of the pharmacological treatment, except a subpopulation of cells in the vicinity of the supramammalary nucleus which were doubly labeled even in control groups. In withdrawn animals numerous TH-negative neurons in the hypothalamus and olfactory bulb expressed Fos-ir. These results indicate that opiate withdrawal induces Fos in noradrenergic or adrenergic cells in the medulla and pons, but not in more rostrally-located dopaminergic neurons, suggesting that activation of noradrenergic neurons, but not dopaminergic neurons, is importantly involved in opiate withdrawal. *Supported by PHS grants DA 06214 , NS 24698 and NS 20643.*

157.14

β-ADRENERGIC ANTAGONISTS BLOCK WITHDRAWAL SIGNS IN MORPHINE AND COCAINE DEPENDENT ANIMALS. G. C. Harris* and G. Aston-Jones. Department of Mental Health Sciences, Hahnemann University, Philadelphia, PA 19102.

Male Sprague-Dawley rats were treated chronically with either cocaine (20 mg/kg/ip/14 days), morphine (incrementing doses of 10 mg/kg/ip per day up to 80 mg/kg and maintained on 60 mg/kg/day) or saline. During morphine and cocaine abstinence (48 hrs), dependent rats showed increased anxiety in a defensive burying paradigm by showing a 4 fold ($p < .01$) increase in burying time relative to saline injected animals ($n = 6$ per group). This withdrawal-induced increase in burying was blocked by both the β1 and β2 antagonist, propranolol (15 mg/kg), as well as, the specific peripheral β1 antagonist, atendolol (15 mg/kg). These drugs (20 - 30 mg/kg) also significantly reduced the aversion to an environment paired with naloxone administration (200 μg) in morphine dependent animals ($n = 5$). Finally, these drugs (20 - 30 mg/kg) eliminated many signs of naloxone-precipitated (500 μg) and abstinence withdrawal, such as: wet dog shakes, teeth chatter, writhing and diarrhea ($n = 7$). These data suggest that many symptoms of opiate withdrawal and some symptoms of cocaine withdrawal can be alleviated through the blockade of peripheral β1-adrenergic receptors. Supported by NIDA Grants DA05387-03 and DA06214.

157.15

EFFECTS OF PRENATAL METHADONE EXPOSURE ON POSTNATAL PRODYNORPHIN AND VASOPRESSIN mRNA EXPRESSION IN THE RAT SUPRAOPTIC NUCLEUS. J.W. Nemitz*Dept. of Anatomy, WV School of Osteopathic Medicine, Lewisburg, WV 24901.

Pregnant Sprague-Dawley rats were made physically dependent to methadone (9mg/kg/day) using subcutaneous osmotic minipumps (Alza). The pregnancies were brought to term and the methadone exposed rat pups were sacrificed at different postnatal days by decapitation. Frozen serial coronal sections (20um) obtained at the level of the hypothalamus were processed for in situ hybridization, using ^{35}S-oligonucleotide probes (DuPont), and emulsion autoradiography. Arginine-vasopressin (AVP) mRNA levels were decreased in the supraoptic nucleus (SON) of methadone exposed rat pups as compared to controls during the first postnatal week. Increases in prodynorphin (DYN) mRNA levels were observed in the adjacent SON sections of neonates exposed to methadone. No labeling for an enkephalin mRNA probe was observed in the SON of either methadone exposed or control pups. Previous work provided evidence that prenatal methadone exposure results in a decrease in oxytocin mRNA expression in hypothalamic nuclei that is rapidly reversed postnatally. The present data indicate that prenatal methadone exposure also inhibits the expression of AVP mRNA in the SON, but for a longer postnatal period. Furthermore the concurrent increase in DYN levels in the SON as a result of methadone exposure is consistent with a proposed modulatory role for DYN. Supported by WVSOM funds.

157.16

DOPAMINE D_2 RECEPTOR SUPERSENSITIVITY WITHOUT RECEPTOR UP-REGULATION IN MORPHINE ABSTINENT RATS. P.L. Reddy, Veeranna and H.N. Bhargava*, Dept. Pharmacodynamics, Univ. Ill. at Chicago, Chicago, IL 60612.

Rats were rendered tolerant to and physically dependent on morphine by implanting 6 morphine pellets during a 7-day period. Each pellet contained 75 mg morphine base. Rats which served as controls were implanted with an equivalent number of placebo pellets. Rats were sacrificed brain and spinal cord were isolated. Brain was dissected into 7 regions. B_{max} and K_d values of ^{3}H-spiroperidol to bind to dopamine D_2 receptors in brain regions and spinal cord of morphine tolerant (pellets left intact at time of sacrificing) and abstinent (pellets removed 18 hr prior to sacrificing) rats were determined. ^{3}H-spiroperidol bound to tissue membranes at a single high affinity binding site. The B_{max} and K_d values of ^{3}H-spiroperidol were unaffected in brain regions and spinal cord of morphine tolerant or abstinent rats. Spontaneous withdrawal of morphine resulted in significant increases in motor activity and stereotypic movements. Dopamine D_2 receptor agonist, 2-bromo-α- ergocriptine induced behaviors were of greater intensity in morphine abstinent rats than in placebo controls. Previously, we had demonstrated that behavioral supersensitivity of D_1 receptors in morphine abstinent rats involved up-regulation of receptors. Evidence is, thus provided for the behavioral supersensitivity of dopamine D_2 receptors without receptor up-regulation (Supported by NIDA grant DA-02598).

157.17

REWARD-SPECIFIC BLOCKADE OF MORPHINE'S EFFECT ON BRAIN-STIMULATION REWARD BY PIMOZIDE BUT NOT SCH 23390. M. Sarkar*, D. Huston-Lyons and C. Kornetsky. Boston Univ. Sch. of Med, Boston, MA 02118.

To further test the hypothesis that dopamine plays an important role in the rewarding effects of morphine sulfate (MS) (2.0 mg/kg sc), we compared the ability of the specific D1 antagonist, SCH 23390 (SCH) (0.00125 - 0.02 mg/kg sc), and the D2 antagonist, pimozide (PIM) (0.05 - 0.4 mg/kg ip), to block the threshold lowering effect of MS on brain-stimulation reward (BSR) to the MFB-LH. Thresholds were determined in 7 male F-344 rats using a discrete trial, rate-independent method. PIM, reversed MS's effect at doses (0.1 - 0.2 mg/kg) that did not significantly alter the threshold alone, suggesting that PIM induced a reward-specific blockade. SCH, in contrast, also reversed MS's effect, but only at doses (>0.01 mg/kg) that significantly raised the threshold when injected alone, suggesting a non-specific blockade of the rewarding effects of MS. Similar non-specific blockade by PIM is seen only at higher doses (>0.2 mg/kg). Thus, to the extent that BSR is a model of drug-induced euphoria, these data suggest that MS's rewarding effects are modified in a *reward-specific* manner by moderate doses of the D2 antagonist, PIM, and only in a *non-specific* manner by the D1 antagonist, SCH. (Supported by NIDA Grant DA02326 and DA00099 to CK).

157.18

CHRONOAMPEROMETRIC MEASUREMENTS OF DOPAMINE LEVELS IN RAT NUCLEUS ACCUMBENS DURING HEROIN SELF-ADMINISTRATION. E. Kiyatkin*, R.A. Wise and A. Gratton. Douglas Hosp. Res. Ctr, McGill Univ. & Ctr Studies Behav. Neurobiol., Concordia Univ., Montréal, Canada.

Heroin has powerful habit-forming or "reinforcing" properties in humans and in lower animals. Mesolimbic dopamine (DA) neurons that innervate nucleus accumbens (NAcc) are thought to mediate the reinforcing effects of many abused drugs, including heroin. In the present study, high-speed chronoamperometry was used to monitor, on 5-6 consecutive daily recording sessions, DA-dependent electrochemical signals in NAcc of rats allowed to lever-press for intravenous heroin (100μg/kg/injection). The electrochemical signal increased gradually after the first drug infusion each day and remained at this elevated level for the rest of the session at which time transient changes in the signal that were time-locked to operant responses were observed. Each response for heroin was preceeded by a reliable increase and followed by a comparable decrease in the electrochemical signal. Doubling the expected dose of heroin reliably potentiated the decrease in signal which then gradually returned to the elevated plateau. Blocking access to the lever at the end of the session caused the electrochemical signal to first increase slightly then to gradually return to the pre-drug baseline; naloxone (1.0 mg/kg i.p.) accelerated the decline of the signal. The present results are generally consistent with the stimulant action of opiates on mesolimbic DA neurons. However, they also suggest a more complex role of this system in opiate reinforcement than has been previously predicted. While extracellular DA levels in NAcc were tonically elevated during heroin self-administration, reinforcement of operant responding by the drug was accompanied by a decrease in extracellular DA, whereas increased DA efflux was more closely associated with the behavioral arousal that preceeded each response. *Supported by FCAR, NIDA and NSERC.*

157.19

INVOLVEMENT OF MESOLIMBIC DOPAMINE NEURONS IN THE INITIATION OF SENSITIZATION TO MORPHINE. Michael Jeziorski* and Francis J. White, Neuropsychopharmacology Lab, Lafayette Clinic, Cellular and Clinical Neurobiology Program, Dept. of Psychiatry, Wayne State University School of Medicine, Detroit MI 48207.

Repeated daily administration of morphine at a moderate dose induces sensitization to the acute stimulatory effects of morphine upon locomotion. Single unit electrophysiological studies have revealed that the basal firing rate of dopamine (DA) neurons within the ventral tegmental area (VTA), the nucleus most extensively implicated in the initiation of this sensitized response, is increased in morphine-sensitized rats (4.7 ± 0.2 Hz) in comparison to control (3.5 ± 0.2 Hz). This elevation persists to a lesser degree in rats withdrawn for 7 days from repeated morphine treatment (4.2 ± 0.2 Hz), mimicking the persistent behavioral response to morphine. We have previously shown that development of the behavioral sensitization produced by 14 day morphine treatment (10 mg/kg/day, i.p.) is blocked by administration of the NMDA receptor antagonist MK-801 (0.25 mg/kg/day, i.p.) prior to each morphine injection. Coadministration of MK-801 and morphine for 14 days also attenuates the increase in basal firing rate of DA cells (4.0 ± 0.2 Hz). Because the actions of morphine within the VTA are likely mediated via GABAergic interneurons, the possibility that alterations in the responses of these neurons are associated with the increase in DA cell activity was also evaluated. DA cells in morphine-sensitized rats displayed a slightly enhanced sensitivity to iontophoretic application of GABA (0.1 M, pH 4.0). Preliminary recordings of non-DAergic neurons within the VTA revealed inhibitory responses to iontophoretic morphine (0.02 M, pH 4.0) which were attenuated in morphine-sensitized rats. [Supported by USPHS grants DA 04093 and MH 40832 (FJW) and NRSA DA 05434 (MJ)].

PSYCHOTHERAPEUTIC DRUGS: CLOZAPINE AND DOPAMINE ANTAGONISTS

158.1

CLOZAPINE AND HALOPERIDOL IN A NOVEL MODEL FOR ASSESSING ANTIPSYCHOTIC POTENCY. N.R. Swerdlow* and M.A. Geyer. UCSD Dept. of Psychiatry, La Jolla, CA 92093

Startle is inhibited or "gated" when the startling stimulus is preceded 30-500 msec earlier by a weak prepulse. "Prepulse inhibition" (PPI) is deficient in schizophrenics and in rats treated with dopamine (DA) agonists. We have reported that the ability of neuroleptics to restore PPI in DA-stimulated rats is a sensitive and specific predictor of clinical antipsychotic potency. The DA agonist apomorphine (APO) potently disrupts PPI, and the ability of neuroleptics to reverse this APO effect correlates highly (0.99) with clinical potency. In the present study, we compared the effects of typical and atypical antipsychotics in this model. Haloperidol and clozapine restored PPI in APO-treated rats; haloperidol followed simple monotonic dose-responsivity, while clozapine exhibited a previously reported "inverted-U" dose-response curve, restoring PPI at low doses, but not high doses. Similarities and differences were also noted in the ability of these drugs to enhance baseline startle gating (increase prepulse effectiveness), and to restore PPI after intra-accumbens DA infusion. The restoration of PPI in DA-activated rats is a predictive model that is sensitive to both typical and atypical antipsychotics, and may also identify important differences in the abilities of these agents to rectify deficits in sensorimotor gating.

158.2

PROPRANOLOL DOES NOT SUBSTITUTE FOR CLOZAPINE IN CLOZAPINE-TRAINED RATS IN A TWO-LEVER DRUG DISCRIMINATION PROCEDURE. A.D. Compton*, B.M. Kelley, J.L. Wiley, and J.H. Porter. Virginia Commonwealth University, Richmond, Virginia 23284.

Although clozapine (CLZ) acts at several types of receptors in vitro and in vivo, the mechanism of action for its discriminative stimulus properties has not yet been determined. Previous research has shown that drugs acting at D1 and D2 dopamine receptors, at 5-HT1A, 5-HT2, and 5-HT3 serotonin receptors, and at alpha nor-adrenergic receptors fail to substitute for CLZ in CLZ-trained rats. The present study examined the effects of propranolol, a beta nor-adrenergic receptor blocker, in stimulus substitution tests with CLZ-trained rats. Six adult male Sprague-Dawley rats (85% BW), trained to discriminate CLZ (5 mg/kg, i.p.) from vehicle in a two-lever drug discrimination procedure, were tested in 15 min sessions under an FR30 schedule of food reinforcement. Stimulus generalization tests with CLZ (1.25, 2.5, 5, & 10 mg/kg, i.p.) produced dose-dependent generalization from the training dose. Propranolol (2.5, 5, 10, 20, 25, & 30 mg/kg, i.p.) produced less than chance levels of responding on the CLZ lever; although, interestingly, 2 of the 6 animals generalized completely across the 4 highest doses. Thus, the exact mechanism of CLZ's discriminative stimulus properties remains to be determined.

158.3

THE DOPAMINE D4 AND D2 RECEPTORS: COMPARISON OF NEUROLEPTIC BINDING AFFINITIES. L.M. Figur, D.L. Evans, N.C. Stratman and R.A. Lahti.* CNS Diseases Research, Upjohn Laboratories, Kalamazoo, MI 49001.

The dopamine D4 receptor, which is considered to be a variant of the D2 receptor, has recently been cloned and then expressed into COS-7 cells. Receptor binding studies demonstrated that clozapine, which is an effective antipsychotic agent but atypical in that it lacks the usual side effects of typical antipsychotic agents, has high selectivity for the D4 vs. the D2 receptor (H.Van Tol *et al.*, Nature 350:610, 1991).

Comparative binding studies have been carried out for a number of interesting dopaminergic agents using membranes from the CHO-D2 and COS-7-D4 cells and ^{3}H-YM--09151-2 as the ligand. It was found that clozapine is selective for the D4 vs. the D2 receptor by a factor of 2.8. Other compounds with D4 selectivity (K_i D2/K_i D4) were (+)apomorphine (8.7), (+)-NPA (2.4) and melperone (1.3). Compounds with selectivity for the D2 receptor (K_i D4/K_i D2) were trifluoperazine (30.1), chlorpromazine (11.9), thioridazine (8.2), SDZ-208-912 (3.9), haloperidol (3.3), and loxapine (2.9). Compounds such as AJ-76 (1.1) and (-)3-PPP (1.6) exhibited low selectivity for the D2 receptor.

The findings are of considerable interest not only because of the selectivity of the atypical antipsychotic agent clozapine for the D4 receptor but because of the selectivity of the typical antipsychotic agents such as chlorpromazine, trifluoperazine, and haloperidol for the D2 receptor. Results may be interpreted to indicate that D2 selectivity confers side effect liability (EPS and TD) and D4 selectivity confers antipsychotic efficacy on neuroleptics.

158.4

EFFECTS OF HALOPERIDOL(HAL) AND CLOZAPINE(CLOZ) ON THE ABILITY OF COCAINE TO INCREASE DOPAMINE RELEASE IN RAT STRIATUM (STR) AND NUCLEUS ACCUMBENS (NA) AS MEASURED BY *IN VIVO* BRAIN MICRODIALYSIS. Baoling Chai[#] and H Y. Meltzer, Case Western Reserve Univ.Sch.of Med., Cleveland, OH 44106

The effects of two antipsychotic agents HAL and CLOZ on DA release and metabolism(dihydroxyphenylacetic acid,DOPAC) in the Str and the N.A.of freely moving rats were investigated using *in vivo* microdialysis.Cocaine(5 mg/kg,s.c.) produced equivalent increases in DA (221% and 244% of baseline level at the maximum level) and decreases in DOPAC(42% and 35%) efflux in the Str and the N.A.,respectively.HAL(2mg/kg,s.c.) 30min prior to cocaine(5 mg/kg,s.c.)increased cocaine-induced DA release(380% and 615%) together with a 210% and 207%increase in DOPAClevel in the Str and the N.A..CLOZ (20mg/kg,s.c.) 30 min prior to cocaine (5mg/kg,s.c.) increased cocaine-induced DA release(456%),produced137% increases in DOPAC levels in the N.A.only.These results suggest that both types of antipsychotics facilitate DA efflux due to cocaine. This would counteract their ability to block the euphoric effects of cocaine. However the D1, D2,and D4 antagonist properties of CLOZ and D2 antagonist properties of HAL would block the effects of DA.

158.5

ACTIONS OF CLOZAPINE AND HALOPERIDOL ON THE EXTRACELLULAR LEVELS OF EXCITATORY AMINO ACIDS IN THE PREFRONTAL CORTEX AND STRIATUM: A COMPARISON TO DOPAMINE. K.D. Youngren,* D.A. Daly, and B. Moghaddam, Department of Psychiatry and Interdepartmental Neuroscience Program, Yale Univ. Sch. of Med., and VA Medical Center, West Haven, CT, 06516.

The technique of intracerebral microdialysis was employed to assess the effects of clozapine and haloperidol on the extracellular levels of excitatory amino acids, aspartate and glutamate, in the striatum and prefrontal cortex of conscious rats. Haloperidol (0.5 mg/kg s.c.) had no significant effect on the levels of excitatory amino acids in either region. Clozapine (12.5 mg/kg s.c.) was without a significant effect in the striatum; however, it significantly decreased the extracellular concentration of both aspartate and glutamate in the prefrontal cortex. We have previously reported that acute and chronic treatment with clozapine alters the outflow of dopamine in the prefrontal cortex to a greater degree than in the striatum. Thus, it seems that clozapine has preferential actions on extracellular levels of both excitatory amino acids and dopamine in the prefrontal cortex. The effect of chronic clozapine, as well as higher doses of acute clozapine, on the outflow of excitatory amino acids is currently under investigation.

158.6

EFFECT OF SCOPOLAMINE (Scop) ON CLOZAPINE (CLOZ) AND HALOPERIDOL (HAL) INDUCED DOPAMINE (DA), EFFLUX IN THE STRIATUM (STR), NUCLEUS ACCUMBENS (NA) AND PREFRONTAL CORTEX (PFC). H. Y. Meltzer*, B-L. Chai and B. Yamamoto, Department of Psychiatry, Case Western Reserve University School of Medicine, Cleveland, OH 44106

CLOZ, an atypical antipsychotic drug (APD), is a potent anticholinergic agent. Nevertheless, in a recent in vivo voltammetric study (Rivest and Marsden, 1991), SCOP, another potent anticholinergic agent, inhibited the CLOZ-, but not the HAL-induced increase in dihydroxyphenylacetic (DOPAC), the DA metabolite, in the rat STR and NA. We now report that CLOZ 20 mg/kg and HAL 1 mg/kg, significantly increased STR DA release as measured by in vivo microdialysis in awake freely moving rats (159.0% and 226.3, respectively). SCOP, 1 mg/kg, had no effect on basal DA release but significantly reduced the effect of CLOZ (69.5%) but not HAL (228.7%) on STR extracellular DA. SCOP treatment also did not affect the ability of CLOZ to increase the release of DA in the NA or PFC. SCOP also did not block the ability of thioridazine (144.8%), another APD with strong anticholinergic properties which has been thought to be atypical, to enhance the release of DA in the STR. These results suggest CLOZ increases striatal DA efflux by an acetylcholine-dependent mechanism which can be blocked by SCOP. CLOZ may be an indirect cholinomimetic agent and a selective antagonist of specific muscarinic receptors. These results suggest that an effect of CLOZ on the cholinergic system to modulate STR DA release may be related to its better profile of effects on the extrapyramidal system compared to typical APD.

158.7

CLOZAPINE AT HIGH CONCENTRATIONS INTERACTS WITH THE CANNABINOID RECEPTOR. D.M. Evans* and A.C. Howlett, Dept. of Pharmacological and Physiological Science, St.Louis Univ. Sch. of Med., St. Louis, MO 63104.

Previous reports have indicated that acute intraperitoneal injection of the neuroleptic clozapine into male Swiss-Webster mice produces hypothermia and ataxia in these animals (Menon et al., Life Sci. 43, 1791-1804 (1988)). Furthermore, bilateral microinjection of clozapine into rat brains results in catalepsy (Fog, R. Int. Pharmacopsychiatr. 10, 89-93 (1975)). These effects are characteristic of animals exposed to cannabinoid drugs (Compton et al., Marijuana, Intnatl. Res. Rep. 1987, pp. 213). In order to determine whether clozapine could interact with the cannabinoid receptor, we assessed the ability of this compound to inhibit the binding of the synthetic cannabinoid [^{3}H]CP55,940 to rat brain membranes *in vitro* using a standard filtration assay. Clozapine produced a dose-dependent decrease in the specific binding of this ligand whilst its vehicle at the appropriate concentrations was without effect. The IC_{50} for inhibition by the neuroleptic approximated 50μM. Furthermore, clozapine was able to inhibit adenylate cyclase activity in membranes prepared from N18TG2 neuroblastoma cells. Inhibition of forskolin-stimulated cyclase activity by 41% was observed in the presence of 200μM clozapine. The data would suggest that, at very high concentrations, some of the effects of clozapine may, at least in part, be due to interaction with central cannabinoid receptors.

Supported by NIDA grants DA-03690 and DA-06912.

158.8

BEHAVIORAL EFFECTS OF CHRONIC CLOZAPINE ARE ALTERED BY IBOTENIC ACID LESIONS OF THE RAT MEDIAL PREFRONTAL CORTEX **G. Hussain, G.E. Jaskiw*, H.Y. Meltzer. Psychobiol. Dept., Case Western Reserve Univ., Cleveland, OH 44106 / Brecksville VAMC, OH.**

Frontal cortex lesions which spare much of the medial prefrontal cortex (MPFC) only modestly influenced the behavioral effects of chronic clozapine (CLZ) treatment (Abstr. Soc. Neurosci. 1991, p 99). However, CLZ may exert unique actions within the MPFC. We now induced ibotenic acid (IA) lesions in the MPFC of male Zivic-Miller rats (220-250 gr). After 21d, rats began a 21d treatment with haloperidol (HAL) 2 mg/kg/d, clozapine (CLZ) 20 mg/kg/d or vehicle in drinking water. 3d after the end of chronic treatment, apomorphine 0.75 mg/kg sc was administered and behavior visually assessed. As expected, HAL pretreatment increased biting but reduced stationary behavior. Compared to SHAM/CLZ animals, IA/CLZ rats also displayed more oral stereotypies and were generally more active. These data indicate that actions within the MPFC may contribute to some of the atypical effects of CLZ. Supported by NARSAD.

158.9

ANTAGONISM OF THE DISCRIMINATIVE STIMULUS EFFECTS OF THE SELECTIVE HIGH-EFFICACY D_1 AGONIST SKF 81297 BY THE ATYPICAL NEUROLEPTIC CLOZAPINE IN SQUIRREL MONKEYS. S. Rosenzweig-Lipson* and J. Bergman. Harvard Medical School/NERPRC, Southboro, MA 01772.

Recent evidence suggests that the behavioral effects of the atypical neuroleptic clozapine may be related to its blockade of D_1 receptors. In the present study, the D_1-related actions of clozapine were evaluated in squirrel monkeys trained to discriminate the selective, high-efficacy D_1 agonist SKF 81297 (0.3 - 1.0 mg/kg) from saline in drug discrimination procedures. Monkeys were trained under a 10-response fixed-ratio schedule of stimulus-shock termination to respond differentially on left and right levers depending on whether drug or saline was injected. Incremental doses of SKF 81297 (0.01 - 1.0 mg/kg) produced dose-related increases in responding on the drug-associated lever in all monkeys. Pretreatment with the selective D_1 antagonist SCH 39166 (0.03 - 0.1 mg/kg) resulted in a dose-dependent rightward shift of the SKF 81297 dose-effect curve, indicative of surmountable antagonism. Pretreatment with clozapine (0.3 - 3 mg/kg) blocked the discriminative-stimulus effects of SKF 81297. However, the effects of clozapine appeared to differ in individual monkeys. In some cases, clozapine produced a rightward shift of the SKF 81297 dose-effect curve, whereas, in other cases drug-appropriate responding was not restored by SKF 81297 up to 3.0 mg/kg. The present results indicate that the behavioral effects of clozapine in monkeys involve its D_1 receptor-mediated actions. Supported by USPHS Grants DA03774, DA00499, MH07658, and RR00168.

158.10

EFFECTS OF LOCAL ADMINISTRATION OF CLOZAPINE IN THE RAT STRIATUM AND PREFRONTAL CORTEX ON DOPAMINE LEVELS. E.A. Pehek* and B.K. Yamamoto. Dept. of Psychiatry, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106

Previous research has demonstrated that systemic administration of the atypical antipsychotic clozapine increased extracellular dopamine (DA) levels in the rat brain and this increase was greater in cortical and limbic areas relative to the caudate-putamen (CP). However, it has not been determined whether these effects were due to direct actions of clozapine on DA nerve terminals in these regions or, alternatively, on their midbrain cell bodies of origin. The aim of the present study was to compare the effects of local infusions of clozapine in nerve terminal and cell body areas on DA levels in the medial prefrontal cortex (MPFC) and CP. *In vivo* microdialysis coupled with HPLC/EC was used to measure extracellular DA every 30 min. in the freely moving rat. After basal DA stabilized, a 1.0 mM solution of clozapine was infused through the probe for one hour and samples were collected for three hours. Data were expressed as percentages of the average baseline values. Administration of clozapine directly into the cortex increased cortical DA levels (239% during the first 30 min. of drug application, 244% during the second 30 min, and 189% 30 min. after termination of the infusion). Likewise, striatal administration increased DA levels in the CP (286%, 156%, and 66%, respectively). These results suggest that systemically administered clozapine may increase DA via direct actions on DA nerve terminals. Further research will examine the effects of clozapine infusions into regions A9 and A10 of the midbrain and will contrast striatal with cortical effects.

158.11

CLOZAPINE AND HALOPERIDOL DIFFERENTIALLY EFFECT COGNITIVE ACTIVATION IN SCHIZOPHRENICS AS MEASURED BY REGIONAL CEREBRAL BLOOD FLOW (rCBF) PET. D. Z. Press*, K. F. Berman, T. Noga, L. B. Bigelow, J. L. Ostrem, D. R. Weinberger. HHMI-NIH RSP and Clinical Brain Disorders Branch, NIMH-DIRP St. Elizabeth's Hosp., Washington, D.C.

Atypical neuroleptics such as clozapine (CLZ) differ from standard neuroleptics such as haloperidol (HAL) both in their pharmacology and their clinical efficacy. Because of the widely varying pharmacologic differences, the basis for the clinical differences remains elusive. To investigate the neural systems underlying these differences we examined cortical physiology with the $H_2{}^{15}O$ method of positron emission tomography (PET) in schizophrenics both while on CLZ (titrated clinically, mean dose 533 mg/day) and on HAL (.4 mg/kg/day) in a crossover, counterbalanced design.

rCBF measurements were taken while subjects performed cognitive tasks (including the Wisconsin Card Sort [WCS], Raven's Progressive Matrices [RAV] and a novel Delayed Alternation [DA] task) and matched sensory-motor control tasks. 15 transaxial slices were acquired with axial and in-plane resolution of 6.5mm after reconstruction. rCBF data were normalized, and both studies were resliced so that they were registered to a single MRI. Regions of interest were defined on the MRI and then applied to both PET studies.

Results from the first 6 subjects show that in the basal ganglia, there was a trend towards decreased blood flow while on CLZ as compared to HAL for almost all tasks. During DA and RAV there was more activation (task - control) in the left dorsolateral prefrontal cortex while on CLZ than on HAL ($p<.05$, $p<.10$ respectively). No such trend was seen during the WCS. On the right a less significant trend in the opposite direction (HAL>CLZ) was seen. CLZ and HAL may have differing effects on tasks mediated by different neural systems.

158.12

CHRONIC HALOPERIDOL TREATMENT REDUCES GLUTAMATE RECEPTOR BINDING IN LIMBIC STRUCTURES OF RAT BRAIN T.Kakigi*, X.M.Gao, O.Shirakawa, C.A.Tamminga University of Maryland, MPRC, Baltimore MD 21228

Haloperidol is a neuroleptic drug with potent antagonist activity at the dopamine D_2 receptor. It is used chronically in the treatment of schizophrenia, with broad antipsychotic activity. Chronic haloperidol treatment causes spontaneous oral dyskinesias called vacuous chewing movements (VCMs) in rat. To understand the pathophysiology of these movements and the extended mechanism of its antipsychotic effect, we have studied neurochemical effects of chronic haloperidol. Previously, we have reported regional changes in D_1, D_2, and GABA-A receptors with chronic haloperidol and their correlation with VCMs. Here we report the effects of chronic haloperidol on glutamate receptor subtypes. Animals were treated with haloperidol (1.5mg/kg/day) in their drinking water for 6 months. NMDA receptors were quantified using ^{3}H-glutamate, AMPA receptors, using ^{3}H-AMPA, both with standard technique. Our results show that chronic haloperidol treatment decreases the ^{3}H-AMPA binding in the nucleus accumbens, subiculum and entorhinal cortex, and decreases the NMDA sensitive ^{3}H-glutamate binding in the CA1, posterior cingulate, parietal cortex, and amygdala. Results from kainate and metabotropic glutamate receptor quantification will be reported. It appears that drug treatment effects glutamate receptors in certain limbic related structures while showing no effect in neocortical areas. We report regionally selective, not generalized, modulation of glutamate receptors by chronic haloperidol. We propose that this is related to the functions of haloperidol which do not show tolerance; and, may be pertinent to postmortem studies in schizophrenia.

158.13

IDENTIFICATION OF PD 139899 AS A NOVEL DOPAMINE AUTORECEPTOR AGONIST WITH ANTIPSYCHOTIC-LIKE ACTIVITY. **L.D. Wise*, D.J. Wustrow, W.J. Smith III, L.T. Meltzer, T.A. Pugsley, T.G. Heffner. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co. Ann Arbor MI 48106.**

PD 139899 was selected from a novel series of aminoalkyl-cyclohexylindoles with dopamine (DA) autoreceptor agonist activity. Structure-activity studies indicate that a trans-cyclohexylethyl side chain and a pyridylpiperazinyl amino moiety provide optimal activity within this series. PD 139889 displays high affinity for DA D_2 receptors *in vitro* with an IC_{50} of 3 nM. In rats PD 139899 activates brain DA autoreceptors as evidenced by reversal of gamma-butyrolactone-stimulated brain DA synthesis and inhibition of nigrostriatal DA neuron firing. Consistent with the profile of other DA autoreceptor agonists, PD 139899 reduces spontaneous locomotor activity (LMA) in mice (ED_{50} = 1.4 mg/kg IP) but even at much higher doses does not attenuate stimulation caused by the direct DA agonist apomorphine. In rats, PD 139899 inhibits LMA (ED_{50} = 2.4 mg/kg PO) but does not cause the LMA activation indicative of stimulation of postsynaptic DA receptors in brain, even at high multiples of its LMA ED_{50}. PD 139899 also does not elicit stereotypy even in the presence of a DA D_1 agonist. In squirrel monkeys PD 139899 inhibits conditioned avoidance responding after oral dosing (ED_{50} = 1.0 mg/kg). Collectively, these results indicate that PD 139899 is a potent DA autoreceptor agonist with antipsychotic-like effects in preclinical tests.

158.14

CHARACTERIZATION OF PD 139899: A NOVEL DOPAMINE AUTORECEPTOR AGONIST WITH ANTIPSYCHOTIC-LIKE ACTIVITY. **T.G. Heffner*, A.E. Corbin, L.W. Cooke, M.D. Davis, S.B. DeMattos, L.M. Georgic, R.G. MacKenzie, F.W. Ninteman, T.A. Pugsley, S.Z. Whetzel, J.N. Wiley, D.J. Wustrow and L.D. Wise. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor MI 48106.**

PD 139899 exhibited effects characteristic of a dopamine (DA) autoreceptor agonist and partial DA D_2 agonist in preclinical tests. In addition to DA D_2 receptor affinity, PD 139899 had weak affinity for DA D_1 receptors as well as $5HT_2$ receptors and had modest affinity for sigma, $5HT_{1A}$ and α_1-adrenergic receptors. The affinity for D_2 receptors was reduced 4-fold by sodium and GTP, a larger shift than seen with DA antagonists but smaller than seen with full DA agonists. PD 139899 also caused DA agonist-like inhibition of forskolin-stimulated adenylyl cyclase activity in GH4C1 cells transfected with the human DA D_2 receptor. Like other DA agonists, PD 139899 inhibited GBL-stimulated striatal DA synthesis in rats and the maximal effect was reduced by pretreatment with the receptor alkylating agent EEDQ. Like DA antagonist antipsychotics, PD 139899 inhibited locomotor activity in rats and caused long-lasting inhibition of conditioned avoidance in squirrel monkeys. But, in contrast to DA antagonists, PD 139899 inhibited striatal DA synthesis and reduced DA overflow as measured with intracerebral microdialysis (ICMD) in rats and squirrel monkeys. Efficacy in rats was maintained in the GBL and ICMD tests during repeated dosing. These findings demonstrate a DA autoreceptor agonist/partial DA agonist mechanism of action for PD 139899 and provide evidence of antipsychotic-like effects.

158.15

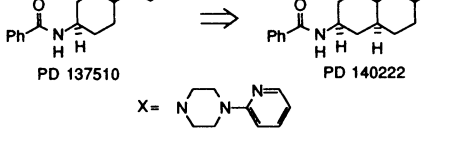
COMPARISON OF PARTIAL DOPAMINE D_2 AGONISTS WITH NEUROPHYSIOLOGICAL AND BEHAVIORAL TESTS. **A.E. Corbin*, C.L. Christoffersen, K.A. Serpa, J.N. Wiley, L.T. Meltzer and T.G. Heffner. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48106.**

Dopamine (DA) D_2 agonists inhibit locomotor behavior in rodents by direct effects on brain DA autoreceptors and may also act via antagonist-like partial agonist actions at brain DA postsynaptic receptors. In order to estimate partial agonist activity of DA D_2 agonists, drugs were compared for maximal inhibition of substantia nigra DA neuron firing and for ability to antagonize the locomotor stimulatory effect of a low postsynaptic dose of the DA agonist apomorphine in rats. For neuronal firing, relative intrinsic activity was: apomorphine, quinpirole > EMD 38362, PD 128483 > terguride > SDZ 912 > haloperidol (inactive). Whereas DA agonists and antagonists inhibited spontaneous locomotor activity (SLA), partial DA agonists and DA antagonists were also effective at reversing apomorphine-stimulated locomotor activity (ALA). The ratio of ALA ED_{50} / SLA ED_{50} was used as an estimate of intrinsic activity, corrected for potency, for the behavioral data. This ratio was 1 or less for DA antagonists and greater than 100 for full DA agonists such as quinpirole. Ranking of compounds on the basis of ALA/SLA ratio gave the results: quinpirole, B-HT 920, (+)-3-PPP >> EMD 38362, PD 128483 > terguride, SDZ 911, (-)-3-PPP > SDZ 912, remoxipride & other DA antagonists. These results indicate close agreement between neurophysiological and behavioral measures of partial DA D_2 agonist activity.

158.16

IDENTIFICATION OF THE ACTIVE CONFORMATION OF A NOVEL SERIES OF BENZAMIDE DOPAMINE D2 AGONISTS. **D.J. Wustrow, W.J. Smith III, D.M. Reynolds, L.D. Wise, R.G. MacKenzie, T.A. Pugsley, T.G.Heffner, L.M. Georgic* and T. Mirzadegan. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor Michigan 48106**

The benzamide PD 137510 was shown to be a dopamine (DA) autoreceptor agonist (Soc. Neurosci. Absts. 17: 689, 1991). In order to probe the conformation of the ethylene chain connecting the cyclohexylbenzamide and pyridylpiperazine portions of PD 137510, a series of rigid analogs were prepared. PD 140222 was identified as being more potent in the DA D2 binding assay (IC_{50} 73 nM Vs 443 nM) than PD 137510. PD 140222 also exhibited potent activity in the mouse locomotor activity screen (ED_{50} 0.10 mg/kg). The inherent conformational stability in PD 140222 suggests the conformation of PD 137510 which is bound by the D2 receptor. The agonist activity of these molecules was further evaluated using inhibition of forskolin-stimulated cAMP accumulation in GH_4C_1 cells transfected with the human D2 receptor. The results will be discussed in terms of a hypothetical model of the D2 receptor.



158.17

ALTERATIONS IN DOPAMINERGIC AND GABAERGIC MECHANISMS OF THE VENTROLATERAL STRIATUM OF RATS PRETREATED WITH DIFFERENT REGIMENS OF CHRONIC NEUROLEPTICS: A MICRODIALYSIS STUDY. A.S. Keys* and G.D. Ellison, UCLA, Department of Psychology, Los Angeles, CA. 90024-1563

Rats given longterm neuroleptic exposure develop dramatically different oral syndromes depending upon whether the neuroleptic is administered continuously or once weekly. The present study utilized microdialysis procedures to examine alterations in the ventrolateral striatum. This area was selected because of its known involvement in the circuitry that mediates bucco-lingual movements of rats pretreated with chronic haloperidol for over a year in these two different regimens. Levels of DA and its metabolites in addition to GABA were assayed using HPLC with electrochemical detection. A time-linked measure of activity was collected on-line during dialysis collection and correlated with probe infusion action. The response to intrastriatal infusions of the selective dopamine d-2 antagonist, raclopride or the GABA agonist, muscimol, in addition to i.p. injections of haloperidol were examined. The results suggest that while differences in response to challenge infusions, as measured by DA and metabolite levels, exist between the control and both neuroleptic treated groups, some differences in the recovery of DA metabolites after challenge infusions exist between the two neuroleptic regimen groups.

158.18

ACUTE ADMINISTRATION OF HALDOL AUGMENTS DOPAMINE'S POSTSYNAPTIC EFFECTS. T.I.Lidsky* & S.P.Banerjee. Inst. Basic Res., S.I., N.Y.

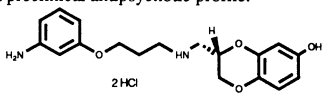
Haloperidol (Hal) can either diminish or facilitate dopaminergic activity by blocking, respectively, postsynaptic or presynaptic D2 receptors. Hal's net effect will be the sum of its pre- and postsynaptic influences. Electrophysiological techniques were used to assess the effects of acute Hal administration on evoked activity in the striatum. Enhanced dopaminergic activity, caused by intravenous cocaine (Coc) or substantia nigra (SN) stimulation, suppressed striatal responses. Hal also caused suppression; dopamine depleting lesions in the SN blocked the effects of Hal. In addition, the suppressive effects of Coc were not attenuated by Hal. Indeed, the suppressive effects of Coc and Hal were additive. Thus, acute administration of Hal actually augments dopaminergic activity due to strong presynaptic actions and weak postsynaptic blocking effects. This must be taken into account in attempting to understand the mechanisms underlying the neural changes evoked by chronic Hal administration that allow this drug to have antipsychotic potency.

158.19

WAY-124,486: CHARACTERIZATION OF A SERIES OF AMINOMETHYLBENZODIOXANS AS DOPAMINE AUTORECEPTOR AGONISTS. G.P. Stack, K.L. Marquis, N.T. Scherer, Y.H. Kang, T.B. Spangler, R.A. Scerni, T.D. Gardner, A.T. Shropshire, R.F. Kucharik, M.J. Piesla, M. Abou-Gharbia*, T.H. Andree. Wyeth-Ayerst Research, CN 8000, Princeton, N.J. 08543.

A series of aminomethylbenzodioxan derivatives was examined both in vitro (for D_2 agonist and antagonist affinity) and in vivo (in the GBL rat model of DOPA accumulation) for the ability to function as dopamine autoreceptor agonists. The compounds were further characterized behaviorally for their effect on mouse locomotor activity, for the ability to induce (either alone or with the D_1 agonist SKF 38393) stereotypy or climbing or to antagonize apomorphine-induced effects, and for the inhibition of conditioned avoidance responding. Structural elements examined include a variety of alkyl, aralkyl and aryloxyalkyl substitutions on nitrogen, hydroxy, alkoxy, alkyl and halo substitutions on the benzodioxan moiety, and the chirality of the aminomethyl function. Dopamine autoreceptor activity was optimized by a hydroxyl function in the 7 position, alkylation of the amine with a 3-(3-aminophenoxy)propyl group and an S configuration at the asymmetric center. These studies (Wasik et al., Gardner et al., Uzzle et al., this meeting) led to the identification of WAY-124,486 (1) as a dopamine autoreceptor agonist with a preclinical antipsychotic profile.

1 WAY-124,486



158.20

WAY-124,486: A NEUROPHARMACOLOGICAL PROFILE OF A DOPAMINE AUTORECEPTOR AGONIST ANTIPSYCHOTIC. T.P. Wasik, R. Scerni, T. Spangler, G.P. Stack, E.A. Muth, and T.H. Andree* Wyeth-Ayerst Research, CN 8000, Princeton, N.J. 08543

WAY-124,486, an aminomethylbenzodioxan, has been investigated in a series of neurochemical assays to determine its ability to function as a dopamine autoreceptor agonist. *In vitro* receptor-binding studies using male Sprague-Dawley rat striatal homogenates have shown WAY-124,486 to have a very high affinity for D_2 agonist binding (IC_{50} = 0.58 nM; quinpirole as radioligand). At D_2 (antagonist), 5-HT_{1A}, alpha$_1$, D_1 and 5-HT_2 receptors, WAY-124,486 possessed the following IC_{50} values (nM): 47, 15, 6, 2006, 8308. WAY-124,486 was capable of inhibiting *in vitro* DOPA accumulation in rat striatal tissue slices up to 90%, with an ED_{50} of 6.2 µM. A submaximal dose of WAY-124,486 (10 µM) that produced a 65% inhibition of *in vitro* DOPA accumulation was partially reversed (80%) by 100 µM haloperidol, a D_2 antagonist. In experiments designed to test the direct effect of WAY-124,486 on soluble tyrosine hydroxylase (TH), WAY-124,486 in concentrations up to 1 mM was unable to block TH activity. Simultaneously run positive controls of dopamine and apomorphine @ 1 mM, however, produced a 100% inhibition. A forskolin-activated *in vitro* model to analyze the regulation of dopamine synthesis through receptor-mediated control of the phosphorylation of tyrosine hydroxylase is currently under development. In the *in vivo* rat NSD/GBL model WAY-124,486 (10 mg/kg, sc) produced a 64% inhibition of DOPA accumulation that was completely reversed by pre-treatment with raclopride (5 mg/kg, sc). The ability of WAY-124,486 to displace quinpirole binding and to suppress both *in vivo* and *in vitro* DOPA synthesis, the latter of which is not mediated by direct action on TH and is reversed by D_2 antagonists, provides strong evidence for the hypothesis that the effects of WAY-124,486 are mediated through dopamine autoreceptors. Neurophysiological (Uzzle et al., this meeting) and behavioral studies (Gardner et al., this meeting) further support these conclusions. This ability of WAY-124,486 to regulate dopaminergic function is consistent with a preclinical profile of antipsychotic activity.

158.21

WAY-124486: A PSYCHOPHARMACOLOGICAL PROFILE OF A DOPAMINE AUTORECEPTOR AGONIST ANTIPSYCHOTIC. T.D. Gardner, A.T. Shropshire, R.F. Kucharik, M.J. Piesla, G.P. Stack, and K.L. Marquis*. Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

WAY-124486, a benzodioxanmethylamine which inhibits DOPA accumulation in the rat (Wasik *et al.*, this meeting), was evaluated in behavioral studies for its potential antipsychotic activity. WAY-124486 reduced mouse locomotor activity with an ED_{50} = .08 mg/kg ip and .03 mg/kg sc. One hour after oral administration, the potency estimate improved when mice were fasted (ED_{50} = 17 mg/kg po) versus free-fed (no effect up to 30 mg/kg po). When fasted and tested immediately, WAY-124486 reduced activity with an ED_{50} = 5.8 mg/kg po. Disruption of mouse rotorod performance (MED = 10 mg/kg ip) was potentiated when combined with ethanol (MED = 1 mg/kg ip). Similarly, traction reflex deficit occurred at an ED_{50} = 4.4 mg/kg ip. WAY-124486 blocked apomorphine-induced stereotypy and climbing in mice at an ED_{50} = 27.3 mg/kg ip and 1.6 mg/kg ip, respectively. At doses up to 30 mg/kg ip, WAY-124486 did not induce stereotypy or climbing when tested alone, with concurrent D_1 agonist treatment, or after reserpine pretreatment. In contrast, WAY-124486 induced contralateral turning (MED = 1 mg/kg ip) in unilaterally 6-OHDA-lesioned rats. WAY-124486 blocked rat conditioned avoidance responding with AB_{50}s of 5.2-11 mg/kg ip. WAY-124486 disrupts prepulse inhibition of acoustic startle. Thus, the dopamine autoreceptor agonist WAY-124486 exhibits a preclinical profile which predicts antipsychotic activity.

158.22

ACUTE EFFECTS OF WAY-124486, A NOVEL DOPAMINE AUTORECEPTOR AGONIST, ON A9 AND A10 DOPAMINE (DA) NEURONAL ACTIVITY. C. W. Uzzle*, K. L. Marquis, T. H. Andree, G. P. Stack and J. T. Haskins. Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543-8000

WAY-124486 (S)-3-[[[3-(3-aminophenoxy)propyl] amino]methyl]-2,3-dihydro-1,4-benzodioxin-6-ol dihydrochloride binds with high affinity to the dopamine (DA) D_2 receptor subtype and is a functional agonist in both *in vitro* and *in vivo* models. Neurochemical studies have shown this agent to inhibit DA synthesis (Wasik, this meeting) and behavioral studies have shown selectivity for the DA autoreceptor versus postsynaptic DA receptor (Gardner, this meeting). It was therefore of interest to compare the agonist effects of WAY-124486 (WAY) with those of apomorphine (APO) on central DA neuronal activity. Standard neurophysiological methods were used for recording. Following acute i.v. administration, both APO and WAY inhibited A10 (VTA) spontaneous neuronal activity. The ID_{50}s were 15.63 µg/kg and 23.66 µg/kg, respectively. These values are not statistically different. When challenged with the D_2 antagonist haloperidol, the agonist effects of both APO and WAY were completely reversed. These data are evidence that the agonist effects of APO and WAY on the A10 DA autoreceptor are mediated via D_2 receptors. Additional testing is underway to determine the effects of APO and WAY on A9 (SNC) spontaneous neuronal activity and to compare these effects with those observed in A10.

158.23

EFFECT OF CHRONIC ANTIPSYCHOTIC TREATMENT ON EXTRACELLULAR DOPAMINE AND GLUTAMATE CONCENTRATIONS IN THE RAT STRIATUM. B.K. Yamamoto* and M.A. Cooperman. Depts. of Psychiatry and Neuroscience, Case Western Reserve Univ., Cleveland, OH 44106

The effect of chronic treatment with haloperidol or clozapine on basal concentrations and K^+-stimulated dopamine and glutamate efflux in the striatum were studied using *in vivo* microdialysis in the awake-behaving rat. Male rats were injected IP once daily for 21 days with haloperidol (0.5 mg/kg), clozapine (20 mg/kg) or vehicle. One day after the last injection, dialysis probes were inserted into the striatum via a guide cannula. Basal concentrations of dopamine were not significantly different between treatment groups. However, depolarization-induced dopamine release with 80 mM K^+ was significantly attenuated in the haloperidol compared to clozapine or vehicle-treated rats. In contrast to the dopamine concentrations, basal concentrations of extracellular glutamate were significantly higher following chronic treatment with haloperidol. K^+-stimulated glutamate efflux was unaffected by haloperidol or clozapine when compared to vehicle-treated animals. Basal and stimulated taurine levels were not affected by chronic antipsychotic drug treatment. These data are suggestive of a depolarization-inactivation of dopamine nerve terminals in striatum as revealed by an attenuation of the local K^+-induced stimulation of dopamine efflux. These results also provide new evidence for a role of extracellular glutamate in discriminating the neurochemical effects of chronic treatment with typical and atypical antipsychotic drugs. Experiments are currently in progress to investigate the effects of chronic neuroleptic treatment on basal and K^+-stimulated dopamine and glutamate efflux in the nucleus accumbens and medial prefrontal cortex.

[Supported by the Scottish Rite Schizophrenia Research Program]

EPILEPSY: ANTICONVULSANT DRUGS

159.1

MODULATION OF CA1 HIPPOCAMPAL NEURONAL EXCITABILITY BY INHIBITION OF GABA UPTAKE WITH NIPECOTIC ACID. A.A.Oyelese,J.D.Kocsis*&R.H.Mattson,Dept of Neurology and Sect. Neurobiology, Yale Medical School, New Haven, CT 06510; and VAMC, West Haven, CT 06516.

The rationale for the use of nipecotic acid (NPA) in clinical trials as an antiepileptic is based on its role as a GABA uptake inhibitor; inhibiting GABA uptake from the synaptic cleft is suggested to prolong its inhibitory effects on post-synaptic membranes, decreasing the hyperexcitability associated with seizure disorders. Synaptic activity was studied by stimulating Schaffer collateral afferents while recording intracellularly from CA1 neurons and extracellularly (field potentials) from the stratum radiatum in rat hippocampal slices. NPA (1.0 mM) application produced a hyperpolarization with a decrease in amplitude of both the EPSP and IPSP in CA1 neurons. With prolonged application (1 hr), neurons tended to slowly repolarize possibly due to GABA receptor desensitization and GABA clearance. These findings were corroborated by the field recordings which showed a decrease in both population EPSP amplitude and a late positivity which corresponds to IPSP activity. Following return to normal Krebs' solution, field potentials showed persistent burst discharge. Intracellular recordings revealed a repolarization and increase in EPSP amplitude with the appearance of burst discharge. This hyperexcitability upon NPA washout may be explained by increased uptake of GABA by glia on the removal of NPA and decreased GABA release by GABAergic interneurons secondary to prolonged depletion of stores by NPA. These results indicate that NPA modulates the excitability of CA1 neurons in agreement with reduced GABA uptake and secondary to accumulation of GABA.

Supported in part by the NIH and Department of Veterans Affairs.

159.2

ANTICONVULSANT ACTIVITY OF CENTRALLY ADMINISTERED D-CYCLOSERINE IS ANTAGONIZED BY 7-CHLOROKYNURENIC ACID. S.L. Peterson*. Dept. of Medical Pharmacology and Toxicology, Texas A&M University Health Science Center, College Station, Texas 77843.

Previous studies in this laboratory have shown the anticonvulsant activity of systemically administered D-cycloserine (DCS) in maximal electroshock seizures (MES) to be stereospecific and antagonized by 7-chlorokynurenic acid. The purpose of the present study was to characterize the activity of DCS in MES after intracerebroventricular (icv) administration.

MES was induced in male Wistar rats by passing a 60Hz, 150mA and 0.2 sec duration current through saline-soaked corneal electrodes. Seizure severity was quantified by tonic hindlimb extension. The animals were implanted with icv injection guide cannulas 4-7 days prior to testing.

The time of peak anticonvulsant activity for DCS occurred 2 hours after icv administration. The approximate ED_{50} for icv administration at 2 hours was 5 μmole. The anticonvulsant activity of 10 μmole DCS (icv) was completely antagonized by 100 nmole 7-chlorokynurenic acid administered icv 5 min prior to seizure test.

The antagonism by 7-chlorokynurenic acid is evidence that DCS may act at the strychnine-insensitive glycine receptor to inhibit MES. (Supported by NIH grant 24566).

159.3

IN VITRO AND *IN VIVO* EFFECTS OF ANTICONVULSANT DRUGS ON PERIPHERAL BENZODIAZEPINE RECEPTORS OF HUMAN LYMPHOCYTES. C.Ferrarese*, C.Marzorati[1], M.Perego[1], G.Bianchi, G.Moretti[1] and L.Frattola. Dept. of Neurology, University of Milan, S.Gerardo Hospital, Monza, and (1)Scientific Institute E. Medea, Bosisio Parini, ITALY.

We investigated possible effects of antiepileptic drugs on peripheral benzodiazepine receptors (PBR) of human lymphocytes. Drug activities were evaluated on *in vitro* binding of PBR ligands ^{3}H-PK11195 and ^{3}H-Ro5-4864. Possible modifications of PBR density and affinity in lymphocytes incubated with drugs *in vitro* or taken from patients under anticonvulsant medications were also investigated.

Various concentrations of phenobarbital (PB), diphenylidantoine (DPH), carbamazepine (CBZ) and valproic acid (VPA) were added to the binding mixture or incubated overnight with lymphocyte primary culture, and subsequently washed before binding experiments. Lymphocytes prepared from 40 epileptic patients under various treatments and from 10 untreated controls were processed to assess PBR density and affinity in intact cells and in membrane preparations.

Displacement curves of antiepileptic drugs revealed that CBZ and PB may bind to PBR at concentrations similar to plasma therapeutic levels, while various drugs did not affect receptor density and affinity after overnight incubation. On the contrary, long-term treatment in epileptic patients induced different modifications of PBR densities.

Such findings may reveal a role of PBR in mediating immuno-endocrine effects of antiepileptic drugs.

159.4

THE EFFECTS OF PHENYTOIN (PHT) ON 4-AMINOPYRIDINE (4AP)-INDUCED CHANGES IN NEUROTRANSMITTER AMINO ACIDS (AA) IN RAT HIPPOCAMPUS IN VITRO. **I.M. Kapetanovic*, W.D. Yonekawa and H.J. Kupferberg, Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892.**

The balance between the effects of inhibitory (GABA) and excitatory [glutamate (GLU) and aspartate (ASP)] neurotransmitter AA may be important in the pathogenesis and potential treatment of epilepsy. Newly synthesized (NEW) AA may be more closely related to neuronally active pools than to total tissue concentrations. 4AP, a voltage dependent potassium channel inhibitor, causes tonic-clonic and electrographic seizures in vivo and evokes epileptiform activity in the in vitro hippocampal preparation. This study examined the effects of 4AP on AA levels in hippocampal slices and a subsequent response to PHT and other anticonvulsants. Isotopic enrichment, after incubation with $^{13}C_6$-glucose, was used to measure NEW AA. Basal and NEW AA were quantitated by GC-MS of the dimethyl t-butyl silyl derivatives. As would be expected, 4AP (50 μM) stimulated production of NEW AA (46, 102, and 31% in GABA, ASP, and GLU, respectively). The effect of 4AP was reversed by 60 μM PHT (corresponding to therapeutic plasma PHT concentration). Similar findings were seen in preliminary studies with several candidate antiepileptic drugs. PHT also inhibited the production of NEW AA in control (non-4AP stimulated) slices, but to a lesser degree. This study shows predictable response of NEW AA to convulsant and anticonvulsant agents and this approach will be evaluated further in terms of mechanistic and screening potential.

159.5

DIFFERENTIAL EFFECTS OF ANTICHOLINERGIC DRUGS AGAINST SOMAN SEIZURES. J.H. McDonough*, T.-M. Shih, and N.L. Adams, USAMRICD, APG, MD 21010.

The nerve agent soman can produce prolonged seizures and brain damage. We have reported that scopolamine exhibits a time-dependent effectiveness in terminating soman-induced seizures. We examined atropine, benactyzine and trihexyphenidyl to see if they also showed time-dependent effects. Rats implanted with cortical and depth electrodes were pretreated with the oxime HI-6 (125 mg/kg, IP), to prolong survival, 30 min before soman (180 ug/kg, SC). At 5, 20 and 40 min after the onset of EEG seizure activity the rats were treated IV with an anticholinergic drug. Anticonvulsant ED50s (mg/kg) for each drug are presented below.

	5 MIN	20 MIN	40 MIN
SCOPOLAMINE	0.07	8.04	>>51.20
ATROPINE	3.02	39.17	not tested
BENACTYZINE	0.14	0.98	5.62
TRIHEXYPHENIDYL	0.16	1.67	6.49

Although all drugs showed time-dependent dose-effect profiles, only trihexyphenidyl and benactyzine were capable of terminating seizures at all times tested. Trihexyphenidyl has N-methyl-d-aspartate (NMDA) antagonist properties which may account for its anticonvulsant effectiveness at long seizure durations. The results indicate that benactyzine may also possess anti-NMDA activity.

159.6

ANTICONVULSANT EFFECTS OF DIAZEPAM OR MK-801 AGAINST SOMAN SEIZURES. T.-M. Shih*, J.H. McDonough and N.L. Adams. USAMRICD, APG, MD 21010.

Both diazepam and MK-801 can modulate seizure activity induced by soman. In this study we investigated whether these drugs showed a time-dependent effectiveness as a function of seizure duration as did anticholinergic drugs. (See accompanying abstract for preparation and procedures.) A fixed dose of each drug, 2X the ED50 for terminating the seizure at 2.5 min, was used. Diazepam (0.6 mg/kg, IV) or MK-801 (0.088 mg/kg, IV) was quite effective in terminating soman seizures at 5 and 20 min. At both times each drug suppressed spike amplitude and produced profound behavioral depression (ataxia). However, they also enhanced the lethal effects of soman by depressing respiration (particularly MK-801). In many cases, seizure activity reoccurred after 1-3 hrs of suppression. In another experiment, scopolamine (0.4 mg/kg, IV) was given at the 20 and 40 min seizure durations followed 2 min later by diazepam or MK-801. Scopolamine protected against the respiratory effects of both drugs, but did not modify the characteristic anticonvulsant or behavioral effects. We conclude that there was no time-dependence to the effectiveness of these two drugs unlike that observed with anticholinergics.

159.7

FLUNARIZINE LIMITS SUSTAINED REPETITIVE FIRING IN VITRO: A POSSIBLE MECHANISM FOR ITS ANTICONVULSANT EFFICACY.

H. Cheung and E.W. Harris. Dept. of Biology, Fisons Pharmaceuticals, Rochester, NY 14623.

Flunarizine, a potent calcium channel antagonist, has been found to possess anticonvulsant efficacy. Despite the wide spectrum of Ca^{2+} channel blocking activities observed in vitro, there is no evidence that the anticonvulsant activity of flunarizine is mediated via neuronal Ca^{2+} channels. The similarity in the convulsant profile of flunarizine and phenytoin suggests that they may have a common mechanism of action. Like phenytoin, flunarizine blocks neuronal Na^+ channels in a use- and voltage-dependent manner, as demonstrated by its ability to limit high-frequency sustained repetitive firing in spinal cord cultured neurones (with an IC_{50} value in the submicromolar range). In addition, flunarizine has also been shown to displace [^{3}H]batrachotoxin binding in the rat brain synaptosomes (IC_{50} = 0.69μM). Both electrophysiological and biochemical evidence suggests that the anticonvulsant action of flunarizine may be mediated by blocking the neuronal voltage-activated Na^+ channels.

159.8

CONVULSANT ACTION AND BINDING PROPERTY OF THE PERIPHERAL-TYPE BENZODIAZEPINE, Ro 5-4864, IN El MICE. Y. Nakamoto, M. Yoshii and K. Yamamoto*. Dept. of Neurophysiology, Tokyo Institute of Psychiatry, Tokyo 156, Japan.

It is well known that "central-type" benzodiazepines (BDZs) such as diazepam and clonazepam act as potent anticonvulsants, whereas "peripheral-type" BDZs such as Ro 5-4864 act as convulsants. Unlike receptors for the central-type, those for the peripheral-type remain to be characterized for their biological roles including proconvulsant action. In the present study, we have investigated how peripheral-type BDZ receptors contribute to seizure disorders in the El mouse, an excellent model of epilepsy, based on behavioral experiments and binding assays. Adult El mice (25-30g) and the control, DDY mice (30-35g), were used. Threshold of seizure in El mice was determined weekly for each animal. Ro 5-4864 induced seizures in El mice at 10 mg/Kg or higher doses (i.p.). In DDY, the action of Ro 5-4864 was three-times less potent. The seizures induced by Ro 5-4864 were inhibited by PK 11195 (10-15 mg/Kg, i.v.), an antagonist for the peripheral-type BDZ receptor. PK 11195 also raised the threshold of seizure in El mice. Binding assays revealed slight decreases in Bmax's of ^{3}H-flunitrazepam and ^{3}H-Ro 5-4864 in the cerebral cortex of El mice. The results suggest that peripheral-type BDZ receptors in the brain play a proconvulsant role in the El mouse, possibly with an altered distribution of both types of BDZ receptors.

159.9

CLASSIFICATION OF NIGRO-TECTAL NEURONS BASED ON RESPONSES TO BENZODIAZEPINE. X. Weng and H.C. Rosenberg, Dept. of Pharmacology, Med. Coll. of Ohio, Toledo OH 43699.

Previous work showed that microinjection of GABA agonists and benzodiazepines (BZs) into substantia nigra had varying effects on different experimental seizures. An explanation was suggested by the observation that nigral neurons were differentiated by their responses to iontophoretic BZ. As some studies suggested that the nigro-tectal pathway was critical in seizure modulation, we hypothesized that the varying effects of intranigral drug on seizures might result from the different responses of nigro-tectal neurons to BZ. Male, Sprague-Dawley rats were anesthetized with chloral hydrate. Nigro-tectal neurons were identified by antidromic activation. Multi-barreled glass electrodes were used to record spontaneous activity of single neurons. GABA and BZs were applied iontophoretically, using several currents for each. Recording sites were marked for later confirmation. As a function of increasing current, both BZs and GABA decreased the rate of neuronal discharge. Most nigro-tectal neurons could be completely silenced by GABA, but with widely varying sensitivities. Based on the maximum effect obtained with BZs, the neurons could be divided into categories. Twelve neurons were inhibited > 75%, while 13 neurons were maximally inhibited < 55% by BZs. This suggested that nigro-tectal neurons can be differentiated by their responses to BZs. The GABA-A/BZ receptors of these neurons will be studied. Supported by DA02194.

159.10

FCE 26749: A NEW AND POTENT ANTICONVULSANT ACTIVE IN EXCITOTOXIN SEIZURE MODELS. M Varasi*, R Maj, A. Bonsignori, N Carfagna, R McArthur and R Fariello. CNS/CVS Research, Farmitalia Carlo Erba, Erbamont Group, 20014 Nerviano, Italy

FCE 26749 [(S)-(+)-2-(4-Benzyl-aminobenzyl) amino-propanamide], exhibits potent anticonvulsant activity in excitotoxin seizure models. On the basis of oral ED_{50} doses determined 60 min after in the rotarod (217 mg/kg) and antagonism of maximal electroshock seizures (9.5 mg/kg) tests, FCE 26749 was selected for further study. FCE 26749 (-60 or -180 min) in mice antagonized the tonic convulsions (ED_{50}'s = 48 and 80 mg/kg; *po*) induced by NMDA (225 mg/kg; *sc*).

Seizures and status epilepticus (SE) in rats induced with kainic acid (KA 10 mg/kg;*ip*) was antagonized by both diazepam (20 mg/kg;*ip* [6/10 and 7/10 rats protected from seizures and SE respectively]) and FCE 26749 (0.5 [1/6, 1/6], 1.0 [4/6, 4/6] and 3.0 [9/12, 10/12] mg/kg;*ip*) administered 15 min before KA. In cortical membrane preparations, FCE 26749 showed little displacement (pKi < 5) of selective ligands for the NMDA, MK-801, glycine, kainate or quisqualate receptor sites. These results indicate that FCE 26749 is a broad spectrum anticonvulsant agent in seizure models resistant to chronic treatment.

159.11

Opiate antagonists have selective effects on morphine-induced potentiation of picrotoxin-induced seizures. W. L. Nores, J. Thomas, V. Kenigs, A. J. Kastin, G. A. Olson*, and R. D. Olson. Department of Psychology, University of New Orleans, New Orleans, LA 70148

The present study evaluated the efficacy of the opiate antagonists Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2), MIF-1 (Pro-Leu-Gly-NH_2), and naloxone against morphine-potentiated picrotoxin-induced seizures. 40 male rats were injected IP with saline, naloxone (3 mg/kg), Tyr-MIF-1 (3.0 mg/kg), or MIF-1 (3.0 mg/kg). After 15 min, they were injected IP with saline or morphine (50 mg/kg). After another 15 min, they received a SC injection of picrotoxin (3.0 mg/kg). The rats were then observed for 45 min and scored on several parameters of different categories of seizures. An ANOVA yielded a significant main effect for the number of focal seizure episodes, $p<.05$, with all combinations of morphine, except the naloxone+morphine group, increasing the number of focal episodes but not differing from each other. Naloxone reduced the number of focal seizures to a level comparable to that of saline. The incidence of generalized clonic seizures was significanct, $p<.05$, and displayed a similar pattern of effects among the groups. Finally, morphine increased the duration of akinetic seizures relative to saline; consistent with their known antiopiate activity, the effect was reduced by all three antagonists. The results suggest that the opiate system interacts with picrotoxin-mediated seizure mechanisms.

159.12

TOLERANCE TO THE ANTICONVULSANT EFFECTS OF PENTOBARBITAL: GREATER TOLERANCE WITH AN ASCENDING-DOSE REGIMEN. L.E. Kalynchuk*, C.K. Kim, J.P.J. Pinel and T. Kippen. Dept. of Psychology, University of British Columbia, Vancouver, B.C., Canada. V6T 1Z4.

In clinical practice, it is common to initiate anticonvulsant therapy at a low dose and to increase it until a therapeutically effective dose is reached. On the basis of the drug-effect theory of tolerance (see Pinel, Kim & Mana, 1990), we predicted that this regimen would promote tolerance development and thus interfere with anticonvulsant therapy. The present study was undertaken to determine the degree of tolerance development to the anticonvulsant effects of pentobarbital with four different dose regimens in amygdala-kindled rats. After kindling, the rats (35 male, Long-Evans) received a convulsive stimulation once every 48 hr for the duration of the experiment. Following five baseline stimulations, the rats received one of four treatments of pentobarbital 1 hr before each of the 20 stimulations of the tolerance-development phase: a constant low dose (10 mg/kg), an ascending dose regimen (10 mg/kg to 20 mg/kg), a constant high dose (50 mg/kg), or saline. On the tolerance test, all rats received a test dose (20 mg/kg) of pentobarbital 1 hr before stimulation. The ascending dose rats displayed significant tolerance to the anticonvulsant effects of pentobarbital whereas the low and high dose rats did not. These results provide further support for the drug-effect theory of tolerance, and suggest that initiating anticonvulsant therapy at doses high enough to block key neural components of convulsions may result in little tolerance to anticonvulsant effects. (Supported by an NSERC grant to JPJP)

159.13

CARBAMAZEPINE AND PHENYTOIN INHIBIT NMDA RECEPTOR-MEDIATED CURRENTS IN THE RAT HIPPOCAMPUS. T.Dalkara, C.Ayata, G.Erdemli and R.Onur. Depts. of Neurology and Pharmacology, Hacettepe Univ. Fac. of Medicine, Ankara, TURKEY. (SPON: ENA).

Involvement of NMDA receptors in epileptic activity is well established. A recent paper* reporting inhibition of NMDA currents in cultured spinal neurons by carbamazepine (1 to 50 µM) prompted us to examine the effects of 4 antiepileptic drugs on the NMDA receptor activity in the rat hippocampus, in situ. The NMDA receptor activity was evoked by high frequencv (5-7 pulses at 100 Hz and 0.1 train per sec.) fimbrial/commissural stimulation. Carbamazepine (n=4) and phenytoin (n=5), at a dose (10 mg/kg) which is protective against maximal electroshock seizures, decreased the amplitude of NMDA receptor-mediated component of the field potential (NMDA-FP) by 51±16 % and 41±7 %, respectivelv, whereas phenobarbital and diazepam (10 mg/kg i.v.) had no appreciable effect. Local application of carbamazepine and phenytoin by pressure injection also inhibited the NMDA-FP by 27±6 % (n=4) and by 25±6 % (n=4), respectively. Extracellular DC potential shifts produced by iontophoretically applied NMDA in stratum radiatum were also antagonized by pressure injection of carbamazepine (70±21%, n=3) and phenytoin (81±27 %, n=9) but not of phenobarbital or diazepam. These data suggest that inhibition of the NMDA receptor-mediated currents may contribute to the antiepileptic action of carbamazepine and phenytoin.

*NeuroReport (1990) 1(Sampler):8-10.

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SYMPOSIUM. RECENT ADVANCES IN NEUROPEPTIDE BIOSYNTHESIS: MOLECULAR AND CELLULAR BIOLOGY OF NEUROPEPTIDE PROCESSING ENZYMES. Lloyd D. Fricker, Albert Einstein Coll. of Med. (Chairperson); Nabil G. Seidah, Clinical Research Inst. of Montreal; Betty A. Eipper, Johns Hopkins Univ.; Stanley J. Watson, Univ. of Michigan.

The field of neuropeptide biosynthesis has undergone an explosive growth in the last few years. Major advances have occurred in the area of neuropeptide-processing endopeptidases; a family of endopeptidases have been identified (furin, PC-1, PC-2, and others). Recent studies on two other neuropeptide-processing enzymes, carboxypeptidase E and the amidating enzyme, have yielded many new results and some surprises; for example, the 'amidating enzyme' is two separate enzymes produced from a single protein precursor, which work together to form C-terminal amide groups. These recent findings will be presented, along with a concise review of the field. **Nabil Seidah** will discuss the cloning of PC-1 and PC-2, and the expression of these enzymes in a variety of cells. The substrate specificities and biochemical properties of the enzymes will be presented. The regulation of the enzymes will also be discussed, with a focus on the significance for neuropeptide biosynthesis. **Lloyd Fricker** will present the cloning of the carboxypeptidase E gene and analysis of the promoter. The regulation of carboxypeptidase E mRNA and enzyme activity will be discussed, along with the implications of this regulation on the processing of neuropeptides in neurons and in non-neuronal cells. **Betty Eipper** will describe the two enzymes whose sequential action is required for peptide amidation, and the single gene encoding both enzymes. The importance of tissue specific alternative mRNA splicing and post-translational proteolytic processing in the generation of soluble and integral membrane forms of these enzymes will be presented. Protein domains which affect the routing through neuroendocrine cells will also be discussed. **Stan Watson** will present the distribution of PC-1, PC-2, furin, CPE, and PAM in brain and other tissues. Emphasis will be placed on whether the enzymes are present exclusively in neuronal cells. The distribution of each enzyme will be compared with the other enzymes, and with the distribution of known neuropeptides, with a focus on the implications for neuropeptide-processing.

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SYMPOSIUM. REGENERATION OF VERTEBRATE SENSORY RECEPTORS. E.W Rubel, Univ. of Washington (chair); P.A. Raymond, Univ. of Michigan; A.I. Farbman, Northwestern Univ.; H.H. Zakon, Univ. of Texas

Loss of sensory function in man and other vertebrates is usually due to destruction or degeneration of the receptor epithelium. The ability to regenerate these receptor surfaces varies markedly across vertebrate classes and across different receptor organs. In this symposium we will explore structural, functional and cellular events associated with the regeneration of vertebrate receptor cells for hearing, balance, vision, olfaction, and electroreception. E. Rubel will discuss recent experiments on regeneration of hair cells in the inner ear of birds. P. Raymond will consider regeneration of rods and cones in the retinas of fish and amphibians. A. Farbman will discuss regeneration of chemoreceptors in mammals. H. Zakon will examine electroreceptors in fish. The emphasis of each presentation will be on recent data attempting to further define the cellular events leading to proliferation and differentiation of the regenerated receptor epithelium.

166.1

AUDITORY MIDBRAIN CELLS IN GOLDFISH: TRANSFORMATIONS OF PERIPHERAL INPUT. R.R. Fay* and J. Lu. Dept. Psychology and Parmly Hearing Institute, Loyola Univ. of Chicago, IL 60626.

Single units from the torus semicircularis (TS) were studied using pipet and indium electrodes in response to tones. Iso-level response areas (RA) were obtained for frequencies between 75 and 1250 Hz. Peristimulus time (PST), inter-spike interval (ISI), period histograms, and scatterplots of inter-spike interval versus spike time (ISIT) were obtained from >150 TS units for comparison with >200 single fibers of the auditory (saccular) nerve (ANF). TS units show less spontaneous activity, greater average sensitivity, sharper tuning (Q10dB up to 5), steeper tuning curve slopes(>200 dB/octave), and more diversity in PSTH patterns than ANFs. While all ANFs phase-lock to the acoustic waveform, only a subset of TS units show phaselocking. TS units that phaselock robustly tend to produce one spike/cycle (entrain) for up to 200 ms with less average error (temporal jitter) than ANFs (ISI standard deviations <40 μs). Some TS units phaselock at both positive and negative phases of the stimulus waveform, as is the case for ANFs. Robustly phaselocked TS units are probably inputs from medullary nuclei. Nonphaselocked TS units fall into several categories based on PSTH shape, similar to some mammal cochlear nucleus units: onset, buildup, pauser, primary-like, and choppers. Choppers have regular interspike intervals (shown in ISIT) that are not phaselocked to the stimulus. Some ANFs show onset and pauser patterns, but not buildup or chopper patterns. In iso-level functions, the frequency producing the most spikes (BF) tends to converge toward 500 Hz as level is raised in ANFs, but not in TS units. This "BF constancy" results in a more secure and robust rate code for frequency in the TS than in the periphery. The origins of this important transformation are being investigated using more complex stimuli. [Supported by NIH, NIDCD]

166.2

INTRACELLULAR RECORDINGS FROM NEUROBIOTIN-LABELED PRINCIPAL CELLS IN BRAIN SLICES OF THE GUINEA PIG MSO. P.H. Smith* and M.I. Banks. Depts. of Anatomy and Neurophysiology and Neuroscience Training Program. Univ. of Wisconsin, Madison, WI. 53706.

We recorded intracellularly from principal cells in the medial superior olive (MSO) of 10 to 20 day-old guinea pigs. Above threshold, cells fired one or a few action potentials at the onset of a depolarizing current pulse. At higher currents, repetitive firing could be elicited. A large hyperpolarizing pulse could generate a membrane potential "sag" back toward rest. Shock stimuli at the brainstem midline could evoke a short latency epsp and a strychnine-sensitive ipsp. As shock strength increased the ipsp currents could act to reduce epsp duration and thus "sharpen" the epsp peak. Shocking the brainstem lateral to the ipsilateral lateral superior olive could elicit similar events. So far, injected cells have been located in the ventral, higher frequency half of the MSO. The dendritic configuration was typically bipolar in the coronal plane. The thick primary dendrites could branch close to the cell body forming multiple thick dendrites that would rarely branch as they headed medially or laterally. In one cell these large thick dendrites terminated in tufts of string-like appendages. In other cells only a few small appendages accentuated the dendritic termination. The dendritic tree could extend for considerable distances outside the MSO-proper. The axon arose from a primary dendrite or cell body and headed dorsally within the body of the MSO. When the axon could be followed past the dorsal aspect of the MSO, axon collaterals were given off that terminated in the dorsal, lower frequency regions of the MSO.

Supported by NIH First Grant NS26285.

166.3

REPRESENTATION OF TONAL CONSONANCE AND DISSONANCE IN THE TEMPORAL FIRING PATTERNS OF AUDITORY NERVE FIBERS: RESPONSES TO MUSICAL INTERVALS COMPOSED OF PURE TONES VS. HARMONIC COMPLEX TONES

M.J. Tramo*, P.A. Cariani, and B. Delgutte Dept of Neurobiology, Harvard Med Sch, Boston, MA 02115, and Eaton-Peabody Lab, Mass Eye & Ear Infirmary, Boston, MA

Tonal dissonance has been attributed to fluctuations in amplitude that give rise to the sensation of beats and roughness. To investigate how beats and roughness are represented in the temporal discharge patterns of auditory nerve fibers, we recorded the responses of single units in Dial-anesthetized cats to stimuli associated with varying degrees of consonance and dissonance. Stimuli were presented at 60 dB SPL and consisted of: 1) two simultaneous pure tones whose frequencies were in the ratios of 1:2 (musical fifth), 2:3 (fourth), 15:16 (minor second), and 32:45 (tritone); and 2) two simultaneous complex tones with fundamental frequencies at the same ratios, each composed of six equal-amplitude harmonics. Post-stimulus time histograms (PSTH) and all-order interspike interval distributions (autocorrelation histograms, AH) were computed. For the pure tone minor second, the most dissonant of the pure tone intervals, the envelope of PSTH for all fibers showed broad local maxima and minima separated by the difference frequency. This beating pattern was reflected in AH by a broad maximum near the difference frequency. For no other pure tone interval was this beating pattern found. For harmonic complex intervals, beating patterns were observed when fiber characteristic frequency (CF) was near two closely spaced components. For the complex minor second, beating patterns were observed over the full range of CFs encompassing the stimulus components. For the fourth and fifth, the most consonant of the complex intervals, relatively weak beating patterns were found only for a small number of units whose CFs were near the most closely spaced components. AH showed prominent peaks at the common fundamental period of these harmonic complexes. For the complex tritone, beating patterns were observed over an intermediate number of CFs. These results indicate that the tonal dissonance of musical intervals correlates with the total number of auditory nerve fibers whose responses show beating patterns, and provide physiological support for the hypothesis that the dissonance of complex tones reflects the sum of the dissonances of adjacent partials.

Supported by DC00071, DC00006, and DC00019

166.4

CORRESPONDENCE OF FUNCTIONAL TOPOGRAPHIES IN CAT PRIMARY AUDITORY CORTEX FOR ACOUSTIC AND ELECTRIC COCHLEAR STIMULATION. M.E. Raggio, C.E. Schreiner* and M.M. Merzenich. Coleman Laboratory, Dept. Otolaryngology, University of California, San Francisco, CA 94143-0732.

The evaluation of electrically-evoked physiological patterns in auditory cortex, and their subsequent changes with meaningful stimuli in an animal model, represents a first attempt at defining the role of cortical representation and representational plasticity in the speech perception of cochlear implant patients. The effects of peripheral electrical stimulation on the primary auditory cortex (AI) of cats was evaluated and compared with the functional topographies for acoustical stimulation in the same animal.

A 'map' of the spatial distribution of neuronal responses in AI of adult cats to basic acoustical stimulus properties such as frequency, bandwidth, and intensity, was obtained with 50 to 100 microelectrode penetrations. The cat was then implanted with a four electrode pair cochlear prosthesis in the scala tympani, and the spatial distribution of response thresholds and temporal responses, with stimulation of each electrode pair, was then mapped at the same locations. The resulting alignment of acoustic and electrical response patterns revealed electrical sensitivity profiles that were cochleotopically appropriate. Comparison of electrical response thresholds with acoustic response thresholds revealed an inverse correlation, i.e. acoustically more sensitive locations were electrically relatively insensitive. (NIDCD N01-0-2401)

166.5

COMPARISON OF C-FOS EXPRESSION WITH 2-DEOXYGLUCOSE-UPTAKE IN THE AUDITORY AND PREFRONTAL CORTEX AFTER ACOUSTIC STIMULATION, CLASSICAL CONDITIONING OR ACTIVE AVOIDANCE TRAINING

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Previous studies using the ^{14}C-2 deoxyglucose method as marker for elevated electrical activity have shown a complex functional organisation of the auditory cortex of gerbils (Meriones unguiculatus) with a parcellation into at least 7 cortical fields, most of which contain tonotopic maps (Scheich, Current Opinion in Neurobiology 1991, 1: 236-247). The 2 DG method was also successful to demonstrate changes in the frequency representation after aversive conditioning (Simonis and Scheich, Eur. J. Neurosci. Suppl. 3, p156, 1990). In the present study we combined 2 DG autoradiography with the immunocytochemical detection of the proto-oncogene c-FOS to analyze the relationship of the two markers in the auditory and prefrontal cortex after acoustic stimulation (900-1100 Hz, frequency modulated, 70 dB/SPL), classical auditory conditioning and active avoidance training.

Compared to naive controls the tone stimulated and conditioned animals showed an increased c-FOS expression in various subfields of the auditory and prefrontal cortex, with the strongest labeling in the active avoidance group. c-FOS expression in some areas corresponds to the 2 DG patterns both in terms of spatial extent and experience dependent activation. This indicates a certain yet complex relationship between the functional measures which depend on the brain area.

Supported by DFG: SFB 45

166.6

FREQUENCY TUNING OF THE HUMAN AUDITORY CORTEX M. Sams* and R. Salmelin. Low Temp. Lab., Helsinki Univ. Technol., 02150 Espoo, Finland.

We employed magnetoencephalographic (MEG) recordings to study frequency tuning of the human auditory cortex. A test sound of 1 or 2 kHz (100 ms, 50 dB SPL) was delivered once per second to the subject's left ear, together with a continuous masker. The masker was either white or notched noise. The notch widths ranged from very wide (4000 Hz) to those inside the critical band. A measurement was started by finding the level of the white-noise masker which just rendered the sound inaudible. Thereafter the masker level was kept constant. The test sounds with different maskers were delivered in separate blocks, in random order.

The N100m response to test stimuli, originating in the auditory cortex, was recorded with a 24-channel neuromagnetometer.

The perceived intensity of the test stimulus increased with the notch width. A parallel effect was seen in the N100m amplitude, plotted as a function of the notch bandwidth. The shapes of the curves measured for the 1-kHz and 2-kHz test sounds resembled those obtained in psychoacoustical experiments studying the shape of auditory filters. The reults show that frequency tuning of the human auditory cortex can be studied noninvasively by MEG.

166.7

A SITE OF PLASTICITY IN THE BRAINSTEM OF THE BARN OWL: REGULATION OF INHIBITION IN RESPONSE TO EARLY MONAURAL OCCLUSION. J. Mogdans* and E.I. Knudsen, Dept. Neurobiology, Stanford University, Stanford CA, 94305.

Monaural occlusion during early life causes adaptive changes in the tuning of units to interaural level differences (ILD) in the barn owl's optic tectum and inferior colliculus. In order to identify the site of plasticity in the auditory pathway, we investigated whether these changes reflect adjustments at the first site of binaural interaction: the posterior division of the nucleus ventralis lemnisci lateralis (VLVp; functional equivalent of the mammalian LSO). VLVp units are excited by sound in the contralateral ear and inhibited by sound in the ipsilateral ear (EI units). Within the nucleus, the strength of inhibition decreases systematically from dorsal to ventral to create a map of ILD.

We compared the maps of ILD in the left and right VLVp of owls raised with one ear plugged. Using dichotic stimulation and two independent measures for the strength of inhibition, we found that units in the VLVp ipsilateral to the occluded ear were inhibited more strongly than units at comparable depths in the contralateral VLVp, indicating a relative shift in the ILD maps. Moreover, the slope of the gradient of inhibition along the dorsoventral axis of VLVp was steeper on the contralateral side. In contrast, excitatory response functions had thresholds and slopes that were the same on the two sides of the brain. These findings indicate that the brain alters the sensitivity of VLVp units to ILDs by regulating the strength of inhibition, and that plastic changes in VLVp are largely responsible for the adaptive changes in ILD tuning observed at higher levels in the auditory pathway.

Supported by a DAAD-fellowship and NIH grant DC 00155-12.

166.8

FREQUENCY DOMAIN ANALYSES REVEALED CONNECTIONS BEYOND STIMULUS LOCKING IN THE AUDITORY THALAMUS A.E.P. Villa*, D.R. Brillinger and F. de Ribaupierre , Institut de Physiologie, UNIL, CH 1005 Lausanne, Switzerland and Department of Statistics, UC Berkeley, Berkeley, CA 94720

Single unit spike trains were simultaneously recorded in the auditory sector of the thalamic reticular nucleus (RE) and medial geniculate body (MGB) of nitrous oxide anesthetized cats. Second order analyses to measure association between 129 pairs of spike trains, A and B, one in RE and one in MGB were carried out. Each unit was studied during spontaneous activity and acoustically evoked activity by white noise bursts. Time domain analyses give the short term firing rate of the B train at a certain time lag after an A spike. This analysis provides an unsatisfactory description of the association between the units if rate effects exist in the presynaptic spike trains. This is particularly true for the bursty units within RE. In a different approach frequency domain coherences were estimated. These reflect the degree of linear time invariant association between the cells as a function of frequency. This method can be extended and the partial coherence analysis *removing* the effects of the point process corresponding to the stimulus onset carried out. Almost half of the pairs (16/34) exhibit a significant difference between partial coherence and coherence during spontaneous activity. The frequencies concerned by these modifications were below 30 Hz in the vast majority of these pairs (75%). These results support the role of RE in selective filtering of the incoming auditory input (Villa et al., Exp. Brain Res. 86:506, 1991). Analyses of the spike trains in the frequency domain led to a physiologically meaningful conclusion: many functional connections in the auditory thalamus are modulated by the stimulus but not necessarily time-locked to its onset.

166.9

QUANTITATIVE ELECTROENCEPHALOGRAPHIC (QEEG) CORRELATES OF HUMAN AUDITORY-CORTEX ANATOMICAL ASYMMETRIES QUANTIFIED USING MAGNETIC RESONANCE IMAGING (MRI): EARLY RESULTS IN THE CNS PROJECT. J. L. Lauter* and E. Plante, Univ. of Oklahoma Health Sciences Cntr., Oklahoma City OK 73190; and Univ. of Arizona, Tucson AZ 85721.

A number of studies, using dissection, CT, and MRI, have described anatomical asymmetries in human auditory cortex around the Sylvian fissure, concluding that some degree of asymmetry is the norm, while symmetry is predictive of pathology, e.g., in language processing. However, there are no physiological data "bridging" between these observations of anatomy on the one hand vs. behavior on the other. Eight neurologically normal young adults were examined with a cross-section of our Coordinated Noninvasive Studies (CNS) Project test battery, including MRI and a qEEG session with both resting and activation conditions. Distinct correlations between MRI and qEEG measures related to periSylvian asymmetries, such as qEEG power asymmetry and coherence, suggested that some purportedly neurologically normal subjects are at risk for subtle neurological dysfunction. MRI/qEEG measures in a second group (four more neurologically normal by report, and three patients: one with a history of stuttering, and two with central-auditory complaints) both supported and extended the original predictions. In general, the data suggest that a variety of "neurologically silent" disorders, such as stuttering, mild learning disorder, central-auditory processing disorder, and substance abuse, may be associated with distinct patterns of anatomical/electrophysiological characteristics in periSylvian cortex.

166.10

EARLY ONSET OF SYNAPTIC TRANSMISSION TO NEURONS IN THE SUPERIOR OLIVARY COMPLEX OF THE RAT. K. Kandler* and E. Friauf, Dept. Animal Physiology, Univ. of Tübingen, 7400 Tübingen, Germany.

Neurons of the rat cochlear nucleus already project to the superior olivary complex (SOC) two days prior to birth, i.e. about two weeks before the ear canals open and physiological hearing starts. Until now it was unknown whether these afferent connections to the SOC function during this period, when the auditory system is still devoid of physiological input. We addressed this question by recording intracellulary from young SOC neurons (postnatal days 2-19) *in vitro* while electrically stimulating afferent fibers in the ventral acoustic stria (VAS) ipsilaterally and contralaterally. Physiologically characterized neurons were identified by intracellular injection of biocytin.

Stable recordings lasting over several hours were obtained from neurons located in the medial nucleus of the trapezoid body, the lateral superior olivary nucleus, the superior paraolivary nucleus, and periolivary nuclei. The neurons had resting potentials from -45 to -73 mV and generated action potentials with amplitudes up to 62 mV during depolarizing current injections. Neurons with resting potentials more negative than -50 mV were not spontaneously active. Stimulation of the VAS resulted in excitatory and inhibitory postsynaptic potentials. Already at P2, EPSPs were able to give rise to action potentials. In most cases, the EPSPs could be blocked by CNQX (20 μM), suggesting that they were mediated by glutamate acting on non-NMDA receptors. EPSPs were observed after ipsilateral stimulation, whereas EPSPs or IPSPs occured after contralateral stimulation. These data indicate that ipsilateral and contralateral inputs converge on SOC neurons shortly after birth and that bilateral synaptic interactions can already take place at least ten days before the onset of hearing.

Supported by the DFG (Fr 772/1-2) and the GKN Tübingen.

166.11

REGRESSIVE EVENTS IN THE DEVELOPING AUDITORY SYSTEM: CALBINDIN-D28K IMMUNOREACTIVITY IN THE SUPERIOR OLIVARY COMPLEX OF RATS. E. Friauf*. Dept. Animal Physiology, Univ. of Tübingen, 7400 Tübingen, Germany.

Calcium-binding proteins, such as calbindin-D28k (CaBP), are widely distributed in the brain and may function to protect neurons against an excess of intracellular calcium. Using monoclonal antibodies against CaBP, we have investigated the distribution of this protein in the superior olivary complex of developing rats. Consistent with the findings of others (e.g., *MR Celio 1990, Neuroscience 35:375-475*), strong CaBP immunoreactivity was found in virtually all somata of the medial nucleus of the trapezoid body (MNTB). Furthermore, immunoreactivity was present in boutons around the neurons of the lateral superior olive (LSO), the medial superior olive, the superior paraolivary nucleus, and the lateral nucleus of the trapezoid body, whereas the somata themselves were unlabeled. As these nuclei are known to receive input from MNTB principal neurons (c.f., *I Sommer et al., this meeting*), it is likely that at least some of the immunoreactive boutons represent terminals of MNTB neurons.

During development, CaBP immunoreactivity in MNTB somata can first be detected at postnatal day 8 (P8), i.e., about four days before the onset of physiological hearing. Surprisingly, heavily immunoreactive LSO neurons are also present at that age. They can be seen throughout the neonatal life and are even present prenatally. Around P6, immunoreactivity in LSO neurons becomes most intense and subsequently decreases until somatic labeling can no longer be found after P21. The transient appearance of CaBP in the LSO shows that there are regressive events during the development of the auditory system and suggests that intracellular calcium may have to be regulated precisely in LSO neurons at a time when synaptic contacts are likely to be rearranged. Supported by the DFG (Fr 772/1-2).

166.12

THE PITCH OF COMPLEX SOUNDS IS SIMPLY CODED IN INTERSPIKE INTERVAL DISTRIBUTIONS OF AUDITORY NERVE FIBERS. P.A. Cariani, B. Delgutte, and N.Y.S. Kiang*. Massachusetts Institute of Technology and Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, 243 Charles St., Boston, MA 02114.

Temporal theories of pitch perception state that the perceived pitch corresponds to the most frequent interspike interval present in the auditory nerve. In order to assess the physiological plausibility of such models, we recorded from auditory nerve fibers in Dial-anesthetized cats, using a wide variety of complex stimuli which have been extensively studied in psychophysical pitch experiments. Stimulus pitches ranged from 80 to 480 Hz.

We computed "autocorrelograms" representing the distribution of all (1st, 2nd, 3rd,...) interspike intervals in a single fiber and summed 40-80 individual autocorrelograms covering a wide CF range to estimate an interval distribution for the entire auditory nerve.

For harmonic stimuli having unambiguous pitches (single-formant vowels and harmonic tone complexes), the most frequent intervals in the aggregate autocorrelogram corresponded to the fundamental period and the pitch period. For inharmonic stimuli with ambiguous pitches (sinusoidally-amplitude modulated (SAM) and quasi-frequency-modulated (QFM) 640 Hz tones), the aggregate distributions showed multiple peaks of roughly equal height at interspike intervals corresponding to the periods of the multiple pitches that would be heard by humans. Interval distributions for psychophysically-similar SAM and QFM tones were similar. Stimuli with weaker pitches (SAM broadband noise and SAM 6400 Hz tones) had less salient peaks in their interval distributions relative to backround.

We also found physiological correlates for the phenomenon of "pitch dominance", wherein the pitch of a tonal complex of lower harmonics (3-5) is heard over the slightly different pitch of a tonal complex of higher harmonics (6-12) of a slightly different fundamental when both complexes are presented simultaneously. Where the pitch of one complex is heard over the other, the interspike intervals corresponding to the pitch which is heard are more frequent than those corresponding to the pitch of the other complex.

These findings suggest that 1) pitch corresponds to the most frequent interspike interval present in the entire auditory nerve 2) ambiguous pitches corrresond to interval distributions with multiple, roughly equal peaks, 3) pitch strength is related to the relative heights of peaks in the interval distribution, and 4) an account of "pitch dominance" can be framed in these terms. Supported by N.I.H. DC 00019 and DC 00006.

166.13

BRAINSTEM PATHWAYS AND BRANCHING PATTERNS OF BIOCYTIN-LABELED OLIVOCOCHLEAR NEURONS IN THE MOUSE.
M.C. Brown* and K.D. Whitley. Depts. of Physiol. and Otol. and Laryngol., Harvard Medical School, and Eaton-Peabody Lab., Mass. Eye & Ear Infirmary, Boston, MA 02114.

Olivocochlear (OC) neurons transmit feedback information from the superior olivary complex in the brainstem to the organ of Corti in the cochlea. We traced OC axons by retrogradely labeling them with extracellular injections of biocytin into the spiral ganglion in CD-1 mice. Tissue was processed with an ABC kit or with an anti-biotin antibody. Two groups of somata were labeled in the brainstem. Lateral olivocochlear (LOC) somata were located entirely within the ipsilateral lateral superior olive. Medial olivocochlear (MOC) neurons were located bilaterally in the medioventral periolivary and rostral periolivary nuclei, and rarely within the lateroventral and dorsal periolivary nuclei. LOC neurons gave off thin axons (diam. 0.5 to 0.7 μm) whereas MOC neurons generally gave off thicker axons (diam. 0.7 to 1.4 μm). From the somata, the axons traveled dorsally to the region of the facial genu, at which point the fibers from contralateral somata crossed the midline. The thick and thin fibers were intermingled from near the ipsilateral facial genu to the ipsilateral vestibular nerve, where they left the brain. There were a few examples of bilaterally projecting thick fibers. Axon branches from thick fibers were often given off to the cochlear nucleus (basal and apical injections) and to the inferior vestibular nucleus (basal injections only). Thin fibers gave off a few branches to the ventral part of the lateral superior olive. Brainstem branches from olivocochlear neurons are a means by which the OC system can exert influence on diverse areas within the central nervous system as well as on the organ of Corti.

(Supported by NIH grants DC00351 and DC00119.)

PAIN: PHYSIOLOGY AND BEHAVIOR

167.1

ADULT HUMAN AND MONKEY DORSAL ROOT AND TRIGEMINAL GANGLION NEURONS: PATCH CLAMP RECORDINGS OF RESPONSES TO NOXIOUS STIMULATION
S.L. Ingram[1], K.J. Burchiel[2] and T.K. Baumann[1,2,*], Department of Pharmacology[1] and Division of Neurosurgery[2], Oregon Health Sciences University, Portland, OR 97201

The mechanisms that are responsible for transduction in primate nociceptors are not known. We studied the membrane responses to capsaicin, resiniferatoxin, and HEPES-buffered physiological salt solution (HBSS) titrated to pH=6.0. Heat stimuli were applied as HBSS (pH=7.35) heated to between 40 and 50°C. Whole-cell tight-seal recordings were made from adult human DRG neurons and monkey (*Macaca mulatta*) trigeminal ganglion neurons, either acutely dissociated or grown in culture for several days. Some responded to all three chemicals, others only to lowered pH. Responses consisted of a depolarization and action potential discharge (current clamp recordings) or a net inward current (up to -3.2 nA) and action current discharge (voltage clamp recordings). In a given neuron, inward currents evoked by capsaicin (10 μM) and resiniferatoxin (100 nM) had similar amplitude. Some capsaicin-sensitive neurons were exposed to heated HBSS to which they also responded with a prolonged net inward current, sometimes associated with action current discharge. Thus these neurons respond to a variety of stimuli known to produce burning pain in humans and should prove valuable in studies of nociceptive transduction.

167.2

PARASPINAL NOCICEPTIVE PRIMARY AFFERENTS.
G.M.Bove, A.R.Light*. Dept. of Physiology, Univ. of NC, Chapel HIll, NC 27599.

Though the paraspinal tissues possess putatively nociceptive elements, nociceptive afferents from this area have not been fully characterized. Single paraspinal Group IV nociceptive afferents from the rat proximal tail were characterized by their C.V. and response to mechanical stimuli. Their receptive fields (RFs) were located through microdissection. Individual fibers were activated from multiple tissues, such as muscle, tendon sheath, and along fascia associated with nerves supplying these structures. Individual RFs within muscle were found to have multiple punctate receptive areas, with mechanical thresholds between 1.5 and 8.5 gm. Background discharge was increased in several units, possibly due to sensitization from repeated mechanical stimulation. These data suggest that individual deep paraspinal nociceptors have multiple functional branches responsive to mechanical stimulation, and that these branches lie in different tissues. This contrasts with the reported discrete receptive fields supplying individual nociceptive Group IV muscle afferents. (Supported by NINDS NS16433 and FCER 90-6-1)

167.3

PLASTICITY OF MECHANICAL HYPERALGESIA IN CHRONIC NEURALGIA AND ACUTE CHEMOGENIC PAIN
M.Koltzenburg, L.K.Wahren and H.E. Torebjörk. (SPON: ENA) Dept. of Neurology,Univ. of Würzburg, Germany, Dept. of Clinical Neurophysiology, Academic Hospital, Uppsala, Sweden

Brush-evoked pain (allodynia) is a hallmark of neuropathic pain and can be experimentally evoked by percutaneous application of the irritants capsaicin (1% in 70% ethanol) or mustard oil (100%) which specifically excite nociceptors and sensitize them to heat. Differential nerve blocks (compression of the superficial radial nerve) determined that the ongoing pain of neuralgia (n=5) and the burning experimental pain (n=14) is conducted by unmyelinated primary afferents whilst brush-evoked pain is signaled by low threshold mechanosensitive Aß-fibres.

Following induction of acute chemogenic pain and in some neuralgia patients, the severity of brush-evoked pain depends critically on persistent C-fibre activity. When nociceptors are sensitized to heat, innocuous warming of the skin from 35° to 40°C raised the level of chemogenic pain (as meassured on visual analogue scales) from 37±4 to 59±8%. Simultaneously, the severity of brush-evoked pain increased and there was a close correlation between both pains in neuralgia (r=.94) and after mustard oil (r=.81). Ongoing chemogenic pain and nociceptor discharge (as evidenced by microneurographic recordings) are abolished by mild cooling and sympatholytic therapy can rapidly eliminate the ongoing pain in some patients. In the absence of background pain, tactile stimuli are no longer painful. The rapid malleability of brush-evoked pain indicates that the central sensitizations which permit allodynia cannot be sustained by central nervous processes alone. This could mean that an abnormal nociceptor function is the primary cause of neuropathic pain and that central changes ensue as normal physiological responses to nocicptive inputs.

167.4

SUSTAINED GRADED PAIN AND HYPERALGESIA FROM EXPERIMENTAL TISSUE ACIDOSIS IN HUMAN SUBJECTS
K.H. Steen & P.W. Reeh*. Dept. of Dermatol., Univ. of Bonn, D-5300 Bonn 1; Dept. of Physiol. & Biocyb., D-8520 Erlangen, Germany.

Nociceptors in rat skin are tonically excited and sensitized by hydrogen ion concentrations that normally appear in inflamed or ischaemic tissues (Steen et al. 1992, J. Neurosci 12: 86-95). Previous psychophysiological investigators, however, concluded algogenic actions of low pH solutions in human skin to be transient (Keele & Armstong 1964, Substances producing pain and itch. London: Arnold).

We used a motorized syringe pump to infuse an isotonic phosphate buffer solution (pH 5.2) at room temperature via sterile filter and cannula into the palmar forearm skin of human subjects (n=6, age 26-42, either sex). This resulted in a localized burning pain sensation (edema and flare response) that was sustained as long as a constant flow was maintained. Flow rates between 1.2 and 12 ml/h were needed to reach individual pain ratings around 20% of a visual analog scale (VAS) extending from "pain-threshold" to "unbearable pain". Increasing the flow in multiples of this basic rate led to approximately log-linear increases in individual pain ratings with reasonable congruence of the slopes. A constant infusion for 30 min of pH 5.2 at a rate producing pain ratings around 20% VAS led to a localized change in mechanical sensitivity. The touch threshold increased - as it did with control infusion of buffered saline (pH 7.4). The punctate force producing a threshold sensation of pain, however, dropped under pH 5.2 from 64 mN (median value, range 45.3-128 mN) to 5.7 mN (range 1.4-32 mN); the final level was usually reached within 15 min.

We conclude that experimental tissue acidosis provides a controllable and harmless method to produce sustained graded pain and hyperalgesia to mechanical stimulation (DFG grant Ste 593/1-1 and Re 704/1-6).

167.5

QUANTITATIVE EVALUATION OF SENSORY DISTURBANCES ACCOMPANYING MUSCULOSKELETAL PAIN. P.Hansson, U. Lindblom and E.Kinnman*.Dept. of Neurology, Karolinska Hospital, S-104 01 Stockholm, Sweden.

Patients with nociceptive musculoskeletal pain sometimes report paresthesia and numbness in or near the pain area although no evidence nor suspicion of peripheral nerve, plexus or root involvement is present. Patients with lateral epicondylalgia and lumbosacral musculoskeletal pain have been examined using quantitative sensory testing (QST) for touch, temperature and pain, to elucidate if such pain may interfere with somatosensory transmission, and perhaps regularly is associated with sensibility disturbances.

Slight hypo- and/or hyperesthesia was encountered in patients with epicondylalgia, however, not of a dignity that it could be mistaken for neuropathic hyperpathia. Single patients with ongoing musculoskeletal pain in the lumbosacral region presented a more pronounced sensory dysfunction profile, mimicing that found in patients with neurogenic pain, but still possible to differentiate by the non-neuroanatomical distribution and transitory nature. Clinically, some patients with musculoskeletal pain and sensory symptoms and/or signs of sensory dysfunction present a diagnostic challenge.

167.6

ADENOSINE INHIBITS CNQX-SENSITIVE EPSCs EVOKED BY DORSAL ROOT STIMULATION J.Li and E.R.Perl*, Dept. of Physiology Univ. of North Carolina, Chapel Hill, NC 27599

The effect of adenosine(ADE) on synaptic transmission between dorsal root input and neurons of the substantia gelatinosa(SG) was examined *in vitro* in transverse spinal cord slices (500μm)from hamster. Drugs were applied through bath superfusion. Excitatory post-synaptic currents (EPSCs) evoked by stimulation of the dorsal roots in whole cell patch recordings were judged to be monosynaptic if the latency was constant at near-threshold excitation. Most of the recorded EPSCs were reversibly blocked by CNQX(2-5μM). More than 1/2 of the recorded EPSCs were inhibited by ADE(30-300μM), an inhibition which could be suppressed by theophylline. ADE depressed all polysynaptic EPSCs; however, it did not affect some monosynaptic EPSCs. N^6-cyclohexyl-adenosine, a specific A_1 agonist, was 10^3 more potent than ADE. ADE produced hyperpolarization of some SG neurons; however ADE did not alter quantal size of spontaneous EPSCs but decreased the spontaneous EPSC frequency. Thus, in spinal SG, ADE appears to inhibit CNQX-sensitive EPSCs initiated by peripheral input, at least partially, through presynaptic A_1 adenosine receptors and to have important actions on SG interneuronal transmission. (Supported by Grant NS 10321 from NINDS.)

167.7

THE EFFECTS OF PRIOR LOCAL ANESTHESIA ON THE EXPRESSION OF THE DEAFFERENTATION SYNDROME FOLLOWING C5 - T2 GANGLIONECTOMIES IN THE RAT B. Iskandar, E. Rossitch, Jr.*, J. Ovelmen-Levitt, and B.S. Nashold, Jr., Div. Neurosurgery, Duke U. Med. Ctr., Durham, N.C. 27710.

There has been controversy as to the importance of injury discharge at the time of nerve sectioning in the subsequent development of a deafferentation pain syndrome. A deafferentation syndrome (DS) has been produced in rats by C5 - T2 ganglionectomies. This was expressed by biting (SM) in the anesthetic, completely denervated zone; and scratching (SCR) in the partially innervated region of the affected limb. In order to reduce or eliminate possible injury discharges, we have applied a long-acting local anesthetic (Marcaine) to the dorsal root ganglia in 9 rats (LAM) for 10 minutes prior to the ganglionectomies. The resultant deafferentation syndrome was then compared to that of 8 ganglionectomy only controls (LAC). Seven of the animals in each group have been observed for at least 30 days post-op. The SM was assessed by means of a point count system, and the extent of scratching noted. Compared to controls, the animals pretreated with Marcaine exhibited the following: 1) the onset of SM was not significantly delayed, but 2) the extent of SM was reduced at 30 days post-op, and 3) the pattern of SM was different. The mean SM score of the LAC rats at 30 days was 9.0 +/- 2.6 , while it was 3.1 +/- 0.83 for the LAM animals. Scratching, however, was found to be more severe in the LAM rats. We conclude that nerve discharges at the time of sectioning may play a role in the expression of the deafferentation syndrome which follows dorsal root ganglionectomies.

167.8

WITHDRAWN

167.9

LOW-THRESHOLD NEURONAL ACTIVITY OF SPINAL DORSAL HORN NEURONS INCREASES DURING REM SLEEP IN CATS. K. Kishikawa*, J.G. Collins, H. Uchida, Y. Yamamori. Dept. of Anesthesiology, Yale Univ. Sch. Med., New Haven, CT 06510

Extracellular recordings of single spinal dorsal horn neurons (n = 19) were made in physiologically intact, awake, drug-free cats during EEG confirmed wakefulness and sleep stages [control awake, drowsy, slow wave sleep, rapid eye movement (REM) sleep]. Both the size of the receptive field (RF) area that was sensitive to light touch and the neuronal response to brushing of that RF area were determined. We were able to accurately map the RF size of 7 neurons during REM sleep. The low-threshold RF size of all seven neurons was increased (mean % increase ± S.D. 52.9 ± 25.6) during REM sleep as compared with control. We studied the response to RF brushing in 15 neurons during REM sleep. Eight of the 15 neurons demonstrated an increased response to brushing during REM sleep, 3 neurons were depressed and 4 unchanged. Response to brushing during drowsy and slow wave sleep states was studied in 16 and 12 neurons, respectively, in each group 4 neurons showed a decreased response to brushing. No other changes were observed. The effects of the intravenous anesthetic propofol (7.5 mg/kg) were studied in 10 neurons after determining sleep effects. The receptive field size was decreased by a mean of 45 ± 26.6% after propofol administration. Seven of these 10 neurons had had their RF size increased during REM sleep. The response to brushing was also decreased by propofol. These results demonstrate that modulation of sensory information within the spinal dorsal horn varies with stages of sleep. Of particular interest, it appears that tonic inhibition of afferent input is reduced during REM sleep. These results also suggest that anesthetic-induced changes in spinal dorsal horn neuronal activity are not simply due to an anesthetic induced loss of consciousness. (Supported in part by NIH GM-29065)

167.10

ORGANIZATION OF LAMINA I TERMINATIONS IN THE POSTERIOR THALAMUS OF THE CYNOMOLGUS MONKEY. A.D. Craig*. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Lamina I nociceptive and thermoreceptive neurons constitute half of the spinothalamic (STT) and trigeminothalamic projections, and their axons ascend in the lateral STT classically associated with pain and temperature sensibility. Previous PHA-L work has identified termination sites in both the lateral and medial thalamus of the cat and the monkey. In this study, the dense projection to the PO/SG region at the caudal aspect of VP of young (ca. 2 kg) monkeys has been examined with multiple fluorescent labeling (using Texas Red, FITC and AMCA). Following iontophoretic injections in lamina I at the trigeminal, cervical or lumbar level in anesthetized animals, (red, green or biotinylated) dextran was found to anterogradely label this projection (but not one other) similarly to PHA-L; thus, double anterograde labeling from different levels was performed in 8 monkeys. In addition, endogenous calbindin or parvalbumin was labeled immunohistochemically with a third fluorochrome. Lamina I terminal fibers are topographically organized within a discrete region identifiable in all planes of section by a confined plexus of calbindin-positive fibers. This region corresponds to a cytoarchitectonic zone that can be resolved in adjacent thionin-stained sections. Control cases indicate that lamina V terminations occur more rostrally in an adjacent region that is part of VP.

These observations support the hypothesis originated by Head and Holmes (1911) that a discrete portion of lateral thalamus subserves the discriminative aspect of specific pain and temperature sensation. (Supported by NIH grant NS25616)

167.11

HUMAN CEREBRAL PROCESSING OF NOXIOUS AND INNOCUOUS STIMULI. R.C. Coghill,* J. Talbot, A. Evans, A. Gjedde, E. Meyer, G.H. Duncan and M.C. Bushnell. U. Montréal and Montreal Neuro. Inst., Montréal, Qué.

The role of human cortex and thalamus in the processing of painful stimuli is currently in dispute. Using positron emission tomography (PET), we demonstrated increases in cerebral blood flow (CBF) in the left primary and secondary somatosensory cortex (SI & SII) and in the left cingulate cortex, all contralateral to the presentation of painful heat (48.2°C) stimuli [Science,251:1355 (1991)]. Other imaging studies have shown activation of left cingulate cortex and thalamus by mildly painful heat (≈46°C) stimuli [Jones et al., Proc.R.Soc.Lond., 244:39 (1991)], but have failed to observe activation of SI or SII [Jones et al.; Apkarian et al., Life Science, (in press)]. Studies using only innocuous vibrotactile stimuli have generally reported activation of SI and/or SII contralateral to the stimulus site. The current study sought to clarify the roles of SI and other brain regions in the processing of somatosensory stimuli by replicating our previous findings, investigating the laterality of nociceptive activation, and comparing the effects of noxious heat and innocuous vibrotactile stimulation within the same group of subjects. Changes in CBF were evaluated by PET following the bolus injection of oxygen-labelled water ($H_2{}^{15}O$) in nine human volunteers. Each underwent scans during the presentation of painful heat (48°C), vibrotactile (150 Hz), and control (35°C) stimuli (12 five-sec repetitions per scan) to the left forearm. We have confirmed significant pain-related increases in CBF in the regions approximating anterior cingulate, SI and SII, all contralateral to the stimulated forearm. In agreement with Jones et al., we also observe pain-related activation in contralateral thalamus. In contrast, significant vibrotactile-related activation appears restricted primarily to contralateral SI and SII. Although similar regions of SI and SII are activated by both vibrotactile and painful stimuli, it appears that mildly painful stimuli are not adequate to elicit detectable CBF changes within these regions. Supported by the Canadian MRC.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS—NEUROPSYCHOLOGY I

168.1

ON THE DEVELOPMENT OF DECLARATIVE MEMORY. R. McKee* and L.R. Squire. VA Med Ctr & UCSD Psychiatry Dept, La Jolla, CA 92093. Infantile amnesia, i.e., the unavailability of recollections from the first three years of life, has traditionally been explained in terms of repression or retrieval failure. Another possibility, based on the idea of multiple memory systems, is that the limbic-diencephalic brain structures that support declarative memory are late-developing and that declarative memories are not formed early in life. By this view, the learning abilities of infants reflect nondeclarative forms of memory (e.g., skills and habits). We studied the performance of normal subjects and amnesic patients on the visual paired-comparison task, which has been used to demonstrate memory in infants. Based on a procedure recommended by B. Malamut, subjects viewed 24 pairs of pictures (2 identical items/pair) and later viewed another 24 pairs of pictures (one novel item and one old item in each pair). Normal subjects exhibited a tendency to look at the novel item in each pair that persisted for a few hours. This tendency was not observed in amnesic patients, indicating that the effect of famiarity on viewing time depends on the limbic-diencephalic structures that are damaged in amnesia and essential for declarative memory. These results and findings in infant monkeys with surgical lesions (Bachevalier, 1990) suggest that visual paired-comparison performance in human infants is supported by declarative memory. If so, performance on this task reflects an early capacity for declarative memory, and the absence or prolonged postnatal development of this system cannot explain infantile amnesia. It is more likely that infantile amnesia results from slow maturation of the neocortex where long-term declarative memories are thought to be stored.

168.2

IMPLICIT LEARNING OF ONE-TRIAL COLOR-WORD "ASSOCIATIONS" IN AMNESIC PATIENTS. G. Musen* & L.R. Squire, VA Med Ctr, & UCSD Psychiatry Dept., La Jolla, CA 92093. Evidence for implicit and rapid learning of verbal associations has been difficult to obtain (Musen & Squire, submitted). We reasoned that nonverbal associations might be more readily detected because the attributes to be associated can be more completely integrated (i.e., a word printed in a color such that the "association" is between two features of the same stimulus). We used a color naming task in which 84 words that were strongly associated with particular colors (e.g., banana) were printed in incongruous colors (e.g., blue banana). Amnesic patients and control subjects were given 5 seconds to view each item and were asked to form an image of the word in the color in which it was printed. At test, subjects were asked to name the colors of 84 test items as rapidly as possible. One third of the items remained in the same color as during study (old), 1/3 were in a different color (recombined), and 1/3 of the words had not appeared at study (new). If new "associations" are acquired, then recombined items should be named more slowly than old items. Both groups of subjects named the colors of the old items significantly more rapidly than the recombined or new items (control subjects: old = 968 ms, recombined = 1029 ms, and new = 1019 ms; amnesic patients: 1029, 1115, and 1112, respectively). Thus, implicit learning of new nonverbal associations (or conjunctions of features) can occur after a single study trial.

168.3

INTACT PROTOTYPE LEARNING BY AMNESIC PATIENTS: EVIDENCE FOR PARALLEL LEARNING OF ITEM-SPECIFIC AND GENERAL INFORMATION B.J. Knowlton* & L.R. Squire. VA Med Ctr. and Dept. of Psychiatry, UCSD, La Jolla, CA 92093.

When subjects study a list of items, they not only learn about the specific items, they also acquire generic information about the items as a group, e.g., a concept about the category that the items belong to. Most theories of concept learning suppose that category-level knowledge is derived from long-term memory for the items that are studied. Our study shows that category-level knowledge can develop normally in the absence of memory for individual items. Amnesic patients and control subjects were shown 40 dot patterns that were distortions of a prototypic pattern. After 5 min, subjects were told that the patterns belonged to a single category and were then shown new patterns (4 repetitions of the prototype, 40 distortions of the prototype, and 40 random patterns). Subjects classified each pattern as to whether it did or did not belong to this category. Control subjects and amnesic patients performed identically. In contrast, in a second study, control subjects were significantly better than amnesic patients at recognizing which specific patterns had been presented. There was also a significant interaction ($p<.05$) of task (classification or recognition) and group (controls vs. amnesics). The results indicate that category-level knowledge about items can develop independently of declarative knowledge about the items themselves.

168.4

IMPAIRED PERCEPTUAL PRIMING AND INTACT CONCEPTUAL PRIMING IN A PATIENT WITH BILATERAL POSTERIOR CEREBRAL LESIONS. M.M. Keane*, H. Clarke, and S. Corkin. Dept. of Brain and Cognitive Sci. and Clinical Research Ctr., MIT, Cambridge, MA 02139.

Dissociations among repetition priming effects in patients with Alzheimer's disease (AD) have revealed that priming is not mediated by a single mechanism. We have proposed that perceptual priming (normal in AD) is supported by memory processes localized to posterior (peristriate) cortices that are relatively preserved in AD, and that conceptual priming (impaired in AD) is supported by memory processes localized to more anterior cortices that are compromised in AD. In the present study, we tested this hypothesis by examining priming in a patient with a severe visuoperceptual impairment consequent to a head injury that caused bilateral posterior cerebral lesions and required a right anterior temporal lobectomy. The hippocampus was spared bilaterally. We predicted that the lesions in this patient would compromise the neural substrate of perceptual priming processes and would spare the neural substrate of conceptual priming processes. Consistent with the first prediction, the patient showed no priming in perceptual identification of words or pseudowords, and reduced priming in within-modality (visual) word-stem completion. Consistent with the second prediction, he showed normal priming in cross-modality (auditory-visual) word-stem completion, and normal priming in category exemplar production. Recognition memory was normal. Coupled with prior findings in AD, these results represent a double dissociation between perceptual and conceptual priming. Further, they constitute the first demonstration of impaired perceptual priming in the face of normal recognition memory. Our findings provide strong evidence for the existence of two cortically mediated memory systems, each supporting a distinct component of repetition priming. These systems are independent of the limbic-diencephalic system supporting recall and recognition.

168.5

NO DREAMS - NO MEMORY: THE EFFECT OF REM SLEEP DEPRIVATION ON LEARNING A NEW PERCEPTUAL SKILL. A.Karni[1,2]*, D.Tanne[2], B.S.Rubinstein[1], J.J.M.Askenasi[2] and D.Sagi[1]. [1]*Dept. of Appl. Math., Weizmann Inst. of Sci., Rehovot and* [2]*Dept. of Neurology, Sheba Med. Cent., Tel-Hashomer, ISRAEL.*

Is REM sleep important for memory consolidation? We combine a novel perceptual skill learning paradigm with specific sleep stage deprivation technique, to investigate the effect of REM sleep deprivation on memory consolidation. The paradigm we use induces a remarkable long-term, retinal input specific, improvement in a basic visual discrimination task which is highly specific for simple physical attributes of the sensory input (retinotopy, monocularity, orientation). This implies discrete, highly specific, local plasticity within the primary visual cortex induced by sensory experience (PNAS 88:4966, 1991). Furthermore, learning in this paradigm is not immediate - it takes about 8-10 hours to "consolidate". It is then not forgotten for at least one year.

Our 2AFC psychophysical masking paradigm was used to measure texture target discrimination thresholds, in consecutive sessions, before and after sleep with polysomnographic recording in a sleep laboratory. Each session was made of some 1000 stimulus presentations where the subjects were required to identify the orientation of a target made of 3 line elements differing only in orientation from the background. Each subject underwent 2 nights of baseline recording followed by 2 nights of sleep stage deprivation, effected by forced arousal (bell or speech) after recording an epoch of the relevant sleep stage.

Results: 1)Performance was improved by 52% (mean, 4 nights) after a normal (6-7 hours) night sleep. 2) REM deprivation (down to 6% of total sleep time) disrupts consolidation (improvement by 0-9%, 2 nights). 3)NREM stage 3-4 sleep deprivation (down to 6% of total sleep time) does not affect normal consolidation (48% improvement, 2 nights). 4)Both types of deprivation did not affect performance on an already trained task.

Depriving REM sleep severely retards processes involved in consolidating the effects of practice ("learning how"). This implies, that processes that consolidate local plasticity within the visual cortex are active during REM sleep.

168.6

VERBS BUT NOT NOUNS: DAMAGE TO LEFT TEMPORAL CORTICES IMPAIRS ACCESS TO NOUNS BUT NOT VERBS. A.R. Damasio*, D. Tranel and H. Damasio. Div. of Behav. Neurol. & Cogn. Neurosci., Univ. Iowa Col. of Med., Iowa City IA, 52242.

In a task designed to elicit the production of *verbs* (n=220), three patients with left temporal damage consistently produced the correct target words, performing no differently from normal controls. However, in a similar task designed to elicit the production of *nouns* (n=360), both patients performed quite defectively, scoring many standard deviations below controls (word frequencies were comparable in the verb and noun sets). The lesions are located outside traditional language areas (left frontoparietal operculum, posterior temporal region, inferior parietal lobule), whose damage is associated with aphasia. The damage is in left anterior and middle temporal lobe (areas 28, 35, 36, 20, and 21). We propose that this sector contains systems for the retrieval of (1) proper nouns, and of (2) common nouns which denote entities of some but not all conceptual categories. Those systems are *not* involved in verb retrieval, nor in the implementation of word-forms. Instead, those systems perform a two-way *lexical mediation* role, e.g., they promote the reconstruction of a word-form triggered by activation of the conceptual structure of the entity that is denoted by that word; they can operate in reverse. We suggest that verb access requires systems in left parietofrontal cortices.

168.7

IMPAIRED FEELING AND AUTONOMIC ACTIVATION FOR VISUAL BUT NOT VERBAL STIMULI FOLLOWING DAMAGE TO RIGHT INFEROTEMPORAL CORTEX. D. Tranel*, H. Damasio and A.R. Damasio. Div. of Behav. Neurol. & Cogn. Neurosci., Univ. of Iowa Col. of Med., Iowa City IA, 52242.

A patient (CB-1614) with a lesion in the right inferotemporal (IT) region developed defects in learning of faces, places, and routes. We conducted experiments in which skin conductance responses (SCRs) were recorded while CB-1614 viewed two randomly ordered series of stimuli. (1) **Nonverbal**: emotionally-charged (*target*) and neutral (*nontarget*) pictures. (2) **Verbal**: high-emotional (*target*) and low-emotional (*nontarget*) words (there were 10 targets and 30 nontargets in each set). CB-1614 had normal SCRs to basic "startle" stimuli (e.g., loud noise). Her SCRs to the nonverbal target stimuli were severely impaired. By contrast, she produced normal SCRs (X=1.68 uS) to the verbal targets. The findings indicate a defect in visually-triggered "emotionality," restricted to nonverbal stimuli. It is interesting to note that the patient's self-report matched the SCR results: she reported a lack of feeling when viewing previously disturbing or exciting stimuli (e.g., watching a provocative movie), but *normal* feelings when reading this type of material. The results suggest that the right IT region plays a role in triggering somatic states to visual-nonverbal stimulation.

168.8

METABOLIC ACTIVATION OF HIPPOCAMPUS AND DORSOLATERAL PREFRONTAL CORTEX BY A SPATIAL WORKING MEMORY TASK: RELATIONSHIP TO DURATION OF DELAY. R.A. Adcock*, H.R. Friedman & P.S. Goldman-Rakic, Sect Neurobiol, Yale Univ Sch Med, New Haven CT.

Both hippocampus (HPC) and dorsolateral prefrontal cortex (PFC) have been implicated in performance of spatial memory tasks in monkeys. Previous studies from this laboratory show that glucose utilization (LCGU) in both areas is enhanced in monkeys performing spatial working memory (SWM) tasks as compared to monkeys performing tasks that do not specifically engage SWM (e.g., Friedman & Goldman-Rakic, J. Neurosci. 8:4693, 1988; Soc. Neurosci. Abstr. 17:1135, 1991). PFC lesions produce impairments on SWM tasks at delays as short as 5s (e.g, Goldman & Rosvold, Exp. Neurol. 27:291-304, 1970); however, studies of HPC lesions have shown that performance is impaired only at longer delay intervals, i.e., 15s and longer (e.g., Zola-Morgan & Squire, Behav. Neurosci. 99:22-34,1985). We used the 2DG method to assess a) whether LCGU in the HPC increases with delay period as predicted by the lesion studies, and b) whether LCGU in the PFC is similarly affected or is less dependent on delay, as lesion studies might suggest.

Monkeys were trained on spatial delayed alternation: two each at 15s and 30s, and three at 5s delay. ^{14}C-2DG (100mCi/kg) was injected for the 45 min 2DG test session, and blood samples were taken for later determination of LCGU using a computerized imaging system. Contrary to prediction, both areas showed overall trends toward increased LCGU at shorter delays. In the HPC, LCGU was enhanced by 24% in the 5s group compared to the 30s group, and 22% over the 15s group. In PFC, LCGU was 33% greater in the 5s group vs the 30s group and 12% greater than the 15s group. These results suggest that for this SWM task, delay duration is not the most important determinant of LCGU for either area and must be reconciled with conflicting data from lesion studies. Supported by MH 38546.

168.9

RECALL OF UNEXPECTED STIMULI WHILE TEMPORAL CORTEX IS SUPPRESSED WITH COLD. James A. Horel*, Department of Anatomy and Cell Biology SUNY HSC Syracuse NY, 13210.

While visual learning is readily disrupted by suppressing inferotemporal cortex (IT) with cold, effects on recall are difficult to produce. Perhaps exposure to the stimuli before the cold is applied causes the animals to withdraw the information from IT. This predicts that if the animal is presented with a visual discrimination that it did not expect during suppression with cold, it would not be able to withdraw this information and could not do the task. One of two well-known discriminations was presented to three monkeys for 10 trials and then the cold was turned on and left on for 60 trials without affecting performance. The unexpected discrimination was then introduced and performance was at chance for 50 trials, whereupon the discrimination was shifted back to the expected pair and performance returned to nearly normal. The same result obtained with expected and unexpected stimuli reversed. This suggests that information the animals anticipate using may be withdrawn from IT and placed in a working memory that is not suppressed by IT cooling. (Supported by NS 18291)

168.10

IMPAIRMENT OF LONG-TERM MEMORY AND SPARING OF SHORT-TERM MEMORY IN MONKEYS WITH LESIONS OF THE HIPPOCAMPAL FORMATION. P. Alvarez-Royo*, S. Zola-Morgan, and L. R. Squire. UCSD Depts. of Neuroscience and Psychiatry, La Jolla, Ca 92093, and V.A. Medical Center, San Diego, Ca 92161.

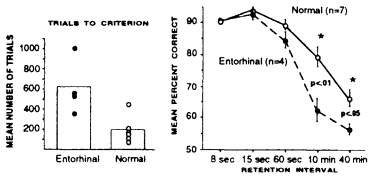
During the last decade, an animal model of human amnesia has been developed in the monkey. Studies using this model have identified structures in the medial temporal lobe that are essential for forming long-term memory (i.e., the hippocampus and the entorhinal, perirhinal and parahippocampal cortices). Recently, an important aspect of these studies has been questioned by Ringo (1991). He suggested that the data from the delayed nonmatching to sample (DNMS) task, which has been extensively used in these studies, have been analyzed in a potentially misleading way and do not support the idea that medial temporal lobe damage produces a selective impairment in long-term memory while sparing short-term memory.

One problem has been that most of the studies analyzed by Ringo were not designed to directly compare short-term and long-term memory. We addressed this issue directly using a computer-controlled apparatus to test monkeys on an automated version of the DNMS task with delays ranging from 0.5 seconds to 10 minutes. We previously reported that monkeys with lesions of the hippocampal formation that include parahippocampal cortex (the H^+ lesion) learned the task normally with a delay of 0.5 seconds, but performed significantly worse than a group of normal animals at delays longer than 30 seconds. In that experiment, however, the delays were presented sequentially. Therefore, differential forgetting or practice with the nonmatching rule could have affected the results. These two groups have now been retested on the DNMS task using a mixed-delay paradigm. The H^+ animals performed normally at a short delay (1 second) and were impaired at long delays (1 and 10 minutes). These data strongly reinforce the idea that the H^+ lesion affects only long-term memory and leaves short-term memory intact. Accordingly, they provide strong confirmation in the monkey of the long-standing idea, based primarily on findings from humans, that short-term memory is independent of medial temporal lobe function.

168.11

SELECTIVE ENTORHINAL CORTICAL LESIONS IMPAIR LEARNING AND MEMORY IN THE MONKEY. B.W. Leonard*, D.G. Amaral, S. Zola-Morgan and L.R. Squire, Salk Institute, San Diego, CA. 92186, Dept. of Psychiatry, UCSD, La Jolla, CA. 92093.

Bilateral medial temporal lobe lesions in monkeys and humans result in a profound impairment of learning and memory. Human neuropsychological studies indicate that damage limited to the hippocampus proper is sufficient to impair memory function. The entorhinal cortex (EC) is an anatomically distinct component of the hippocampal formation that acts as an interface through which the hippocampus and other hippocampal fields communicate with the neocortex. As part of an ongoing program designed to determine the relative contributions to memory function of the separate components of the medial temporal lobe, we have prepared a group of monkeys (*M. fascicularis*) with aspiration lesions intended to include only the EC. Learning and memory were assessed postoperatively with a standard battery of tests. Here we report the results from 4 monkeys on the visual, trial-unique delayed non-matching to sample (DNMS) test. EC animals were impaired at learning DNMS at a standard 8 sec delay ($p<.01$). Across longer retention intervals, performance of EC animals was also impaired (group effect, $p=.056$; group X retention interval interaction, $p<.05$; based on the 4 longest delays). These results show that bilateral lesions intended to damage entorhinal cortex and spare perirhinal and parahippocampal cortices are sufficient to impair memory function.



168.12

RETROGRADE AMNESIA IN RHESUS MONKEYS: DIFFERENTIAL RETENTION OF HABITS AND MEMORIES? R. C. Saunders,* Clinical Brain Disorders Branch, NIMH, St Elizabeths Hospital, Washington, D.C. 20032.

Retention of information acquired prior to the onset of amnesia has been difficult to evaluate in human amnesic subjects. In animals we can evaluate retrograde amnesia more easily because we can control and assess retention of information learned before and after the onset of the amnesia. In experiment 1 monkeys (*Macaca mulatta*) were trained on several lists of 20 object discrimination pairs in two ways. A list for concurrent discrimination learning was presented either multiple times (5 to 10) during a single testing session or only once (24 hr concurrent). Learning of each list was to the same 90% criterion level. One list of each type was presented at 8-9 month, 4-5 month, and 1 month intervals before the removal of the medial temporal lobe area. Retention of object discriminations tested before surgery was at a high level averaging 84.5%, with no differences between lists. In contrast, post-op retention was dramatically reduced to near chance levels on all lists (58%) and were not differentiated by type of training or by length of interval before the onset of amnesia. In experiment 2, monkeys were given several object discrimination pairs to be learned one at a time. After reaching a 90% performance level they were over-trained for 500 trials before surgery. Monkeys showed perfect post-op retention on each pair. In experiment 3 a paired-comparison recognition memory task was used (Saunders, et al., Neurosci. Abstr., 1990). In contrast to the results of experiment 2, post-op retention of pre-operatively acquired information was at chance (55%). These data taken together demonstrate that 1) a severe retrograde amnesia may be present and not temporally-limited, and 2) retrograde amnesia after medial temporal lobe removal may show a selective defect for 'memories' and not 'habits' similar to that seen in anterograde amnesia.

168.13

SEVERE RETROGRADE AMNESIA IN A PATIENT WITH TRAUMATIC BRAIN INJURY. H.J. Markowitsch[1], Pasquale Calabrese[1,2], Michael Haupts[2] and W. Gehlen[2] (SPON: European Neuroscience Association), [1]Dept. Psychology, University of Bielefeld, D-4800 Bielefeld, [2]Neurology Dept., University Hospital Langendreer, D-4630 Bochum, Germany

We describe a 45-year old patient who at the age of 41 years fell from a horse and received severe head injury. The patient was comatose for 6 weeks. His brain damage included the ventral half of the right frontal lobe, the temporal polar horns bilaterally, and (as contre coup), the area of the left temporo-parietal cortex.
The patient, a former industrial manager, was of average intelligence, had a normal short-term memory span, but severe problems in the long-term memory domain, especially in the retrieval of remote memories. He was given a comprehensive test battery to assess his intelligence, learning and memory, his emotional status, problem solving abilities, and language functions. The memory tests included priming tasks, skill learning, and tests of his old memory.

We conclude from our results that similar to the case described by Kapur et al. (Brain, 1992, 115, 73ff), the temporo-polar cortex may play a crucial role for long term memory retrieval.

VISUAL CORTEX: ANATOMICAL STUDIES

169.1

THE INHIBITORY INPUT TO THE PERIKARYON AND INITIAL AXON SEGMENT OF THE LAYER II/III PYRAMIDAL CELL OF RAT VISUAL CORTEX. A. PETERS,* Department of Anatomy and Neurobiology, Boston Univ. Sch. of Med., Boston, MA 02118.

The inhibitory input to the cell body and initial axon segment of pyramidal cells is complex. It is provided by at least four different types of local circuit neurons, all of which are GABAergic. The chandelier cells provide input to the axon initial segment, and the other neuronal types provide input to the cell body. Their terminals are distinctly different and because of their characteristics they have been termed large, medium-sized and dense terminals (Peters and Harriman, J. Neurocytol., 19:154, 1990). To determine the origins of these terminals, their features have been compared with those of labelled neurons. It is then evident that the medium-sized and dense terminals resemble those of multipolar neurons with unmyelinated axons, while the dense terminals resemble those of VIP-positive bipolar cells. The source of the large terminals could not be determined. From an analysis of 50 profiles of layer II/III pyramidal cells it is calculated that the average neuron of this type forms 65 axosomatic synapses. 43 of these synapses are medium-sized and are provided by the axon terminals of the medium-sized multipolar neurons, while 12 of the synapses are of the dense kind, which are associated with bipolar cells. The remaining 10 synapses are formed by large terminals. How the inhibitory inputs provided by the different types of non-pyramidal cells differ in function is not known. **Supported by NIH grant NS 07016.**

169.2

FUNCTIONAL AND STRUCTURAL TOPOGRAPHY OF LATERAL INHIBITORY CONNECTIONS IN CAT VISUAL CORTEX (AREA 18) Z.F.Kisvárday[1*], D.-S.Kim[2], U.T.Eysel[1], T.Bonhoeffer[2] [1]Dept. Neurophysiol. Ruhr-Univ.Bochum, PO Box 102148., F.R.G. [2]Max-Planck Inst.Brain Res., 6000 Frankfurt 71, F.R.G.

GABAergic large basket cells in layers III-V provide long-range horizontal connections over several mm. In order to know the relationship between the axonal topography of large basket cells and the topography of orientation specificity over their influenced area we used a combination of optical imaging and single cell tracing in the same tissue. A craniotomy was made above area 18 at Horsley-Clarke co-ordinate A5 and an area of 9.6x7.2 mm^2 of the exposed cortex was studied with the optical recording technique, revealing orientation domains. Then, at several sites, *biocytin* was extracellularly delivered in to the superficial layers using positive current of 0.7-0.9 μA at 1 Hz.
The labelling was revealed with the ABC method, axonal fields of 3 biocytin labelled large basket cells were reconstructed from adjoining large horizontal sections in layer III. The distribution of these axonal fields was compared with the distribution of the orientation map of the same area. For alignment, identified electrode penetrations were used as landmarks.
The results show that the axonal field of each large basket cell establishes inhibitory connections over regions representing the whole range of orientations, similar and different, to that of the parent soma. It is thus reasonable to assume that the same large basket cell provides so-called iso- as well as cross-orientation inhibition depending on the topography of its target cells in relation to the topography of the orientation map.
Z.F.K. and U.T.E are supported by the Deutsche Forschungsgemeinschaft.

169.3

Biocytin injections, guided by optical imaging, reveal relationships between functional architecture and intrinsic connections in monkey visual cortex. R. Malach*, Y. Amir, E. Bartfeld and A. Grinvald Weizmann Inst. Rehovot, Israel 76100.

How are the patterns of intrinsic horizontal connections related to the functional architecture of the cortex? In this study we explored the relationship between patterns of intrinsic connections and the detailed functional architecture. Our approach combined the techniques of tract tracing and optical imaging. Tracing of intrinsic connections with the anterograde tracer biocytin revealed an intriguing diversity of stereotyped patterns of clustered projections. These patterns ranged from highly asymmetric, oval shapes to nearly symmetric "rosette"- like forms. Similarly, individual clusters varied greatly in their shape and size. High resolution optical imaging provided detailed maps of ocular dominance columns and iso-orientation domains which formed pinwheel-like structures around orientation singularity points. Furthermore, optical imaging enabled us to select the precise sites of biocytin injections in the functional map. Our results provide examples of remarkable specificity of intrinsic connections between functional domains. Interestingly, injections that overlapped singularity points, including all orientations, did not produce diffuse labeling. Rather, clear cut, clustered projections were observed. These clusters overlapped with a diverse set of neighboring functional columns.

169.4

CORRELATION BETWEEN PATTERNS OF LATERAL CONNECTIVITY AND PATTERNS OF ORIENTATION PREFERENCE IN MONKEY STRIATE CORTEX.

G. G. Blasdel,*T. Yoshioka , J.B. Levitt, J.S. Lund. Department of Neurobiology, Harvard Medical School, Boston, MA 02115; Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

We explored structure function correlations in differentially imaged regions of macaque striate cortex by making small iontophoretic injections of biocytin into the upper layers. Most of the resulting injections were 100-200 μm across, and gave rise to patchy projections that spread as far as 2.3 mm laterally. After each animal had been perfused, and the injections had been recovered, patterns of transport and orientation were compared. In some cases patchy projections were found in regions that preferred similar orientations; in others they occurred in regions that preferred different orientations. One possible explanation for this is that injections into slightly different vertical compartments label different circuits. It is also conceivable that patterns of transport such as these are driven by constraints other than orientation preference. Iso-orientation slabs appear correlated with the anisotropic spread of projections since both appear elongated along axes perpendicular to the ocular dominance columns (see Blasdel, 1992; Yoshioka et al, 1992). (Supported by EY05403 and the Office of Naval Research (G.B.), and by EY05282, EY08098, EY06275, and ARVO/Alcon.)

169.5

INTRINSIC CONNECTIONS OF OWL MONKEY STRIATE CORTEX: DIFFERENCES BETWEEN CYTOCHROME OXIDASE (CO) BLOBS AND INTERBLOBS. *V.A. Casagrande *, J.A. Mavity-Hudson, and J.G. Taylor*. Depts. of Cell Biol. and Psych., Vanderbilt Univ., Nashville, TN 37232

Previous work showed that the CO-blobs in layer III of striate cortex in 2 diurnal (macaque and squirrel monkey) and 1 nocturnal (bushbaby) primates receive indirect input (via layer IV) from *both* the LGN parvocellular (P) and magnocellular (M) pathways (Casagrande et. al.,'90; Lachica et.al.'92). However, connections of interblobs showed species differences; in the diurnal simians interblobs receive indirect input only from the P pathway while in the nocturnal prosimian interblobs receive input only from the M pathway. We asked if the species differences in interblob connections were a reflection of phylogenetic (simian verses prosimian) or visual niche (diurnal verses nocturnal) differences. We made HRP or biocytin injections into layer III of 6 nocturnal simians (owl monkeys) and compared the laminar patterns of retrogradely labeled cells. Results show that, as in the other primates, injections in either blob or interblob layer IIIA in owl monkey labeled cells mainly in IIIB and V, and injections into blob centers in IIIB labeled cells in *both* IVα (M target) and IVβ (P target), as well as cells in the other subdivisions of layer III, and layers V and VI. In contrast, following interblob IIIB injections, the majority of labeled cells were restricted to IVα, as in the bushbaby. Many cells were also labeled in IIIA, dorsal divisions of V and VI. Results suggest that CO blobs in primates get input from all LGN pathways and are concerned with basic aspects of vision that transcend primate niche specializations. Interblobs receive either M input (via IVα) in nocturnal primates or P input (via IVβ) in diurnal primates suggesting that interblobs perform a function that must take into account visual niche requirements. Supported by EYO1778 (VAC) and core grants EY08126, HD15052.

169.6

CYTOSKELETAL PROFILE OF NEURONS INVOLVED IN THE M AND P PATHWAYS OF THE MACAQUE VISUAL CORTEX. S. B. Kupferschmid[1]*, R. Gattass[2], L. G. Ungerleider[2], J. H. Morrison[1]. [1]Fishberg Research Center for Neurobiology, Mt. Sinai School of Medicine, NY, NY 10029; [2]Lab. of Neuropsychology, NIMH, Bethesda, MD 20892

A monoclonal antibody directed against a non-phosphorylated neurofilament protein, SMI32, labels pyramidal cells in the monkey visual cortex in areal specific patterns. In V1, SMI32-immunoreactivity (ir) labels the cells of layer 4B and the Meynert cells of layer 6. In visual association areas V2, V3, V4 and MT, the SMI32-ir neurons are predominantly in layers 2 and 3. Area MT is unique in having a band of SMI32-ir neurons in layer 6. Because SMI32 also labels the retinal alpha ganglion cells and the magnocellular cells of the lateral geniculate nucleus in addition to the MT projecting cells of V1, SMI32 may be a selective marker of components in the M pathway of the monkey visual cortex.

To test this hypothesis we made injections of distinct retrograde tracers into areas V4 and MT of 2 cynomolgus monkeys. The brains were processed for the immunofluorescent distribution of SMI32 and the proportion of double labeled cells (SMI32 + retrograde labeled/total retrograde labeled) was quantified on a regional and laminar basis. The projections from layers 4B and 6 of V1 to MT were 88% and 92% double labeled, respectively, and from layers 2 and 3 of V2 to MT 74%. The projection from layers 2 and 3 of V2 to V4 was 49% double labeled; from V3 to V4 47%. Of the cells in V2 containing both FB and DY labels, 92% were also recognized by SMI32. These cells probably represent at least part of the anatomical basis for the physiologically identified magnocellular contribution to V4 (Nealey et al., '91). Preliminary studies (Kupferschmid et al., '91) have shown that cells projecting back to V1 and V2 from V4 and MT have a much lower proportion of SMI32-ir. Thus, our data suggest forward and feedback pathways differ in cytoskeletal profiles and that the M pathway has a particularly high representation of SMI32-ir neurons. Supported by AG06647 and AHAF.

169.7

OPTICAL IMAGING OF ORIENTATION MAPS IN V1, V2, AND MT OF NEW WORLD MONKEYS. R. B. H. Tootell*, D. Malonek and A. Grinvald, Depts. Neurobio., Harvard Med. Sch., Boston, Mass. 02115 and Weizmann Inst., Rehovot, Israel 76100.

The many visual cortical areas exposed on the lateral surface of Aotus (owl) and Saimiri (squirrel) monkeys are ideal for exploration of cortical functional organization by imaging of intrinsic signals. In this study, the main stimulus was an achromatic, moving (2-10^0/sec) rectangular wave grating, presented binocularly at or near zero disparity, at different directions/orientations (45^0 apart). Such stimuli produced orientation-specific patches in V1 and V2 similar to those previously observed in macaque; a 180^0 representation with a center-to-center spacing of ~500 um and 750 um, respectively.

In owl monkey MT, such stimuli also produce radially-arranged functional maps, with center-to-center spacings of ~800 um. These images were sufficiently robust so that iso-orientation maps were obtained by subtracting a "blank" stimulus, rather than a grating at the orthogonal orientation. Tests with other stimuli (see Malonek et al, 1992) suggested that the organization is specific for orientation rather than axis-of-motion, and that it coexists with other architectures, including one for direction.

To begin exploring the relationship between these optical maps and earlier DG data on the functional organization of owl monkey MT (Tootell and Born, 1990), DG and optical maps of orientation were successfully obtained in MT in the same monkey, in response to identical stimuli. Supported by EY07980 and the Schilling Foundation.

169.8

RELATIONSHIP OF ISO-DIRECTION AND ISO-ORIENTATION MAPS REVEALED BY OPTICAL IMAGING IN OWL MONKEY AREA MT. *D. Malonek* , R.B.H. Tootell and A. Grinvald*, Depts. of Neurobiology, Weizmann Inst. of Science, Rehovot 76100, Israel, and Harvard Med. School, Boston, Mass. 02115.

Previous electrophysiology and deoxyglucose studies suggest that several columnar systems co-exist in the primate area MT. To clarify the yet-unknown relationship between the functional architecture of various functional domain, we used optical imaging based on intrinsic signals.

To generate iso-direction maps, random dots moving at different directions, covering 360°, were used as a visual stimuli. We found that cells in MT selective for direction of motion are clustered, forming what we term iso-direction domains. Inspecting the spatial distribution of iso-direction domains, 180° apart, revealed that they did not overlap. This result suggests a 360° organization, in agreement with previous electrophysiological and double-label 2-DG studies.

To explore the relationship between iso-direction and iso-orientation domains we stimulated with gratings having different orientations and different directions of motion. Iso-orientation (termed "axis-of-motion" in other studies) maps were first generated. We found that iso-orientation domains produced by opposite directions of motion exhibited a large overlap. To obtain the iso-direction maps, from gratings rather than from random dots stimuli, we calculated the difference between two maps resulting from gratings moving in opposite direction. Such maps were periodic, but were weaker (2-3 fold) than the iso-orientation maps. The present results, indicating large overlap of clusters responding to different axis-of-motion, are thus different from the model of the functional architecture of area MT in another primate species, suggested by Albright et al., (1984). Supported by the Schilling Foundation.

169.9

WIDESPREAD FEEDBACK CONNECTIONS FROM AREAS V4 AND TEO. K.S. Rockland, *[1] K.S. Saleem,[2] and K. Tanaka[2]. [1]Dept. of Neurology, Univ. of Iowa, Coll. Med., Iowa City, IA 52242 and [2]RIKEN, Wako, Japan.

Feedback connections in the ventral visual pathway from TE to V1 are generally thought to be serial and reciprocal in nature. That is, area TEO projects densely to V4. In turn, area V4 projects densely back to V2. In order to characterize further these feedback connections, PHA-L injections were made in V4 or TEO in two macaque monkeys. Injections in area V4 (ventral prelunate gyrus:PLG) in fact demonstrated dense connections to area V2, to what is probably V3 (in the depth of the LS), and also to a large sector of dorsolateral V1. Connections to areas V2 and V3 concentrated in layers 1, 2, and 6, with occasional terminations in 3a or 5. Those in area V1 were in layer 1, but could occur in layers 2, 6, or 5. Connections from area TEO were directed to area V4 in the ventral PLG. There, they terminated densely in layers 1, 2, 3a, and 6, and occasionally in 5. Sparser connections were directed to area V2 and to area V1 (in the ventrolateral operculum). Connections to V1 were in layer 1; those to V2 in layer 1 and occasionally 2 and 5. Three serially reconstructed axons in V1 (from TEO, n=1; or from V4, n=2) traveled 4-7 mm in layer 1. Injections of fluorescent dyes into V1 and V2 confirmed the connections from areas V4 and TEO (labeled neurons were mainly in layers 6 and 3a). We conclude that feedback connections from V4 and TEO are widely distributed rather than merely reciprocating feedforward connections. These connections all target layer 1, but additional layers can be involved, possibly as a function of distance (EY07058 and NS19632, and the Carver Trust)

169.10

EXTENSIVE VISUAL FEEDBACK CONNECTIONS FROM VENTRAL INFEROTEMPORAL CORTEX. K.L. Douglas* and K.S. Rockland. Dept. of Neurology, Univ. of Iowa, Coll. Med., Iowa City, IA 52242.

Previous work has demonstrated that inferotemporal cortex in the macaque projects densely to area V4. In order to investigate further this relatively inaccessible cortex, injections of PHA-L were made in ventral area TE (in the depth of the occipitotemporal sulcus:OTS). As expected, this injection produced labeled axons in the dorsal prelunate gyrus (primarily layer 1) and in more posterior regions of the OTS (primarily in layers 1, 2, 3a, and, to a lesser extent, 5 and 6). There were also, however, labeled axons in dorsolateral V1 (layer 1) and V2 (layers 1, 2, and, sparsely, 5). Serial reconstruction of one axon in V1 showed 3 branches primarily in layer 1, with 2 of these continuing into V2. Branches measured 2.8, 4.8, and 5.6 mm dorsoventral (DV) x 1.5-2.4 mm AP. A second axon, in V2, had a single trunk which traveled in layer 1 for 7.0 mm DV x 1.8 mm AP. There was a short collateral (0.3 mm) in layer 5. This axon branched in the white matter to send a separate collateral to a second focus within V2 (layers 1 and 2). Injections of fast blue into V1 and diamidino yellow into V2 confirmed the connections from cortex in the OTS (labeled neurons were mainly in layers 6 and 3a). A few double-labeled neurons were evident. As V1 is not reported to project to anterior TE cortex, we conclude that feedback connections need not be strictly reciprocal. These connections all target layer 1, but shorter-distance connections, in particular, seem to have more involvement of other layers (NS19632, EY07058 and the Carver Trust)

169.11

MODULAR SEGREGATION OF VISUAL PATHWAYS IN OCCIPITAL AND TEMPORAL LOBE VISUAL AREAS IN THE MACAQUE MONKEY. D. J. Felleman*, E. McClendon and Ki Lin, Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX , 77030.

Area V4 consists of modules which receive input from either thin or interstripe compartments of V2 (DeYoe et al.,'88; Zeki and Shipp,'89) and from segregated regions of occipital and temporal cortex (DeYoe and Sissola,'91). V4 outputs to the PIT are also organized into segregated, interdigitated modules (Felleman and McClendon,'91). We now wish to determine: 1) how many types of modules are contained within V4 or PIT, 2) what are their relative sizes, 3) what are their input-output relationships, and 4) whether other V4 targets contain segregated modules. In the current experiments, 2 fluorescent retrograde tracers were injected into V4 sites separated by 2-4 mm. The precise patterns of labeling were digitized from unfolded, tangential sections of occipital and temporal cortex and were correlated with patterns of cytochrome oxidase and myeloarchitecture. V4 injections were characterized by their inputs from V2 thin- or interstripe compartments. In 2 cases, segregated clusters of labeled cells were found in the same V2 stripe, suggesting that V4 contains more than 2 types of modules. PIT projections to V4 arose from several clusters of cells of variable size, ranging from 6-8 mm across to less than 2 mm. V4 "interstripe" injections tended to label large, irregular zones in PIT, while dual V4 "thin-stripe" injections labeled several smaller foci in PIT and interdigitating zones of VOT. In 2 cases, WGA-HRP was also injected into PIT to determine the specificity and reciprocity of occipito-temporal pathways and to correlate the pattern of intrinsic connections in PIT to the extrinsic V4 pattern. This led to exquisite patterns of local, patchy, reciprocal connections which extended 5 mm or more from the injection sites. WGA labeled terminals and cell clusters in PIT, VOT, V3A and V4 often coincided with V4 fluorescent labeled cell clusters, thus demonstrating the reciprocity and specificity of these modular connections. Supported by EY-08372, the Sloan Foundation and the Whitehall Foundation.

169.12

CALCIUM-BINDING PROTEINS DEFINE SPECIFIC CORTICAL AND SUBCORTICAL COMPONENTS OF THE DOLPHIN VISUAL SYSTEM. I.I. Glezer*, P.R. Hof and P. J. Morgane. Dept. Cell Biol. Anat. Sci., CUNY Med. Sch., New York, NY 10031 Sch., Mt. Sinai Med. Sch., Fishberg Ctr. for Neurobiology, New York, NY 10029 and Worcester Fndn. Exp. Biol., Shrewsbury, MA 01545.

The calcium-binding proteins, calbindin D-28k (CB), calretinin (CR) and parvalbumin (PV) were studied immunohistochemically in primary visual cortex and subcortical structures (lateral geniculate nucleus, LGN and superior colliculus, SC) of dolphin (*Tursiops truncatus*) and macaque (*Macaca mulatta*). In the dolphin, in magno and parvicellular layers of the LGN almost all neurons are CB-positive; CR-positive neurons are found only in magnocellular layers, whereas PV-positive neurons are very sparse. The same relationships between the three calcium-binding proteins are found in the SC. Thus, specific subcortical pathways in the dolphin visual system are subserved mostly by CB- and CR-positive neurons. This correlates with the high concentrations of CB and CR-positive neurons in the upper cortical layers (I and II) which are considered major thalamocortical input layers in dolphin neocortex. Sparsely distributed PV-positive neurons are found mostly in layer IIIc/V. The rarity of PV-positive neurons in visual cortex and specific subcortical nuclei correlates with the general agranularity of the dolphin neocortex and absence of layer IV. In contrast, in the macaque, predominant thalamocortical neurons are PV-positive, whereas specific CB-positive neurons are much less numerous. These findings may reflect significant differences in phylogenesis of sensory systems between highly adapted aquatic and terrestrial mammals. Supported by NSF grant BNS-89-03717, by grant PSC-CUNY RF-662232, by MBRS/CRS Program of CCNY and by the Brookdale Foundation.

OTHER FACTORS AND TROPHIC AGENTS: GENERAL I

170.1

CILIARY NEUROTROPHIC FACTOR (CNTF) ALTERS THE THRESHOLD OF HIPPOCAMPAL PYRAMIDAL NEURON SENSITIVITY TO GLUTAMATE TOXICITY: SYNERGISTIC EFFECTS OF MONOSIALOGANGLIOSIDES. S.D. Skaper*[1], A. Negro[2], R. Dal Toso[1] and L. Facci[1]. [1]Fidia Research Labs and [2]Advanced Technology Division, Fidia S.p.A., Abano Terme, Italy.

CNTF is a multifunctional protein which promotes neuronal survival and also controls cell division of neuronal precursors, neurotransmitter and glial cell differentiation. Because trophic factors may influence excitotoxin-associated neurodegeneration, CNTF was examined for its ability to alter glutamate-induced hippocampal injury. Using cell cultures prepared from E18 rat hippocampus, recombinant human CNTF limited the loss of pyramidal neurons subsequently exposed to 200uM glutamate from DIV 5-6 (ED50: 5 ng/ml). CNTF was equally effective when added at least 48hr prior to glutamate. Subthreshold amounts (2-3 ng/ml) of CNTF and 10uM monosialogangliosides (GM1 or its inner ester form siagoside) given together were almost as active as 30 ng/ml CNTF in preventing glutamate-induced death. Facilitation of such trophic factor effects by gangliosides makes the latter useful tools in the study of CNS plasticity and repair processes.

170.2

CILIARY NEUROTROPHIC FACTOR (CNTF) PREVENTS AXOTOMY-INDUCED DEGENERATION OF ADULT RAT SUBSTANTIA NIGRA DOPAMINERGIC NEURONS. T. Hagg* and S. Varon

Dept. of Biology 0601, University of California San Diego, La Jolla, CA 92093

After a discrete but complete stereotactical transection of the adult rat nigrostriatal pathway with an extrusion micro-knife, approximately half of the substantia nigra compacta dopaminergic neurons undergo degeneration over a 2 week time period. The extent of degeneration was quantified by counting in the compacta region the number of neurons immunostained for their transmitter enzyme tyrosine hydroxylase and the number of cresyl violet/Nissl stained neurons larger than 10 µm in diameter. To test trophic factors which might affect the nigral degeneration we designed an infusion-cannula where solutions can be continuously administered directly into the brain tissue between the transection and the injured substantia nigra neurons. A 2-week infusion with vehicle did not cause excessive damage or alter the extent of the axotomy-induced degeneration. In sharp contrast, infusion with recombinant human CNTF (a gift from California Biotechnology, Mountainview CA) almost completely prevented the disappearance of cresyl violet stained neurons. However, CNTF did not prevent the loss of staining for tyrosine hydroxylase. This finding appears similar to that seen previously with the axotomized septal cholinergic neurons, where CNTF prevented their degeneration and losses of low affinity NGF receptor, but not the loss of choline acetyltransferase (Hagg, Neuron 8:145-158, 1992). These results provide evidence that CNTF is a general neurotrophic or neuroprotective factor and may have relevance for the future treatment of Parkinson's disease which is caused by degeneration of these substantia nigra neurons. Support: NINCDS grant NS-16349 and 27047.

170.3

EXPRESSION OF HUMAN CILIARY NEUROTROPHIC FACTOR *B*-GALACTOSIDASE DURING DEVELOPMENT.

F. Altruda, G. Stefanuto E. Hirsch, L.Silengo, L. Callegaro[1], and A. Negro[1]* Dept. of Genetics, University of Turin, [1]Advanced Technology Div., Fidia S.p.A. Abano Terme, Italy.

Ciliary Neurotrophic factor (CNTF) is not only a potent survival factor for a variety of embryonic neurons in culture, but also influences the differentiation of both developing neurons and glial cells. In order to evaluate the possible role of CNTF in neurous system development, it is important to determine which cells produce CNTF and when they begin to do so. Expression of CNTF has been previously examined using probes for its mRNA and immunoreactive protein. We have attempts a more precise analysis by use of transgenic animals. The 5' flanking region of the human CNTF gene was used to direct expression of a bacterial *B*-galactosidase reporter gene in various subset of neurons in transgenic mice. The spatial and temporal patterns of expression of these transgenes during embryogenesis and in adults will be reported.

170.4

STIMULATION OF INSULIN-LIKE GROWTH FACTOR-II EXPRESSION IN HUMAN NEUROBLASTOMA CELLS. **D.M. Martin*, and E.L. Feldman.** Department of Neurology and The Neuroscience Program, The University of Michigan, Ann Arbor, MI 48104.

Insulin-like growth factor II (IGF-II) has been implicated in regulating proliferation and differentiation of cultured neuroblasts. In human fetal brain and in neuroblastoma cells, IGF-II mRNA is highly expressed and may act as an autocrine growth or survival factor. In the current study, we report increased expression of IGF-II mRNA and protein in the human neuroblastoma cell line SH-SY5Y after exposure to serum-free media (SFM). Using Northern analysis, we observed a two-fold increase in the 6.0 kb IGF-II mRNA transcript after 24 hours in SFM. Levels of this IGF-II mRNA transcript returned to basal three days after addition of media containing 10% calf serum. IGF-II mRNA increased 30-fold after a three day incubation in SFM. The increase in IGF-II mRNA after exposure to SFM was most dramatic at lower plating densities. In addition to changes in mRNA, we observed a four-fold elevation of IGF-II protein after a three day incubation in SFM, measured by radioimmunoassay of conditioned SFM. SH-SY5Y cells continue to divide in SFM, based on time lapse photography and a steady rise in ^{3}H-thymidine incorporation over a three day incubation period in SFM. Taken together, our data support a role for IGF-II in autocrine mediated growth of neuronal cells, and suggest a feedback mechanism for IGF-II production.

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170.5

EXPRESSION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS IN HUMAN NEURONAL CELLS. **E. Feldman*, A. Randolph, D. Yee[1] and D. Martin.** Univ. of Michigan Med. Center, Ann Arbor, MI 48109, [1]Univ. of Texas Health Sci. Center, San Antonio, TX 78284.

SH-SY5Y neuroblastoma cells provide a good *in vitro* model for examining the action of the neuronal mitogen, insulin-like growth factor II (IGF II). SH-SY5Y cells express IGF II, which acts as an autocrine growth factor to promote SH-SY5Y cellular growth. IGF II action is modulated by a family of high affinity binding proteins, designated IGFBPs. IGFBPs influence IGF II receptor binding and regulate IGF distribution among tissue compartments.

In the present study, we examined the gene and protein expression of IGFBPs in SH-SY5Y cells cultured in the presence and absence of serum. In some experiments, γ-interferon was added as a neuronal differentiating agent. After collecting conditioned media, total RNA was analyzed by RNase protection or Northern blotting. Dr. S. Shimasaki, La Jolla, CA and Dr. D. Powell, Houston, TX, provided the human cDNA clones IGFBP 2,4,5 and 1,3, respectively. Under all conditions, IGFBP 2 was the major transcript present with four fold less gene expression of IGFBP 4 and 5. No IGFBP 1 or 3 mRNA was detected on Northern analysis or RNase protection. Ligand blotting demonstrated abundant IGFBP 2 in conditioned media, with lesser amounts of IGFBP 4. Cells grown in serum free media had a 30-fold increase in IGF II gene expression, with no corresponding change in IGFBP 2 gene expression. In the presence of γ-interferon, IGF II gene expression decreased two-fold while IGFBP expression increased. These findings suggest a differential regulation of IGF II and IGFBP gene expression during neuronal growth.

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170.6

INSULIN AND IGF-I INDUCE RAPID AND SUSTAINED CHANGES IN cFOS mRNA EXPRESSION IN CULTURED FETAL NEURONS. K.A. Heidenreich* and L. J. Robinson. Dept. of Pharmacol., Univ. of Colo. Hlth. Sci. Ctr., and the Denver VA Med. Ctr., Denver, CO 80262

Insulin and IGF-I support the growth and differentiation of fetal neurons in culture. One of the earliest cellular changes elicited by many growth factors is the induction of immediate early genes. In this study, we examined the ability of insulin and IGF-I to induce c-fos, a prominent member of the immediate early gene family, in post-mitotic neurons from day 8 chick forebrains cultured in fetal calf serum. Northern analysis of total RNA using a full-length 32P-labeled mouse cDNA probe (1.8 kB) revealed a single mRNA species of 2.2 kB induced 3-4 fold by insulin, IGF-I, and TPA (0.1μM). The dose-dependent induction of c-fos mRNA by insulin and IGF-I (1-250 ng/ml) was detected within 5 min and remained elevated in the presence of growth factor for at least 3 hr. Inhibition of protein synthesis by cycloheximide (100μM) also increased c-fos mRNA levels and was synergistic with insulin and IGF-I. Basal levels of c-fos mRNA were very low and constant during the 5 days of culture. These data suggest that insulin and IGF-I support neuronal differentiation in part by the induction of transcriptional programs initiated by c-fos.

170.7

ESTROGEN REGULATION OF THE INSULIN-LIKE GROWTH FACTOR-I (IGF-I) SYSTEM IN THE RAT PITUITARY. K.M. Michels*, W-H. Lee, A. Seltzer, S. Zorad, C.A. Bondy and J.M. Saavedra. Section on Pharmacology, LCS, NIMH and the Developmental Endocrinology Branch, NICHHD, NIH, Bethesda, MD 20892.

We used in vitro binding of [^{125}I]IGF-I and quantitative autoradiography along with in situ hybridization of riboprobes for IGF-I, type 1 IGF receptor and binding protein 2 (BP2) to investigate the effect of ovariectomy and estrogen replacement on the pituitary IGF system of rats.

Ovariectomized (OVX) and male rats had less binding in the anterior and posterior pituitary compared to estrus phase females. Estrogen replacement restored binding to levels similar to the intact female. Binding was highest during proestrus and significantly lower through the rest of the cycle.

No change in IGF-I receptor mRNA was detected. Expression of the BP2 mRNA was lower in the male and OVX female anterior and posterior pituitary compared to the estrogen-replaced female and was highest at proestrus. IGF-I mRNA expression in the anterior pituitary was lower for males and OVX compared to estrus phase females while no difference was detected in the posterior pituitary. Estrogen replacement restored mRNA expression to that seen in intact females. IGF-I mRNA expression was highest at estrus and proestrus and lowest at metestrus.

These results show that, in the pituitary, estrogen independently regulates, and is necessary for full expression of, several components of the IGF-I system.

170.8

CO-REGULATION OF TGFß-1 AND FIBRONECTIN mRNA IN RESPONSE TO BRAIN INJURY. G.M. Pasinetti*, N.R. Nichols, Dugich-Djordjevic M.M., Morgan T.E., Laping N., and C.E. Finch. Andrus Gerontology Center and Dept. of Biological Sciences, University Southern California, Los Angeles, CA 90089-0191

Striatal responses to cortical deafferentation in adult rat brain include elevation of fibronectin (FN) mRNA and TGFß-1 mRNA 3 and 10 days postlesioning as assessed by northern blot hybridization assay. In situ hybridization combined with immunocytochemistry confirmed northern blot data. Striatal TGFß-1 mRNA signal co-localized with immunostained microglia (OX-42, CR3 complement receptor) which overlapped anatomically with the distribution of degenerating afferent fibers. FN mRNA signal was sparsely distributed in the deafferented striatum and overlapped with the distribution of increased FN immunoreactivity associated with blood vessels and with punctate deposits in the striatal neuropil on adjacent tissue sections. In hippocampus, TGFß-1 mRNA and FN mRNA showed a similar coordinated schedule of changes by 3 and 10 days after colchicine injection, as assessed by in situ hybridization assay. This study suggests in vivo co-regulation and differential cellular distribution of FN and TGFß-1 mRNA following brain injury. Intracerebral infusion of TGFß-1 will ascertain the functional role of TGFß-1 on FN mRNA. Supported by United Parkinson Foundation and Alzheimer Association to GMP and NIH grant AG-7909, AG-9793 to CEF.

170.9

LOCALIZATION OF ACIDIC FIBROBLAST GROWTH FACTOR IN SPECIFIC SUBCORTICAL NEURONAL POPULATIONS. F.P. Eckenstein*, A.Stock, K.Kuzis, R.Nishi and W.R.Woodward. Dept. of Cell Biology&Anatomy, Dept. of Neurology, Oregon Health Sciences Univ., Portland OR 97201.

The effects of fibroblast growth factors (FGF) *in vitro* include the stimulation of mitogenesis in a variety of non-neuronal cell types and the promotion of the survival of various central and peripheral neuronal populations. The precise physiological role of FGFs *in vivo* is currently not known. As a step toward understanding the role of FGFs in the nervous system we determined the distribution of acidic FGF (aFGF) in the rat central nervous system (CNS). The levels of aFGF in dissected areas of the nervous system were quantified using a biological assay method and the cellular distribution of aFGF was determined in tissue sections using immunohistochemical methods. Acidic FGF was found to be localized within specific neuronal populations in the CNS and was absent from non-neuronal cells. Neurons found to contain aFGF immuno-reactivity were: magnocellular neurons in the septal area and nucleus basalis, some additional defined neuronal cell groups in the subcortical telencephalon, specific neuronal populations in the hypothalamus, the thalamus, the substantia nigra, the reticular formation, the pons, motor and sensory neurons. Only a very limited number of neurons in cerebral cortex and hippocampus contained aFGF immunoreactivity. A significant overlap of neuronal populations known to express the low-affinity nerve growth factor receptor (LNGFR) with populations containing aFGF-immunoreactivity was demonstrated by staining neighboring tissue sections for aFGF or LNGFR.

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170.10

NEURONAL SURVIVAL: COMBINATORIAL EFFECTS OF FGF, DEPOLARIZATION AND SUBSTRATES. M.E. Schmidt* and S.B.Kater, Program in Neuronal Growth and Development, Dept. of Anatomy and Neurobiology, Colorado State University, Fort-Collins, Colorado 80523

This study tests the survival promoting effects of specific agents alone, and in combination, in E10 chick ciliary ganglion neurons. Three classes of agents were used: Trophic factors (FGF), depolarization (high potassium) and surface substrate (laminin and Collagen IV). We found that while depolarization and FGF could independently promote survival, their effect was supra-additive when presented in combination. Furthermore, FGF and depolarization were shown to promote survival via separate pathways. These survival promoting effects showed striking differences depending upon the substrate. Although neither laminin (4.3±0.9% survival; at 24 hours) nor collagen (3.7±0.8% survival) promoted survival on their own, FGF mediated survival was highly dependent upon substrate: FGF survival on laminin was 76.5±1.8% and only 6.6±0.9% on collagen. In contrast, depolarization mediated survival did not appear to be substrate dependent (survival was 85.4±3.4% on collagen and 93.7±2.4% on laminin). Interestingly, although FGF was not able to promote survival on a collagen substrate, when added together with depolarization, FGF dramatically enhanced depolarization mediated survival ($P<0.001$), suggesting that depolarization may prime cells to respond to FGF. Taken together, this study reports two findings that are consistent with the growing idea that neuronal survival is regulated by the complex interactions with several different cues. First, we show that FGF and depolarization act through different pathways and act synergistically when added in combination. Second, we show that substrate can act as an important modulator of the combinatorial action of various survival promoting agents.

170.11

CYTOKINE DEVELOPMENTAL REGULATION OF IMMORTALIZED HIPPOCAMPAL NEURONAL PROGENITOR CELLS. M.F. Mehler*, R. Rozental, M. Dougherty, D.C. Spray, and J.A. Kessler. Albert Einstein College of Medicine, Bronx, New York 10461.

The mechanisms governing progressive neuronal lineage commitment and successive stages of terminal differentiation are largely unknown. Significant parallels exist between "neuropoiesis" and hematolymphoid development, the latter regulated by an array of multifunctional cytokines. To establish an experimental system to study a possible role for cytokines in neuronal development, we investigated growth factor requirements for differentiation of a well-defined neuronal precursor population. Dissociated murine embryonic (E17) hippocampal cells were immortalized using retrovirally-mediated gene transfer with a temperature-sensitive mutant form of the SV40 large T antigen. Clonal cell lines propagated at 33°C exhibited immature cellular forms that expressed the neuronal precursor protein nestin. These cells, when cultured at 39°C (with breakdown of thermolabile large T) in defined media supplemented with selected interleukins, exhibited neurite outgrowth, immunoreactivity for the 200 kD phosphorylated form of neurofilament and appearance of action potentials that were tetrodotoxin (TTX)-insensitive. Pretreatment of cultured cells with basic fibroblast growth factor or concurrent application of transforming growth factor alpha resulted in further morphologic maturation and the generation of action potentials and inward current in response to depolarizing steps that were reversibly sensitive to TTX administration. These studies demonstrate the feasibility of utilizing clonal neuronal progenitor cell lines for the characterization of successive stages of neuronal maturation. Further, they suggest that cytokines which control selective stages of hematopoiesis may also regulate the maturation of neurons from their progenitor cells.

170.12

PURIFICATION AND PROTEIN SEQUENCING OF GLIAL GROWTH FACTORS -I AND -II . A.D.J. Goodearl*, J.B. Davis, N.F. Totty, J.J. Hsuan, Q.T. Nguyen, M.D. Waterfield, M. Otsu and P. Stroobant Ludwig Institute for Cancer Research, 91 Riding House Street, London W1P 8BT (UK)

Glial Growth Factor (GGF) has been identified as an active component present in extracts of brain in many vertebrates, and also bovine pituitary glands, that stimulates cell division in rat Schwann cells and cortical astrocytes. GGF activity has been observed in certain human Schwann cell tumours and is also expressed in a nerve-dependent fashion during limb regeneration in the newt (Brockes (1987) Meth. Enz. 147 217 and refs. therein).

Purification of GGF from whole bovine pituitary glands was undertaken using a Schwann cell proliferation assay to measure GGF activity. Two protein species were identified, named GGF-I (approx. MW 33 kDa) and GGF-II (approx. MW 59 kDa) and were purified to apparent homogeneity as judged by silver stained SDS-PAGE. Both purified proteins are mitogenic for neonatal rat Schwann cells at subnanomolar concentrations in vitro, effects potentiated by cAMP-elevating agents .

Considerable amounts of peptide sequence from both preparations have been obtained including a highly homologous peptide sequence found in each preparation, suggesting that these molecules are related at the primary structure level. Computer database searches have revealed no significant sequence similarities between the GGFs and other growth factors. GGF-I and GGF-II may thus represent founding members of a new family of mitogenic growth factors in the nervous system.

170.13

MOLECULAR CLONING OF BOVINE GLIAL GROWTH FACTORS

M. Marchionni, F. Danehy, D. Misumi, M. Chen, A. Goodearl, I. Hiles, M. Otsu, P. Stroobant, M. Waterfield, E. Gamzu*, and D. Gwynne Cambridge NeuroScience, Inc., Cambridge, MA and Ludwig Institute for Cancer Research, London, UK.

Glial growth factors I and II are potent mitogens for cultured Schwann cells and are present during limb regeneration in the newt (see Brockes et al (1987) Meth. Enz. 147, 217). These factors may have therapeutic utility in treatment of peripheral neuropathies and in nerve regeneration following injury. Bovine pituitary GGF-I and GGF-II were purified to apparent homogeneity leading to several novel peptide sequences (Goodearl et al, unpublished). Using sets of degenerate oligonucleotide probes that represented similar peptide sequences found in both the GGF-I and the GGF-II preparations, one 20 kb bovine genomic DNA was identified and cloned. Five distinct mRNAs have been isolated by RT-PCR reactions and by screening cDNA libraries from bovine posterior pituitary. Sequence analysis show multiple splicing patterns and that these mRNAs contain several GGF-I and GGF-II peptide sequences suggesting that this gene encodes GGF-I and/or GGF-II. Selected clones have been expressed to verify activity. Comparisons to sequence databases indicate that the GGFs represent a new family of growth factors. Southern blot analysis at reduced stringency suggests the presence of related sequences in several mammalian genomes. In situ hybridization studies are in progress to elucidate further the expression pattern of GGF-coding sequences.

170.14

BIOCHEMICAL ISOLATION AND CHARACTERIZATION OF MUSCLE DERIVED DIFFERENTIATION FACTOR (MDF): A NOVEL FACTOR(S) WHICH INDUCES EXPRESSION OF THE TYROSINE HYDROXYLASE GENE IN NON-CATECHOLAMINE NEURONS IN CULTURE. L. Iacovitti* and X. Du, Dept. of Neurology, Hahnemann Univ., Philadelphia, Pa., 19102.

Our previous studies have demonstrated that incubation of brain non-catecholamine neurons overnight with the soluble portion of an extract of rat striatal muscle cells (L6 clonal line) will dramatically induce the appearance of the catecholamine (CA) enzyme tyrosine hydroxylase (TH) in brain non-CA neurons grown in culture (Iacovitti et al, 1989, 1991). Because of its ability to direct the biochemical differentiation of these neurons without affecting cell survival, the substance(s) responsible for this activity has been termed muscle-derived differentiation factor(s) or MDF. We have recently used bovine heart to isolate and biochemically characterize MDF. Standard purification procedures, including ammonium sulfate fractionation, DEAE–cellulose ion exchange chromatography, sulfate sephadex G-100 chromatography, ultrafiltration and immobile phase chromatography have been utilized. Column fractions have been bioassayed in culture: mouse striatal neurons have been incubated with individual column fractions overnight. Cultures are then fixed and the number of TH containing neurons determined immunocytochemically. These procedures have yielded a 20,000 fold purification of MDF: the final products, as determined by SDS-PAGE under nonreducing conditions, include 84kD, 82kD, 78kD and 64kD molecular weight species. Inductive activity could not be electroeluted from the gel. We are therefore raising polyclonal antisera against each of these four molecules for neutralization studies. The ability to directly neutralize biological activity or to prevent TH induction following immunoprecipitation with a specific antibody is critical for the final identification of MDF. Supported by NIH NS24204-04 and the Ben Franklin Foundation.

171.1

MECHANISM OF DARK ADAPTATION: EVIDENCE FOR SUSTAINED ACTIVATION OF THE VISUAL CASCADE BY BLEACHED PHOTOPIGMENT MC Cornwall[1] and GL Fain[2*]. [1]Dept Physiol, BU Sch Med, Boston, MA 02118 & [2]Depts Ophthal & Physiol Sci, UCLA, LA, CA 90024.

Using methods pioneered by Hodgkin and Nunn (*J. Physiol.* **403**:439-471, 1988), we have examined the effects of steady backround intensity (I_B) and bleaching on the velocities of the PDE and cyclase in rods isolated from the retina of the tiger salamander. Backgrounds accelerate both PDE and cyclase velocities. This effect increases with increasing I_B (and so with *decreasing* receptor sensitivity, S_F). PDE and cyclase velocities are also increased by the bleaching of a substantial fraction (>10%) of the visual pigment. The acceleration of enzyme velocites by bleaching persists in darkness in an isolated rod as long as we have waited (1-2 hours) and is accompanied by a sustained decrease in S_F. The increases in PDE and cyclase velocities (and the decrease in S_F) can be reversed by the addition of liposomes containing 11-cis retinal. These experiments indicate that bleached pigment (probably just opsin) activates PDE, though with an efficiency perhaps 10^{-6} that of Rh^*. They also suggest that the mechanisms of desensitization by bleaching and backgrounds may share some fundamental similarities.

171.2

COBALT IONS OF LOW LEVELS SELECTIVELY BLOCK ROD PATHWAY IN CARP RETINA. X.L.Yang* and L.L.Yuan. Shanghai Institute of Physiology, Chinese Academy of Sciences, Shanghai 200031, China

It is well established that synaptic transmission in the outer plexiform layer of the retina is generally blocked by extracellular cobalt ions of high levels (more than 1 mM). In superfused, isolated carp retinas we found, however, that cobalt ions of low levels (8-12 μM) showed differential effects on rod and cone synaptic activity. With the application of 10 μM cobalt, the rod-driven horizontal cells hyperpolarized and their light responses were suppressed, whereas neither membrane potential nor light responses of the cone-driven horizontal cells were affected. Furthermore, scotopic ERG b-waves, which have dominant contribution from the rods were suppressed by 10 μM cobalt, but cone-contributed photopic b-waves remained unaffected. These results suggest that the rod pathway in the outer plexiform layer is selectively blocked by cobalt of the low concentrations. The glutamate-isolated receptor potentials (PIII) were unaffected by 10 μM cobalt in either scotopic or photopic conditions, indicating that cobalt must act on the synapse between the rods and rod horizontal cells.

171.3

QUANTITATIVE FITS OF AN AMACRINE MODEL OF DIRECTIONAL SELECTIVITY TO RABBIT DATA. N. M. Grzywacz* and F.R. Amthor. Smith-Kettlewell Inst., 2232 Webster St., San Francisco, CA 94115 and Dept. Psychol. and NRC, Univ. Alabama at Birmingham.

On-Off directionally selective (DS) ganglion cells of the rabbit retina respond in a DS manner to edge motions spanning as little as 3 to 5 μm. Their directionality is maintained over at least three decades of speed. When stimulated with periodic gratings, these cells' preferred-direction is invariant to spatial frequency, except sometimes for high-spatial frequencies, which in any event elicit small responses. We show that a recently proposed model of retinal directional selectivity (Borg-Graham and Grzywacz, 1992) accounts for this robust directionality. The model postulates that an amacrine-cell dendrite receives excitatory and inhibitory synapses throughout its length. This dendrite would then make an excitatory asymmetric synapse via its distal end onto the DS ganglion cell. The inhibitory synapses would have slow, sustained kinetics and would be of a shunting-inhibition type.

The model accounts for the DS responses to small motions through the pattern of activation of inhibitory synapses. During null-direction motion, their position between the excitatory synapses and the amacrine dendrite's output allows them to shunt out almost all excitatory currents. Furthermore, speed robustness results from sustained inhibitory synapse activation during arbitrarily slow null-direction motions. While the spatial period of periodic gratings is larger than the dendrite, preferred-direction responses remain strong. However, the model's inhibition reduces them at high spatial frequencies and the preferred direction changes to reduce the spatial frequency along the original preferred--null axis. In addition to these results, this model is consistent with apparent-motion, pharmacological, and patch-clamp data (Borg-Graham and Grzywacz, 1992). We conclude that this symmetric-inhibitory asymmetric-amacrine model can account for retinal directional selectivity.

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171.4

NEUROTENSIN MODULATES QUANTAL TRANSMISSION BETWEEN PAIRS OF RETINAL AMACRINE CELLS. E. Gleason, S. Borges and M. Wilson*. Department of Zoology, University of California, Davis, CA 95616.

Synaptic transmission between isolated pairs of GABAergic amacrine cells has been examined in sparse cultures of chick retina. In normal saline (3mM, $[Ca]_o$), discrete quantal outward currents can be observed in the presence of TTX when the postsynaptic cell is voltage-clamped at 0mV and the presynaptic cell is held negative to -40mV, or else left unclamped. Quantal currents rise quickly (less than 3 msec) and decay with a mean time constant of 17 msec. Mean peak quantal conductance was about 254 pS, equivalent to the simultaneous opening of only 15, 17 pS $GABA_A$ channels. A net flux of $32x10^3$ Cl^- ions crosses the membrane per mV of driving force.

While the time intervals between spontaneous quanta are generally random (Poisson distribution), bath applied neurotensin (300 nM) causes quanta to arrive in periodic bursts separated by tens of seconds. Fura-2 ratio imaging of amacrine cells reveals that neurotensin induces $[Ca]_i$ oscillations that are prevented by the phospholipase inhibitor 4'-bromophenacyl bromide, or by the phospholipase C inhibitor U-73,122 but not its inactive analog U-73,343. In addition, the protein kinase C inhibitor H-7, has no effect on the induced $[Ca]_i$ oscillations. These results suggest that neurotensin, a peptide that has been localized in some amacrine cells of the retina, modulates release of GABA through IP_3 control of intracellular calcium in postsynaptic cells. Were this control of calcium local, it might provide a functional explanation for the reciprocal and serial synapses common in the inner retina. Supported by EY04112.

171.5

GLUTAMATE, GABA AND GLYCINE IMMUNOREACTIVITY IN THE MACAQUE MONKEY RETINA

M Kalloniatis[1] and RE Marc*[2]: [1]Department of Optometry, University of Melbourne, Parkville 3052, Australia and [2]Sensory Sciences Center, University of Texas, 6420 Lamar Fleming, Houston, Texas 77030.

The putative neurotransmitters, glutamate, GABA and glycine are though to mediate "fast" synaptic transmission in the vertebrate retina. It is still uncertain whether these three neurotransmitters are found in the majority of retinal neurons and if distinct subpopulations of neurons, for example, some bipolar cells or ganglion cells, are exclusively GABAergic or glycinergic. We conducted serial section post-embedding silver-intensified immunogold detection of anti-glutamate, anti-GABA and anti-glycine immunoglobulin binding in the monkey retina.

All photoreceptor cells (rods and cones) were immunoreactive for glutamate. Of all the somas located in the inner nuclear layer (INL) [N=344], 73% were immunoreactive for only one of the three neurotransmitters. Within the central retina, all horizontal cells were immunoreactive for GABA with no glutamate immunoreactivity. Bipolar cell bodies were immunoreactive for glutamate, and as in previous studies, distinct subpopulations of bipolar cells were weakly immunoreactive for GABA or glycine. From the serial sections, it was possible to show that these bipolar cells were always also strongly immunoreactive for glutamate. Cell bodies in the amacrine cell layer were immunoreactive for either GABA or glycine, with a small population of cells being immunoreactive for both (1%). Co-localization of the neurotransmitters in the INL were; glutamate and GABA (8%) and glutamate and glycine (8%). Two distinct groups of presumed GABAergic amacrine cells were observed; one showing no glutamate immunoreactivity and the other showing weak glutamate immunoreactivity. Cell bodies in the ganglion cell layer (GCL) [N=432] were immunoreactive for glutamate (89%) confirmed to be ganglion cells using an anti-glutamine antibody, with a subpopulation of large soma ganglion cells (3%) also being weakly immunoreactive for GABA. The subpopulation (9%) of displaced amacrine cells in the GCL were only immunoreactive for GABA. No glycine immunoreactive cell bodies were found in the GCL.

Virtually all the neural cells (90%) in the INL and all the cell bodies in the GCL were immunoreactive for one or more of the "fast" neurotransmitters. The cell bodies showing no immunoreactivity in the INL (10%) probably reflect Müller's cell bodies. We conclude that the vast majority retinal neurons use one or more of these three putative neurotransmitters for "fast" neurotransmission.

171.6

NBQX SELECTIVELY BLOCKS THE NULL RESPONSE MECHANISM OF DIRECTIONALLY SELECTIVE RABBIT RETINAL GANGLION CELLS. E.D. Cohen* and R.F. Miller Dept. Physiol., Univ. of Minnesota., Minneapolis, MN 55455

We examined the role of NMDA and non-NMDA EAA receptors on on-off directionally selective ganglion cells in a superfused retina eyecup preparation of the rabbit. When the non-NMDA antagonists NBQX (1-10μM) or DNQX (10μM) were bath applied to the retina, direction selectivity to a moving slit was abolished and the DS ganglion cell responded equally well to preferred and null movement. However, NBQX/DNQX did reduce, by 20-40%, the number of impulses when compared to the magnitude of the preferred discharge under control conditions. Similar to the findings of Massey and Miller (J.Neurophys.63:1 '90) application of the NMDA antagonist D-AP7 (200-250μM) had no effect on the DS mechanism, but did reduce the number of impulses evoked by moving stimuli. The combination of NBQX and D-AP7 abolished the light-evoked responses of DS cells. It appears one action of NBQX/DNQX is to force the preferred response to be subserved by NMDA receptors. In contrast, the inhibition underlying the null response appears to depend more critically on NBQX/DNQX sensitive mechanisms. Presumably the NBQX-sensitive inhibitory site is at the level of amacrine cells. Intracellular recordings from amacrine cells show that the light evoked responses of these neurons typically become more transient in NBQX/DNQX raising the possibility that differences in phasic-tonic response components could contribute to loss of the null mechanism in the DS ganglion cell. This idea, together with other competing hypotheses are presented. Supported by NIH grants F32EY0617 to E.D.C. and R01EY03014 to R.F.M.

171.7

DIRECT VISUALIZATION OF THE DENDRITIC AND RECEPTIVE FIELDS OF MOTION SELECTIVE RETINAL GANGLION CELLS. G. Yang and R.H. Masland.* Harvard Medical School, Boston MA 02114.

Directionally selective ganglion cells receive excitatory inputs from the starburst amacrine cells. Other things being equal, the receptive field of a directionally selective ganglion cell should thus equal the width of its dendritic arbor plus the width of all of the starburst cells that synapse upon it. We used a new experimental technique to compare the ganglion cells' dendritic and receptive fields.

Isolated rabbit retinas with ganglion cells labeled by retrograde transport of Fast Blue were mounted in a fluorescence microscope. The activity of a directionally selective ganglion cell was recorded extracellularly (with the microscope off) and its receptive field plotted, using stimuli projected via the microscope condenser. The identified cell was then injected with Lucifer Yellow. The boundary of the receptive field was then projected through the same optical system, so that it appeared superimposed upon the dendritic arbor of the injected cell.

For a sample of 33 directionally selective ganglion cells, the mean diameter of the receptive field center exceeded the diameter of the dendritic field by 8.0 $\pm$ 2.4%. This is much less than the spread of the overlapping starburst amacrines at the eccentricities studied. Either the spread of activity along the amacrine cell's dendrites or their synaptic connectivity must be quite limited.

171.8

NOISE ANALYSIS OF ß GANGLION CELL MICROCIRCUITRY

Michael A. Freed* LNP, NINDS, NIH, Bethesda, MD 20892

The ß ganglion cell in sublamina b of the cat retina has an On-center receptive field similar to those of presynaptic bipolar cells. This suggests bipolar cells contact ganglion cells at excitatory, sign-conserving synapses. We used an eyecup preparation to test this idea: we impaled with intracellular electrodes 2 On-ß ganglion cells. Flashing, red slits of light (1 s, 647 nm) produced depolarizing center responses and also increased voltage noise variance. This suggests presynaptic neurons open channels with a reversal potential positive to the dark potential, and thus are predominantly excitatory.

Two intervals of noise (250 ms, 512 points) were extracted from each digitized response: one during the sustained portion of the response and one in the dark. Although somatic spiking was inactivated by somatic depolarization, lower amplitude events persisted. These were probably spikes from the initial segment, and would confound noise analysis. To avoid this hindrance, only those intervals of noise which had symmetrical, unimodal, amplitude distributions were studied further. The changes in voltage noise variance ($\Delta\sigma^2 \approx 10\ nV^2$) and mean voltage ($\Delta V \approx 1$ mV) indicated an elementary event amplitude ($\approx \Delta\sigma^2/\Delta V$) on the order of 10 µV. Assuming the time course of the elementary events was determined by the time constant of the cell ($\tau \approx 10$ ms), the frequency of elementary events ($\approx \Delta V^2/\Delta\sigma^2\ \tau$) was about $10^4\ s^{-1}$. The frequency of captured photons was about $10^9\ s^{-1}$ (area = 70 µm x 400 µm; $I = 6.3$ log(photons $\mu m^{-2}\ s^{-1}$)). This suggests, under mesopic conditions, elementary events are not captured photons but either channels or synaptic quanta. These results support previous results (1991 Neurosci Abst #143.6) by indicating infrequent synaptic events (≈10 per integration time) from a large number of synapses (200).

171.9

RABBIT RETINAL GANGLION CELL SURROUND MECHANISMS: PHYSIOLOGY AND PHARMACOLOGY.

DK MERWINE[1] & FR AMTHOR[2]*. [1]Optometry, [2]Psychology/NRC, U. Alabama B'ham 35294

We have previously shown that surround stimulation lowers the slope of the center mechanism's response vs contrast function - consistent with a nonlinear center-surround interaction. In addition, the function's saturation level is reduced with no change in threshold. These data can be fit with a shunting-inhibition model originally developed for ON-OFF DS cells (Amthor & Grzywacz, 1991).

We have now analyzed the pharmacological mechanisms of surround inhibition in rabbit retinal ganglion cells. Both 3 µM strychnine or picrotoxin increase the cell's spontaneous activity; however, strychnine, a glycine antagonist, is shown to lower, while the GABA antagonist, picrotoxin, raises the response function's saturation level. Strychnine also reduces the effectiveness of the surround, while picrotoxin has no effect on surround inhibition.

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171.10

REDOX MODULATION OF GLYCINE-EVOKED CURRENT IN RAT RETINAL GANGLION CELLS.

Zhuo-Hua Pan* and Stuart A. Lipton. Department of Neurology, Children's Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Disulfide bonds and/or sulfhydryl group(s), which exist on all known ligand-gated channels, have been shown to play an important functional role in the regulation of neurotransmitter responses (e.g., for NMDA-evoked currents, see S.Z. Lei et al., *Neuron*, June 1992). We report here that glycine responses are also modified by sulfhydryl reagents. The whole-cell patch clamp technique was used to examine the effects of sulfhydryl reagents on glycine-induced current of solitary rat retinal ganglion cells in vitro. Extracellular application of the disulfide reducing agent dithiothreitol (DTT; 2-5 mM) significantly suppressed responses evoked by glycine (10-100 µM), while the oxidizing agent 5,5-dithiobis-2-nitrobenzoic acid (DTNB; 500 µM) slightly potentiated the responses. The DTT effect was not voltage dependent; nor did it interfere with inhibition by strychnine (100 nM). Similar to DTT, reduced glutathione (GSH; 5 mM) decreased glycine-evoked currents. In contrast, oxidized DTT (2-5 mM) or oxidized glutathione (GSSG; 5 mM) modestly enhanced glycine responses. *N*-ethylmaleimide (NEM; 1-10 mM), which irreversibly alkylates sulfhydryl groups, enhanced glycine-induced currents, especially after applying DTT to expose free sulfhydryl (-SH) groups. Taken together, these results suggest that regulatory sulfhydryl group(s) exist on the extracellular surface of the glycine receptor, influencing physiological function.

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171.11

CORRELATIONS IN THE RESPONSES OF RETINAL GANGLION CELLS TO VISUAL STIMULI.

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As a first step in analyzing how the ganglion cell population encodes visual information, we have attempted to determine whether different cells respond to visual stimuli independently of each other.

A multielectrode array was used to record action potentials from the ganglion cell layer of the isolated salamander retina under spatially uniform illumination modulated in intensity as a 1Hz square-wave. The cross-correlogram between the spike trains of two cells often showed a central peak 20-40 ms wide, containing up to twenty times as many spikes as the corresponding interval one stimulus period removed. The excess correlation decreased with increasing separation between the cell bodies, declining to roughly half at 200µm. Similar correlations were observed in darkness and during flickering white noise stimuli. The excess correlations are not expected if a common light stimulus were processed independently by different ganglion cells. Instead, the results indicate that ganglion cells may receive input from a common stochastic signal source. The short time scale of the correlations suggests that the signal does not arise from shared photoreceptor inputs. The correlations may be generated by reciprocal connections between ganglion cells or common input from spiking amacrine cells.

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171.12

THE CONTRAST GAIN CONTROL OF PRIMATE M GANGLION CELLS: Spatial Organization And Temporal Properties.

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Previously, we have shown that the temporal frequency response of primate retinal ganglion cells (RGCs) that project to the magnocellular layers of the LGN (M cells) is modified by the overall contrast in the visual stimulus (Benardete *et al., Vis. Neurosci.* '92). Work on the cat retina established that a similar process, the contrast gain control, was responsible for this change in dynamics (Shapely & Victor, 1981). Here we investigated the spatial extent and the temporal frequencies that influence the contrast gain control.

RGC activity was monitored as synaptic (S) potentials in the LGN of anesthetized and paralyzed macaque monkeys. The contrast of sinusoidal gratings and/or a spot and an annulus produced on a CRT was temporally modulated with a sum of 8 sinusoids in order to calculate temporal transfer functions. In addition, we used a hybrid m-sequence method to measure the impulse response of various parts of the M cell receptive field (Benardete *et al.*, ARVO '92).

Experiments with sinewave gratings show that the contrast gain control signal comes from retinal elements with a fine spatial scale (similar to the receptive field center or smaller). These elements cover a large area of the receptive field, coextensive with the classical receptive field center and surround. Experiments with small, centered spots and annuli suggest that the contrast signal is pooled from a large number of these elements to modify the M cell temporal frequency response. We are using data from the spatial and temporal analyses to construct a model that accounts for the dynamical properties of the M cell.

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171.13

PERMEATION OF CALCIUM IONS THROUGH KAINATE RECEPTOR-COUPLED CHANNELS IN RAT RETINAL GANGLION CELLS. Dongxian Zhang* and Stuart A. Lipton. Dept. of Neurology, Children's Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

The Ca^{2+} permeability and current-voltage (*I*-V) relation of non-NMDA receptor subunits is controlled by a glutamine to arginine substitution in the second transmembrane segment (Verdoom et al., *Science* 1991;252;1715; Hume et al., *ibid.* 253:1028). Non-NMDA receptors permeable to Ca^{2+} have been found in several types of neurons, including retinal bipolar cells (Gilbertson et al, *ibid.* 251;1613). We investigated the permeation of Ca^{2+} through kainate receptor-coupled ion channels in cultured rat retinal ganglion cells (RGCs) by using patch-clamp and calcium-imaging techniques. With physiological Hanks' solution in the bath and CsCl/TEACl in the patch pipette, RGCs displayed a nearly linear *I*-V curve for kainate responses. In 5/27 RGCs, with extracellular Na^+ replaced by the impermeant ion *N*-methyl-D-glucamine and Ca^{2+} as the only permeant cation, an inward current was observed at a holding potential of -60 mV. The reversal potential shifted to -45 mV in 2.5 mM external $[Ca^{2+}]$, and to near 0 mV in 70 mM external $[Ca^{2+}]$. In contrast, for 22/27 RGCs no Ca^{2+} influx was observed. The permeation of Ca^{2+} through kainate-gated channels in a subpopulation of RGCs was further supported by fura-2 calcium imaging measurements showing that kainate increased intracellular $[Ca^{2+}]$ in a subpopulation of RGCs independent of their voltage-dependent Ca^{2+} channels. Therefore, glutamate may affect $[Ca^{2+}]_i$ in RGCs via both NMDA- and non-NMDA-gated ion channels.

171.14

X - TYPE CAT GANGLION CELLS SHOW NONLINEAR OSCILLATIONS OF THE GENERATOR POTENTIAL DETERMINING THE REGULARITY OF SPIKE BURSTING. A. W. Przybyszewski*, M. J. M. Lankheet, and W. A. van de Grind Dept. of Physiology, Freie Universitaet Berlin, Arnimallee 22, 1000 Berlin 33, Germany & Utrecht Biophysics Research Institute, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

The classification of ganglion cells into X - and Y - types is based on the linear and nonlinear spatial summation properties (Enroth-Cugell and Robson, 1966). It will be shown that the generator potential of X-type ganglion cells shows *strong nonlinear* behaviour in the time domain.

Ganglion cell activity was recorded intracellularly in the optically intact *in situ* eye. The light stimuli were spots of different sizes modulated sinusoidally in intensity. The mean illumination level was in the photopic range. Recorded signals were convoluted with the wavelet function (Przybyszewski, 1991) to separate the generator potential (GP) from the spikes. Fourier analysis of the GP of on-center X-ganglion cells showed resonance for a frequency between 8 and 16Hz, and for 0.7 deg spot size. The analysis of the GP in 3D phase-space showed other faster oscillations which were local and not directly synchronized in time with the stimulus. The GP of X-cells is strongly nonlinear for the spot sizes comparable to the size of the receptive field center, temporal frequency 8Hz and for the high light intensities (53 - 530cd/m^2). Such oscillations are very similar to those described by the van der Pol equation. The strong nonlinearities uncovered in the GP of X-cells produce the observed precise timing of spike bursts.

EXCITATORY AMINO ACIDS: RECEPTORS III

172.1

DEVELOPMENTAL EXPRESSION OF NMDA RECEPTOR SUBTYPES. H. Monyer* and P. Seeburg. Center for Molecular Biology, Heidelberg, Germany.

A new family of NMDA receptors whose members (NR2A, NR2B and NR2C) are between 55% and 70% identical but only 20% identical to other excitatory amino acid receptor subunits has been cloned and characterized recently (Monyer et al., *Science*, in press). Heteromeric channels formed from one of these subunits plus NMDAR1 (Moriyoshi et al., 1991, *Nature* 354:31) differ in their gating behavior, Mg2+ sensitivity and the effect of glycine. There is an abundant but differential expression of all subtypes in the adult and developing rat brain as visualized by in situ hybridization. We have now identified a new subtype, NR2D. NR2D is heavily expressed in the embryonic rat brain and spinal cord followed by a substantial decline during development. The physiological properties of this subunit will be discussed.

172.2

Ca^{2+} PERMEABILITY OF RECOMBINANT NMDA RECEPTOR CHANNELS. N.Burnashev, H.Monyer, R.Schoepfer and P.Seeburg*. MPI für Med. Forschung and ZMBH, 6900 Heidelberg, Germany.

Ca^{2+} permeability was measured for heteromeric NR1/NR2A and NR1/NR2C receptor channels assembled from recently cloned subunits transiently expressed in 293 cells using patch clamp techniques in whole-cell mode. The reversal potentials for the NMDA-activated whole-cell current shifted from about 0 mV in normal, high Na^+ extracellular solution to positive potentials around +20 mV in high (110 mM) Ca^{2+} extracellular solution for both receptor subtypes. The reversal potentials in high Ca^{2+} extracellular solution are close to those of native NMDA receptor channels in these conditions and they are identical to reversal potentials determined for homomeric AMPA selective GluR-channels engineered to carry asparagine at the Q/R site in the putative TM II segment. Point mutations in homologous position in NMDA receptors revealed that this site is involved in control of Ca^{2+} permeability.

172.3

SINGLE CHANNEL ANALYSIS OF A MUTANT NMDA RECEPTOR WITH ALTERED ION-CHANNEL PROPERTIES FOR MAGNESIUM IONS. R.Schoepfer*, P.Seeburg, T.Kuner N.Burnashev, W.Günther and P.Ruppersberg. ZMBH and MPI für Med. Forschung, 6900 Heidelberg, Germany

Ionotropic glutamate receptors are divided into two classes: NMDA (N-methyl-D-aspartate) and non-NMDA receptors. Recent molecular cloning of subunits for both classes defines them as members of one gene family.

The NMDA receptors display several distinctive features: The channels are highly permeable for Ca^{2+}. Mg^{2+} in the extracellular milieu causes a voltage dependent block of the ion channel. This Mg^{2+} property is considered to be of major biological significance and is thought to be involved in some cases of long term potentiation.

The abstract by N.Burnashev presents a NMDA receptor subunit mutant in the presumed channel lining segment with altered Ca^{2+} properties. We analysed this mutant with respect to the magnesium block. Wild-type and mutant NMDA receptor subunits were first expressed transiently in 293 cells. Agonist-induced whole cell currents were measured and Mg^{2+} dependent parameters determined. For single channel analysis recombinant receptors were expressed in Xenopus oocytes and analyzed by patch-clamping.

We found that the mutant has altered Mg^{2+} dependent channel properties.

172.4

CHROMOSOMAL LOCALIZATION OF GLUTAMATE RECEPTOR GENES: CANDIDATES FOR FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS AND OTHER NEUROLOGICAL DISORDERS OF MICE AND MAN

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To seek genes underlying hereditary neurological disorders, eight glutamate receptor (GluR) genes were localized to mouse chromosomes by genetic linkage using interspecific backcrosses, and two of these were also assigned to human chromosomes.

The mouse *Glur-5* gene was mapped to Chr 16 using a mouse GluR5-3 cDNA that appears to encode a novel C-terminal isoform of GluR5 (Bettler et al., Neuron 5: 583). The gene is 3.9 centiMorgans (cM) telomeric to the *App* gene and 2.4 cM centromeric to *Sod-1*. The strong conservation of this linkage group between mouse and man predicted that the homologous *GLUR5* gene would be found on human Chr 21q21-q22.1. Localization to Chr 21 was confirmed by analysis of human-rodent somatic cell hybrids. The *GLUR5* gene thus becomes a candidate for a familial form of amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease), which has been mapped telomeric to *APP* and close to *D21S58* on Chr 21 (Siddique et al., N. Engl. J. Med. 20:1381). The expression of the rodent *Glur-5* gene correlates with the pathophysiology of ALS. Gene dosage effects of *GLUR5* could also play a role in Down's syndrome pathophysiology.

A clone of mouse GluR6c cDNA, encoding a novel C-terminal isoform of subunit GluR6 (Egebjerg et al., Nature 351: 745), was mapped to mouse Chr 10, close to loci that are defined by neurological mutants *waltzer* and *Jackson circler*. *Glur-1* maps close to *shaker-2*, *vibrator*, *tipsy* and *spasmodic* on Chr 11; *Glur-2* is close to *spastic* on Chr 3; and *Glur-7* is near *clasper* on Chr 4.

172.5

HETEROGENEITY OF Ca^{2+}-PERMEABLE AMPA/KA RECEPTORS IN CULTURED PURKINJE NEURONS. J.R. Brorson*, S.J. Gibbons, and R.J. Miller. Department of Pharmacological and Physiological Sciences, The University of Chicago, Chicago IL 60637.

We have shown that in cultures of rat cerebellar neurons enriched for Purkinje cells, most of the neurons express Ca^{2+}-permeable non-NMDA receptors activated by kainate (KA). Whole-cell sustained currents were evoked by bath application of non-NMDA agonists in Na^+-free solution with potencies of AMPA> domoate> KA; EC_{50} values were 4.3, 19, and 171 μM, respectively. Domoate and KA evoked much larger maximal sustained Ca^{2+} current responses than those evoked by AMPA (mean currents of 211, 224, and 38 pA, respectively), consistent with the rapid inactivation produced by AMPA. The magnitudes of currents evoked by domoate from cell to cell correlated closely with those evoked by KA, whereas those evoked by AMPA did not. Furthermore, coapplication of AMPA with 100μM KA in most cells diminished, but in some cells increased, the Ca^{2+} currents relative to those evoked by KA alone. Fura-2 Ca^{2+} imaging also suggested that the Ca^{2+} influxes induced by applications of KA and AMPA in Na^+-free media did not correlate closely. These results are best explained by the expression of Ca^{2+}-permeable non-NMDA receptors heterogeneous in their agonist selectivity. GluR1 and GluR2/3 expression have been immunocytochemically identified in these cells; the detection of Ca^{2+}-permeable KA/AMPA receptors may be explained on the basis of certain combinations of these subunits.

172.6

EVIDENCE THAT ASTROCYTES POSSESS FUNCTIONAL CA^{2+} PERMEABLE KAINATE RECEPTORS James A Holzwarth*[1], James R Brorson[1], Simon J Gibbons[1], Robert J Wenthold[2] and Richard J Miller[1] [1]Dept. of Pharm and Physiol Sciences The University of Chicago Chicago, Il 60637. [2]Lab of Neuro-Otolaryngology NIH Bethesda, Md.

Astrocytes are a heterogeneous population of cells. Some types of astrocytes have been shown to possess excitatory amino acid receptors of both metabotropic and ionotropic types. The present study was undertaken to determine if astrocytes express kainate receptors which are permeable to Ca^{2+} ions. We cultured glia from various regions of the brain, and these cells were replated for greater purity. We then examined cells for kainate responses using the intracellular Ca^{2+} indicator, fura-2.

In subsets of astrocytes from both the cortex and the hippocampus, we observed responses to kainate (30μM) using both fura-2 microfluorimetry and imaging. These cells responded with an increase in $[Ca^{2+}]_i$ to kainate, even in the absence of extracellular Na^+. The magnitude of the response in Na^+ free medium was similar to that seen in Na^+ containing medium. These results suggest that this Ca^{2+} influx is not a consequence of the activation of voltage gated Ca^{2+} channels by Na^+ dependent depolarization, but rather is via Ca^{2+} permeable kainate receptors. Quisqualate failed to give a Ca^{2+} mobilization response in the same cells, but in another set of cells did give a Ca^{2+} mobilization response.

In some cortical and cerebellar astrocytes we observed staining using antibodies for the GluR4 receptor, but little evidence for the GluR1, 2 and 3 receptor subunits. In contrast in neurons we observed staining using antibodies for the GluR1 and 2/3 receptor subunits, but there was little evidence for GluR4 in comparison to astrocytes. It has been reported that GluR4 containing receptors which lack GluR2 subunits are permeable to Ca^{2+}. Since glia seem to lack GluR2 subunits and possess the GluR4 subunit, these reults suggest a possible basis for the kainate induced Ca^{2+} influx seen in these cells.

172.7

B-AMYLOID (25-35) OR SUBSTANCE P (1-11), BUT NOT *B*-AMYLOID (1-40), STIMULATE [^{3}H]MK-801 BINDING TO RAT CORTICAL MEMBRANES IN THE PRESENCE OF GLUTAMATE AND GLYCINE. D.O. Calligaro*, P.J. O'Malley and J.A. Monn. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

We have observed that substance P (1-11) is able to simulate [^{3}H]MK-801 binding in the presence of submaximal concentrations of glutamate and glycine. Substance P (1-11) has sequence homology with *B*-amyloid (25-35). This 11 amino acid segment has been reported to mediate the neurotrophic and neurotoxic effects of B-amyloid (1-40) on cultured neurons. This study examined whether *B*-amyloid protein is also able to activate the NMDA operated ion channel when measured by the open channel blocker [^{3}H]MK-801. Micromolar concentrations of *B*-amyloid (25-35) or substance P (1-11) stimulated [^{3}H]MK-801 binding in the presence of low concentrations of glutamate (1 uM) and glycine (0.02 uM). Full length *B*-amyloid (1-40), up to 100 uM, had no effect. Similar to other NMDA receptor complex antagonists (5,7-dichlorokynurenic acid, CGS-19755 and arcaine), substance P-[D-Arg1,D-Trp7,9,Leu11] completely inhibited the stimulated [^{3}H]MK-801 binding. Substance P (6 uM) increased the IC50 of 5,7-dichlorokynurenic acid, but not that of CGS-19755 on [^{3}H]MK-801 binding, suggesting a glycine site interaction. These data may help explain some of the reported effects of *B*-amyloid proteins on cultured neurons. Whether these observations have any relevance to Alzheimer's disease will remain unclear until more in known about the presence of *B*-amyloid fragments in vivo.

172.8

PHOSPHORYLATION AND REGULATION OF GLUTAMATE RECEPTORS. T.R. Soderling*, H. Yamamoto, E. McGlade-McCulloh, S.E. Tan, and D. Brickey. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.
Regulation of glutamate receptor/ion channels may be important in synaptic plasticity. This study was designed to investigate whether glutamate receptors (GluR) can be regulated by phosphorylation. Purified CaM-kinase II and PKC (10-100nM), but not PKA, catalyzed rapid in vitro phosphorylation of GluR which had been immunoprecipitated from Sf9 cells expressing GluR1. In isolated synaptosomes, stimulation of CaM-K II and PKC, but not PKA, stimulated phosphorylation of GluRs. These results are consistent with the existence of consensus phosphorylation sites for PKC and CaM-K II, but not PKA, on the major intracellular loop of GluR1-4. Using whole-cell patch clamp recording from cultured hippocampal neurons, "run-down" of kainate current was partially prevented by inclusion of 5mM Mg^{2+}/ATP in the recording pipette. Kainate current was further enhanced by protein phosphatase inhibitors when Mg^{2+}/ATP was in the pipette; 1.25μM okadaic acid in the perfusion medium or 10μM microcystin-LR in the pipette. We are currently exploring the effects of PKC and CaM-K II in the recording pipette. The above results are consistent with regulation of glutamate ion channels by protein phosphorylation. Supported by NIH grant NS27037

172.9

DISTRIBUTION OF NMDA RECEPTORS ON DENDRITES OF RAT HIPPOCAMPAL NEURONS. T. A. Benke*† and K. J. Angelides†‡. Departments of Molecular Physiology and Biophysics† and Neuroscience‡, Baylor College of Medicine, Houston, TX 77030.

The NMDA subtype of glutamate ligand-gated ion channels is unique in its ability to permit the influx of calcium ions upon activation. Calcium is thought to act then as a second messenger. Since the activation of the NMDA class of glutamate ligand-gated ion channels is implicated in a number of processes such as synaptic plasticity, epilepsy, and ischemic cell death, the location of these receptors on neurons may be critical to their understanding.

Using Bodipy-conantokin-G, a fluorescent derivative of conantokin-G which specifically blocks NMDA-type glutamate receptors, and a lipophilic membrane permeable dye (DiI) to outline dendritic morphology, we have examined the distribution of these receptors in living cultured hippocampal CA1 neurons and in hippocampal slices. Digital imaging shows that NMDA receptors are clustered and segregated on cell somata and at regions of dendrites associated with synaptic contact sites. Fluorescence photobleach recovery (FPR) studies of these clusters shows that 25% of the receptors are mobile in the membrane. This feature could provide the neuron with additional plasticity by the recruitment of receptors to active zones. Confocal microscopy of slices shows that NMDA receptors are associated with some but not all dendritic spines.

Using the latest information available on NMDA channel kinetics, we are incorporating this anatomical information into mathematical models of different neurons from the hippocampus to gain more insight into how the distribution of NMDA receptors that we have determined can affect the integrative properties of neurons. These models provide information on the time and voltage dependent changes of intracellular calcium concentration, which provides a direct conceptual link with how the distribution of NMDA receptors is involved in calcium dependent processes.

172.10

EXPRESSION OF ENDOGENOUS NMDA RECEPTOR MRNA WITHOUT FUNCTIONAL CHANNELS IN PC12 CELLS. Nikolaus J. Sucher*, David L. Deitcher, Hilmar Bading, Michael E. Greenberg, Connie L. Cepko & Stuart A. Lipton. Dept. of Neurology, Children's Hospital, Dept. of Genetics, Dept. Microbiology & Molecular Genetics; Harvard Medical School, Boston, MA 02115.

Recently, the cloning of a cDNA encoding the rat NMDA receptor (NMDAR1) has been reported (Moriyoshi et al., *Nature* 354,31;1991). Expression of NMDAR1 mRNA has been demonstrated by *in situ* hybridization in neurons throughout the brain. Using northern blot hybridization, we found that NMDAR1 mRNA is expressed at high levels in rat pheochromocytoma (PC12) cells. An NMDAR1 probe hybridized to a band of ~4.4 kb with RNA from undifferentiated as well as nerve growth factor-treated PC12 cells. A ~4.4 kb band was also detected in hippocampal RNA but not in RNA from astrocytes or rat adrenals from which PC12 cells presumably arose. No hybridization to PC12 RNA was detected with a probe for the non-NMDA receptor GluR1. To investigate whether PC12 cells translate NMDAR1 mRNA into functional receptors, we performed patch-clamp recordings and fura-2 digital Ca^{2+}-imaging. However, we could not detect NMDA-evoked whole-cell currents or increases in intracellular Ca^{2+} in PC12 cells. Thus, detection of NMDAR1 mRNA by northern or *in situ* hybridization does not necessarily indicate that a functional receptor is being made. Moreover, our results indicate that the expression of NMDA-operated channels may be regulated by post-transcriptional mechanisms. The discovery that PC12 cells express NMDAR1 mRNA may afford the possibility of studying in a widely available cell line the transcriptional and post-transcriptional regulation of the NMDA receptor.

172.11

NMDA RECEPTORS ON BRAIN CAPILLARY ENDOTHELIAL CELLS ARE COUPLED TO ORNITHINE DECARBOXYLASE. H. Koenig[1], M. Spatz[2,*], C.Y. Lu[1], A.D. Goldstone[1], J.J. Trout[1], Neurol. Svc, VA Lakeside Med. Ctr., Dept. of Neurol., Northwestern U. Med. Sch., Chicago, Il 60611, & [2]LNNS, NINDS, NIH, Bethesda, MD 20892.

We recently reported evidence showing the presence of NMDA receptors on rat brain capillaries (BC) (Soc. Neurosci. Abstr. 17:7, 1991). BC NMDA receptors are coupled to ornithine decarboxylase (ODC), the regulatory enzyme of polyamine synthesis, upmodulate capillary transport of $^{45}Ca^{2+}$, 2-[^{3}H]deoxyglucose & horseradish peroxidase in a Ca^{2+}- & polyamine-dependent manner, & mediate blood-brain barrier breakdown induced by cold injury. We have now investigated endothelial cells (EC) cultured from neonatal & adult rat cerebral capillaries. The basal ODC activity of 4 h serum-deprived neonatal & adult BCEC was approx. 200 & 100 pmol/h/mg respectively. NMDA (50 µM) evoked a rapid (<15 sec) increase in ODC activity which peaked (2-fold) at 2 min, & was still elevated at 5 min. NMDA-induced activation of ODC was concentration-dependent with a maximum response at 50 µM, & was blocked by the competitive NMDA antagonist 2-amino-5-phosphonovalerate (AP5, 200 µM) & the ODC inhibitor α-difluoromethylornithine (DFMO, 10 mM). These results demonstrate that NMDA receptors are expressed in cultured BCEC, are coupled to ODC, & thus regulate polyamine synthesis. (Supported by the VA Research Service & NIH grant NS 18047)

172.12

ANALYSIS OF THE mRNA ENCODING THE AMPA RECEPTOR IN SINGLE PURKINJE CELLS. E. AUDINAT, B. LAMBOLEZ, P. BOCHET, J. ROSSIER* and F. CREPEL Lab. Neurobiologie, CNRS, 91405 Orsay and Inst A. Fessard, CNRS, 91198 Gif/Yvette, France.

Several subunits of the AMPA subtype of the glutamate receptor have been recently cloned. These subunits named GluR1, 2, 3 and 4 exist in two versions (flip and flop) generated by alternative splicing. The AMPA receptor is likely to be a pentameric structure composed of combinations of the GluR1-4 subunits. However, the subunit composition of native AMPA receptors involved in single neuron responses is still unknown. To answer this question we combined whole cell recording and amplification, by means of the polymerase chain reaction (PCR), of the mRNAs harvested from the single neuron under investigation. Purkinje cells from cerebellar slice cultures were recorded in the whole cell configuration of the patch clamp technique. The cell cytoplasm was then collected into the patch electrode and the pipette content used for reverse transcription and PCR amplification of the mRNAs encoding the AMPA receptor subunits of each recorded neuron. Our results show that each single Purkinje cell expresses the mRNAs encoding the five following subunits: the flip and flop versions of GluR1 and GluR2 as well as GluR3flip, GluR2 being the most abundant. GluR3flop and GluR4flip were scarcely expressed in half of these neurons whereas GluR4flop was never detected. This suggests that the AMPA receptors of single Purkinje cells are heterogenous with respect to their subunit composition.

RECEPTOR MODULATION, UP AND DOWN REGULATION I

173.1

PHOSPHORYLATION AND DESENSITIZATION OF THE HUMAN β_2-ADRENERGIC RECEPTOR EXPRESSED IN SF9 CELLS. P. Chidiac, B.Mouillac, M. Caron, M. Dennis and M. Bouvier*. Département de Biochimie, Université de Montréal, Montréal, Canada.

Postranslational modifications, such as phosphorylation and palmitoylation, are involved in β_2-adrenergic receptor (β_2AR) regulation. Such modifications are difficult to study in mammalian cells due to the relative scarcity of the receptor protein. Recombinant baculovirus expression in Sf9 cells provides an alternative system which allows purification of large amounts of proteins. We have used this system to express an epitope-tagged human β_2AR (c-mycβ_2AR). The epitope consists of 10 amino acids from human c-myc protein and is attatched to the N-terminus of the β_2AR. The c-mycβ_2AR displayed the appropriate pharmacology as determined by binding assays, and promoted an isoproterenol-sensitive activation of adenylyl cyclase. Stimulation of the cells by 1 µM isoproterenol for 10-30 minutes resulted in desensitization of the agonist-stimulated adenylyl cyclase activity, with decreases of up to 40% in maximal enzymic stimulation. This desensitization profile could be partially mimicked by the membrane-permeable cAMP analogue dibutyryl cAMP, suggesting the involvement of protein kinase A. The desensitization of the response was accompanied by an agonist-promoted increase in phosphorylation level of the c-mycβ_2AR. The increase was observed both in alprenolol-sepharose affinity-purified receptors and in receptors immunoprecipitated with an anti-c-myc monoclonal antibody (9E10). Sf9 cells infected with recombinant baculovirus encoding G protein-coupled receptors thus provides a useful system to study aspects of regulation and post-translational modification of these receptors.

173.2

SUBTYPE-SPECIFIC INTRACELLULAR SORTING OF ADRENERGIC RECEPTORS. M. von Zastrow, R. Link, D. Daunt, X.-M. Guan* and B.K. Kobilka. Dept. of Molecular and Cellular Physiology, HHMI, Stanford Med. Ctr., Stanford, CA 94305. A remarkable feature of the G protein-coupled receptor superfamily is the conservation of multiple subtypes, some of which have nearly identical functional properties. These observations raise the question of whether other functional parameters, apart from ligand binding and G protein coupling, may differentiate receptor subtypes. Simultaneous immunocytochemical localization of β_2 and different epitope-tagged α_2 adrenergic receptor subtypes expressed in the same cells revealed that these receptors differ substantially from one another in their intracellular targeting and in the regulation of their sorting by agonists. One α_2 receptor subtype was colocalized with β_2 receptors in the plasma membrane of untreated cells. Treatment of cells with the common adrenergic agonist norepinephrine resulted in a rapid sorting of β_2 receptors to endosomes without redistribution of α_2 receptors. Another α_2 receptor subtype was localized to both the plasma membrane and a vesicular intracellular compartment which was distinguishable, in the same cells, from vesicles containing internalized β_2 receptors. Subtype-specific sorting reveals an additional dimension of functional complexity in the G protein-coupled receptor superfamily.

173.3

HOMOLOGOUS DOWN-REGULATION OF M_1 AND M_2 MUSCARINIC ACETYLCHOLINE RECEPTORS IN TRANSFECTED FIBROBLAST B82 CELLS. Hongbing Wei, Henry I. Yamamura and William R. Roeske* Departments of Pharmacology and Internal Medicine, The University of Arizona Health Science Center, Tucson, AZ 85724.

Murine fibroblast cell lines which had been transfected with the m1 or m2 genes were used to study the homologous regulation of mAChR. The cells were pretreated with (+)cismethyl-dioxolane (CD), carbachol (CCh), (-)YM796 or atropine for up to 24 hours. The muscarinic full agonist CD resulted in the loss of the M_2 binding sites more rapidly than that of the M_1 binding sites measured by [^{3}H](-)MQNB binding. After 24 hr exposure of the cells to CD, the densities of the M_1 and M_2 receptors decreased 76-78%, and the M_1 receptor mediated phosphoinositide hydrolysis or the M_2 receptor mediated inhibition of forskolin-stimulated cAMP formation was attenuated. A 24 hr exposure of the cells to the M_1 selective muscarinic partial agonist, (-)YM796, induced a 45% reduction in the density of the M_1 receptors without significantly alterating the functions of the M_1 and the M_2 receptors. The antagonist atropine did not influence the densities of the M_1 or M_2 receptors. These results suggest that the M_2 receptors may be more sensitive to homologous down-regulation than the M_1 receptors in the early stages of exposure to CD. The M_1 selective partial agonist (-)YM796 induced down-regulation of the M_1 receptors but did not significantly influence the M_2 receptor density or the M_1 and M_2 receptors. Supported by USPHS grants.

173.4

INTERNALIZATION OF MUSCARINIC RECEPTORS IN RESPONSE TO PHORBOL ESTER AND AGONIST. P.S. Goldman* and N.M. Nathanson. Dept. of Pharmacology, Univ. of Washington, Seattle, WA 98195.

Previous studies in our lab and in others have indicated that activation of protein kinase C (PKC) by phorbol ester causes internalization of m1 but not m2 receptors. PMA caused greater internalization of m1 than m2 receptors when expressed transiently in COS-7 cells. The effects of internalization by PMA and carbachol were measured with a chimeric m1/m2 receptor in which the 17 amino acids at the carboxy terminal end of the third cytoplasmic loop up to the carboxy terminal end of the m1 receptor have been replaced with the corresponding region of the m2 receptor. The m1/m2 receptor did not internalize in response to PMA suggesting that the carboxy terminal end of the m1 receptor is important in PKC activated internalization. The m2 but not the m1 receptor was internalized in response to carbachol when expressed in COS-7 cells. These data suggest that the mechanisms for PMA- and carbachol-induced internalization of the m1 receptor are different, and that a part of the mechanism involved in m1 agonist-induced internalization is not present in COS-7 cells. The m1/m2 receptor hybrid did internalize in response to carbachol in COS-7 cells implying that the carboxy terminal end of the m2 receptor is sufficient for the m2 specific pathway for internalization of the receptor by agonist. Studies are underway to further localize the regions responsible for PKC- and agonist-mediated internalization and to determine if phosphorylation of the receptors is required for internalization.

Recent studies have shown that tyrosine residues located in the carboxy terminal tail of a number of different receptors are involved in the internalization and or downregulation of these receptors by agonist. Mutant mAChRs in which single tyrosine residues in their carboxy tails were replaced internalized normally in response to agonist and current work involves determining the ability of these mutant receptors to be downregulated.

173.5

AGONIST-DEPENDENT PHOSPHORYLATION OF THE SEROTONIN 1C RECEPTOR IN RAT CHOROID PLEXUS AND *XENOPUS* OOCYTES. J. Hurley*, J. Liu, L.S. Bye, M. Baez[1] and L. Yu Dept. of Med. and Mol. Genetics, Indiana Univ. School of Medicine, Indpls, IN 46202; [1]Lilly Research Laboratories, Lilly Corp. Center, Indpls, IN 46285.

The serotonin 1c (5-HT_{1C}) receptor, a G protein-coupled receptor, is present at very high density in choroid plexus, where its function and regulation are not well understood. The 5-HT_{1C} receptor contains putative phosphorylation sites for several protein kinases and may be regulated by phosphorylation in a manner analogous to other G protein-coupled receptors. Two methods were used to address this question. Freshly dissected rat choroid plexus was incubated in radiolabeled inorganic phosphate prior to treatment with 5-HT. *Xenopus* oocytes expressing the mouse 5-HT_{1C} receptor were injected with [γ-^{32}P]ATP 10 min. prior to vehicle or 5-HT treatment. Membrane proteins were analyzed by SDS-PAGE and autoradiography. Time and dose-dependent increases in phosphorylation of the 5-HT_{1C} receptor were observed. Anti-peptide antibodies directed against the mouse 5-HT_{1C} receptor, which recognize both the mouse and the rat receptors, were used to identify the phosphorylated receptor. These methods should be useful to investigate the role of phosphorylation in the regulation of serotonin receptor function.

173.6

A NOVEL ARRESTIN EXPRESSED IN CEREBRAL CORTEX IS SIMILAR TO THE BETA-ADRENERGIC ARRESTIN. C.M. Craft*[1], D.H. Whitmore[2], R. Gonzalez, Jr.[1,1]Lab. Mol. Neurogen., Dept. of Psychiatry, U TX Southwestern Med. Sch. & VA Med. Ctr, Dallas, TX 75235, [2]Dept. Biology, U TX, Arlington, TX 76019.

Arrestin (AR) proteins, S-antigen (SAG) and β-adrenergic arrestin (βAR), are involved in the inactivation of light-activated rhodopsin and homologous desensitization of β-adrenergic receptors after receptor phosphorylation. Differential expression of mRNAs and proteins encoding multiple ARs occurs in retina, pineal gland, cerebral cortex, cerebellum and sympathetically innervated tissues. Although antigenic epitopes are shared and highly conserved between members of the family, separate genes encode the individual members. Primers designed from these conserved domains between SAG and BAR generated products by polymerase chain reaction (PCR) amplification of cDNAs synthesized from mRNA from cerebral cortex and pineal. PCR subclones of SAG and BAR were detected; plus a unique AR (CAR) was isolated independently from both mRNAs. The carboxy terminus (200 amino acids) of CAR shares 74% identity with BAR. Alternative processing of CAR excludes a conserved SAG/BAR 3' exon and modifies the predicted secondary structure. CAR's distribution, evolutionary relationship with the other ARs, and interaction with membrane receptors are being examined.

173.7

SELECTIVE UP-REGULATION OF ALPHA-2B ADRENOCEPTORS AFTER BRAIN NORADRENERGIC LESIONS. G.A. Ordway*, J.D. Brodkin, and S.M. Jaconetta. Depts. of Psychiatry & Pharmacology, Case Western Reserve Univ. & MetroHealth Med. Ctr., Cleveland, OH 44109.

The location and function of alpha-2 adrenoceptors are poorly understood. Both alpha-2A and alpha-2B subtypes have been described in rat brain, and have high and low affinity, respectively, for oxymetazoline (OXY). We determined the effect of lesioning catecholaminergic neurons on concentrations of OXY-sensitive and OXY-insensitive binding of ^{3}H-rauwolscine in rat brain. OXY competition curves are GTP-sensitive so all binding was performed in the presence of 250 uM GTP. Competition curves for inhibition of ^{3}H-rauwolscine binding by OXY in rat cerebral cortex were best described by a two-site model ($p < 0.005$). OXY demonstrated a 100-fold difference in affinity for its high and low affinity binding sites ($IC50_H$ = 20 nM; $IC50_L$ = 2086 nM). Binding to OXY-sensitive and OXY-insensitive alpha-2 receptors was determined in rat cerebral cortex 3 weeks following intraventricular injections of saline (SHAM), 6-hydroxydopamine (6OHDA), and 6OHDA with desipramine pretreatment to protect noradrenergic neurons (6OHDA+DMI). Binding to OXY-sensitive alpha-2A-like adrenoceptors was not affected by either lesion (in fmol/mg protein: SHAM 12±2; 6OHDA 9±2; 6OHDA+DMI 10±2). In contrast, OXY-insensitive alpha-2B-like binding was elevated (+32%, $p<0.01$) in 6OHDA lesioned rats compared to sham-lesioned rats and to 6OHDA+DMI rats (in fmol/mg protein: sham 30±2; 6OHDA 40±2; 6OHDA+DMI 30±2). OXY-insensitive alpha-2B-like adrenoceptors are selectively up-regulated in cortex following destruction of noradrenergic neurons. Lack of change in OXY-sensitive alpha-2A-like adrenoceptors suggests that either these receptors are not synaptic, or alternatively, that these receptors are located on and post-synaptically to noradrenergic neurons.

173.8

IRREVERSIBLE BLOCKADE OF DOPAMINE RECEPTORS BY FLUPHENAZINE-N-MUSTARD INCREASES D_2 DOPAMINE RECEPTOR mRNA AND PROENKEPHALIN mRNA IN RAT STRIATUM. J.F. Chen*, Z.H. Qin and B. Weiss. Div. of Neuropsychopharmacology, Dept. of Pharmacology, Medical College of PA, Philadelphia, PA 19129.

An understanding of the long-term factors that influence dopamine and neuropeptide systems at the level of gene expression is a key to uncovering the role of these systems in dopamine-associated diseases. This study examined the effects of irreversible blockade of dopamine receptors by fluphenazine-N-mustard (FNM) on D_1 and D_2 dopamine receptors, D_1 and D_2 dopamine receptor mRNAs and proenkephalin mRNA in rat striatum. Sprague-Dawley rats were treated with FNM (20 µmole/kg, i.p.) daily for 5 days. The animals were sacrificed 20 hr after the last injection, and brain sections at the level of the corpus striatum were prepared. D_1 and D_2 dopamine receptors were detected by receptor autoradiography, using [^{3}H]-SCH23390 and [^{3}H]-spiperone, respectively. D_1 and D_2 dopamine receptor mRNAs and proenkephalin mRNA were determined by in situ hybridization histochemistry, using radiolabelled oligodeoxynucleotide probes. FNM treatment caused a 90% reduction in D_2 dopamine receptors and a significant increase in D_2 dopamine receptor mRNA in striatum. By contrast, FNM treatment caused only a 20% inhibition of D_1 dopamine receptors and no significant change in D_1 dopamine receptor mRNA in striatum. FNM treatment also significantly increased proenkephalin mRNA in striatum. These results showing that transcripts for the D_2 dopamine receptor and proenkephalin are increased following irreversible blockade of dopamine receptors suggest not only that these systems can be modulated by dopaminergic input but also that these two systems interact at the level of gene expression. (Supported by MH42148).

173.9

THE EFFECT OF NEUROTENSIN ON DOPAMINE TRANSMISSION IN THE STRIATUM: EVIDENCE FOR A SUBSTRATE OF NEURONAL PLASTICITY BASED ON PRE- AND POSTSYNAPTIC NEUROTENSIN-DOPAMINE RECEPTOR INTERACTIONS. W.T.O'Connor*, K.Fuxe, T.Antonelli, P.Osborne, S.Tanganelli, L.F.Agnati & U.Ungerstedt. Depts. of Pharmacology, Histology and Neurobiology, Karolinska Inst., Stockholm, Sweden.

The major mechanism underlying the neuroleptic action of the tridecapeptide neurotensin (NT) appears to be an interaction with dopamine (DA) receptor mechanisms based on biochemical binding and behavioral experiments. In vivo microdialysis was used in conscious rats to investigate the effects of local perfusion with NT on the sensitivity of striatal DA D1 and D2 receptors for their selective agonists by monitoring extracellular DA and GABA levels in the awake unrestrained male rat. Perfusion with NT (10nM) counteracted the inhibitory effects of the DA D2 agonist pergolide (500nM) on DA and GABA levels. In contrast, NT (10nM) significantly enhanced the reduction of DA levels after perfusion with the D1 agonist SKF 38393 (5000nM) and this combined treatment also resulted in a significant increase in the extracellular striatal levels of GABA. These results provide in vivo evidence that NT regulates central DA transmission by reducing pre and postsynaptic DA D2 and enhancing D1 receptor sensitivity possibly through an antagonistic NT/D2 receptor-receptor interaction. This heteroregulation has the potential to substantially increase the plasticity within the DA synapse.

173.10

Chronic Elevation of Protein Kinase A (PKA) Enhances $GABA_A$ Receptor Currents Composed of $\alpha\beta\gamma$ Subunits and Not $\alpha\beta$ Subunits. T.P. Angelotti*[∇], M.D. Uhler[+], and R.L. Macdonald[#@], Depts. of Pharmacology[∇], Biochemistry[+], Neurology[#], and Physiology[@], Univ. of Michigan, Ann Arbor, MI 48109.

Expression of α1, β1, and γ2S $GABA_A$ receptor (GABAR) subunit cDNAs in L929 (intermediate PKA), Cα12 (high PKA), and RAB10 cells (low PKA) produced functional GABAR with similar GABA and diazepam pharmacology, as assayed by whole-cell patch clamp analysis. The major difference was the magnitude of the currents; GABARs expressed in Cα12 cells were 3-4 times larger than those expressed in the other two cell lines, at all concentrations of GABA. Co-expression of only α1 and β1 subunits in the same cells produced non-cooperative GABA concentration-response curves, but no enhancement of currents were seen in Cα12 cells. Substitution of a β1 subunit mutagenized to remove the consensus PKA phosphorylation site (Ser^{409} --> Ala) also produced functional GABARs in all three cell lines after expression with α1 and γ2S subunits. This result suggested that the mutation did not effect expression. More importantly, the size of the whole-cell currents in all three cell lines were similar; the mutation reversed the enhancement seen in Cα12 cells. Single-channel patch clamp analysis of the expressed α1, β1 (WT or Mutant), and γ2S GABARs in all three cell lines revealed similar main and subconductance levels, and open, closed, and burst durations.

Supported by a grant from the Lucille P. Markey Charitable Trust Fund

174.1

MOLECULAR CLONING AND CHARACTERIZATION OF A MOUSE AND RAT GENE ENCODING THE NPY-1 RECEPTOR. C.Eva*,R.Sprengel°,A. Oberto,S.Ricci Gamalero and E.Genazzani. Institute of Pharmacology, Univ. of Torino, Italy and °ZMBH, Univ. of Heidelberg, Germany.

Neuropeptide Y is an important central and peripheral modulator of neural and endocrine functions that interacts with at least two distinct receptors, named Y1 and Y2. We isolated from a rat cDNA library a novel G protein-coupled receptor belonging to the neuropeptide receptor family. Expression patterns of the receptor encoding mRNA in rat brain showed significant similarities with the localization of the Y1 receptor. Transient expression of this G protein-coupled receptor in 293 cells reveals the ligand binding selectivity of a Y1 receptor with high affinity for the selective analog (Leu31,Pro34)NPY. NPY stimulation of this receptor leads to a decrease of cAMP content, stimulation of membrane phospholipids and mobilization of intracellular calcium. The analysis and sequencing of the NPY-1 receptor gene, isolated from a mouse genomic library, shows that this gene lacks introns in the coding region and that regulatory elements potentially important for transcription can be found in the sequence of the putative promoter of this gene.

174.2

HIGH LEVEL EXPRESSION OF NEUROPEPTIDE Y (NPY) BINDING SITES IN CELLS INFECTED WITH A RECOMBINANT VACCINIA VIRUS. P. Walker *, M.Munoz, M.-C. Combe, E. Grouzmann, H. Herzog#, L. Selbie#, J.Shine#, H.R. Brunner, B.Waeber and R.Wittek¶ .Division of Hypertension, CHUV, 1011 Lausanne, Switzerland. # Gavran Institute, Sidney, Australia, ¶ Institute of Animal Biology, 1015 Lausanne Switzerland.

Neuropeptide Y (NPY) is a 36 amino acid peptide first isolated by Tatemoto[1]. Numerous studies point to a role of NPY in cardiovascular regulation[2]. NPY effects are mediated through stimulation of specific cell surface G protein-coupled receptors. The sequence of a human NPY receptor as recently been published[3]. To allow biochemical studies of the receptor and its interaction with the ligand, we have developed a potent expression system for NPY receptors using recombinant vaccinia virus. A NPY receptor cDNA was fused to a strong vaccinia virus promoter and inserted into the viral genome by homologous recombination. Recombinant viruses were isolated and tested for their ability to induce NPY binding site expression following infection of several mamalian cell lines. Using saturation and competition binding experiments we measured a B_{max} of 5 to 10 X10^6 NPY binding sites / cell with a Kd of about 20 nM. Cross-linking studies identified a single labelled protein of 45 KD in agreement with the size of the receptor deduced from the nucleic acid sequence. Labelling of infected cells with a fluorochrome-labelled NPY indicated that the recombinant protein intergrates into the cell membrane.

1 K.Tatemoto, Proc.Natl Acad.Sci.USA.,**79**, 5485-5489, 1982
2 P.Walker et al., Trends in Pharmacology, **12**, pp111-115, 1991
3 H.Herzog et al., Proc.Natl Acad.Sci.USA, 1992, in press

174.3

SUBSTITUTED NPY ANALOGUES EVOKE Y1 AND Y2 RECEPTOR-MEDIATED REPONSES: IDENTIFICATION OF SIGNAL EPITOPES. **Lars Grundemar*[1,2], John L. Krstenansky[3] and Rolf Håkanson[2], Departments of [1]Clinical Pharmacology and [2]Pharmacology, Univ. of Lund, Lund, Sweden and [3]Marion Merrell Dow Res. Inst. OH, USA.**

A series of substituted neuropeptide Y (NPY) analogues were studied with regard to their ability to activate Y1 receptors (guinea pig caval vein) and Y2 receptors (rat vas deferens).

An anlogue with increased alpha-helicity in the 14-30 region, ESALL-NPY was about equipotent with NPY (1-36) at Y1 and Y2 receptors, respectively. Centrally substituted, linear NPY analogues with 8-aminooctanoic acid (Aoc) in the mid molecule region (hairpin loop), (Aoc $^{8-17}$)NPY and (Aoc$^{5-24}$)NPY), were rather potent at the Y2 receptor, however, being 30-100 times less potent than NPY at the Y1 receptor. A shorter peptide, (Aoc$^{2-27}$)NPY, was virtually inactive at either receptor type. Cyclic analogues (with S-S bonds bringing N- and C-termini together) of (Aoc $^{8-17}$)NPY, C7-NPY of (Aoc$^{7-20}$)NPY, C5-NPY and of (Aoc$^{5-24}$)NPY,C2-NPY were about 10 times less potent than NPY at the Y2 receptor, and 25-50 times less potent at the Y1 receptor. The cyclic analogues appeared more potent than the corresponding linear analogues at the Y1 receptor, but equipotent at the Y2 receptor. A truncated C2-NPY analogue, (Des-S^3, Des-K^4)C2-NPY was 20 times less potent than NPY at the Y2 receptor, however, inactive at the Y1 receptor.

The results suggest that fairly extensive N-terminal truncation of the NPY molecule does not greatly impair recognition by the Y2 receptor, while the Y1 receptor needs an intact N-terminal end in order to become fully activated. Some centrally truncated analoges were rather potent at the Y1 and Y2 receptors, implying that the hairpin loop is not essential for receptor recognition. Conceivably, it may impose constraints on the N- and C-terminal ends, thereby facilitating Y1, but not Y2 receptor activation.

174.4

LOCALIZATION OF HIGH AFFINITY Y_2 RECEPTORS FOR NEUROPEPTIDE Y AND PEPTIDE YY IN THE RAT POSTERIOR PITUITARY.

S. P. Sheikh, E. L. Stuenkel, J. D. Mikkelsen, P. J. Larsen, M. Treiman', T. W. Schwartz and M. Møller. Dept. of Clinical Biochemistry, Rigshospitalet 6321, and Depts. of Medical Anatomy & Physiology, Panum Inst. University of Copenhagen, Denmark, and Dept. of Physiology, University of Michigan, Ann Arbor, MI 48109.

NPY has been reported to regulate secretion of several transmitters and hormones in the hypothalamo-pituitary axis. To investigate whether NPY affects pituitary hormone secretion by a direct effect on pituitary nerve terminals we looked for NPY receptors on the rat pituitary gland. Receptor autoradiographic techniques at the light microscopic level revealed specific labeling in the posterior but not anterior lope. Using a preparation of isolated posterior nerve terminals a NPY receptor of surprisingly high affinity with $K_d = 14$ pmol $\pm$ 9 (n = 8) and $B_{max} = 494 \pm 68$ fmol/mg protein was observed. Data from inhibition experiments utilizing specific NPY analogues and fragments classified these binding sites as belonging to the Y_2 type of NPY and PYY receptors (n = 6). By contrast, primary cell cultures of rat pituicytes did not specifically bind the PYY radiolabel. Thus, NPY and PYY receptors are present on the pituitary nerve terminals, and not on cultured pituicytes. In order to identify the structure, that contains the NPY binding sites we used the technique of receptor autoradiography at an electron microscopic level. Preliminary data shows that specific autoradiographic silver grains are localized over a distinct population of nerve terminals, whereas others are not labeled. We are currently in the process of identifying vasopressin and oxytocin immunoreactive nerve terminals on the receptor autoradiographs.

174.5

CLONING OF A POTENTIAL NEW SUBTYPE OF THE BOMBESIN/GRP RECEPTOR FAMILY FROM THE HUMAN COLON ADENOCARCINOMA CELL LINE WiDr. E. Giladi*, S. Nagalla and E.R. Spindel, Division of Neuroscience, Oregon Regional Primate Research Ctr, Beaverton, OR 97006

The bombesin like peptides comprise a large family of peptides common to amphibians and mammals that function as growth factors, neurotransmitters and paracrine hormones. Three subfamilies of bombesin-like peptides have been described; the bombesin/GRP subfamily, the ranatensin/NMB subfamily and the phyllolitorin subfamily. To date two mammalian bombesin-like peptides and their cognate receptors have been characterized, gastrin-releasing peptide (GRP) and neuromedin B (NMB). The phyllolitorins have so far only been characterized in amphibians. Recently, Cardona *et al.* (Br.J.Cancer 63:14,1991) reported that the WiDr colon carcinoma cell line showed a strong calcium response to phyllolitorin. In order to characterize the bombesin receptor subtype from WiDr cells, poly(A) RNA was prepared and injected into *Xenopus* oocytes and phyllolitorin induced changes in intracellular calcium were measure luminometrically. A 2-3-fold increase compared to control oocytes was measured. RNA size-fractionated on sucrose gave up to a 6-fold increase in light output in specific RNA fractions. A cDNA library constructed from the fractionated RNA fractions was screened with the human GRP receptor probe and one hybridizing phage was isolated. Sequence analysis revealed this cDNA encoded the C-terminus of a receptor highly related to the GRP and NMB receptors. Amino acid homology was 88% and 64% with the human GRP and NMB receptors, respectively. RNA blot analysis showed hybridization to a 9.5 kb band in WiDr RNA, the same size as the GRP receptor mRNA. Studies are currently in progress to isolate the entire cDNA to allow expression of this receptor and characterization of its pharmacologic specificity.

174.6

EXPRESSION OF THE GASTRIN-RELEASING PEPTIDE (GRP) AND GRP RECEPTOR GENES IN DEVELOPING RHESUS MONKEY LUNG. K. Li*, S.R. Nagalla and E.R. Spindel Oregon Regional Primate Research Center, 505 NW 185th Ave., Beaverton OR 97006.

High levels of the mammalian bombesin-like peptide, gastrin-releasing peptide (GRP), is expressed in developing human lung. Rabbits and rats do not show this developmental pattern of expression and, thus, cannot be used to characterize the role of GRP in lung development. In this study we investigated the expression of the GRP and GRP receptor genes in fetal, neonatal and adult rhesus monkey lung. By RNA blot analysis, GRP mRNA was first detectable in fetal monkey lung at 63 days gestation, reached highest levels at 80 days gestation and then declined to near adult levels by 120 days gestation. In monkey lung, *in situ* hybridization localized GRP mRNA to neuroendocrine cells just as in human fetal lung. A surprising finding in this study was that during early stages of development (between 63 to 80 days gestation) GRP mRNA was present not only in the neuroendocrine cells, but also in cells of budding airways. Immunohistochemical studies showed that bombesin-like immunoreactivity was present in neuroendocrine cells, but not in the budding airways. Thus, the GRP mRNA is apparently present but not translated in airway progenitor cells. This is similar to the many human lung neoplasms which also express but do not translate the GRP mRNA.

PCR was used to clone a fragment of the monkey GRP receptor. RNAse protection analysis using this probe demonstrated that the GRP receptor is expressed in monkey lung by 63 days gestation, plateaus from 80 to 140 days gestation and only low levels are present in adult lung. *In situ* hybridization suggested that GRP receptors are expressed in the airway epithelial cells. Thus, both a source of GRP and target cells to respond to GRP are present in developing monkey lung.

174.7

EXPRESSION CLONING AND PHARMACOLOGICAL CHARACTERIZATION OF A RAT BRAIN SOMATOSTATIN RECEPTOR cDNA.
H. Lübbert*, F.-W. Kluxen, C. Bruns, D. Hoyer, L. Rohrer, R. Schüle, K. Kaupmann. Preclinical Research, Sandoz Pharma Ltd., 4002 Basel, Switzerland.

We have used an expression-cloning strategy to isolate a cDNA encoding a somatostatin receptor from rat cortex and hippocampus. A positive clone was identified by autoradiography after binding of radio-labeled somatostatin to COS-1 cells previously transfected with pools of cDNA clones. The deduced amino acid sequence of the receptor displays sequence and structural homology to the family of G-protein coupled receptors. After expression of the receptor cDNA in COS cells a single high affinity binding site was found with a K_D of 51 pM and a B_{max} of 157 fmol/mg protein. Several somatostatin receptor types have been postulated based on pharmacological criteria. The affinity of various SRIF analogs to the receptor cloned here was analyzed and resembled closely their effects on growth hormone release from pituitary cells. The distribution of the RNAs encoding this and other somatostatin receptors has been analyzed in rat tissues, tumors and cell lines by *in situ* hybridization, RNA blotting and quantitative RT-PCR analyses. Of the tissues examined, the mRNA encoding the above mentioned somatostatin receptor was found in hippocampus, cortex, and pituitary as well as in a pancreas tumor and an exocrine pancreas derived cell line, AR4 2J, but not in kidney. Thus, the pharmacology and tissue distribution of this receptor corresponds to that described for the somatostatin receptor type which inhibits growth hormone release. Similar characterizations are performed for other cloned somatostatin receptor types.

174.8

EVIDENCE FOR A NONCLASSICAL NK-1 RECEPTOR ON MICROGLIA
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This study examined whether microglia possess SP receptors. Microglia isolated from primary rat brain cultures were incubated with Bolton-Hunter labeled I^{-125} SP and various concentrations of cold SP or cold analogs. Results were analyzed by an exact weighted nonlinear regression. The data best fit a single receptor model with a K_d of $5.3x10^{-7}M$ (SE $\pm$ $0.4x10^{-7}M$) and 35,000 (SE $\pm$ 3500) binding sites per cell. The binding site bound NK-1 selective agents such as the peptide SP methylester and the nonpeptide CP-96,345, while failing to bind NK-2 or NK-3 selective analogs. The binding heirarchy was not typical for NK-1 receptors, however, with SP>SP methylester, very weak binding for physalaemin and none for neurokinin A. The K_d was also much larger than for classical NK-1 receptors. Similar results have been reported for macrophages (Hartung et al., 1986, J. Immunol., 3856-3863). These data indicate the presence of a nonclassical NK–1 receptor on microglia and other cells of monocyte-macrophage lineage.

174.9

Desensitization of Human Endothelin A and Neurokinin A Receptors
R. Kris*, C. Cyr, S. Josiah and V. Chu. N.Y.U. Med. Center, 550 1st Ave. New York, N.Y. 10016

Endothelin is the most potent endogenous vasoconstrictor known at this time and is implicated in several normal and abnormal physiological conditions including the pathogenesis of myocardial infarction, broncho-constriction, high blood pressure and acute renal failure. The human endothelin receptor A (ETA) has recently been cloned and belongs to the rhodopsin-like superfamily of receptors that contain seven putative hydrophobic transmembrane domains.

The response of G-protein coupled receptors is modulated by homologous desensitization. The endothelin response is probably tightly regulated since it is such a potent vasoconstrictor. The extent of desensitization and resensitization of endothelin responsiveness was therefore studied. Human neurokinin A (NKA) and human serotonin II (5-HT2) receptors were utilized for comparison to ETA in desensitization experiments. These three receptors are coupled to G proteins which mediate phosphotidyl-inositol hydrolysis.

Xenopus laevis oocytes injected with the three different receptor mRNAs all show homologous desensitization but exhibit variable rates of recovery. Human 5-HT2 receptors recover within 2 to 5 minutes while human NKA receptors recover after 20 to 30 minutes. The human ETA receptors have a prolonged desensitization period lasting 2 to 3 hours. Such a prolonged recovery period is unique to this class of receptors and may serve to regulate some of the deleterious effects of the receptor.

174.10

DIFFERENT BINDING EPITOPES FOR SUBSTANCE P AND THE NON-PEPTIDE ANTAGONIST, CP-96,345 ON THE NK_1 RECEPTOR.
U. Gether[A], T.E. Johansen[A], R.M. Snider[B], J.A. Lowe III[B], S. McLean[B]*, S. Nakanishi[C] and T.W. Schwartz[A]. [A]Lab of Molecular Endocrinology, Rigshospitalet 6321, Univ of Copenhagen, Denmark, [B]Dept of Medicinal Chemistry, Central Research, Pfizer Inc., Groton, CT 06340 and [C]Inst. for Immunology, Kyoto Univ. Japan.

Non-peptide ligands for neuropeptide receptors are being discovered in many systems through file screening programs. However, the structural basis for their binding to the peptide receptors is unknown, since they have no chemical resemblance to the natural peptide ligands. CP-96,345 is a selective, high affinity non-peptide antagonist of the NK_1 (substance P) receptor. We constructed a series of chimeric receptors by systematically exchanging segments of the NK_1 receptor with homologous segments of the NK_3 receptor, which normally does not bind the non-peptide ligand. In this way a domain of the NK_1 receptor was localized which was not only necessary but also sufficient for high affinity binding of the non-peptide antagonist as transfer of this domain to the NK_3 receptor conveyed CP-96,345 binding activity. By exchanging smaller segments of the NK_1 receptor with corresponding NK_3 segments, it was found that CP-96,345 binds to two subdomains around the junction between extracellular loop 2 and the top of transmembrane segment V and a small, third domain just outside the top of transmembrane segment VI. The binding of the peptide ligands, substance P and eledoisin, was unaffected by these chimeric exchanges. It is concluded that major epitopes for the binding of CP-96,345 are localized at the top of transmembrane regions V and VI of the NK_1 receptor and surprisingly, that these epitopes are not important for the binding of the natural ligand, substance P.

174.11

MOLECULAR CHARACTERIZATION OF THE NK1 ANTAGONIST SPECIES SELECTIVITY ON RAT AND HUMAN RECOMBINANT RECEPTORS.
L. Pradier*, L. Emile, E. Habert, L. Mercken, J. Le Guern, V. Moras, I. Loquet, J. Clot, V. Fardin, A. Doble, J.F. Mayaux. Rhône Poulenc Rorer, 13 Quai J. Guesde, 94400 Vitry, France.

The neuropeptide Substance P, member of the tachykinin family, is a potent vasodilatator and smooth muscle contractant. SP has also been proposed as a candidate for pain neurotransmission. Recently, two non-peptide competitive antagonists for NK1 receptors, the high affinity SP receptors, have been described: CP96345 and RP67580. Unlike SP, those compounds show strong species selectivities in binding studies with RP67580 being more potent on the rat NK1 receptor and CP96345 on the human receptor (Fardin et al., 1992, *Brit. J. Pharm.* **105**:80P). However, the recent cloning of the NK1 receptor from the two species has revealed few differences in their primary sequences. We have used a transient expression system in COS1 cell line to study the binding properties of recombinant rat and human NK1 receptors as well as multiple rat/human hybrids. Point mutants were also constructed in order to identify the respective residues involved in the strong and opposite species selectivities observed in binding. This study has allowed a fine characterization of the residues involved in the ligand binding cleft on the NK1 receptor.

175.1

A NOVEL GABA RESPONSE FROM ROD-DRIVEN HORIZONTAL CELLS OF THE WHITE PERCH RETINA. H. Qian and J. E. Dowling*. Biol. Lab. Harvard Univ. Cambridge, MA 02138.

GABA is the main inhibitory neurotransmitter in the central nervous system. Two classes of GABA receptors ($GABA_a$ and $GABA_b$) have been identified and their responses extensively studied. Here, we report a GABA response from rod-driven horizontal cells (H4) of the white perch retina that does not fit either class.

Whole-cell patch clamp recording techniques were used to monitor GABA-induced membrane currents from solitary rod-driven horizontal cells. When the recording pipette was filled with KCl and the cell held at -70 mV, an inward current of ~100 pA was induced by the application of 100 uM GABA to the cell. The response was characterized by a slow time course of recovery (time constant of 15.9 sec at -50 mV) and it did not desensitize during prolonged (30 sec) exposure to GABA. A membrane conductance increase accompanied the response. The reversal potential followed closely the chloride equilibrium potential when extracellular chloride was replaced by isethionate. This finding, coupled with the fact that the response was blocked by picrotoxin, indicates this GABA response is mediated by the opening of chloride channels. The response could not be elicited with either muscimol or baclofen, and it was not blocked by bicuculline, phaclofen or hydrosaclofen, agents that interact with $GABA_a$ or $GABA_b$ receptors. However, the response could be evoked in H4 horizontal cells by *cis*-aminocrotonic acid (CACA), a putative agonist of a third class of GABA receptor, termed $GABA_C$ receptors (Draw et al., Neurosci. Lett. 52:317, 1984) and observed in oocytes expressing retinal RNA (Polenzani et al., PNAS 88:4318, 1991)

175.2

TWO $GABA_A$ SYNAPTIC RESPONSES IN THE RAT HIPPOCAMPUS. R.A. Pearce.* Departments of Anesthesiology and Anatomy, University of Wisconsin, Madison, WI 53706.

Molecular biological and biochemical studies have shown that multiple $GABA_A$ subtypes exist, but corresponding physiological differences in synaptically evoked currents have not been described. Here, two anatomically segregated $GABA_A$ synaptic responses in rat hippocampal CA1 neurons are described that have distinct physiological, pharmacological, and functional properties.

Monosynaptic $GABA_A$ IPSCs in hippocampal CA1 cells *in vitro* were recorded with single electrode voltage clamp using standard sharp electrodes filled with CsAc or CsCl and QX-314 (50 μM) to block the $GABA_B$ phase of the evoked response. APV (40 μM) and CNQX (20 μM) were added the perfusion medium to block excitatory synaptic transmission. The decay phase of the IPSC in response to stratum radiatum stimulation was best fit by the sum of two exponentials with time constants 3-8 ms and 30-70 ms, and both components were blocked by bicuculline (10 μM). The relative amplitudes of the fast and slow current decay components ($GABA_{A,fast}$ and $GABA_{A,slow}$) depended on stimulus location: stimulation near or directly on stratum pyramidale evoked $GABA_{A,fast}$, whereas stimulation of stratum laculnosum-moleculare evoked $GABA_{A,slow}$. Reversal potentials of the two components were the same with CsAc electrodes. With CsCl electrodes $GABA_{A,slow}$ was shifted by a smaller amount than $GABA_{A,fast}$, suggesting that it enters at a more distal dendritic location. Furosemide (600 μM) selectively and reversibly blocked $GABA_{A,fast}$ but not $GABA_{A,slow}$. In slices with synaptic transmission intact, blocking $GABA_{A,fast}$ with furosemide resulted in an enhanced EPSP and multiple action potentials in intracellular current clamp recordings, and double population spikes in extracellular recordings. However, the early IPSP remained, and paired pulse depression of the population spike was not blocked. Thus, $GABA_{A,fast}$ enters near the cell body and limits the response to excitatory input to a single spike, whereas $GABA_{A,slow}$ mediates a longer lasting and more distal inhibition. The receptors mediating these distinct inhibitory responses may represent subtypes of the $GABA_A$ receptor.

175.3

SYNCHRONOUS ACTIVITY INDUCED BY 4-AMINOPYRIDINE (4AP) IN THE HUMAN NEOCORTEX MAINTAINED IN VITRO. M.Avoli*, D. Mattia, G.G.C. Hwa and A.Siniscalchi. MNI & McGill University, Montreal, QC, Canada

Extracellular and intracellular recordings were made in vitro from slices of human neocortex obtained from patients who underwent surgery for epileptic or non-epileptic conditions. Regardless of the type of pre-existing pathology, perfusion with medium containing the convulsant 4AP (50μM) induced the appearance of spontaneous, mainly negative-going, field potentials that lasted 200-500 ms, occurred at 0.05-0.1 Hz and attained largest amplitude 800-1,000 μm from the pia. This type of activity persisted in the presence of the excitatory amino acid receptors antagonists CPP and CNQX, but was abolished by the $GABA_a$ agonist baclofen (50-100μM). 4AP-induced field potentials were associated intracellularly with a sequence of events that depending on the membrane potential could consist of: (i) initial EPSP; (ii) short-lasting hyperpolarization; (iii) long-lasting depolarization (LLD; 4-30mV; up to 800 ms); and (iv) late, long-lasting hyperpolarization LLH). Perfusion with the excitatory amino acid receptor antagonists CPP and CNQX blocked the early EPSP, but did not modify the other components of the sequence that was thus initiated by a hyperpolarization. LLDs disappeared during perfusion with the $GABA_A$ antagonist bicuculline methiodide (BMI, 5-10μM). In the presence of BMI spontaneous, LLHs (400-600ms) that were presumably due to the activation of post-synaptic $GABA_a$ receptors still occurred. These results indicate that in the human neocortex: (i) 4AP induces synchronous, spontaneous events that mediated by GABA presumably released from inhibitory interneurons; (ii) this mechanism does not require the participation of excitatory synaptic potentials, but (iii) it is under the control of pre-synaptic $GABA_a$ receptors.

175.4

PROPERTIES OF SYNAPTICALLY ACTIVATED, WHOLE-CELL $GABA_B$ CURRENTS. T.S. Otis*, Y. De Koninck, and I. Mody, Dept. of Neurology & Neurol. Sciences, Stanford Univ. Sch. of Med., Stanford, CA.

Monosynaptically evoked $GABA_B$ currents were recorded in the presence of 10 µM CNQX, 40 µM D-AP5, and 75 µM picrotoxin with whole-cell voltage clamp techniques in dentate gyrus granule cells of 400 µm thick, coronal, adult rat brain slices maintained at 34-35°C. Molecular layer stimulation evoked slow outward currents (at $V_h \approx$ RMP) sensitive to the $GABA_B$ antagonist CGP 35348 (200-800 µM). The conductance was also blocked by 2-OH-saclofen, intracellular Cs^+ or QX-314. Peak $GABA_B$ current was linearly related to V_h in the range from -110 mV to -49 mV, and reversed at -97.9 mV with a mean slope conductance of 1.52 nS. The kinetics of the current could be fit by the product of fourth power exponential activation and double exponential inactivation processes. The mean activation time constant was 45.2 ms, while the mean inactivation time constants were 110.2 ms and 516.2 ms. The kinetics were not voltage dependent (V_h: -45 mV to -95 mV), and Q_{10} values ranged from 1.8 to 2.3.

Paired pulse depression (PPD) of the $GABA_B$ current was similar in time course and magnitude to the PPD of the $GABA_A$ current. The time course of the PPD differed from that of the postsynaptic $GABA_B$ current. The PPD was not voltage dependent, nor were the kinetics of a test current altered by a preceeding conditioning current. Similarly, partial (50%) blockade of the current by CGP 35348 had no effect on the kinetics. These results are consistent with the hypothesis that PPD results from a presynaptic mechanism, which must be different from the postsynaptic $GABA_B$ conductance. Supported by NIH grant NS-12151, the Klingenstein Foundation (I.M.), a Howard Hughes Predoctoral Fellowship (T.S.O.), and the Canadian MRC (Y.D.K).

175.5

MODULATION OF SYNAPTIC GABA CURRENTS IN BRAIN SLICES: NON-STATIONARY FLUCTUATION ANALYSIS. Y. De Koninck*, T.S. Otis and I. Mody. Dept. of Neurology & Neurological Sciences, Stanford Univ. Sch. of Med., Stanford, CA.

Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded using whole-cell patch clamp techniques in dentate gyrus granule cells of 400 µm thick hemisected coronal adult rat brain slices maintained at 34-35°C. The recordings were made in the presence or absence of 1 µM tetrodotoxin with fast glutamatergic transmission blocked by CNQX and D-AP5. Our previous studies have shown that such sIPSCs are solely generated by activation of $GABA_A$ receptors, and are modulated by temperature, barbiturates, benzodiazepines and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). We have now examined the kinetics of the subsynaptic receptor/channels based on a non-stationary fluctuation analysis method (Robinson *et al.*, *Biophys. J.*, 59: 290, 1991).

Under control conditions (V_h = -60 to -80 mV) the average monoexponential decay time constant (t_D) of sIPSCs was 4.9 ms and the corresponding mean open time of the channels (t_{open}) was 1.4 ms. At room temperature (22-23°C), t_D was 22.9 ms while t_{open} was 2.6 ms. Modulation of sIPSCs decay kinetics by Na-pentobarbital (PB) and by increases in $[Ca^{2+}]_i$ was investigated at 34-35°C. PB (50 µM) increased t_D to 24.3 ms and t_{open} to 2.6 ms. During recordings with Ca^{2+}-filled (1-10 µM buffered or 20-50 µM unbuffered) patch electrodes, t_D and t_{open} progressively (30 min) increased to 9.5 ms and 2.0 ms respectively without accompanying changes in sIPSC rise time or amplitude. In addition, a non-stationary fluctuation analysis was performed on isolated stimulus-evoked $GABA_B$ receptor mediated responses.

Supported by NINDS grant NS-27528 and the Klingenstein Foundation (I.M.), a Postdoctoral Fellowship from the Canadian MRC (Y.D.K.) and a Howard Hughes Predoctoral Fellowship (T.S.O.).

175.6

ZINC INDUCES SPONTANEOUS BURSTING OF INHIBITORY NEURONS IN RAT NEOCORTEX. J.J. Hablitz* and F.M. ZHOU. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

Zinc is a negative modulator of NMDA and $GABA_A$ receptors in cultured neurons. We have examined the effect of zinc on synaptic potentials in rat neocortical slices. Intracellular recordings were obtained from layer II-III pyramidal neurons. At RMP, weak stimulation produced EPSPs while stronger stimulation produced EPSP-IPSP complexes. IPSPs were depolarizing since RMPs were > -80 mV. Bath application of 50-300 μM zinc did not affect weak EPSPs nor I-V relations. IPSPs were enhanced in amplitude and duration while responses to iontophoretically applied GABA were not affected by zinc. When EPSPs were blocked by excitatory amino acid (EAA) receptor antagonists, directly evoked IPSPs were enhanced by zinc and giant $GABA_A$- and $GABA_B$-mediated IPSPs occurred spontaneously. Giant depolarizing $GABA_A$-mediated events could trigger action potentials in some neurons. Picrotoxin (100 μM) greatly reduced or blocked the depolarizing events. All effects of zinc were reversible upon washing. These results demonstrate that zinc has a novel effect in neocortical neurons, namely, an enhancement of GABA-mediated synaptic responses. The occurrence of spontaneous giant IPSPs in the presence of EAA blockers suggests that GABAergic neurons can be synchronized by a non-EAA mechanism. (NS18145)

175.7

FUNCTIONAL AND PHARMACOLOGICAL PROPERTIES OF $\alpha_1\beta_2\gamma_2$ AND $\alpha_5\beta_2\gamma_2$ ISOFORMS OF THE RAT $GABA_A$ RECEPTOR. P. Avenet*, P. Granger, C. Faure-Halley, D. Graham, S. Arbilla, H. Depoortere B. Scatton and S.Z. Langer. Dept. of Biology, Synthélabo Recherche (L.E.R.S), 31 Ave. P.V-Couturier, 92220 Bagneux, France.

Recombinant $\alpha_1\beta_2\gamma_2$ (R-α_1) and $\alpha_5\beta_2\gamma_2$ (R-α_5) forms of the $GABA_A$ receptor were expressed in human embryonic 293 kidney cells by transfection with $GABA_A$ subunit cDNAs and investigated using the whole cell configuration of the patch-clamp technique. At a holding potential of -20 mV, fast superfusion with GABA (1-300 µM) elicited Cl^- currents in 44 out of 89 R-α_1 cells (mean intensity 125 pA, GABA 1 µM, n=13) and in 41 out of 92 R-α_5 cells (mean intensity 70 pA, GABA 1 µM, n=13). The GABA concentration dependency for both recombinant receptors was similar (K_D=3 µM). Both receptor types slowly desensitized with 1-10 µM GABA and showed a faster desensitization with 100-300 µM GABA which could be resolved into two exponential components. In 40% of the R-α_1 cells and 41% of the R-α_5 cells, diazepam and zopiclone (1 µM) potentiated the GABA (1 µM) induced current by a factor ranging between 1.2 and 2.6. Zolpidem (1 µM) increased the Cl^- current to a similar extent in R-α_1 recombinants but had no effect in R-α_5 cells. Thus, although R-α_1 and R-α_5 receptors exhibit similar functional properties, zolpidem, unlike diazepam and zopiclone, does not interact with $GABA_A$ receptors containing the α_5 subunit.

175.8

CHARACTERIZATION AND FUNCTIONAL EXPRESSION OF THE PROMOTER FOR THE HUMAN β1 SUBUNIT OF THE $GABA_A$ RECEPTOR. S.J. Russek and D.H. Farb*, Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA 02118.

The remarkable heterogeneity and ubiquitous distribution of the $GABA_A$ receptor in the central nervous system distinguishes it as part of an important neurotransmitter system that is amenable to the study of receptor function and regulation. Genes coding for the subunits of the $GABA_A$ receptor are differentially expressed in the CNS. A number of the $GABA_A$ receptor genes display a preferential expression in only one region of the brain, while expression in other brain regions is barely detectable (Wisden et al., (1992) *J. Neurosci.* 12: 1040). In particular, the highest expression of the β1 gene is seen in the hippocampus, making it a prime candidate for the study of region specific gene regulation. Using a novel form of the polymerase chain reaction (PCR), we have isolated a 600 bp region upstream to the 5' end of the reported full length human β1 cDNA (Schofield et al., (1987) *Nature* 328: 221). This PCR product was cloned into a reporter vector that contains the gene for firefly luciferase. Transfection of the reporter construct into primary cell cultures of embryonic chick brain demonstrates that there is a functional promoter in the 600 bp PCR product. We have identified several possible regulatory elements that may be necessary for the expression of the β1 gene in the vertebrate nervous system.

175.9

PROTRACTED DIAZEPAM TREATEMENT INDUCES A DOWN REGULATION OF $GABA_A$ RECEPTOR FUNCTION. P. GIUSTI*, N. ASTI§, M.M. ZANELLATO§ and R. ARBAN§. Department of Pharmacology, University of Padova, 35131 Padova, §FIDIA Research Laboratories, 35031 Abano Terme (PD), Italy.

$GABA_A$ receptor down regulation was assayed in rats undergoing a chronic treatment with diazepam. Changes were measured in the anticonvulsant action of this benzodiazepine (BZ) against a slow intravenous infusion of bicuculline (0.11 µmol/min), or the β-carboline ester β-CCM (0.28 µmol/min) to assess the functional state of GABA binding site and the coupling between the BZ modulatory sites with the GABA binding sites, respectively. Animals receiving protracted doses of diazepam (8.75, 17.5, 35, and 88 µmol/kg; 3 time day, p.o.) showed increased sensitivity to the convulsant actions of bicuculline and β-CCM when compared to naive animals receiving the same doses of diazepam. Tolerance to the anticonvulsant effect of diazepam against bicuculline and β-CCM-induced seizures occured after 68, 35, 9, and 4 days, respectively in animals repeatedly treated with 8.75, 17.5, 35, and 88 µmol/kg. The K_d and the B_{max} values for diazepam in vivo binding in the forebrain and the drug plasma levels (measured by HPLC) did not differ between control and treated animals.

The results are consistent with the hypothesis that chronic diazepam treatment may be accompained by an alteration in the molecular mechanisms underlying sentivity of the $GABA_A$ receptor complex to GABA and BZs.

175.10

FULL AND PARTIAL MODULATION OF NEUROSTEROIDS ON GABA ACTION IN NATIVE AND RECOMBINANT $GABA_A$ RECEPTORS. G. Puia, I.Ducic, S. Vicini* and E. Costa, FGIN, Georgetown University, Washington, DC.

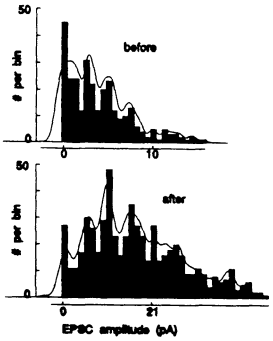
Certain naturally occurring steroids (neurosteroids) have been found to modulate $GABA_A$ receptor function. In previous studies, we have shown that the neurosteroids 3α-OH-DHP (5α-pregnan-3-α-ol-20-one) and THDOC (5α-pregnan-3-α-21-diol-20-one) potentiate GABA-activated Cl^-currents elicited on native or recombinant heterooligomeric (αβ, αβγ) as well as homomeric (β) $GABA_A$ receptors. Using the patch-clamp technique, we describe the modulation by 3α-OH-DHP and pregnenolone sulfate (PS) of GABA anionic current in cells expressing recombinant $GABA_A$ receptors including different molecular forms of α (α1 through α6) and γ (γ1 through γ3) subunits. With our experimental conditions we fail to show important differences in neurosteroid efficacy and potency in relation with the structural $GABA_A$ receptor differences. Furthermore, 3α-OH-DHP and 3β-OH-DHP compete in modulating GABA- activated Cl^-currents both in native and in α1β1γ2 $GABA_A$ receptors . Moreover the activity of 3α-OH-DHP and diazepam, barbiturate and PS summates algebrically. Since the modulation by PS and 3α-OH-DHP are independent of each other, we suggest that the "putative" sites for the allosteric positive and negative modulation of GABA action by the two compounds may be different. Interestingly in cortical neurons and reconstituted α1β1γ2 receptors PS has a biphasic modulatory action ; i.e. at 1µM decreases by 43± 10 % while at 10 nM potentiated by 35 ± 5 % (mean ± SE, n=5 cells) GABA-activated Cl^- currents. Evidence for differential binding characteristics of neurosteroids in frontal cortex and spinal cord (Gee and Lan, Mol. Pharm. 1991 40:995) prompted us to further investigate and to describe the modulation by neurosteroids of GABA-activated Cl^-currents in outside-out macro-patches excised from diverse brain regions.

LONG-TERM POTENTIATION I

176.1

INCREASED PRE- AND POSTSYNAPTIC QUANTAL MEASURES DURING LTP IN CA1 HIPPOCAMPUS. R. Malinow*, A. Jones and D. Liao. Dept Physiology and Biophysics, University of Iowa, and Dept Mathematics, Reed College.

The modification responsible for long-term synaptic potentiation is not known. To elucidate possible changes we have resolved quantal levels of transmission before and after induction of LTP. LTP was induced by pairing presynaptic activity (with no change in stimulus frequency) and postsynaptic depolarization. The potentiated transmission could be completely blocked with APV (n=10). After induction of LTP, we find an increase in both quantal content and quantal amplitude, consistent with combined pre- and postsynaptic modifications. On average, 61% of LTP can be accounted by presynaptic enhancement. The increase in either quantal amplitude or quantal content varies significantly among different experiments, but is inversely correlated with its initial value. These results may help to reconcile the different views concerning the site of LTP expression.



Amplitude distributions before and during LTP. Note amplitude axis change.

176.2

ALTERED RESPONSES TO PRESYNAPTIC MANEUVERS DURING LONG-TERM POTENTIATION OF MINIATURE EPSC FREQUENCY IN CULTURED HIPPOCAMPAL NEURONS. A. Malgaroli* & R. W. Tsien, Department of Molecular and Cellular Physiology, Stanford CA 94305.

Recordings of miniature excitatory postsynaptic currents (minis) were made from hippocampal cells in culture to gain new information about presynaptic changes during the expression of LTP. LTP of mini frequency was induced by direct application of glutamate or hypertonic solution (0 Mg^{2+}) (Malgaroli & Tsien, Nature in press). Like the amplitude of evoked responses, the frequency of minis was strongly increased. The rise in mini frequency (TTX present) was induced by activation of postsynaptic NMDA receptors and persisted up to the end of the recording. The potentiation was not abolished by removal of external Ca^{2+}, indicating that it cannot be explained by a sustained elevation of Ca^{2+} entry. We wondered whether the synaptic enhancement might result from increased efficiency of other steps in neurosecretion, such as those controlling vesicle availability or vesicle fusion. To gain insights into the nature of the presynaptic change, we monitored quantal vesicular release induced by variations in the tonicity of the bathing medium, a classical presynaptic intervention. Before potentiation, brief challenges with hypertonic solutions(500-600 mosm)increased mini frequency 50-100-fold above basal levels; hypotonic media(250 mosm) produced only a mild decrease. During mini frequency potentiation, the fold response to hypertonicity was much reduced while the fold response to hypotonic media was accentuated. Thus, the top of the dose-response curve is little changed, while the foot of the tonicity dose-response curve appears to be shifted toward lower values. The fact that hypertonicity occludes the mini frequency potentiation indicates that both interventions act along the same presynaptic secretory pathway. A possible explanation of the shift in the tonicity dose-response curve would be an increase in the sensitivity of the secretory machinery to $[Ca^{2+}]_i$.

176.3

Approaches to Quantal Analysis at the CA3-CA1 Synapse in Rat Hippocampal Slices

Felix E. Schweizer*, Julie A. Kauer, David D. Friel, Richard W. Tsien. Stanford University Medical Center, Stanford, CA 94305

We tried to apply the methods of quantal analysis (QA) to study long-term potentiation (LTP) at the CA3-CA1 synapse in the rat hippocampus. To this goal we constructed amplitude distribution histograms of EPSCs recorded with whole cell voltage clamp from CA1 pyramidal cells. For a majority of experiments there was at least one epoch containing 100-1000 EPSCs from which histograms exhibiting equally spaced peaks could be constructed. Inclusion of more events in the histograms made the peaks less pronounced or let them disappear altogether. We broke the experiment apart into consecutive independent epochs and tried to evaluate whether the peaks reflect sampling artifacts or whether they reflect quantal behavior of the synapse(s). To test for equally spaced, quantal peaks we measured the maximal power in the Fourier spectrum of either mean-subtracted histograms or of the crosscorrelation between two halves of the dataset. To determine the statistical significance of peaks in the entire experiment, we carried out Monte Carlo simulations. Samples taken from either smooth or peaky, simulated distributions were taken, histograms constructed and the respective scores determined. The distribution of scores from simulated peaky and smooth simulations showed considerable overlap, indicating that sampling artifacts can introduce spurious peaks into smooth datasets and smooth out peaks in genuinely peaky datasets. Experiments in which peaks are statistically significant will yield information about quantal size, quantal content and the statistic of release.

176.4

CHANGES IN PAIRED-PULSE FACILITATION (PPF) SUGGEST PRESYNAPTIC INVOLVEMENT IN THE EXPRESSION OF LTP IN HIPPOCAMPAL AREA CA1. *P.E. Schulz* & D. Johnston*, Dept. of Neurology & Div. of Neuroscience, Baylor Col. of Med., Houston, Tx 77030.

Despite intensive investigation, there is still disagreement as to the locus of change underlying LTP expression. It has been hypothesized that if LTP expression includes a presynaptic locus, then changes should be observed in other forms of presynaptic potentiation. PPF is a potentiation of a second population excitatory postsynaptic potential (pEPSP) when it follows shortly after a first and is thought to be mediated presynaptically by residual calcium. Thus, if LTP expression includes a presynaptic locus, then PPF may be partially occluded by LTP. Extracellular recordings were made in rat hippocampus area CA1, in 375μm thick slices, with picrotoxin and 3.0 or 4.5mM $[Ca^{2+}]_e$ in the bath. Increased extracellular calcium and post-tetanic potentiation (PTP) are thought to increase pEPSPs via a presynaptic mechanism. It was confirmed that both forms of potentiation decreased PPF indicating that PPF expression is sensitive to changes in transmitter release. Slices were repeatedly tetanized until LTP was saturated at a single stimulus intensity that initially yielded a 1.3mV pEPSP. PPF was compared between baseline and after LTP saturation. LTP saturation was associated with a decrease in PPF. The decrease in PPF associated with LTP was additive with the decrease associated with increased $[Ca^{2+}]_e$ and PTP. Once LTP was saturated, further tetani were not associated with additional changes in PPF suggesting that the PPF changes were related to LTP. PPF at baseline, and the change in PPF between baseline and saturation, did not correlate with the magnitude of LTP expressed. We conclude that the mechanism of LTP expression includes at least a presynaptic locus, but these results do not exclude possible additional postsynaptic mechanisms. (AG00432, AG08664, MH44754, MH48431)

176.5

A NOVEL ACTION OF ENDOGENOUS OPIOIDS IN THE INDUCTION OF HIPPOCAMPAL MOSSY FIBER LTP. *S.H. Williams* & D. Johnston*, Division of Neuroscience, Baylor Colllege of Medicine, Houston, Tx 77030.

The mossy fiber system of the rat hippocampus contains a high concentration of both dynorphin and enkephalin. Previous studies have suggested these peptides are important for mossy fiber LTP induction, possibly acting by producing disinhibition during high frequency stimulation (HFS). We have tested this hypothesis in the hippocampal slice preparation.

Intracellular recordings were made from CA3 neurons at 32-34°C. $GABA_A$ inhibitory synapses were blocked by 10 μM bicuculine and picrotoxin. The NMDA antagonist APV was routinely added to the saline. The percentage change in EPSP or EPSC amplitude was determined by comparing pretetanus values to 15 min post-tetanus. In control slices LTP was elicited in 12/15 experiments. In contrast if 1 μM naloxone (NAL) was added prior to HFS, LTP was obtained in only 2/9 experiments. The mean increase in EPSP amplitude in control was 75±18%, compared to the a small decrease of 13±4% in the NAL group. If slices were given HFS prior to NAL perfusion, LTP was not affected, indicating that the action of naloxone is specific to the induction step of LTP. NAL did not affect LTP induction in the commissural/associational input to CA3 (n = 4, no APV present). These data suggest that endogenous opioids are important modulators of induction of mossy fiber LTP. The mechanism of action of opioid peptides, however, appears to be independent of $GABA_A$-type inhibition, counter to previous proposals. These data suggest that the opioids released from mossy fiber terminals can act via a previously unsuspected and extremely novel mechanism. (MH44754, NS11535.)

176.6

A PHYSIOLOGICAL ROLE FOR DYNORPHIN AT MOSSY FIBER SYNAPSES IN THE HIPPOCAMPUS. M. G. Weisskopf*, R. A. Zalutsky and R. A. Nicoll Depts. Pharmacol. and Physiol., UCSF, San Francisco, CA 94143-0450.

In the hippocampal slice preparation tetanic stimulation delivered in s. granulosum of the dentate gyrus produces robust long-term potentiation (LTP) of synaptic transmission at the mossy fiber-CA3 synapse. Such stimulation also produces a transient depression, lasting 10-20 minutes, in a second, separate set of mossy fiber-CA3 synapses.

We have used field potential recording of mossy fiber synaptic action in the CA3 region to elucidate the mechanism of this heterosynaptic depression. Dynorphin A, the most abundant opioid peptide in mossy fibers, when applied exogenously (100 - 500 nM), reduced mossy fiber synaptic responses, but had no effect on the responses evoked by commissural/associational afferents. The dynorphin-induced depression is likely to result, at least in part, from a presynaptic action, since the depression is associated with an increase in paired-pulse facilitation. In addition, dynorphin had no effect on the excitability of CA3 pyramidal cells recorded intracellularly. Finally, the tetanus-induced heterosynaptic depression was blocked by naloxone, indicating that this effect is largely mediated by a synaptically released endogenous opioid peptide.

In summary, high frequency stimulation of mossy fibers releases, in addition to glutamate, an opioid peptide, most likely dynorphin, which diffuses to neighboring mossy fiber synapses and inhibits the release of glutamate. Such an action would provide a transient inhibition surrounding the potentiated pathway.

176.7

INCREASE IN POSTSYNAPTIC NON-NMDA RECEPTOR SENSITIVITY INDUCED BY NMDA IN HIPPOCAMPAL SLICES. T. Manabe and R.A. Nicoll* Depts. Pharmacol. and Physiol., UCSF, San Francisco, CA 94143.

NMDA application enhances excitatory synaptic transmission in the CA1 region of the hippocampus. Although this enhancement is generally transient, recent reports indicate that it is closely related to long-term potentiation (LTP). We have shown that during NMDA-induced potentiation an increase in the size of miniature EPSCs (mEPSCs) occurs, which correlates with the change in the size of evoked responses. Furthermore, the NMDA-induced enhancement of mEPSC size shares two key properties with LTP: it requires postsynaptic depolarization and a rise in the concentration of postsynaptic calcium.

An increase in mEPSC size is usually interpreted as resulting from an increase in postsynaptic transmitter sensitivity. However, it could also be explained by an increase in the amount of transmitter in presynaptic vesicles. To address this issue we repeatedly applied AMPA, a selective non-NMDA receptor agonist, to the dendritic region of CA1 pyramidal cells in guinea pig hippocampal slices before and after NMDA application. The response to AMPA, monitored by whole-cell voltage clamping, showed a significant increase during NMDA-induced potentiation of mEPSC size. Thus, it is concluded that NMDA-induced potentiation in hippocampal slices is mediated, at least in part, by an increase in postsynaptic sensitivity. The results also suggest that LTP, during which mEPSCs increase in size, is expressed, in part, by a similar mechanism.

176.8

EFFECTS OF PRESYNAPTIC MODULATION OF SYNAPTIC TRANSMISSION AND LTP ON DUAL-COMPONENT EXCITATORY SYNAPTIC CURRENTS IN THE HIPPOCAMPUS. D. J. Perkel* and R. A. Nicoll Depts. Pharmacol. and Physiol., UCSF, San Francisco, CA 94143.

NMDA and non-NMDA ionotropic glutamate receptors are thought to be colocalized at postsynaptic sites. A postsynaptic modification, or a change in transmitter concentration in the synaptic cleft could change the relative receptor contributions to synaptic events, since the two receptor types have very different sensitivities to glutamate. It is not known whether changes in release probability affect cleft transmitter concentration, and controversy exists over the effects of LTP on the two receptor components of synaptic events. We have used "blind" whole-cell recording from guinea pig CA1 pyramidal cells in slices to examine the effect of presynaptic manipulations or of LTP on Schaffer collateral synaptic currents. Postsynaptic neurons were held at +30 mV to allow simultaneous measurement of the two components and to reduce space-clamp artifacts.

Reduction of synaptic currents by 4-fold by baclofen (10 μM), or similar enhancement by theophylline (50-100 μM) led to parallel changes in late (NMDA) and early (non-NMDA) components. LTP was induced by pairing synaptic stimuli with depolarization to +30 mV. In cells that showed LTP (as measured upon return to -80 mV), there was a marked increase in the ratio of non-NMDA to NMDA receptor contribution. In cells not showing LTP, a small parallel increase (25%) in both components was seen, similar to the growth of the NMDA component in cells showing LTP, likely due to frequency facilitation caused by starting stimulation.

Our data suggest that pharmacological modulation of release is all-or-none at individual synaptic clefts. In contrast, LTP could result from a postsynaptic change or a change in cleft transmitter concentration.

176.9

NMDA RECEPTOR-INDEPENDENT POTENTIATION OF SYNAPTIC TRANSMISSION IN HIPPOCAMPAL CA1 CELLS. D.M. Kullmann, T. Manabe, D.J. Perkel, S. du Lac* & R.A. Nicoll. Depts. Pharmacol. & Physiol., UCSF, San Francisco, CA 94143.

Calcium influx via NMDA receptor gated channels is necessary for the induction of long-term potentiation (LTP). To assess whether a rise in postsynaptic calcium is *sufficient*, we have attempted to maximize calcium influx into CA1 cells via voltage-sensitive calcium channels, in the presence of 50 mM D-2-amino-5-phosphonovaleric acid (D-APV) to block NMDA receptors. Using either conventional intracellular recording or whole-cell patch-clamping *in vitro*, a transient potentiation of excitatory transmission from stratum radiatum stimulation was obtained by delivering repeated depolarizing pulses to the postsynaptic cell. This potentiation typically decayed to baseline within 30 minutes and did not require presynaptic stimulation during the voltage steps. It was, however, greatly enhanced by raising the extracellular calcium concentration from 2.5 to 4 - 5 mM, or by including an ATP regenerating system in the patch-pipette solution: a 2 - 3 fold potentiation of transmission was reliably obtained, but this still decayed with a similar time course. The potentiation was completely abolished by including the calcium chelator BAPTA in the pipette solution, or by adding 20 mM nifedipine to the perfusion medium, confirming that a rise in intracellular calcium was necessary. If D-APV was omitted from the extracellular solution, and LTP was elicited by pairing presynaptic stimulation with postsynaptic depolarization, this did not reduce the amplitude of the transient potentiation produced by depolarizing pulses delivered after a delay of 20 minutes.

Calcium influx via dihydropyridine-sensitive channels can lead to a potentiation of transmission, but this differs from LTP in several respects.

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176.10

EXAMINATION OF TEA-INDUCED SYNAPTIC ENHANCEMENT REVEALS THE ROLE OF Ca^{2+} CHANNELS IN THE INDUCTION OF LTP IN AREA CA1 OF RAT HIPPOCAMPUS. Y.-Y. Huang and R.C. Malenka*. Depts. of Psychiatry & Physiology, U.C. San Francisco, CA 94143.

The role of voltage-dependent Ca^{2+} channels (VDCCs) in the induction of LTP in area CA1 of rat hippocampal slices was determined by examining the relationship between the synaptic enhancement induced by application of tetraethylammonium (TEA; 25 mM) and tetanus-induced LTP. As previously reported (Aniksztejn & Ben-Ari, Nature, 349:67), the TEA-induced synaptic enhancement (TEA-SE) was unaffected by blockade of NMDA receptors with D-APV (25-50 μM). It was blocked by the VDCC antagonist nifedipine (10-12 μM) or by intracellular injection of the Ca^{2+} chelator BAPTA and could be mimicked by direct activation of VDCCs with repetitive depolarizing current pulses (Perkel et al., Soc. Neurosci. Abst. 17:2). Nifedipine had no effect on tetanus-induced (100 Hz, 1 sec), NMDA receptor-dependent LTP.

Saturation of LTP with repetitive tetani reduced the magnitude of TEA-SE (by 55%; n=9). Similarly, increasing synaptic transmission by applying TEA reduced the magnitude of subsequent tetanus-induced LTP (by 61%; n=7). Like LTP, the TEA-SE did not significantly affect paired-pulse facilitation (ISI=50 msec).

These results suggest that dihydropyridine sensitive VDCCs do not normally contribute to the induction of LTP even though their repetitive activation can result in an increase in synaptic strength. The mutual occlusion of TEA-SE and LTP suggest that they either share a common expression mechanism or activate a common intracellular process. One hypothesis to explain these results is that VDCCs are absent from dendritic spines and do not contribute significantly to the rise in Ca^{2+} required for LTP induction. However, repetitive activation of VDCCs may cause a large rise in Ca^{2+} that overcomes endogenous buffering mechanisms and activates processes within the spine. The lack of complete occlusion of one form of synaptic enhancement by the other raises the possibility that there may also be some mechanistic differences between TEA-SE and LTP.

176.11

THE TIME COURSE OF INCREASED POSTSYNAPTIC CALCIUM NECESSARY FOR LTP REVEALED BY PHOTOSENSITIVE CALCIUM CHELATORS. B. Lancaster*, R.S. Zucker & R.C. Malenka#. Dept. of Molec. & Cell Biology, U.C. Berkeley, CA 94720 and # Depts. of Psychiatry and Physiology, U.C. San Francisco, CA 94143.

Experiments were performed to quantify the temporal limits of the rise in postsynaptic Ca^{2+} which is required for LTP induction. This was achieved by manipulation of the timing between the LTP-inducing tetanus and an instantaneous increase in Ca^{2+} buffering capacity produced by rapid, flash photolysis of the photolabile chelator diazo-4 (Adams et.al., J. Am. Chem. Soc. 111:7957).

In hippocampal area CA1 two independent pathways were stimulated and simultaneous recordings were made of field epsps and intracellular epsps using the whole cell configuration (Cs gluconate based solution, pipette tips filled with 1.0-2.5 mM diazo-4). Photolysis was induced with a xenon arc flashlamp. If a tetanus (1 sec, 100-200 Hz) to the control path produced LTP, a tetanus was given to the test path within 2 minutes. Photolysis of diazo-4 after the control tetanus prevented induction of LTP by the test pathway in the same cell (n=16) whereas field epsps showed LTP. The minimum delay for photolysis which did not affect LTP induction was 2.5 secs from the start of the tetanus. Photolysis immediately at the end of a tetanus blocked LTP (n=5). Intermediate (1.0-2.0 secs) delays gave mixed results including a decrementing, short term potentiation. The results indicate that a tetanus-induced rise in postsynaptic Ca^{2+} lasting 2.0-2.5 secs is sufficient to generate LTP.

We thank Drs. S. Adams and R. Tsien for providing diazo-4. Supported by NS15114 to RSZ and MH45334, MH00942 to RCM.

176.12

DENDRITIC Ca^{2+} ACCUMULATIONS ACCOMPANYING TEA-INDUCED LTP IN HIPPOCAMPAL CA1 NEURONS IN BRAIN SLICE. J.J. Petrozzino*, S.-C. Sun, and J.A. Connor. Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

Brief exposure of hippocampal slices to tetraethylammonium (TEA) induces Ca^{2+}-dependent, NMDA receptor-independent LTP (LTP_K) of synaptic transmission (Aniksztejn & Ben-Ari, *Nature* 349:67-69, 1991). We combined microfluorometric fura-2 imaging of guinea pig hippocampal CA1 pyramidal cells with intracellular and field recordings to evaluate the spatio-temporal distribution of postsynaptic Ca^{2+} during LTP_K. Test stimuli were applied at 0.05 Hz to stratum radiatum in the presence of 100 µM D,L APV at both 23 and 35 °C. TEA (15-25 mM, 10 min) produced increases in the initial slope of both the field and intracellular EPSP ($EPSP_f$ and $EPSP_i$, respectively) measuring 183±12% ($EPSP_f$; mean±SEM, n=8) and 250±35% ($EPSP_i$; n=5) of control. LTP_K was followed for 30-120 min ($EPSP_f$) and 30-80 min ($EPSP_i$) after TEA washout. During TEA exposure cell firing was intense, and dendritic and somatic Ca^{2+} levels were transiently elevated to 1-5 µM from resting levels of <100nM (8/10 cells tested). In 6 of these 8 cells, Ca^{2+} returned to near basal levels within 2-20 min of TEA washout. Application of TEA in APV-free saline produced LTP of similar amplitude and duration, but more sustained Ca^{2+} elevations were observed. These results establish that large, transient postsynaptic Ca^{2+} accumulations accompany LTP_K in hippocampal CA1 pyramidal cells, and support a role for postsynaptic Ca^{2+} in the induction of LTP_K.

BRAIN METABOLISM AND BLOOD FLOW II

177.1

PROTEIN KINASE C (PKC) AND CEREBRAL VASODILATORY RESPONSES IN THE STREPTOZOTOCIN (STZ)-TREATED RAT. D.A. Pelligrino* and A. Sharp. Dept. of Anesthesiology, Univ. of Illinois-Chicago, Chicago, IL 60616

Previous work indicated a selective impairment of cerebral vasodilatory responses in chronically hyperglycemic, diabetic (CHD) rats. This suppression appears to include 2 separate receptor-mediated vascular relaxation processes: *1)* endothelium-derived nitric oxide (NO) release (e.g., mediated by muscarinic or purinergic-P_2 receptors) and *2)* an NO-independent mechanism, mediated by the ß-adrenergic receptor (ß-AR). In this study, we tested the hypothesis that chronic hyperglycemia-induced PKC activation can account for the suppression in both *1* and 2. Male S-D rats were prepared and studied under fentanyl/N_2O anesthesia, paralysis, and mechanical ventilation. The reactivity of pial arterioles (40-60 μm in diameter), observed through a closed cranial window, was assessed using intravital microscopy/videometry. Vessel diameters were measured with a video micrometer, in CHD (5 mo. post-STZ) vs non-diabetic (ND) rats. These measurements were made at the end of each of the following periods of cortical suffusions of artificial CSF solutions (37° C; PCO_2, PO_2, pH constant; infusion rate=1 ml/min), with or without added test agents: *a)* 10 min CSF ; *b)* 10 min sodium nitroprusside (SNP) (10^{-6} M); *c)* 10 min acetylcholine (Ach) (10^{-6} M); *d)* 10 min CSF; *e)* 10 min isoproterenol (ISO) (10^{-4} M); *f)* 20 min CSF; *g)* 30 min of the PKC inhibitor staurosporine (STAURO) (10^{-7} M); *h)* repeat *b-e* (includes STAURO in CSF). In ND rats, Ach, ISO, and SNP produced increases in arteriolar diameters of 12, 15, and 26%, respectively. In CHD rats, Ach and ISO produced 5% and 1% *reductions*, respectively, in diameters, but SNP induced a 31% increase. In the presence of STAURO, the Ach and ISO responses were partially restored (Ach=+6%, ISO=+9%) and the SNP response was unaffected (+30%). STAURO produced no changes in Ach or ISO responses in ND rats. These results suggest that PKC activation in CHD rats suppresses receptor-dependent NO release *and* desensitizes ß-ARs.

177.2

HYPOTHERMIC CEREBRAL VASODILATION IS MEDIATED BY NITRIC OXIDE (NO). J.W. Kuluz[1], R. Prado, B. Watson, M Ginsberg, R. Busto, A. Martinez*, C Schleien[1]. Depts Pediatrics[1] and Neurology. Univ of Miami School of Medicine, Miami, FL 33136

We have found that selective brain cooling (SBC) increases CBF in rats to 115±26% above baseline. To test whether this response is mediated by NO, we measured CBF in rats by laser-doppler at baseline, for 30 min after a 15mg/kg infusion of L-NAME (a potent NO synthase inhibitor), during 20 min of SBC and during 30 min of rewarming (RW) while rectal temp was kept 37-38°C. Mean CBF, MAP and brain temp (BT) for each 5 min period was compared to baseline by ANOVA (* p<.05). **Results:** L-NAME increased MAP by 12% and decreased CBF by 25%. During SBC, CBF increased to 98±7% *of* baseline, or 31% *above* CBF after infusion of L-NAME. During RW, CBF decreased to baseline.

Time	CBF %Baseline	MAP mmHg	BT°C
Baseline	100±14	125±3	37.2±.0
30'p L-NAME	*75±6	*140±5	37.1±.0
20' of SBC	98±7	*141±7	*29.7±.2
30' of RW	*71±5	*143±6	36.7±.3

Conclusions: These results suggest that hypothermic cerebral vasodilation is mediated by NO. Production of NO during SBC may protect the brain against ischemic insult.

177.3

INHIBITORS OF NITRIC OXIDE SYNTHESIS ATTENUATE THE CEREBROVASODILATION ELICITED BY HYPERCAPNIA WITHOUT AFFECTING LOCAL METABOLISM C. Iadecola, Dept of Neurology, Univ. of Minnesota, Minneapolis, MN 55455.

Nitric oxide (NO), is an important neural signal and a potent vasodilator that may regulate the cerebral circulation. We have recently demonstrated that inhibition of NO synthesis substantially attenuates the cerebrovasodilation elicited by hypercapnia, an effect that is stereospecific and reversed by co-application of arginine (PNAS, 89:3913, 1992). In this study we sought to determine whether NO-synthase inhibitors attenuate the cerebral blood flow (CBF) response to hypercapnia by decreasing local cerebral glucose utilization (lCGU). Rats were anesthetized (chloralose 80mg/kg) and artificially ventilated. Arterial pressure and blood gases were monitored. The sensory cortex was exposed and superfused with aerated Ringer (pH 7.3-7.4; 37C°) with or without the NO-synthase inhibitor N^{ω}-nitro-L-arginine (L-NA). CBF was measured autoradiographically at the site of superfusion using 14-C-iodoantipyrine or 14-C-2-deoxyglucose as tracers, respectively. At normocapnia, CBF was 83±19 ml/100g/min at the site of superfusion and 90±14 in the contralateral cortex (p>0.05). L-NA superfusion (1mM) decreased resting local CBF from 83±19 to 55 ml/100g/min (p<0.001; n=5). However, L-NA did not affect resting lCGU (Ringer: 69±8μmol/100g/min; L-NA: 81±7; p>0.05). During superfusion with Ringer, hypercapnia (pCO_2=61±4mmHg) increased CBF bilaterally and symmetrically (+161±30%; n=5; p<0.001 ANOVA). L-NA attenuated the CBF increases elicited by hypercapnia by 87±6% (n=5) while the local cerebrovasodilation elicited by topical papaverine (1mM) (n=5) was unaffected (+213±15; p>0.05 from Ringer). Thus, NO synthesis is required for maintaining resting CBF and for the cerebrovasodilation elicited by hypercapnia. Since NO-synthase inhibition does not affect lCGU, these effects are independent of local metabolic activity. The results add further support to the hypothesis that arginine-derived NO is also involved in the regulation of the circulation of the brain. (Supported by the American Heart Association of Minnesota and the Minnesota Medical Foundation).

177.4

BREAKTHROUGH OF CEREBRAL BLOOD FLOW (CBF) WITH HYPERTENSION: EVIDENCE FOR ACTIVE CEREBRAL VASODILATATION. W.T. Talman*, D. Nitschke Dragon, H. Ohta. Dept. of Neurology, VAMC and Univ. of Iowa, Iowa City, IA 52242.

Acute elevation of mean arterial pressure (MAP) above 150 mmHg leads to loss of autoregulation, rapid decrease of resistance (CVR) and increase of CBF. We have shown that breakthrough in rats is nearly eliminated by sinoaortic denervation (SAD). We have extended those studies to determine if pharmacological attenuation of the baroreflex interferes with breakthrough as does SAD and if nitric oxide (NO) may mediate vasodilatation with breakthrough. Changes in CBF were assessed by laser flowmetry during control periods and during infusion of phenylephrine (PE), angiotensin II (AII), or vasopressin (AVP) in 15 intact rats; during infusion of PE in 13 rats after SAD; and during infusion of PE in 11 intact animals after an i.v. bolus of L-nitroarginine (LNA). Animal temperature, arterial pCO_2 and pO_2, and rate of rise of MAP were carefully controlled. Breakthrough of CVR and CBF occurred in intact rats when MAP was raised to 160±8 or 179±3 mmHg by PE or AVP respectively but did not occur in intact rats treated with AII or with PE after LNA despite equal or greater increases of MAP. Breakthrough also did not occur in rats after SAD, which, like treatment with AII, eliminated baroreflex responses to increased MAP. Rats with SAD and removal of the superior cervical ganglia bilaterally likewise did not demonstrate breakthrough. Change in pulse pressure from basal to maximal levels of MAP, change in MAP with respect to time, and pCO_2 did not significantly differ between groups where breakthrough occurred and those where it did not. Thus, breakthrough of autoregulation during acute hypertension may be dependent on the arterial baroreflex and mediated by release of NO. Support: VA Merit Review and Clinical Investigatorship (WTT) and NIH HL32205 and HL14388.

177.5

PRESERVATION OF CEREBROVASCULAR DILATION FOLLOWING CORTICAL SPREADING DEPRESSION IN ANESTHETIZED RABBITS. David W. Busija*, Dept. of Phys. & Pharm., Bowman Gray Sch. of Med., Wake Forest Univ., Winston-Salem, NC 27157

We examined responses of rabbit pial arterioles to three different stimuli before and after induction of cortical spreading depression (CSD). In urethane-anesthetized rabbits equipped with a closed cranial window, we measured pial arteriolar diameter during baseline conditions, during topical application of 10^{-7}M calcitonin-gene related peptide (CGRP), topical application of 10^{-4}M acetylcholine, and inhalation of 10% CO_2 in air (arterial hypercapnia), before CSD and 30, 60, and 120 after CSD. CSD was induced by localized application of a 5% KCl solution anterior to the arteriole being measured. Average baseline diameter was approximately 100μm. During CSD, arteriolar diameter increased to a peak value that was 51±5% above baseline (n=26). Prior to CSD, arteriolar diameter changed 47±7% (n=9) during hypercapnia, 42±10% (n=7) during 10^{-7}M CGRP, and 61±13% (n=6) during 10^{-4}M acetylcholine. After CSD, arterioles dilated during hypercapnia by 36±7% (n=8) after 30 minutes, 44±9 (n=6) after 60 minutes, and 61±10% (n=5) after 120 minutes. Following CSD, arterioles dilated during CGRP (10^{-7}M) application by 55±10% (n=7) at 30 minutes, 51±19% (n=8) during 60 minutes, and 30±5% (n=5) at 120 minutes. After CSD, acetylcholine (10^{-4}M) dilated arterioles by 53±7% (n=9) at 30 minutes, 41±6% (n=13) at 60 minutes, and 53±15% (n=10) at 120 minutes. Arteriolar responsiveness was not changed significantly to any of these stimuli by prior CSD. We conclude, that dilator capacity of pial arterioles is still intact in urethane-anesthetized rabbits following CSD. (Supported by HL46558 and HL30260)

177.6

CORTICAL CEREBRAL BLOOD FLOW (CBF) CYCLING. John L. Williams, Mary Shea, Datong Wei, and Stephen C. Jones.* Cerebrovascular Research Laboratory, Cleveland Clinic Foundation, Cleveland, OH 44195.

Temporal oscillations of CBF and of other cerebral parameters have been noted with a frequency of 0.1 Hz, but only in a small fraction of the animals. Suspicion that this phenomena is dependent on the type of anesthesia led us to investigate the occurrence of CBF cycling with changes in mean arterial blood pressure (MABP) with different anesthetics.

Fifteen Sprague-Dawley rats (370 ± 8 g, SEM) were anesthetized with either pentobarbital (n=5, 40-50 mg/kg), chloralose (n=5, 60 mg/kg), or halothane (n=5, 1-0.5%). Body temperature was maintained at 37°C. Femoral arterial and venous catheters were placed, and a tracheotomy was performed, permitting artificial ventilation with 30% O_2 and 70% N_2. A closed cranial window was formed over a 3 mm diameter craniotomy. MABP (126 ± 3 mmHg), $PaCO_2$ (36 ± 2 mmHg), PaO_2 (139 ± 6 mmHg), and pH (7.43 ± 0.03) were controlled and stabilized. CBF was determined using laser Doppler flowmetry. MABP was transiently and repeatedly lowered by exsanguination. Fast Fourier analysis of selected 60 s flow recordings (n=38) was performed.

CBF cycling was observed, independent of the type of anesthesia, in 14/15 animals. In 13 animals, cycling was induced when MABP was reduced to 71 ± 3 mmHg. The mean frequency and amplitude were 0.102 ± 0.004 Hz and 6.5% ± 0.7%. There were no correlations over the entire group between frequency or amplitude and MABP.

These oscillations occur at MABPs that are near the lower limit of autoregulation. At this pressure, CBF oscillations would suggest that vasoconstrictive and dilatory forces are no longer in balance, but alteratively vying for control.

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177.7

AN ELECTROPHYSIOLOGICAL CORRELATE RELATED TO SPONTANEOUS AND EVOKED ELEVATIONS OF CEREBRAL BLOOD FLOW IN RAT CEREBRAL CORTEX. E. Golanov* and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

In the cerebral cortex, blood flow (rCBF) is regulated by intrinsic neural networks many of which, when excited, increase rCBF independently of metabolism. To identify electrophysiological correlates associated with spontaneous and/or induced changes in rCBF, rCBF was measured over parietal cortex by laser doppler flowmetry (LDF). Forty five rats were paralyzed, ventilated and anesthetized with 1.5% isoflurane, measuring EEG and arterial pressure. During steady-state conditions, rCBF spontaneously oscillates with brief elevations of rCBF (147.1±2.6% of control) lasting 11.7±0.5 sec. The average periodicity was 0.1-0.025 Hz. Oscillations of rCBF were always associated with a characteristic pattern of EEG activity consisting of bursts of high-amplitude (0.75±0.04 mV), low frequency (5.6±0.2 Hz) activity varying in burst duration. The onset and peak of each oscillation followed the onset of each burst with constant latencies (1.7±0.1 and 3.8±0.1 sec, respectively). The frequency of bursts and the oscillations co-vary with depth of anesthesia. Bursts of EEG appear simultaneously and synchronously in different cortical areas. Stimuli increasing rCBF independently of metabolism (e.g. hypoxia, hypercapnia, or brainstem or cerebellar stimulation) simultaneously and proportionally increase EEG bursts to fusion and rCBF. We conclude that spontaneous oscillations of rCBF are tightly coupled to a burst event in the cerebral cortex. This appears triggered by a subcortical pacemakers providing synchronous and simultaneous patterns of cortical neuronal activity. The results suggest the existence of cortical neurovascular modules related to mediating primary vasodilation.

177.8

REGIONAL CEREBRAL BLOOD FLOW, PLASMA CATECHOLAMINES, AND EEG DURING HYPOGLYCEMIA AND RECOVERY. R.M. Bryan,* M.Y. Eichler, C.N. Niederman, T.D. Johnson, W.T. Woodward, and J.L. Williams. Department of Anesthesiology, Baylor College of Medicine, Houston, TX, 77030.

Regional cerebral blood flow (rCBF), plasma catecholamines (PC), and EEG were measured during the onset of insulin-induced hypoglycemia (HG) and recovery. Male Long-Evans rats were fasted overnight and surgically prepared using isoflurane. Each rat was immobilized by securing the hindquarters with a plaster cast and allowed to recover from anesthesia. Regional CBF (using ^{14}C-isopropyliodoamphetamine), PC, and EEG were measured during control conditions (plasma glucose, PG, =171 mg/dl), HG (PG=29 mg/dl), five (PG=213 mg/dl), and 30 (PG=134 mg/dl) minutes of recovery (IV injection of glucose). The EEG showed a characteristic high-amplitude, slow-wave pattern during HG. Plasma epinephrine in the normoglycemic control rats was 529 pg/ml and increased by 4.5 times when PG had fallen to 50 mg/dl. Plasma epinephrine (E) steadily decreased toward baseline over the next 90 minutes as the HG became more severe. Unlike E which showed a very large increase during the early stages of HG, plasma norepinephrine (NE) significantly increased by 60% only during the latter stages of HG (PG=35-40 mg/dl). NE remained elevated during much of the recovery period. Regional CBF increased 55-82% in most regions, but did not change in regions lacking a blood-brain barrier. During the recovery, rCBF returned to normal within five minutes in those regions where it had increased. We conclude that (a) rCBF immediately returns to normal values upon recovery from HG; and (b) increases in E are probably not responsible for the increase in rCBF during hypoglycemia since it peaked and was returning toward baseline when rCBF increased.

177.9

Pituitary adenylate cyclase activating peptide (PACAP): a VIP-like peptide with vasodilator actions in the cerebral circulation P.J. Goadsby*, R. Ekman† and L. Edvinsson‡ Department of Neurology, The Prince Henry Hospital, Sydney AUSTRALIA and Departments of Otorhinolaryngology† & Experimental Research‡, Malmo General Hospital, Malmo SWEDEN.

Pituitary adenylate cyclase activating peptide (PACAP) is a VIP-like peptide that has been recently isolated from ovine hypothalamus. VIP, peptide histidine isoleucine (PHI), PACAP, PACAP-related protein ,secretin and glucagon all belong to a super-family of neuroendocrine peptides with generally vasodilator properties. The existence of PACAP in neurons in the central nervous system raises the possibilty that, like VIP, it may be present in nerves on cerebral vessels. To study cerebral blood flow cats were anesthetised with α-chloralose (60mg/kg, ip) and prepared for physiological monitoring including cardiovascular parameters to assess the need for supplemental anesthesia. Cerebral blood flow (CBF) was measured using laser Doppler flowmetry (CBF_{LDF}; Goadsby, Am J Physiol, 260;R255,1991). PACAP was injected directly into the brain in 1μl aliquots. To examine the cerebral vessels cats were deeply anesthetised and brain vessels harvested. Double immunostaining for VIP and PACAP using rabbit antibodies was used. Local injection of 1μl of vehicle did not significantly alter resting CBF although injection of PACAP resulted in a concentration-dependent increase in CBF_{LDF} with a mean maximum increase of 18±6% at 5μg in 1μl. The double staining technique revealed that PACAP-immunoreactive nerve fibers are a subset of VIP-immunoreactive fibers. These data represent an initial characterisation of the distribution and action of a novel VIP-like vasodilator peptide, PACAP, in the cerebral circulation.

CEREBELLUM I

178.1

INCREASED EXPRESSION OF CORTICOTROPIN RELEASING FACTOR IN INFERIOR OLIVARY NEURONS EVOKED BY OPTOKINETIC STIMULATION (OKS) IN THE RABBIT N.H. Barmack* and P. Errico, RS Dow Neurological Sciences Inst., Good Samaritan Hosp. & Medical Ctr., Portland, OR 97209.

Neurons located in the dorsal cap (DC) of the inferior olive are excited by low velocity optokinetic stimulation (OKS) in the posterior-anterior direction of the contralateral eye. These visual olivocerebellar neurons have been implicated in the modification of eye movements. A neuropeptide, corticotropin releasing factor, CRF, has been found in all olivary neurons. Prolonged, unidirectional, binocular OKS increases the level of CRF mRNA in DC neurons by a factor of 12. Using an antibody to CRF, we have studied the expression of CRF in DC neurons after prolonged OKS. Rabbits were partially restrained within an optokinetic drum that rotated at an angular velocity of 5 deg/sec. An antiserum to rat CRF was used to immunohistochemically label brainstem sections of stimulated rabbits. The density of reaction product within individual olivary neurons in slide-mounted sections was analyzed with a computer-based densitometer. Binocular OKS for 48 hr caused a 20-70% increase in the optical density of "stimulated" DC neurons, measured relative to the optical density of control olivary neurons in the same section. This elevated expression of CRF lasted approximately 48 hr. If there was a delay of 18 hr following binocular OKS prior to sacrifice, during which the rabbits were deprived of pattern vision then both DCs were densely labeled. Monocular OKS for 48 hr in the anterior-posterior direction caused no change in CRF expression in either DC. But, if a "null-stimulated" eye was deprived of pattern vision for 18 hr prior to sacrifice, then the DC contralateral to the "null-stimulated" eye was densely labeled. We infer that stopping prolonged "null-stimulation" causes a rebound in activity in the stimulated DC. This "rebound" must have its origin at the level of the DC or more peripherally in the optokinetic pathway.

178.2

COMPLEX SPIKE ACTIVITY OF PURKINJE CELLS IN THE PIGEON FLOCCULUS: RESPONSES TO ROTATIONAL FLOWFIELDS PRODUCED BY A PLANETARIUM PROJECTOR. D.R. Wylie* and B.J. Frost. Dept. of Psychology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The flocculus receives visual signals from the accessory optic system, which is involved in the analysis of visual flowfields resulting from self-motion and the production of compensatory movements to maintain gaze stabilization. We recorded the complex spike activity of floccular Purkinje cells in anesthetized pigeons in response to optokinetic stimuli produced by a rotating "planetarium projector". This projector consisted of a light inside a cylinder which was pierced with numerous small holes. A pen motor oscillated the cylinder about its long axis. This apparatus was placed above the bird's head and the resultant rotational flowfield was projected onto screens surrounding the bird. The rotation axis of the planetarium could be placed at any orientation in space. Two types of responses were found. **VA or yaw** neurons (n = 43) were maximally excited by rotation of the planetarium about the vertical axis in the direction producing forward and backward motion in the ipsilateral and contralateral eyes, respectively. Rotation about the vertical axis in the opposite direction maximally inhibited **VA** neurons, and rotation about horizontal axes generally resulted in no modulation. **H-135i** neurons (n = 34) were maximally excited by counterclockwise (as viewed from the bird's ipsilateral eye) rotation of the planetarium about a horizontal axis at 135° azimuth on the ipsilateral side (beak = 0° azimuth). Clockwise rotation about this axis maximally inhibited **H-135i** neurons, and rotation about orthogonal axes, (i.e. the vertical axis or a horizontal axis at 45° azimuth on the ipsilateral side), did not produce modulation. These results suggest that the reference frame of the optokinetic signals to the flocculus is similar to that of the vestibular canals. That is, **VA** and **H-135i** neurons would respond best to optic flow resulting from a head rotation maximally stimulating the ipsilateral horizontal canal and the ipsilateral anterior canal, respectively.

178.3

SPATIAL PROPERTIES OF FLOCCULUS NEURONS IN THE DECEREBRATE CAT. K.D. Powell*, K.J. Quinn, L.D. Barke, B.W. Peterson, J.F. Baker. Dept. of Physiology, Northwestern Univ., Chicago, IL 60611.

An intact flocculus is necessary for VOR adaptation. We are studying the spatial properties of neurons in the anterior third of the flocculus. Response properties are more complex than previously expected. Responses of 34 neurons were recorded during 0.5 Hz rotations in 4-11 vertical planes and yaw in decerebrate cats to calculate a plane of maximum activation. Connections were determined by electrical stimulation of bilateral labyrinth and ipsilateral superior vestibular nucleus. Contrary to expectation, only three of the 34 neurons had a preferred plane of activation within 20° of the plane of the ipsilateral anterior canal-contralateral posterior canal pair. All three had type II response to rotation. Three neurons were primarily horizontal canal neurons, two type II and one type I response. One neuron's response plane was within 20° of the ipsilateral posterior canal-contralateral anterior canal pair plane and was type II. The remaining neurons received convergent input from two canals (21 neurons) or 3 canals (5 neurons). Many of the 34 neurons were activated by labyrinth stimulation at 4-6ms, often on top of a N3 field potential suggesting that they were Purkinje cells. In some cases this identification was supported by complex spikes and/or histological reconstruction. Velocity step tests were performed on 9 neurons. Only 4 of 9 neurons had push-pull responses, 2 type I and 2 type II. One neuron was type III and none were type IV. The remaining 4 neurons responded only in one direction, excitatory (3 neurons) or inhibitory (1 neuron) to ipsilateral rotation. The signals in the flocculus, rich in convergence and complicated response patterns, could provide the substrate for VOR direction adaptation and phase shifts. Supported by DC01559, EY06485, EY07342.

178.4

TRANSIENT CEREBELLAR CLIMBING FIBER ACTIVITY DURING MOTOR LEARNING: RELATIONSHIP TO KINEMATICS. C.L. Ojakangas* and T.J. Ebner, Depts. of Neurosurgery, Physiology & Grad. Program in Neuroscience, Univ. of Minnesota, Mpls., MN 55455.

Cerebellar Purkinje cells were recorded during a visually guided two-dimensional arm movement task in which primates used a manipulandum to place a cursor in target boxes displayed on a video screen. When the relationship between the cursor and hand position was altered, the animals adapted their movements to the new gain over 100-200 trials. The peak tangential velocity and movement amplitude gradually changed to match the new gain while the time to peak velocity remained constant. Complex spike (CS) activity increased during the learning process just prior to or after movement onset, the same time period in which the kinematics were scaled. To determine the relationship of the CS occurrence to the kinematics and simple spike (SS) discharge, the movement trials were sorted into complex spike and non-complex spike trials for 20 cells with significant CS responses. Arm kinematics and simple spikes were aligned on the CS occurrence. For the non-CS trials a Monte-Carlo type simulation was done to achieve a comparable alignment of the data. Results indicate that the CS is followed by a change in the tangential velocity. Both increases (30%) and decreases (55%) have been observed. In 75% of the cells changes in velocity preceded the CS activity. These changes in velocity were accompanied by differences in the SS activity in the CS and non-CS trials. These results suggest a role for the CS in the detection and/or correction of small errors in the movement characteristic of learning. Supported by NIH Grant R01-NS18338.

178.5

PURKINJE CELL EYE AND HEAD VELOCITY SENSITIVITY ARE NOT ALTERED DURING VOR ADAPTATION. R. Baker, A. M. Pastor R. R. de la Cruz, and J. I. Simpson*, Dept. Physiology and Biophysics, NYU Med. Ctr., New York, NY 10016.

Electrical stimulation of the goldfish vestibulo-cerebellum produces conjugate ipsilateral horizontal eye movements. Biocytin injection into Purkinje cells, identified by climbing fiber responses to visual motion, demonstrated a characteristic soma-dendritic arborization, recurrent axon collaterals and projections to the vestibular nuclei. To further elucidate the role of the vestibulo-cerebellum in motor execution and/or learning, Purkinje cell simple spike discharges were quantified in terms of eye and head velocity sensitivity during the optokinetic reflex and visual cancellation of the VOR, respectively. Purkinje cells could be classified into three distinguishable, but overlapping, groups. For the first group, discharge frequency was largely correlated with eye velocity, and in the second group, with head velocity. The third group consisted of two sub-types that had nearly equal sensitivity to eye and head velocity, but in one case, at a VOR gain of 1.0, signals cancelled, and in the other, they added. Purkinje cells representative of all three groups were recorded for 3-24 hrs during eye/head velocity modification of VOR gain from -0.6 to 2.5, representing eye velocity changes up to 50 deg/s. At any time during adaptation, the observed simple spike modulation of Purkinje cells varied in strict accordance with VOR performance. However, the eye and head velocity sensitivity characterizing each Purkinje cell remained constant irrespective of the VOR gain state. These findings demonstrate that Purkinje cell activity is correlated with movement execution, even throughout acquisition of the adapted VOR response. The invariance of Purkinje cell sensitivity to head and eye velocity clearly indicates that VOR learning must occur in the brainstem pathways, not in the vestibulo-cerebellum.

178.6

THE ABILITY OF MOTOR CORTEX STIMULATION TO EVOKE VIBRISSAL MOVEMENTS IS MODULATED BY A 10 HZ SIGNAL ARISING IN THE INFERIOR OLIVE. E. J. Lang*, I. Sugihara, and R. Llinás. Dept. of Physiology & Biophysics, New York University Medical Center, 550 First Avenue, New York, N.Y. 10016.

Synchronized and rhythmic activity in the olivocerebellar system has been shown to facilitate activity generated in other brain regions, increasing the probability of movement execution. This suggests that the ability of motor cortex activation to generate movements should depend on its phase relationship to olivary activity. Thus, if the motor cortex is stimulated at a frequency different from the 10 Hz frequency of the IO, beating patterns reflecting the difference between the two frequencies should occur. Trains of 500 µsec pulses were applied to the motor cortex using a bipolar stimulating electrode. The evoked vibrissal movements were recorded using a high speed VCR. The pattern of vibrissal movements depended on the frequency of the stimulation. In control animals 10 Hz trains produced movements whose amplitudes tended to increase monotonically throughout the train, 20 Hz trains produced alternating large and small amplitude movements, and 25 Hz trains produced four small amplitude movements for each large one. These beating patterns can be explained as the interference between the train frequency and a 10 Hz signal.

To demonstrate that the 10 Hz modulation arises from the IO we micro-injected picrotoxin and lidocaine into the IO. Picrotoxin injections synchronize olivary activity and slow the oscillation frequency to 5-6 Hz. Injections of picrotoxin led to changes in the beating patterns as would be predicted from changing the IO's frequency from 10 to 5 Hz. Lidocaine injections abolished the beating patterns. Furthermore, beating patterns were absent when the IO had been destroyed by 3AP. NIH grant NS13742.

178.7

RHYTHMIC OLIVO-CEREBELLAR CONTROL OF SKILLED TONGUE MOVEMENT IN RELATION TO PATTERNED HYPOGLOSSAL NERVE ACTIVITY. J.P. Welsh*, E.J. Lang, I. Sugihara and R. Llinás. Dept. Physiology & Biophysics, NYU Medical Center, 550 First Avenue, NY, NY 10016.

We have combined simultaneous recordings of the activity of the medial and lateral branches of the hypoglossal nerve (XIIn) with multi-electrode recording of Purkinje cell complex spikes (CSs) during conditioned tongue protrusion in the rat. Rats were trained to protrude their tongue 6 mm in response to a tone in order to obtain water reinforcement. The occurrence of the tone reset olivary rhythmicity and subsequently produced rhythmic CS activity in ensembles (25-30) of Purkinje cells whose frequency varied in different rats (n=4) from 6-8 Hz. The frequency of olivo-cerebellar oscillation produced by the tone in each of the rats predicted within 0.3 Hz (96% accuracy) the rhythm of the tongue protrusion. Cross-correlation of the CS activity of cell ensembles with different movement parameters indicated that rhythmic CS firing was more highly correlated to the time that the tongue leaves the target than to either the time of tongue arrival or mouth opening. The medial and lateral branches of the XIIn manifested highly patterned activity consonant with their respective roles in protrusion and retraction. Both branches manifested 2 burst of activity during the movement, the latter of which occurred within 20 ms of the time of maximal protrusion. CSs reliably preceded the second burst in the lateral XIIn by 30 ms. Because the time the tongue remains at the target varies (15-70 ms), the finding that CSs are more tightly time-locked to tongue withdrawal than to tongue arrival indicates that CSs are not driven by the immediate sensory consequences of the tongue contacting the target. Rather, rhythmic olivo-cerebellar activity engaged by the conditioned stimulus prior to movement makes available a central oscillation that is used by the motor system of the trained rat to coordinate patterned and precisely timed motor activity when the tongue reaches the target. (Supp. NS08844 and NS13742).

178.8

THE ROLE OF CAT RED NUCLEUS DURING REACHING KM. Horn*, P.L.E. van Kan and A.R. Gibson. Barrow Neurol. Inst., Phoenix, AZ, 85013.

Reaching out and grasping an object is accompanied by high rates of neural discharge in the intermediate cerebellum and its output target, the magnocellular red nucleus (RNm). In contrast, simpler movements made while operating single joint manipulanda fail to elicit high discharge rates. Here, we report the results of several experiments designed to analyze the role of the RNm in the control of reaching.

Five cats were trained to reach out and retrieve a lever while we recorded neural discharge in RNm. To date, we have recorded 130 forelimb neurons, and essentially all discharged at high rates during various phases of the reach. Patterns of discharge are highly characteristic for individual cells, and several classes are recognizable. The response patterns for two of the cell classes are reciprocal and match the EMG patterns of specific distal forelimb muscles (extensor digitorum communis and palmaris longus). In contrast, no close correspondence between patterns of neural discharge and activation patterns of proximal muscles is apparent.

Reversible inactivation of the RNm with either lidocaine or GABA severely alters reaching behavior. Interestingly, reaching out and grasping is impaired whereas the retrieval of the lever is relatively normal. Often, the cats must make several attempts to grasp the lever, and the grasp itself has a heavy reliance upon the wrist rather than the toes or claws. These results indicate that the output of the intermediate cerebellum through the RNm is especially important for limb coordination required to reach and grasp.

178.9

DIFFERENCES IN THE PROPERTIES OF SIMULTANEOUSLY RECORDED RESPONSES IN THE CEREBELLAR NUCLEI DURING THE ACQUISITION AND PERFORMANCE OF SEQUENTIAL FORELIMB MOVEMENTS IN THE CAT. J.R. Bloedel*, V. Bracha, M.S. Milak, F. Kolb, J.D. McAlduff. Barrow Neurological Institute, Phoenix, AZ 85013.

Experiments were performed using chronic multiple single unit recordings to test the hypothesis that neurons in the various cerebellar nuclei are modulated differently during the acquisition and performance of complex forelimb movements. In this task cats reached for a manipulandum on cue and moved it through one of a variety of templates. Each template required the execution of 2-3 straight movements performed sequentially in varying directions. Recordings were performed simultaneously across the cerebellar nuclei with 24 microwires coupled to a newly developed adjustable electrode system. The kinematics of the ipsilateral forelimb movements were assessed using an active LED system and EMG was recorded from specific forelimb muscles. Data processing techniques were developed to represent graphically the changes in neuronal activity in each nucleus as the tasks were learned and subsequently performed. To date the data show that the activity of dentate neurons is highly related to specific features of the movement sequence. Neurons in the fastigial nucleus discharge primarily in a broad monotonic manner beginning just prior to the lift off of the reaching limb. During learning, the amplitude of the responses in each nucleus was related to specific phases of task acquisition. However, qualitative features of the responses only changed minimally. NIH Grant R01 NS21958.

178.10

PARALLEL FIBER INPUT MODULATES PURKINJE CELL RESPONSES TO ASCENDING FIBER GRANULE CELL INPUT. E. De Schutter* and J.M. Bower.
Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

We have constructed a detailed compartmental model of a Purkinje cell, based upon anatomical and physiological data. We used this model to explore the response to synchronous synaptic inputs, expected to result from activation of synapses from the ascending part of the granule cell axon, during continuous background asynchronous inputs from the parallel fibers. Dendritic Ca^{2+}-channels amplify synaptic inputs so that the response is independent of the dendritic location of synchronous inputs, i.e. the cell will always fire an action potential within 10 ms (De Schutter and Bower, Analysis and Modeling of Neural Systems 1992).

To quantify these effects, we examined the somatic EPSP caused by synchronous synaptic inputs at 6 locations in a model with active dendritic membrane and a passive soma. Averaged peak amplitudes of EPSPs (100 samples) were 2.3 to 4.8 mV, corresponding to an amplification of 1.4 to 4.9 compared to a completely passive model. EPSPs from distal inputs were amplified more than those from proximal inputs, so that attenuation of distal inputs was greatly diminished compared to the passive model. However, the amplitude of single EPSPs was quite variable, with an average standard deviation of 52%. The random, asynchronous excitatory and inhibitory inputs to the model are the only possible source of this variability. The modulation by parallel fibers was independent of the location of the synchronous inputs. For identical patterns of random inputs the size of EPSPs generated from different synchronous input locations was correlated by 78 to 98%.

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178.11

CEREBELLAR ATAXIA WITHOUT CHANGES IN FUSIMOTOR (γ) CONTROL OF PROPRIOCEPTION. M. Gorassini*, A. Prochazka & J.L. Taylor. Div. Neuroscience, University of Alberta, Edmonton, AB, CANADA

The cerebellum has been implicated in the control of muscle spindle sensitivity via the γ fusimotor system[1,2]. This has never been confirmed in intact animals, though abnormal motor cortical responses to proprioceptive input seen in monkeys with cerebellar dysmetria[3] are consistent with aberrant fusimotor control. Disordered proprioceptive feedback could well contribute to dysmetria or hypotonia. To test these ideas, we studied the firing of 8 muscle spindle afferents (7 Ia, 1 II) in 5 intact cats before and during 4-6 min blockade of interpositus or dentate nuclei (1.5-2.5 μL, 2% Lidocaine). Spindle sensitivity was assessed in three situations. Before blockade in the resting cat it was low, indicating little γ action; in gait, moderate Ia and II sensitivity and bias suggested moderate γ_s action; in imposed movements, Ia sensitivity was high, indicating strong γ_d action[4]. During unilateral interpositus blockade, marked ataxia and dysmetria occurred in ipsilateral limbs. Despite aberrant EMG's in parent muscles, spindle sensitivities in the three situations described above were normal, except for reduced γ_d action in imposed movements in one case. The lack of effect of cerebellar dysfunction was unexpected and suggests that the cerebellar interpositus and dentate nuclei are not primarily responsible for fusimotor control. Furthermore, the results show that severe dysmetria and ataxia can occur in the presence of normal proprioceptive feedback.

1) Gilman, S. (1969) Brain, 92:621-638. 2) Vitek, J.L. (1984) Ph.D. thesis, U. of Minnesota. 3) Hore, J. & Flament, D. (1988) J. Neurophysiol. 60:1285-1302. 4) Prochazka, A (1989) Progr. Neurobiol. 33:281-307.

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178.12

A NEW INDEX OF CEREBELLAR DYSMETRIA IN HUMANS. R. G. Jacoby, D. Rotella, W. Darling, M. Rizzo*. Departments of Neurology and Exercise Science, The Univ. of Iowa, Iowa City, IA 52242.

We evaluated aspects of limb trajectory, timing, and accuracy in 3-D space in 6 patients with cerebellar lesions. We used an optoelectronic technique (the WATSMART system) to evaluate their ability to smoothly transport each hand from a midline start position to distal targets located on the right, middle and left. The digitized data were low-pass filtered at 10 Hz. The onset and termination of movements were determined by velocity threshold criteria. As expected, the limb on the side of the cerebellar lesion performed much worse, and exhibited intention tremor primarily in the deceleration phase. The major finding was that the endpoint variability of the initial movement towards the target with the hand ipsilateral to the lesion was 3-25 times greater than for comparable movements with the contralateral limb. Nevertheless, the durations of these movements were similar for each limb. Endpoint variability of the initial movement to targets appears to provide a sensitive quantitative index of cerebellar dysmetria in humans.

STAINING, TRACING AND IMAGING TECHNIQUES I

179.1

MECHANISMS OF DISTRIBUTING SODIUM CHANNELS IN MYELINATED AXONS. Barry W. Hicks* and Kimon J. Angelides. Dept. of Molecular Physiol. and Biophysics, Baylor Coll. of Med., Houston, TX 77030.

In mature, myelinated axons, sodium channels (NaChs) reach a density as high as 5,000/μm^2 at the node of Ranvier with an internodal density of <100/μm^2. How are NaChs accumulated and maintained at the node? It has been reported that NaCh distribution on axons of sensory neurons is altered in the presence of Schwann cells (SCs), and that NaChs may travel by surface lateral diffusion to axonal sites after insertion into the somal plasma membrane of olfactory neurons. To test this hypothesis and explore the mechanisms responsible for SC-dependant NaCh redistribution, we examined the movement of surface NaChs on living dorsal root ganglion neurons in the absence and presence of SCs. Previous FPR studies on cultures of these neurons indicate that only about 20% of the NaChs are mobile. The time scale for most FPR experiments is in the seconds to minutes range. In contrast, production of compact myelin can take 2-3 weeks in culture. To determine if the small mobile fraction detected by FPR is capable of traveling large distances on the axonal surface, NaCh movements were examined in a longer time frame using time-lapse fluorescent video microscopy. Movements of individual NaChs were probed with a monoclonal antibody to the NaCh covalently bound to fluorescent latex microspheres. Bulk NaCh movements were examined by labeling channels with the same antibody and Fab fragments of TRITC conjugated goat anti-mouse antibodies. After bleaching large regions on the axonal surface with an argon laser, fluorescence recovery was filmed. In both cases little movement of NaChs on the axonal surface was observed. Although localized redistribution on the axon may occur, these results suggest that the high nodal density of NaChs does not arise by diffusion of channels from the soma to the distal regions of the axon.

179.2

HEMOSTASIS REGULATION IN GUINEA PIG BRAIN. Mark Fisher*, Milo Lipovac, Aldana Martin, Vicky Wong, Berislav Zlokovic.
Departments of Neurology and Neurological Surgery, USC School of Medicine, Los Angeles, CA 90033 (USA).

Abnormalities of hemostasis are prominently associated with ischemic stroke. However, little is known of hemostasis regulation in brain. We are investigating hemostasis proteins and their regulation in the brain of the young (six weeks of age) guinea pig. As part of an ongoing study, we have used acetone-fixed frozen sections taken from cerebral hemispheres of two animals. Endothelium of blood vessels of all sizes expressed von Willebrand Factor and stained consistently using antibody to tissue plasminogen activator (provided by Nicholas Seeds, U. of Colorado). Brain parenchyma was immunoreactive for tissue factor while this procoagulant protein was infrequently observed in association with blood vessels. Brain sections were not immunoreactive for tumor necrosis factor-alpha, an important regulator of hemostasis in vitro. In summary, these findings appear to represent baseline expression of several important endothelial and hemostasis-related proteins in guinea pig brain. The guinea pig subjected to a variety of stroke risk factors should prove useful for understanding brain-hemostasis interactions and their relation to stroke. (Supported by NIH R01 NS20989 and TRDRP 2RT0071)

179.3

THREE-DIMENSIONAL IMAGING OF LIVING NEURONS AND GLIA WITH THE ATOMIC FORCE MICROSCOPE. V. Parpura*, E. Henderson and P. G. Haydon. Signal Transduction Training Group, Dept. of Zoology and Genetics, Iowa State Univ., Ames, IA 50011.

The atomic force microscope (AFM) collects information from a substrate by scanning a small probe over the surface of interest. Measurements of the deflection of the probe are displayed as a topographic map. The AFM can image samples at extremely high, and in some cases atomic, resolution and has sufficient dynamic range to allow imaging of molecules to a group of cells. In this study we have used the AFM to directly image hippocampal neurons and glia. Using chemically fixed cells it was possible to reconstruct three-dimensional cell structure and detect subcellular features such as the nucleus, mitochondria and filaments. Imaging of neuronal growth cones revealed that their height increases in a step-like manner.

We have found it possible to image living cells with the AFM. By repeatedly scanning a single living cell we observed the movement of filaments beneath the cell membrane. Additionally, by controlling the force applied to the scanning tip, nanosurgery was performed to selectively sever neurites or to remove cells from the culture substrate. Neurons and glia were differentially sensitive to the tip-applied forces, neurons being removed with 5-36 nN (n=5) and glia with a force of 60-84 nN (n=5). Thus, the atomic force microscope offers the opportunity to gain three-dimensional information about living cells, to observe the behavior of cellular components by imaging through the intact cell membrane and to perform nanosurgery.

179.4

AN MRI-BASED STEREOTACTIC ATLAS FROM 250 YOUNG NORMAL SUBJECTS. A.C. Evans, D.L. Collins and B. Milner.* Montreal Neurological Institute, 3801 University St., Montréal H3A 2B4, Québec.

Blood flow (CBF) subtraction studies of cognitive activation with PET have increased rapidly in recent years, usually using young healthy volunteers. 3-D image data are mapped into a standardized anatomical coordinate space (Talairach and Tournoux, 1988). The Talairach atlas consists of single sections at 4mm intervals obtained post-mortem from a single brain, that of a right-handed 60-yr old European female. We have constructed an average 3-D brain volume from MRI data (64 2mm-thick planes: T_1 sequence) acquired from 250 young healthy volunteers (age 23.4 ± 4.0 ; 179 males ; 71 females). All data were mapped into Talairach space by identification of the bicommissural plane and resampled on to a 3-D grid with spacing of 1.34mm x 1.72mm x 1.50mm in X,Y and Z. Following intensity normalization, the average young normal brain was obtained by voxel summing. Separate maps were also produced for males and females. All major features were clearly defined and the atlas exhibits remarkable detail in the central regions where the dorsomedial nucleus of the the thalamus was visible. The mean position of the primary sulci, e.g. central, cingulate, occipitoparietal, calcarine, were well-defined while the more variable secondary cortical anatomy was, as expected, less apparent. Known left-right size differences in frontal and occipital lobes (petalia) were confirmed. This database is a first step in the construction of a 3-D probability map of gross neuroanatomy across a large population.

179.5

RADIOIODINATED 5-IODO-6-NITROQUIPAZINE AS AN IN VIVO LIGAND FOR BRAIN SEROTONIN TERMINALS. A. Biegon, C. A. Mathis, S. Taylor and W. Jagust*, Lawrence Berkeley Laboratory, Univ. Calif. Berkeley, CA 94720.

The feasibility of using radioiodinated 5-iodo-6-nitroquipazine (INQUIP) as an in vivo imaging agent for brain serotonin terminals and uptake sites was examined by ex vivo autoradiography in the rat. Following the tail vein injection of 25μCi [^{125}I]INQUIP (specific activity 2175Ci/mmole, ~100μCi/kg), animals were decapitated and their brains removed, frozen and sectioned in 20μ sections, which were apposed to tritium sensitive film for 7-21 days with commercial ^{125}I standards. Autoradiograms were quantified using a computerized image analysis system. Initial brain uptake was high (1.5 ± 0.2% injected dose/g brain at 5 min, n =3) and the proportion of specific binding increased with time up to 12 hours postinjection.Thus, the frontal cortex-to-cerebellum radioactivity concentration ratios increased from 1.2 ± 0.1 at 5 min to 11 ± 1 at 12 hrs (n = 3). The distribution of radioactivity by 4-6 h following injection was similar to the distribution of serotonin uptake sites and terminals derived from in vitro autoradiographic and immunohistochemical studies. Coinjection of paroxetine (2mg/kg), a selective 5HT uptake blocker, resulted in a ~95% decrease in brain radioactivity. INQUIP brain uptake was also substantially blocked by coinjection of fluoxetine and sertraline (2mg/kg) but not by LSD (0.4mg/kg) nomifensine, GBR12909 or DMI (2mg/kg). A lesion of serotonin terminals (2X10mg/kg PCA i.p.) resulted in a ~90% decrease in brain radioactivity. These results support the use of suitably labeled INQUIP for in vivo SPECT imaging of serotonergic terminals in the human brain.

CELL LINEAGE AND DETERMINATION II

180.1

IDENTIFICATION OF A NOVEL NEURON SPECIFIC NUCLEAR PROTEIN BY GENE TRAPPING IN ES CELLS AND CHIMERA TRANSGENESIS. W. Jing, and A. Peterson*. Laboratory of Developmental Biology, Royal Victoria Hospital, H5, McGill University, 687 Pine W., Montreal, Quebec, Canada H3A 1A1

We have employed a gene trapping approach using mouse embryonic stem (ES) cells to identify genes that, on the basis of their temporal and spatial expression patterns, may play important roles in nervous system development. The gene trap construct contains a promoterless LacZ gene ligated at its 5' end to a splice acceptor site derived from the mouse En-2 gene. This construct also contains, as a positive selectable marker, a neo^r gene driven by the β-actin promoter. Integration of this construct into an intron of a gene expressed in ES cells in the correct orientation and frame results in a fusion transcript encoding a protein with β−galactosidase activity. Four such β-galactosidase positive clones have been obtained (1/450 neo^r colonies) and one, when injected into mouse blastocysts, produces chimeras in which the trapped gene is expressed throughout the nervous system and in no other tissue. All neuronal populations in both the central and peripheral nervous system contain positive cells, indicating that the trapped gene is expressed pan-neuronally. Moreover, the protein encoded by this gene contains a nuclear localization signal suggesting the possibility that it plays a role in the function or organization of neuronal nuclei. Molecular characterization of this gene is in progress and is expected to reveal further clues to its normal function and ultimately, to the regulatory elements that confer its pan-neuronal expression profile.

180.2

EXPRESSION OF A NEWLY IDENTIFIED PROTEIN TYROSINE KINASE IN THE DEVELOPING MOUSE RETINA

Xianjie Yang and Constance Cepko*, Department of Genetics, Harvard Medical School, Boston, MA 02115

Protein tyrosine kinases play critical roles in transducing environmental signals to cells. We have cloned a putative protein tyrosine kinase from a neonatal mouse cDNA library. The 4.2 Kb cDNA clone encodes the mouse homolog of the human JAK-1 kinase, which represents a new class of non-receptor protein tyrosine kinases. The main feature of this class of kinases is the presence of an additional phosphotransferase-like domain immediately N-terminal to the highly conserved tyrosine kinase catalytic domain. Analyses of mRNA using Northern blots indicate that the RNA level of mouse JAK-1 increases between embryonic stages and neonatal stages in the eye and brain. In situ hybridization studies analyzing the mRNA expression pattern using different regions of the JAK-1 cDNA are correlated with the immunohistochemical staining patterns at the cellular level using a polyclonal antibody generated against a mouse JAK-1 fusion protein. As early as embryonic day 14, JAK-1 can be detected in axons of mouse retinal ganglion cells. In the adult mouse retina, JAK-1 is expressed in the retinal ganglion cells, amacine cells and horizontal cells. This pattern of expression is also observed in the chick retina. In the adult brain, JAK-1 is expressed most abundantly in the olfactory bulb, which correlates with a high level of expression in an olfactory cell line (OBL21). Thus, JAK-1, a newly identified protein kinase with two catalytic domains, is expressed in the mouse central nervous system, and may play a role in the differentiation of a subset of neurons.

180.3

F5 PROTEIN, A NOVEL MARKER OF ACTIVATED T CELLS, IS LOCALIZED IN DENDRITES AND PERIKARYA OF MATURE NEURONS

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F5, a cDNA clone with novel sequence, was initially isolated from IL-2 stimulated helper T cell line. Using Northern blot and *in situ* hybridization, we demonstrated that F5 mRNA is expressed exclusively in lymphoid tissues and in mature neurons within the nervous system. An open reading frame of 1125 bp encodes a 42 kDa protein with multiple potential phosphorylation sites. The objective of this study was to elucidate the biochemical properties of the F5 protein.

Antiserum was raised against C-terminal 18 amino acid peptide of the deduced F5 protein. This antibody detected a single band with apparent molecular weight of 42 kDa on Western blot of mouse brain extract. It specifically bound to an *in vitro* translation product with 42 kDa. Subcellular fractionation of adult mouse brain demonstrated that F5 immunoreactivity was present in membrane fractions, especially in synaptosome/ mitochondria fraction, but almost absent in cytosolic fraction. Immunoperoxidase staining showed that within the neurons, perikarya and dendrites were stained but axons were not. C-terminal peptide, which contains a consensus sequence for a protein kinase C (PK-C) phosphorylation site, served as a substrate for an PK-C assay.

F5 is a novel class of specific marker for mature neurons. The C-terminus of the F5 protein can be phosphorylated by PK-C. The biochemical properties and intracellular localization of the F5 protein suggests that it functions in receptor anchoring or signal transduction.

180.4

CHARACTERIZATION OF *NOTCH* HOMOLOGUE cDNAS FROM GOLDFISH RETINA. S. A. Sullivan, B.L. Largent*, & P.A. Raymond. Dept. of Anatomy & Cell Biology, University of Michigan, Ann Arbor, MI 48109.

Mutations at the *Notch* locus affect the developmental fate of cells in the neurogenic region of the *Drosophila* embryo, and in the developing retina of the adult fruit fly. Several vertebrate *Notch* homologues have been identified and shown to be expressed in developing nervous systems. In *Xenopus* tadpole retinas, *Xotch* mRNA localizes to a zone of cell proliferation and differentiation. The goldfish retina differs from that of amphibians in that it grows throughout adult life, and retains the capacity to regenerate after injury. New cells arise from two sources: all retinal cell types except rod photoreceptors arise from the germinal zone (which corresponds to the proliferation zone of *Xenopus* tadpoles); rods are produced at scattered points throughout the retina from proliferating rod precursor (RP) cells. During normal retinal growth RP cells produce rods exclusively, but in regenerating retinas RP cells can assume different fates. We are interested in the factors influencing the developmental fates of germinal zone cells and of RP cells. Using low-stringency library screening with *Xotch* probes concomitantly with the PCR, we have cloned two *Gotch* cDNAs. Clone 8X1 contains 150bp that encode part of the EGF-like repeat region [amino acid identity = *Notch* 69%; *Xotch* 90%; Rat 85%; Human 87%]. Clone 10X1 contains 1100bp encoding the entire cdc10/ankyrin-like repeat region [amino acid identity = *Notch* 58%; *Xotch* 65%; Rat 69%; Human 67%]. Preliminary digoxygenin-labelled RNA *in situ* studies reveal significant levels of *Gotch* expression in the adult goldfish retina.

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180.5

Neuroepithelial stem cells: identification and differentiation in vitro and in vivo.

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The central nervous system in vertebrates is derived from a sheet of epithelial precursor cells. Fate mapping and transplant experiments show that the immediate precursors to neurons are multipotential cells that commit to a specific fate occurs in the hours immediately following the last S phase of the stem cells. These data lead to a model where the many cell types in the brain are a consequence of the response of multipotential stem cells to local signals in the embryonic neuroepithelium. The specific expression of the nestin promoter allows these cells to be identified and manipulated in transgenic mice. Neurotrophins interact with stem cells in both hippocampus and cerebellum. Transplants of primary and immortal stem cells suggest that the extracellular signals drive these cells to appropriate neuronal fates. These results suggest that stem cells can be manipulated in powerful new ways.

180.6

IMMORTALIZED, TEMPERATURE-SENSITIVE, DIENCEPHALIC CELLS EXPRESS MAP2 AT A NON-PERMISSIVE TEMPERATURE, BUT NOT AT THE PERMISSIVE TEMPERATURE FOR TRANSFORMATION.

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Cells from the diencephalon of the E13 rat fetus were grown in culture and tranfected with SV40 large T antigen temperature-sensitive mutant, carried by a retrovirus vector (sai2 producer cell line: a kind gift from Dr. C. Cepko). The cell were selected with Geneticin, and cultured for 4 weeks. Those cells expressing MAP2 were identified by immunocytochemistory at a non-permissive temperature(33-35°C), but not at the permissive temperature. The cloned cells differentiated at the non-permissive temperature, and divided at the permissive temperature. The cloned cell line stained negative for neurofilament (68kD), GFAP, vimentin and TH and positive for c-fos, ß-tubulin, tau, MAP2 and SV40LT. This cloned, temperature-sensitive cell line could become a useful molecular biological model for studies of neuronal differentiation. It might also prove to be of value as a source of donor cells for neuronal transplantation in models of neurodegenerative diseases.

180.7

ESTABLISHMENT AND CHARACTERIZATION OF A NEURONAL CELL LINE OBTAINED BY c-myc IMMORTALIZATION OF MOUSE MESENCEPHALIC CELLS.

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A cell line was obtained from E11 mouse mesencephalon in primary cell cultures (di Porzio et al. Nature 288, 370, 1980) by infection with replication defective retrovirus produced in ψ2 fibroblasts containing mouse c-myc and neomycin resistance genes under MoLTR (Blondel et al. Oncogene 5:857-65, 1989). Selection of transformed cell was carried in medium containing 400 µg/ml G-418. Five clones were isolated; their growth rate was initially rather slow. Subsequently, a few clones with fast duplication time in 10% FBS were isolated (**mes-c-myc-fast-A** and **-B**). Without FBS these cells practically ceased to divide and about 50-60% acquired a neuron-like morphology whereas the remaining population showed less distinct morphology. Both cell types were vimentin-positive in the presence or absence of serum, but only the neuron-like population grown without serum was stained with anti-neurofilamnet (NF) antibodies. NF expression was confirmed by PCR amplification using specific primers for the gene encoding for the 200 kDa NF subunit.

Phenotypic expression under different gowth conditions will be shown. This cell line can provide a useful tool to study CNS neuron differentiation, and neuron replacement in aging brain and CNS degenerative diseases.

180.8

MICROGLIA PROGENITOR CELLS IN ASTROGLIA CULTURES.

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When mouse astroglia cultures of newborn mice were nutritionally deprived for up to 10 days, macrophage-like cells identified as microglia developed (Hao et al., Int. J. Dev. Neurosci. 9:1-14, 1991). Such cultures contained microglia progenitor cells which did not express Mac-1 or F4/80 and could not be distinguished from astroglia. It seems that microglia progenitor cells do not proliferate, but give rise to microglia by transformation. Once the microglia are formed then in the presence of CSF-1 they proliferate extensively and assume a specific phenotype.

Using limiting dilution analysis, it was found that in the presence of astroglia-spent medium, 2% of the cells formed microglia; in the presence of CSF-1, 3.8%; and 12.5% in the presence of CSF-1 and a STO cell feeder layer. CSF-1 and a feeder layer or still unknown factor(s) produced by mesodermal cells, appear to be required for the formation of microglia. Analysis of microglia formation in osteopetrotic mice (*op/op*) which lack CSF-1, also indicated that a factor in addition to CSF-1 is required for microglia formation.

Thus, in cultures initiated from newborn animals at least 12.5% of the cells are microglia progenitor cells which transform into macrophage-like cells in the presence of trophic factors and proliferate when CSF-1 is present.

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180.9

PHENOTYPIC CHARACTERIZATION OF C6 GLIAL CELLS.

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The C6 glial cell line has been used as a model system for the study of glial derived neurotrophic factors and the regulation of glial cells by extracellular substrates and growth factors. This cell line has been reported to express a glioblastic phenotype during early passages which can be influenced to express either astrocytic or oligodendrocyte phenotype. Later passages appear to be more committed to the astrocytic phenotype. The C6 cell line, however, has not been characterized by its antigenic phenotypic expression. In this study, the characterization of the expression of glial fibrillary acidic protein (GFAP), A2B5, galactocerebroside (GalC), fibronectin and laminin was determined from C6 cells grown under serum and serum-free conditions.

C6 cells were grown in DMEM with 10% fetal bovine serum or in Opti-MEM-I supplemented with 27 nM Sodium selenite, 20 nM progesterone and 10 µM hydrocortisone (passage 41-43). The serum-free cultured cells were grown on 8 ug/cm^2 rat tail type I collagen. After fixation (with or without permeabilization) the cells were exposed to a specific antibody and detected by use of either immunohistochemical or immunofluorescent techniques.

Under both culture conditions the C6 cells were GFAP, A2B5, GalC, laminin and fibronectin positive. However, the GFAP expression of the C6 cells exhibited a population of darkly staining and lightly staining cells. C6 cells cultured under these conditions expressed a mixed oligodendrocyte-type 2 astrocyte phenotype. A cell population which expresses this phenotype (O4/GalC/GFAP) has been reported to occur, although infrequently, in primary human temporal lobe white matter cultures (Armstrong et.al. J. Neurosci. 12:1538-1547,1992). *This project was supported by BRSG S07 RR05408-30 awarded by the Biomedical Research Support Grant Program, National Center for Research Resources, National Institutes of Health.*

180.10

NEUROTROPHIN-3 IS BOTH MITOGENIC FOR NEURAL CREST (NC) CELLS AND STIMULATES SURVIVAL AND/OR NEURONAL DIFFERENTIATION OF POSTMITOTIC NC PROGENITORS.

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Neurotrophin-3 (NT-3) is mitogenic for cultured avian NC cells (Kalcheim et al, PNA'S 89, 1661-65, 1992). We now report that this factor also promotes the surivial and/or differentiation of a subset of postmitotic NC precursors into neurons expressing the HNK-1, A2B5 and neurofilament epitopes. NC cells derived from 48 hr clusters were grown on top of mesodermal cells. As early as one day after coculture, a 2-fold increase in neuronal number over controls was measured upon treatment with 1 ng/ml NT-3. No further stimulation was obtained when higher factor concentrations were used. Localization experiments using specific anti NT-3 antibodies which do not cross react with either NGF or BDNF, revealed that NT-3 immunoreactivity is expressed by neuroepithelial cells of quail neural tubes both in vitro and in sections of E3 and E4 embryos. Staining was also evident on the fibers of developing central neurons, i.e; in the early marginal zone and ventral roots. We propose that NT-3, a factor expressed in the early avian CNS, has multiple effects both on the proliferation and differentiation of distinct NC cells, which depend on the state of commitment of the responsive progenitors.

180.11

NON-NEURONAL CELLS INHIBIT CATECHOLAMINERGIC DIFFERENTIATION OF PRIMARY SENSORY NEURONS. G. Fan* and D. M. Katz. Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

Sensory neurons exhibit marked phenotypic heterogeneity, including expression of diverse transmitter traits. To elucidate underlying developmental mechanisms, we are studying expression of the catecholamine synthesizing enzyme tyrosine hydroxylase (TH) in sensory ganglion cells of the rat. Although some mature sensory neurons are catecholaminergic (CA), most ganglion cells only transiently express TH during early embryogenesis (E10.5-15.5; Katz & Erb, 1990; Jonakait, et al., 1984). The lack of CA expression at later stages appears to be due to modulation of this phenotype by factors related to cell aggregation (Katz, J. Neurosci. 11: 3991, 1991). For example, 20-40% of E16.5 trigeminal (TG) and jugular ganglion neurons express TH in dissociate cell culture even though TH is not detectable *in vivo* at this age. Moreover TH levels are inversely related to cell density; a four-fold increase in density leads to a 30-50% drop in the percentage of TH cells. This density-dependent effect appears to be mediated by ganglionic non-neuronal cells (NNC), because 1) plating E16.5 TG neurons at low density on top of NNC monolayers mimicked the effect of high cell density, 2) treatment with NNC conditioned-medium (NNCM) reproduced the effect of coculture with NNC and 3) plating neurons at high density in the absence of NNC had no effect on TH levels. The inhibitory effect of NNCM was mimicked by Ciliary Neurotrophic Factor (CNTF, 10 ng/ml) and Leukemia Inhibitory Factor (LIF, 5-20 ng/ml), two peptides known to inhibit TH expression in sympathetic neurons, suggesting that these or related molecules, may mediate NNC effects on the sensory CA phenotype. Ganglionic NNC may therefore play an important role in shaping sensory transmitter diversity by modulating expression of specific molecular phenotypic traits. Supported by HL-42131 (DMK) and the Dysautonomia Foundation.

180.12

THE EVALUATION AND PURITY OF MYOBLAST CULTURES FROM CHILDREN vs.ADULTS BY IMMUNOSTAIN AND FLOW CYTOMETRY. Q. Fang*, M. Dockter, P.K. Law.Cell Therapy Research Foundation, Memphis, TN 38117 and Dept. of Medicine, University of Tennessee, Memphis, TN 38163.

Myoblast Transfer is an experimental therapy being developed for hereditary myopathies. Its success relies on the purity and vigor of the donor myoblasts. The selection of donor thus becomes an important factor.

Muscle biopsies obtained from ten donors, aged between eleven to forty, were processed for myoblast culture. Purity of the ten cultures were assessed by immunostain with Leu 19, a monoclonal antibody that reacts with human myoblasts and regenerating muscle fibers, using the FACS flow cytometry.

Donor	Age(yr)	Myoblast Purity(%)
1	40	81.23
2	12	93.56
3	15	93.74
4	11	96.46
5	31	75.12
6	13	97.83
7	18	92.20
8	36	86.68
9	12	95.86
10	35	89.22

Myoblast purity was between 75.12% and 89.22% from father donors. It was between 92.20% and 97.83% from brother donors. The result indicates that purer myoblast cultures can be obtained from younger donors. (Supported by PHS NS 26185 to PKL)

180.13

NEUROFILAMENT EXPRESSION AND PRIMARY NEURONS IN THE EMBRYONIC ZEBRAFISH. R.C. Marcus*, L. Stec and S.S. Easter, Jr. Dept. of Biology and Neuroscience Program, University of Michigan, Ann Arbor, MI 48109.

Neurofilaments (NF) are neuron-specific intermediate filaments useful as markers of neuronal differentiation. We examined NF expression in the embryonic zebrafish using an antibody generated against rat NF (RMO44, kindly provided by V. Lee). Labeled embryos at 24, 36, 48 and 72h post fertilization, were viewed as wholemounts and in sections.

At 24h many identified neurons were labeled in the spinal cord; Rohon-Beard cells, CaPs, some RoPs and MiPs, and CoPAs. Two bilateral pairs of neurons were labeled in the hindbrain, Mauthner cells and a pair further rostral in rhombomere two. Neurons in the trigeminal ganglion were also labeled. By 36h labeled reticulospinal neurons were located in seven clusters in the hindbrain. In the midbrain, 2-3 cells in the nucleus of the MLF labeled per side. At 48 and 72h there were more labeled neurons in the trigeminal ganglion, hindbrain clusters and in the nucleus of the MLF, but except for one or two axons traced rostral of the nucleus of the MLF at 48h, no other label was found in the mid and forebrains.

Labeled cells correspond closely, but not exclusively, to "primary neurons", a developmentally distinct population of cells. Cells in the mid and forebrains are generated and extend axons at times similar to labeled neurons in the hindbrain and spinal cord, indicating that RMO44-labeling does not depend on neuronal birthdate or axonal outgrowth. The restricted labeling pattern of the antibody makes it a useful marker for the study of identified neurons and may provide a useful addition to the study of primary neurons.

Supported by NIH grants EY00168 and EY07022, and NSF 027832.

180.14

MULTIPLE MECHANISMS FOR THE INDUCTION OF ECTOPIC SENSILLA IN THE WING OF *DROSOPHILA*. S.S.Blair* and E.Rulifson. Dept. Zoology, U. Wisconsin, Madison, WI 53706.

The positioning of sensilla precursors in the developing wing blade of *Drosophila* is thought to arise through a multi-step process: positional information roughly defines patches of proneural cells; these cells express proneural genes (*scute* and *achaete*) rendering them competent become sensilla; refinement defines single or multiple sensilla within these patches; finally, the sensillar and non-sensillar identity of the cells is maintained. Mutations can thus result in the formation of ectopic sensilla by interfering with different steps in this process. We examined the timing and localization of several mutations in order to understand their effects in terms of this model.

We have found that *hairy* and *Hairy wing* act late in development, interfering throughout the wing with maintenance after normal sensillar specification has taken place. *Notch*, on the other hand, induces ectopic sensillar cells only within regions corresponding approximately to regions of proneural gene expression; *Notch*^{ts} experiments suggest that *Notch* is required for maintenance as well as refinement. Finally, *shaggy* induces the formation of novel proneural regions, apparently interfering with expression or interpretation of positional information; the ectopic expression of the margin-specific gene *vestigial* in *shaggy* clones is consistent with a novel partial transformation towards a marginal identity

CELL SHAPE AND DIFFERENTIATION II

181.1

CROSS-LINKING OF MEMBRANE-ASSOCIATED GM1 BY B SUBUNIT OF CHOLERA TOXIN MODULATES $[Ca^{2+}]i$ AND DIFFERENTIATION IN SENSORY NEURONS. D. Milani*, M.-C. Minozzi, L. Petrelli, D. Guidolin, S.D. Skaper and P.E. Spoerri. Fidia Research Laboratories, Abano Terme, Italy.

The B subunit of cholera toxin, which binds specifically to GM1 ganglioside on cell surfaces, has been shown to modulate intracellular free calcium concentration, $[Ca^{2+}]i$, and cellular growth. To explore a role of such changes in $[Ca^{2+}]i$ in the growth regulatory function of cell-associated GM1 in neurons, chicken embryonic day 8 dorsal root ganglion neurons were exposed to B subunit. Incubation of naive cultures with 1 ug/ml of B subunit produced modest increases in $[Ca^{2+}]i$ in 37% of cells, as measured by fura-2 imaging. GM1 pretreatment of cells for 48 h increased even further the elevations in $[Ca^{2+}]i$ and the percentage of responding neurons observed after B subunit exposure. Such changes in $[Ca^{2+}]i$ were accompanied by fine alterations in morphology, mostly affecting the branching of neurites; these effects were more pronounced in GM1-pretreated neurons. Immunogold electron microscopy using anti-B subunit depicted extensive aggregations of immunoreactive gold particles on neuronal surfaces, especially in cells treated with GM1. These results suggest that modification of cell membrane ganglioside distribution together with modulation of Ca^{2+} homeostasis can influence differentiation of neuronal cells.

181.2

ASSEMBLY OF THE 66-kDa NEUROFILAMENT PROTEIN (NF-66) in vitro AND ITS ASSOCIATION WITH NF-L, NF-M and NF-H PURIFIED FROM BOVINE SPINAL CORD. B.J. Balin* and M.E. Miller. Med. Coll. of Penn., Dept. Path. & Lab Med, Div. Neuropath., 3200 Henry Avenue, Phila., PA 19129.

NF-66, also known as alpha-internexin, has been characterized as a 66-kDa mammalian neurofilament (NF) protein whose expression in developing rat brain precedes that of the low molecular weight NF protein (NF-L). NF-66 is thought to assemble into 10nm-diameter intermediate filaments *in vitro*, although the nature of the assembly process remains unclear. Likewise, the ability of NF-66 to associate and/or polymerize with the low (NF-L), middle (NF-M) and high (NF-H) M, NF proteins has not been delineated. Therefore, this study was initiated to determine the reassembly properties of NF-66 regarding its formation into 10nm filaments and its polymerization capabilities with other well-characterized NFs. NF-66 was isolated from bovine spinal cord using established biochemical extraction and isolation procedures (Balin et al., Brain Research (1991) **556**:181-195), and purified utilizing HPLC DEAE anion exchange and hydroxyapatite column chromatography. Initial experiments of the reassembly of NF-66 (0.8mg/ml) against 0.1M MES buffer, pH 6.8 at room temperature for 2hr revealed that ~10 nm-diameter filaments of varying length were formed. Immunoelectron microscopy revealed labeling of these filaments by a monoclonal antibody to intermediate filament antigen (IFA). Furthermore, our preliminary studies of the association of NF-66 with GFAP, a type III intermediate filament, indicate that NF-66, indeed, may co-assemble with other 10nm-diameter intermediate filaments. Co-assembly experiments utilizing monoclonal antibodies to NF-66 and NF-L, NF-M and NF-H are in progress to determine the exact nature of NF-66 assembly and/or association with other NFs. These studies suggest that NF-66 can self-assemble *in vitro* under defined conditions and that the interactions of NF-66 with other NFs may prove important to the development of the axonal NF network. Supported by PHS grant AG10160.

181.3

KINESIN IS REQUIRED FOR NEURONAL MORPHOGENESIS J.R.Mancillas* and G. DeFeo. Dept. of Anatomy and Cell Biology, UCLA School of Medicine, BRI & Molecular Biology Inst. UCLA, Los Angeles, CA 90024.

Differentiated neurons display a unique, complex cytoarchitecture and a high degree of regional specialization. Converting differentially expressed genetic information into a distinct neuronal morphology requires the well regulated spatial distribution of macromolecules and cellular organelles. To evaluate the role of the motor protein kinesin in the translocation of components required for neuronal differentiation, we analyzed the morphology of neurons in specimens of C. elegans containing mutations in the gene encoding the kinesin heavy chain. We observed abnormalities in axonal morphology (frequent prominent varicosities and variations in caliber), neurite length, neuritic branching patterns, microtubule distribution, and axonal trajectories. E.M. analysis reveals cytoskeletal abnormalities associated with some of the defects observed, suggesting inappropriate assembly of the cytoskeletal scaffold during neurite extension and branching. Our findings suggest previously unreported roles for kinesin in the development or maintenance of neuritic structure and in the formation of the stereotypical patterns of axonal projections. Since the base of the growth cone is the primary site of neurite assembly, kinesin may be involved in the establishment of the precise spatial arrangement of cytoskeletal proteins during neuronal morphogenesis by translocating cellular constituents essential for normal growth cone dynamics or by a direct participation in the interactions between cytoskeletal elements when the neuritic scaffold is being erected.

181.4

DELETION OF KINESIN HC TAIL DOMAIN SELECTIVELY DISRUPTS ITS ROLE IN NEURONAL MORPHOGENESIS N. Patel* G. DeFeo and J. R. Mancillas. Molecular Biology Inst. UCLA, and Dept. of Anatomy and Cell Biology, UCLA School of Medicine, Los Angeles, CA 90024

Kinesin heavy chain (KHC) consists of 3 domains: a motor domain containing the microtubule-binding and ATPase sites, a stalk implicated in dimerization and a tail domain believed to be the site of interactions with transported organelles and macromolecules. We have cloned and sequenced the C. elegans homologue of KHC, unc-116, and analyzed the phenotype of 2 mutant alleles. Kinesin defective mutants display an 80% penetrant embryonic lethality, gross morphological abnormalities, severe impairments in various behaviors and a discrete set of abnormalities in neuronal cytoarchitecture. A weak mutant allele, e2281, displays only mild abnormalities in locomotion and defects in neuronal morphology. We have identified the e2281 mutation as a transposon (TC5) insertion into the coding region of unc-116. The site of insertion was localized between RodII and the tail domain. Sequence analysis suggests that the mutant gene encodes a truncated protein product missing the entire tail domain, which is replaced by 19 amino acids encoded in the transposon. The restricted phenotype of e2281 suggests that absence of the tail domain causes the defects in neuronal morphogenesis in all alleles. They may be due to the disruption of kinesin-mediated interactions between cytoskeletal elements in the growth cone that require the tail domain. Translocation of cellular components involved in functions not disrupted in e2281 may be mediated by the Rod II region of the stalk domain.

181.5

DEVELOPMENTAL TIME COURSE OF THE EXPRESSION OF A NEURON-SPECIFIC β-TUBULIN IN FROG. V.L. Miller*, A. Frankfurter and S.A. Moody. Depts. Neuroscience, Biology and Anatomy & Cell Biology, Univ. Virginia, Charlottesville, VA 22908.

β-Tubulins are encoded by several genes whose proteins are distinguished by unique sequences at the carboxy terminus. Class III β-tubulin is neuron-specific in mammals and birds, but antibodies that specifically recognize this isotype do not stain frog or fish brain. A gene that encodes a neuron-specific β-tubulin in frog recently has been sequenced (Good et al. 1989 Nucleic Acids Res. 17:8000), and is homologous to the mammalian Class II β-tubulin:

human: Y Q Q Y Q D A T A D E Q G E F E E E G E E D E A

Xenopus: Y Q Q Y Q D A T A D E Q G E F E E E E * * D E A

Developmental Northern analysis shows that the mRNA for this gene is first detectable at Xenopus stage 17, during neural tube closure (Richter et al. 1988, PNAS 85:8086). Using an antibody raised against a peptide sequence (underlined above) in the C-terminus of mammalian Class II β-tubulin (Banerjee et al.,1988,J.Biol.Chem.263:3029) we stained a developmental series of Xenopus embryos with standard PAP-immunohistochemical procedures. Protein staining is not detected until st 21 (closed neural tube), where it appears in the longitudinal and commissural tracts of the hindbrain and spinal cord, and in the trigeminal ganglion cells and their processes. The only other cell bodies detected were those of Rohon-Beard and extramedullary cells. Staining in the axonal tracts was coordinated with their known time course of development. Retinal ganglion cells and axons stained by st 38. The pattern of staining in the tadpole (st 41-48) was in peripheral neuron cell bodies and axons, central neuron axons only, and a few scattered cells in the gut. This antibody is an excellent marker of pioneer axons in the frog. Our results suggest that in embryonic frog Class II, rather than Class III, β-tubulin is the neuron-specific gene product. Supported by HD07192, NS23158 and NS21142.

181.6

PROCESS FORMATION BY MAP2-TRANSFECTED NON-NEURONAL CELLS TREATED WITH CYTOCHALASIN. B. Weisshaar, K. Edson and A. Matus*. Friedrich Mieschcr Institute, 4002 Basel, Switzerland.

The neuron-specific microtubule-associated protein MAP2 and the embryonic version MAP2c are believed to influence neuronal morphogenesis by promoting the formation and stabilization of microtubules in neuronal processes. To investigate the underlying molecular mechanism, rat cDNAs for both high-molecular weight MAP2 and low-molecular weight MAP2c were expressed in non-neuronal cells by transfection. In the human hepatoma cell-line PLC, which does not express endogenous MAP2, the exogenous MAP2 protein binds to microtubules and leads to their bundling in a concentration dependent manner. If MAP2-transfected cells were then treated with the actin-depolymerizing drug cytochalasin B, processes formed on these cells. These processes contained bundled microtubules, and time-lapse video microscopy suggested that the processes push out from the cell-body when the cortical actin cytoskeleton is depolymerized. Process formation was completely reversible when cytochalasin was washed out from the culture medium. This suggests that MAP2 does not only promote the formation and stabilisation of microtubule bundles, but also that these bundles have an inherent ability to lead to process formation when they are not inhibited by the cortical actin cytoskeleton.

181.7

MAP 2-IMMUNOREACTIVITY AND CYTOCHROME OXIDASE-ACTIVITY IN THE RETICULAR NUCLEUS OF THE HUMAN FETAL THALAMUS. N. Ulfig (SPON: European Neuroscience Association) Dept.Anatomy, J.W.Goethe-University, D-6000 Frankfurt/M., F.R.G.

Microtubule-associated proteins (MAP) play an important role in assembly and stabilization of microtubules. Three subforms are distinguished within the MAP group 2: high-molecular-weight MAP 2a and 2b are only found in mature neurons, low-molecular-weight MAP 2c is found in immature neurons. MAP 2 is found in soma and dendrites. Dendritic development is controlled by afferent input. Functional synaptic input can be demonstrated indirectly with the aid of cytochrome oxidase (CO)-activity (Wong-Riley, TINS 12: 94-101, 1989). Frozen sections of human fetal thalami (age: 15th-23rd week) are incubated with anti-MAP 2 (marks all MAP 2-subforms) and anti-MAP 2ab. In unfixed cryostat sections CO-activity is demonstrated histochemically. In the 16th week the distribution of MAP 2- and MAP 2ab-immunoreactive structures in the reticular nucleus is similar so large amounts of MAP 2c are no longer visible. Adjacent thalamic nuclei reveal much more MAP 2- than MAP 2ab-immunoreactive structures which indicates that considerable amounts of MAP 2c are present. In numerous reticular neurons a high CO-activity is detectable in the 16th week whereas adjacent thalamic nuclei reveal only a very slight activity. In the 20th week the other thalamic nuclei do no longer show significant amounts of MAP 2c, they display higher amounts of CO-activity so these nuclei are later differentiated than the reticular nucleus. (Support: P.C.Weill-Stiftung)

181.8

REDISTRIBUTION OF DREBRIN TO SUBMEMBRANOUS REGION FROM CYTOPLASM BY NEURONAL DIFFERENTIATION. H.Asada[1],T.Shirao[1]*,M.Toda[2],S.Toya[2],and K.Uyemura[1], Department of [1]Physiology and [2]Neurosurgery, Keio University School of Medicine, Tokyo 160, JAPAN.

Drebrins are developmentally-regulated actin-binding proteins (Shirao,et al, NeuroReport,1992). In this study, we demonstrated that cultured human SY5Y neuroblastoma cells contain drebrin E (embryonic type), by Western blotting analysis of two-dimensional gel electrophoresis. Confocal microscopy demonstrated that patchy drebrin immunoreactivity appeared scattered in the cytoplasm of control cells. Two weeks after retinoic acid treatment (10^{-5}M), expression of neurofilament proteins was increased in differentiated SY5Y cells. In parallel with this neuronal differentiation, subcellular localization of drebrin changed to submembranous regions. This drebrin subcellular localization was consistent with those in primary cultured neurons. A close relationship between drebrin and F-actin in subcellular localization was observed. F-actin within undifferentiated SY5Y cells was continuously present in stress fibers and drebrin was discontinuously present in the same regions. In differentiated SY5Y cells, however, drebrin was localized continuously with F-actin. F-actin in the submembranous region, which binds to drebrin, was more stable against cytochalasin-D, an actin depolymerizing agent, than F-actin, which does not bind to drebrin, in the stress fibers. [Partly supported by Ichiro Kanehara Foundation]

181.9

INDUCTION OF CELL PROCESS OUTGROWTH WITH SUBMEMBRANOUS ACTIN FILAMENTS IN FIBROBLASTS BY DREBRIN A OVER-EXPRESSION. T. Shirao, H. Asada, P. Kaub, K. Yamaguchi* and K. Uyemura. Department of Physiology, Keio University School of Medicine, Tokyo 160; Natl. Inst. for Physiol. Sci. Okazaki 444, JAPAN.

Neuron-specific actin-binding protein, drebrin A, was expressed in parallel with neurite outgrowth and synapse formation during brain development (*Dev. Brain Res.* **29**: 233,1986). In adult it localized in the dendrite (*Brain Res.* **413**:374,1987). Over-expression of drebrin A in fibroblasts induced the formation of highly branched processes from the cell perimeter (*NeuroReport*, **3**:109,1992). We proposed that drebrin plays important roles in neuronal morphogenesis and in remodeling of neuronal connections in the adult.To investigate cellular functions of drebrin A, fibroblast cell lines were transfected with a drebrin A expression plasmid,which contained either the ß-actin promoter or the metallothionein promoter. In the transfected cells, expressed drebrin A is co-localized with F-actin. Transfected cells that expressed a large amount of drebrin A changed the subcellular localization of actin filaments and extended a number of processes from a round cell body. The percentage of process-bearing transformants is in parallel with induction of drebrin A expression by cadmium ion. Thus we conclude that drebrin A contributes directly to neurite outgrowth. [Supported by grant from the Ministry of Education, Science and Culture in Japan.]

181.10

DIFFERENTIATION AND NEUROFILAMENT EXPRESSION IN IMMORTALIZED CELL LINES DERIVED FROM RAT MESENCEPHALON. H. Takashima*, M. Marone †, H. M. Geller † and W. J. Freed. NIMH Neurosci. Centr. at St. Elizabeth. Washington D.C. 20032. †Dep. of Pharmac. UMDNJ-RWJMS, Piscataway, NJ 08854.

Several immortalized cell lines have been established from the E12-E14 ventral mesencephalon of the rat using a retroviral vector containing the temperature-sensitive mutant (tsA58) of SV40 large T antigen. At the permissive temperature (33°C) most clones appeared polygonal with only occasional short processes and were positive for SV40 T antigen. Several of the clones stopped growing at the non-permissive temperature (39.5°C) and extended processes. A cell line derived from E14, designated K1, was confirmed to be a single clone by Southern blotting. One subclone (K1S) which shows contact inhibition developed neurofilament-positive processes at 39.5°C. When differentiation was further stimulated with a mixture of bFGF, IBMX and dibutyryl cAMP at 39.5°C with 10% FCS, this clone developed elongated processes and a neuron-like multipolar morphology. Expression of neurofilament was promoted by adding conditioned medium from rat brain culture. Another subclone (K1K) which does not show contact inhibition developed a similar morphological change including development of processes, when cultured with bFGF, IBMX and dibutyryl cAMP, but was negative for neurofilament. Characterization of neurofilament expression in additional clones isolated from E12-E13 rats is in progress. Supported in part by NIH grant P01 NS 21469.

181.11

DEVELOPMENTAL DIFFERENTIATION OF EMBRYONIC HUMAN MESENCEPHALIC DOPAMINERGIC NEURONS IN VIVO AND IN VITRO. P. Almqvist*, F. Souverbie, E.-B. Samuelsson, H. Pschera[1], Å. Seiger and E. Sundström. Depts. of Geriatric Medicine and Obstetrics & Gynecology[1], Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge, Sweden.

The present study emphasize to characterize the morphological and neurochemical differentiation of mesencephalic dopaminergic neurons from human embryos, derived from elective first trimester abortions. Mesencephalic tissue was taken for analyses of dopamine (DA) content using HPLC-ED and for tyrosine hydroxylase (TH) immunohistochemistry, as well as for primary culture. TH-immunoreactive neuroblasts were first seen in the ventral mesencephalon at 5 weeks postconceptional age. Detectable levels of DA were found at the same age and increased exponentially during the first trimester.

Mesencephalic cells and cells from target areas (striatum, cerebral cortex) were cultured in separate but connected wells in order to supply dopaminergic neurons with diffusible, trophic factors. Basic fibroblast growth factor (bFGF) added to the growth medium was found to further improve survival and differentiation of TH-immunoreactive cells. Dissociated, mesencephalic primary cultures were plated at 5 - 9 weeks of gestational age and maintained *in vitro* for several weeks without even brief exposure to antibiotics. TH-immunoreactive neuroblasts with large rounded cell somata, each with a single axonal process and several minor neurites, were detected in cells from 5 week old embryos after 1 week in culture. Neurites extended successively with time in culture, developed varicosities and formed an extensive network. TH-positive cells migrated rapidly to associate with highly proliferative supportive cells. Mesencephalic tissue older than 8 weeks did not survive in long-term culture. For detection of monoamines, cultured mesencephalic cells were harvested and subjected to HPLC-ED analysis. With addition of TH-cofactor tetrahydrobiopterin (BH_4) to the culture medium, DA could be detected at 5 + 2.5 weeks, at an approximate concentration of 0.5-1 ng/ 500-1000 TH-immunoreactive neurons. In conclusion, TH-expression and DA-synthesis are maintained in 5 - 8 week old mesencephalic cells during differentiation *in vitro*.

FORMATION AND SPECIFICITY OF SYNAPSES III

182.1

COMPLEX EXTRACELLULAR MATRIX BUT NOT LAMININ INCREASES VESICLE DENSITY IN SYNAPTIC PROFILES OF SYMPATHETIC NEURONS IN VITRO. M. Loegering, D. Higgins, + and M.I. Johnson*. Univ. of Ariz. Col. of Med., Tucson, AZ 85724 and SUNY +, Buffalo, NY 14214.

Although well-studied at the neuromuscular junction, the role of extracellular matrix (ECM) in synaptogenesis in the autonomic nervous system has received little attention. Our previous studies found that complex ECM increases vesicle numbers in axonal varicosities of cultured sympathetic neurons. These studies have now been extended to include an analysis of the effect of a major component of ECM, laminin. Nonglial-cell-containing dissociated embryonic rat sympathetic neurons were grown on polylysine or laminin adsorbed to polylysine. Polylysine cultures received either defined medium or defined medium containing 50 µg/ml of ECM (Matrigel, Collaborative Research, Inc.). Laminin grown cultures remained on defined medium. After 3 weeks, the cultures were processed for electron microscopy and subjected to analysis by stereology. Vesicle density within varicosities found in polylysine or laminin cultures (on defined media) was less than neurons treated with ECM (1820 ± 186 or 1766 ± 261 vs. 5903 ± 544 vesicles/μM^3; mean ± SEM; $p < .0001$). Varicosities along the axons were increased with ECM treatment (2.5 ± 0.5 vs. 0.8 ± .2 varicosities/mm; $p < .004$). More varicosities (1.28 ± .24) were found within 3 µM of an index varicosity in the ECM group than in the polylysine group (.45 ± .12; $p < .002$). Varicosity clustering on laminin, however, was similar to the ECM group (1.75 ± .14 per index varicosity; $p > 0.05$). We conclude that laminin appears to affect clustering of varicosities but some other component(s) contained in the ECM result in the vesicle density changes. [Supported by grants NIH-NS15070 (MIJ), and NSFB-BNS8909373 (DH)].

182.2

WITHDRAWN

182.3

WITHDRAWN

182.4

AGRIN GENE EXPRESSION IN DEVELOPING CHICK CILIARY GANGLION. W.S. Thomas*§, D.K. O'Dowd†§, M.A. Smith.§ Dept. Anat. and Neurobiol§.; Dept. Dev. and Cell Biol.†, UC Irvine, CA 92717.

We are interested in identifying factors that regulate agrin gene expression. As a first step, we have begun to examine agrin expression in chick ciliary ganglion (CG), a parasympathetic ganglion whose normal targets include both smooth and striated muscle cells in the eye. Using oligonucleotide primers derived from a chick brain agrin cDNA (Tsim et al., 1992) we have identified agrin transcripts in embryonic CG by PCR. Previous studies have shown that active and inactive forms of agrin are products of alternate splicing of a single gene. Transcripts encoding active agrin contain a 33bp exon that is absent from those encoding inactive forms of the protein (Ruegg et al., 1992). Our PCR analysis indicates that CGs express mRNAs containing and lacking this 33bp exon, consistent with the presence of both active and inactive forms of agrin in the ganglion. We also report the presence of a transcript that contains an additional 24bp exon, contiguous with the 5' end of the 33bp sequence, that may also encode an active agrin protein. *In situ* hybridization demonstrates that agrin transcripts are present as early as 8d and throughout embryonic development in both neurons and glial cells in the ganglion. In addition, levels of expression of transcripts that contain the 33bp exon alone, are high at 8d in ovo and decline with development. These studies demonstrate that the agrin gene is expressed in parasympathetic neurons that innervate striated and smooth muscle cells. Expression levels of at least one agrin transcript are developmentally regulated, the highest level coincident with the onset of synapse formation in the periphery. Supported by NIH NS-27563.

182.5

AGRIN EXPRESSION IN THE DEVELOPING RAT M. Ferns, J. Campanelli, W. Hoch, F. Rupp, R. Scheller, & Z.Hall*. Dept. Physiol. UCSF, SF, CA 94143-0444; and HHMI, Dept. Molecular. & Cellular Physiol. Stanford Univ. Stanford, CA 94305.

Agrin, a component of the synaptic basal lamina at the adult neuromuscular junction, is thought to be the neurally-derived factor responsible for the initial clustering of AChRs at developing synapses. Agrin expression is not limited to motoneurons however, raising the question of whether agrin in other cells of the CNS or in muscle also participates in synaptogenesis, or serves other functions. To address these questions we have examined agrin gene and protein expression in brain, spinal cord and muscle in the developing rat. Northern blotting of tissue extracts at all stages revealed a single agrin transcript of 8.2 kb in the three tissues, with the mRNA being more abundant in brain and spinal cord than in muscle. In each tisssue, the transcript was at its highest levels prenatally and declined during the postnatal period. Interestingly, whilst expression of the transcript in spinal cord was relatively constant during the time of initial synapse formation (embryonic day (E)14-birth), expression in limb muscle peaked early in neuromuscular development, at E16. The agrin protein was identified as a single band of 200kD in immunoblots, and in preliminary experiments showed a pattern of expression corresponding to that seen for the mRNA. Immunostaining of limb sections showed that agrin immunoreactivity is initially widespread within early stage muscles (E14-16) and with development progressively becomes restricted to the synaptic regions. We conclude that agrin expression in the spinal cord and brain is highest during synaptogenesis. Muscle also expresses agrin during this period, although its widespread, extrasynaptic distribution prenatally suggests that it may have roles other than in clustering of receptor. Such different biological activities and roles for agrin may be related to the occurrence of multiple forms of the protein generated by alternative RNA splicing.

182.6

AGRIN GENE EXPRESSION IN ADULT RAT CNS AND ITS REGULATION BY SEIZURE ACTIVITY. L.T. O'Connor*, J.C. Lauterborn, C.M. Gall, and M.A. Smith. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Agrin is an extracellular matrix protein thought to play a critical role in development and maintenance of the neuromuscular junction. Detection of agrin mRNA in brains of adult electric rays and embryonic rats and chickens raises the possibility that agrin may also play a role in synaptogenesis in the CNS. As a first step towards understanding agrin's role in the CNS, we have used probes derived from a rat agrin cDNA (Rupp et al., 1991) to analyze the regional and cellular distribution of agrin mRNA in the adult rat brain and to examine whether changes in synaptic activity can influence its expression. Northern blot analysis shows a single band of approximately 8kb present in RNA isolated from all regions of adult brain examined. Consistent with this observation, *in situ* hybridization studies using ^{35}S-labelled cRNA probes reveal that agrin mRNA is widely distributed throughout the adult brain; expressed in cholinergic as well as non-cholinergic areas. We have also observed that levels of agrin mRNA can be influenced by seizure activity. For seizure studies, an electrolytic lesion is placed in the hilus of the dentate gyrus that induces a period of recurrent limbic seizures for 8-10 hrs. Rats sacrificed 24hrs postlesion exhibited increased levels of hybridization in the caudate, septum, and intermediate layers of neocortex but markedly reduced levels in superficial layers of neocortex and piriform cortex. These findings demonstrate that agrin is widely expressed in adult CNS and its expression can be influenced by changes in synaptic activity induced by seizures. Supported by NIH NS-27563 to M.A.S., NS-07351-01 to L.T.O., and NS-26748 to C.M.G.

182.7

BIOCHEMICAL CHARACTERIZATION OF A PUTATIVE AGRIN RECEPTOR FROM *TORPEDO* ELECTRIC ORGAN Jianyi Ma* and Justin R. Fallon. Worcester Fdn. for Exper. Biol., Shrewsbury, MA. 01545

The putative agrin receptor is a plasma membrane protein that binds agrin and is likely to mediate agrin-induced AChR clustering on cultured myotubes. Our previous biochemical characterization of the putative agrin receptor from *Torpedo* electric organ indicates that this receptor is an integral membrane protein distinct from the AChR that is selectively concentrated in postsynaptic-rich membrane fractions. Here, we report the further biochemical characterization of the putative agrin receptor. Agrin binding to postsynaptic-rich membranes is unaffected by pretreatment of the membranes with phosphatidylinositol phospholipase C. The solubilized putative agrin receptor from *Torpedo* electric organ selectively binds to several lectins that recognize galactose, N-acetylglucosamine and α-linked mannose, but not to lectins that recognize fucose. In addition, agrin, but not the putative agrin receptor binds to heparin, indicating that agrin binding to membranes is not homophilic. We have determined the optimal conditions of calcium concentration, pH, ionic strength and detergent for agrin binding to the solubilized putative agrin receptor. Agrin affinity columns bind the putative agrin receptor, but not the AChR. Taken together, these results indicate that the agrin receptor is a transmembrane glycoprotein that is distinct from agrin itself and the AChR. We are currently using agrin affinity methods to identify and purify this receptor. Supported by the NIH and the March of Dimes.

183.1

METABOLIC KINETICS OF ENDOGENOUS NERVE GROWTH FACTOR. K. Siminoski* and B. Tobin. Department of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2S2.

Nerve growth factor (NGF) is produced in most tissues of the body and is supplied locally to target neurons. To assess the endogenous whole-body utilization and metabolism of NGF, we have administered tracer doses of ^{125}I-labelled growth factor to 5 rats and monitored serum levels for up to 4 days. Intact NGF was determined by TCA precipitability.

Intact NGF was cleared from plasma with 3 slopes, consistent with 2 distinct non-plasma NGF compartments. An average endogenous NGF molecule was in the body 1120+/-270 min, of which 70+/-20 min were spent in the blood. After first entering the circulation, NGF exited and subsequently re-entered 1.8+/-0.4 times. Fractional catabolic rate was 1.3+/-0.3%/min.

NGF metabolites accumulated until about 4 hours, and represented a maximum of 4.4+/-0.4% of the administered NGF dose. Subsequently, degraded NGF disappeared from plasma with 2 sequential slopes, the final one paralleling the terminal slope of intact NGF clearance. Radiolabel accumulation in urine lagged behind plasma disappearance; all urine NGF was degraded, and the excretion rate was directly related to serum levels of degraded NGF.

We have demonstrated that radiolabel tracer methods may be useful in assessing whole body NGF kinetics. Accurate analysis requires monitoring intact NGF for at least 36 hours, and the degraded NGF pool at least 72 hours.

183.2

INSULAR CORTICAL GRAFTS WITH NGF ACCELERATES RECOVERY OF LEARNING AND REESTABLISH THE ChAT ACTIVITY. M. Escobar*, R. Tapia, J.C. López, N. Jiménez and F. Bermúdez-Rattoni. Instituto de Fisiología Celular, UNAM, P.O. Box 70-600, México, D.F.

It has been demonstrated that insular cortex (IC) is involved in the acquisition of Conditioned Taste Aversion (CTA) and Inhibitory Avoidance (IA) tasks. We have recently shown that cortical brain grafts produced a significant recovery of the ability to learn after 60 days post-graft in IC lesioned rats. In the present work we evaluated the role of the nerve growth factor (NGF) in the recovery of CTA and IA by cortical grafts. Results showed that IC, but not occipital grafts with NGF promote recovery of learning at 15 days post-graft in both tasks. Biochemical analysis showed that choline acetyltransferase (ChAT) activity in the homotopic but not heterotopic grafts plus NGF, had a similar pattern of activity as the intact control animals. Measures of glutamic acid decarboxylase (GAD) activity at 15 days post-graft did not show any differences between groups. These findings suggest that NGF should be associated with homotopic grafts, to produce a faster and stable recovery of learning abilities in IC lesioned rats. Moreover the ACh, but not GAD activity may play an important role in the grafts mediated behavioral recovery.

Supported by DGAPA-IN204689 and CONACyT 0178-N19107

183.3

ACUTE AND CHRONIC INTRAVENTRICULAR INFUSIONS OF NMDA INDUCE BDNF AND NGF mRNA SELECTIVELY IN RAT DENTATE GYRUS GRANULE CELLS. B.J. Gwag* and J.E. Springer, Department of Neurology, Hahnemann University School of Medicine, Philadelphia, PA 19102-1192.

Events associated with seizure activity and kindling increase mRNA expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) -a member of the NGF family of neurotrophins- in the central nervous system (CNS). Recently, we found that activation of NMDA receptors induces NGF mRNA expression exclusively in dentate gyrus (DG) granule cells. This increase in NGF gene expression was observed in the absence of both seizure activity and neurotoxicity. The present study investigated whether gene expression of BDNF is also influenced by excitatory transmission via NMDA receptor activation. We also tested whether the expression of mRNA for these two neurotrophins is influenced by long- term (7 days) infusions of NMDA.

In the first set of experiments, a single, non-toxic dose of NMDA was infused into the lateral ventricle of adult rats, and levels of BDNF mRNA were analyzed over a 24 hour period using in situ hybridization histochemistry. NMDA infusions maximally induced the expression of BDNF mRNA in DG granule cells at 4 hours following infusions, and returned to control levels within 24 hours. In addition, the induction of BDNF mRNA by NMDA was blocked by prior treatment with AP5 (an NMDA antagonist). In the long-term infusion experiment, animals received a single permanent intraventricular cannula attached to an osmotic minipump containing NMDA or AP5. Following 7 days of NMDA infusions, BDNF mRNA levels were significantly enhanced in DG granule cells, while only a slight increase in NGF mRNA levels was observed. The gene expression of both neurotrophins was unchanged following acute or chronic infusions of AP5. These studies provide further evidence that the regulation of genes encoding NGF and BDNF in DG granule cells can occur through NMDA receptor activation. Supported by PHS grants AG-08969 and NS-30248 (JES).

183.4

ANGULAR BUNDLE STIMULATION INDUCES NEUROTROPHIN GENE EXPRESSION IN DENTATE GYRUS GRANULE CELLS. J.E. Springer*, B.J. Gwag, and F.M. Sessler, Departments of Neurology (JES and BJG) and Physiology and Biophysics (JES and FMS) Hahnemann University School of Medicine, Philadelphia, PA 19102-1192.

We have previously demonstrated that N-methyl-D-aspartate (NMDA) receptor activation increases NGF mRNA expression exclusively in dentate gyrus granule cells. The induction of NGF mRNA, which occurs following intraventricular or intrahippocampal infusions, is observed in the absence of seizure activity, and is time- and dose-dependent. In the dentate gyrus, NMDA receptors are present primarily in the outer third of granule cell dendrites, and innervated by putative glutamatergic afferents projecting from entorhinal cortex via the angular bundle and perforant path.

Because the neurotrophins NGF and BDNF are thought to influence the function and survival of basal forebrain cholinergic neurons, we hypothesize that the activity of entorhinal cortical afferents on hippocampal formation play an important role in transneuronal plasticity. To test this, rats received unilateral high frequency stimulations of the angular bundle and the expression of NGF and BDNF mRNA was analyzed using in situ hybridization histochemistry. Some of the stimulation parameters used in this study were sufficient to produce long-term potentiation (LTP), but not seizure activity. At lower intensities of angular bundle stimulation, gene expression of both neurotrophins was induced exclusively in ipsilateral dentate gyrus granule cells. At higher intensities of stimulation, induction was observed bilaterally, with a greater hybridization signal occurring on the ipsilateral side. Pretreatment with AP5 (NMDA antagonist) effectively blocked induction of NGF and BDNF mRNA, but pretreatment with CNQX (non-NMDA antagonist) had no such effect. These studies demonstrate that activation of angular bundle, using parameters sufficient to produce LTP, results in the increased expression of mRNA encoding NGF and BDNF. We speculate that NMDA receptor stimulation in vivo may ultimately impact the function and survival of neurotrophin-sensitive cholinergic neurons. Supported by PHS grants AG-08969 and NS-30248 (JES).

183.5

LOCAL AND DISTANT SOURCES OF NERVE GROWTH FACTOR (NGF) MAY PROVIDE TROPHIC SUPPORT FOR NGF-DEPENDENT CHOLINERGIC BASAL FOREBRAIN NEURONS. J.M. Conner* and S. Varon, Department of Biology, 0601, UCSD, La Jolla, CA 92093

The idea that NGF primarily acts as a target derived trophic agent was established in the PNS and accumulating evidence has suggested that a similar role may exist within the CNS. Our previous attempts to localize NGF within the normal adult rat CNS by immunohistochemical methods demonstrated that NGF immunoreactivity (NGF-ir) was exclusively found in the mossy fiber region of the hippocampal formation and within the NGF-dependent basal forebrain cholinergic neurons. A lack of staining among potential NGF sources within cholinergic basal forebrain innervation territories suggested that cells which produce NGF did not accumulate the antigen in quantities large enough to be detected by our immunohistochemical techniques. In the present investigation, an attempt was made to force the accumulation of NGF within the cell bodies of NGF producing cells by pretreating animals with the neuritic transport inhibitor, colchicine. Following a 48 hour colchicine treatment, NGF-ir cells were detected within all cholinergic basal forebrain innervation territories- including cortex, olfactory bulb and hippocampal formation. In addition, numerous NGF-ir neurons were detected within the medial and lateral septum, horizontal limb of the diagonal band/magnocellular preoptic region, and nucleus basalis. Colocalization of NGF and low affinity nerve growth factor receptor within the same section further demonstrated that NGF-ir neurons within the basal forebrain were not the NGF-sensitive cholinergic ones. These results indicate that NGF producing cells are distributed among all basal forebrain cholinergic cell populations, as well as within their innervation territories, and suggest that both local and distant supplies of trophic factors may play a role in supporting NGF-dependent cholinergic cell populations of the basal forebrain. Supported by NINCDS grants NS-16349 and NS-27047.

183.6

HUMAN NERVE GROWTH FACTOR (NGF) SELECTIVELY IMPROVES SPATIAL MEMORY DEFICITS IN AGED RATS VIA STIMULATION OF THE BASAL FOREBRAIN CHOLINERGIC SYSTEM (BFCS). C.A. Fleischman[1], M.C. Gustilo[1], A.L. Markowska[2], L.K. Gorman[1], L.E. Burton[1], D. S. Olton[2], D.L. Price[1] and V.E. Koliatsos*[1]. [1]Neuropathology Lab. and [2]Dept. of Psychology, The Johns Hopkins University, Balto., MD 21205. [3]Genentech Inc., San Francisco, California.

In rats, NGF ameliorates age-associated decline in certain types of memory [*Nature* 329: 65, 1987]. The present study extends the previous findings and establishes the selectivity of NGF effects. Fisher-344 rats, pretested on two tasks of spatial memory and on tasks assessing sensorimotor skills, were treated with recombinant human NGF for one month and subsequently retested. Significant improvement was observed in spatial memory but not in sensorimotor skills. ChAT activity in hippocampus and neocortex and sizes of cholinergic neurons in all sectors of the BFCS were significantly increased. Effects of NGF on catecholaminergic and intrinsic cortical markers will be discussed. The present findings emphasize the potential of NGF as a therapeutic agent for disorders involving the BFCS, including Alzheimer's disease.

183.7

BEHAVIORAL EFFECTS OF NERVE GROWTH FACTOR (NGF) IN BOTH YOUNG AND AGED RATS. D.S. Olton*, A.L. Markowska, V. Koliatsos, R. Henshaw, S. Sinco, L.E. Burton, And D. Price. Department of Psychology and Laboratory of Neuropathology, The Johns Hopkins University, Baltimore, MD 21218; Genentech Inc., San Francisco, CA 94080.

The behavioral effects of nerve growth factor (NGF) were assessed in Fischer-344 rats in three tests: recent memory in a T-maze, place learning in a water maze, and sensorimotor skills. Each rat was trained in each task, given continuous infusion of NGF via an osmotic minipump or a control procedure (CON), and tested again three-four weeks later. Two doses (total amount infused) of NGF were given: 60 and 240 micrograms. Two age groups were tested: 4 months old (4MO) and 23 months old (23MO). The resulting four groups are identified by an abbreviation giving first the age and then the procedure. In 23MO-NGF rats, both doses improved performance in the place learning task and the recent memory task, but not sensorimotor skills. In 4MO-NGF rats, the low dose of NGF did not improve performance. These data indicate that continuous infusion of NGF can have significant behavioral effects.

183.8

DEVELOPMENTAL EXPRESSION OF *trk* mRNA IN THE RAT BASAL FOREBRAIN

Y. LI, D.M. HOLTZMAN, R. RIOPELLE, AND W.C. MOBLEY*, Dept. of Neurology and the Neuroscience Program, UCSF, San Francisco, CA 94143, Dept. of Neurology, Queens Univ. of Kingston, Ontario, K7L3N6

Recent data indicate that the product of the *trk* gene, p140^{trk}, is a critical component of the NGF receptor. We studied *trk* mRNA expression in rat basal forebrain in postnatal day (PD) 0, 4, 7, 11, 16, 21, 30, and adult animals, using *in-situ* hybridization histochemistry. Frozen sections of rat brain (18 mm) were hybridized utilizing radio-labeled sense and antisense *trk* riboprobes. *Trk* mRNA was first detected at low levels at PD 0 and slightly increased by PD 7. A sharp increase of *trk* mRNA was seen at PD 11, and high levels of expression were maintained through PD 21. By PD 30, mRNA levels began to decrease and reached lower levels in adult animals. Studies on choline acetyltransferase (ChAT) mRNA showed similar expression patterns in the same region. *Trk* expression correlated well with the specific activity of NGF high affinity binding. Our findings indicate that *trk* expression in the rat CNS is developmentally regulated and that its pattern of expression is consistent with it serving as part of the high affinity NGF receptor.

183.9

PHARMACOLOGICAL STIMULATION REVEALS NGF-INDUCED INCREASES OF HIPPOCAMPAL CHOLINERGIC FUNCTION MEASURED IN VIVO IN RATS WITH PARTIAL FIMBRIAL TRANSECTIONS. E.O. Junard*, P.A. Lapchak, D.J. Jenden[1], and F. Hefti, USC, Andrus Ger. Ctr., and [1]Dept. Pharmacol., UCLA, Los Angeles, CA.

Recent pharmacological studies have shown that chronic recombinant human (rh) NGF treatment enhances hippocampal cholinergic markers measured in vitro following partial fimbrial transections. The present study determined whether the effects of chronic rhNGF administration are also evident when hippocampal cholinergic function is measured in vivo. Partial fimbrial transections did not significantly alter the levels of ACh or [^{3}H$_4$]ACh in the hippocampus, possibly due to functional compensation by surviving cholinergic terminals. In animals chronically treated with rhNGF (icv, 1.0 µg qod for 21 days) the levels of ACh, choline, [^{3}H$_4$]ACh and [^{3}H$_4$]choline accumulated in the hippocampus on the lesioned side were not different from those on the unlesioned side. However, lesion and/or rhNGF-induced changes in cholinergic parameters became evident when animals were challenged using stimulants. First, in rhNGF-treated animals administered pentylenetetrazole (10mg/kg) 2 minutes prior to measuring in vivo cholinergic parameters, we observed an increase (422-505%) in the hippocampal content of [^{3}H$_4$]choline in both lesioned and unlesioned hippocampi. However, there was no concomitant increase in the level of [^{3}H$_4$]ACh. Second, in rhNGF-treated animals administered lithium chloride (3 mmol/kg) 20 hours prior to pilocaprine (30 mg/kg) we observed a 50% increase in the content of ACh in the hippocampus of the lesioned side. These results suggest that rhNGF-induced differences in the functional performance of hippocampal cholinergic neurons may only be manifest during behavioral or pharmacological stimulation.

183.10

NERVE GROWTH FACTOR CONJUGATED TO AN ANTI-TRANSFERRIN RECEPTOR ANTIBODY CROSSES THE BLOOD BRAIN BARRIER: EVIDENCE FROM INTRAOCULAR SEPTAL TRANSPLANTS. ACh.Granholm*, C.Bäckman[1], B.Hoffer[2], L.Walus[3], F.Bloom[4] and P.Friden[3]. [1]Dept. Basic Science, School of Dentistry, and [2]Dept of Pharmacology, University of Colorado HSC, Denver, CO 80262, [3]Alkermes, Inc., 26 Landsdowne Street, Cambridge, MA 02139, and [4] Dept. Neuropharmacol., Scripps Clin. and Res. Fnd., La Jolla, CA 92037. Nerve Growth Factor (NGF) has been shown to affect survival of cholinergic neurons in the medial septal nucleus. However, the delivery of this protein to the brain is impeded by the blood brain barrier (BBB). Our purpose was to investigate if an anti-transferrin receptor antibody (OX-26) could function as a carrier of NGF across the BBB. Fetal rat septal tissue (E18) was grafted into the anterior eye chamber of adult albino rats. An intact BBB was found in the grafts two weeks postgrafting. The experimental group was injected intravenously with OX-26-NGF conjugate, and the control groups with 1)saline, 2)NGF and 3)OX-26 at 2,4,6 and 8 weeks postgrafting. The growth of grafts treated with OX-26-NGF was significantly enhanced as compared to all control groups. After eight weeks the grafts were studied using immunohistochemistry with choline acetyltransferase, laminin and neurofilament antibodies. The overall densities of nerve cells and glia were similar in all groups. However, the survival rate of cholinergic neurons in OX-26-NGF treated grafts appeared to be much higher than in controls. These results suggest that the delivery of NGF across the BBB can be achieved using an anti-transferrin receptor antibody as a carrier.

183.11

INCREASED EXPRESSION OF NGF AND bFGF mRNAs IN RAT BRAIN FOLLOWING MAXIMAL ELECTROSHOCK . P. Follesa, K. Gale and I. Mocchetti . Depts. Anatomy & Cell Biology and, Pharmacology, Georgetown Univ., Med. Center, Washington DC 20007.

Little is known about the regulation of neurotrophic factor biosynthesis in the mature CNS. We have previously found that focally evoked seizures rapidly increase nerve growth factor (NGF) and fibroblast growth factor (bFGF) mRNAs in selected brain regions. In this study, we examine the effect of maximal electroshock (MES)-evoked seizures on the regional pattern of NGF and bFGF mRNA expression. Three MES seizures (induced via corneal electrodes) were evoked at 20 min intervals. Each seizure lasted for not more than 15 sec. 5 h after the first MES, 2-4 fold increases in bFGF mRNA and NGF mRNA were observed in both hippocampus and entorhinal cortex. Previous evidence has implicated corticosteroids in the regulation of growth factor expression. To determinate the role of corticosteroids in the mediation of the MES effect on NGF and bFGF mRNA levels, we repeated the experiments in adrenalectomized rats. In adrenalectomized rats, MES increased NGF and bFGF mRNA to the same extent as that observed in sham operated rats, indicating that adrenal steroids are not required for the seizure-evoked increase in NGF and bFGF mRNA. Our results, together with our previous findings, suggest that the induction of NGF and bFGF mRNA following convulsive seizures is associated with the activation of neuronal circuits. This induction may represent an adaptive response which may be important for protecting and mantaining the integrity of the brain circuitry. Supported by H.H.S. grants NS29664, NS 20576.

183.12

INCREASED HIPPOCAMPAL NGF-LIKE ACTIVITY FOLLOWING MEDIAL SEPTAL LESIONS IS DETECTED BY A BIOASSAY BUT NOT BY AN ELISA. K.A. Crutcher*, S. Liang and J. Weingartner.
Department of Neurosurgery, Univ. of Cincinnati, Cincinnati, OH 45267.

Nerve growth factor-like immunoreactivity (NGF-LI) has been reported to increase in the rat hippocampal formation (hf) following septal denervation, an increase that may contribute to sympathohippocampal sprouting. The increase has been found in separate laboratories using either an ELISA or a bioassay. However, there are discrepancies between these reports, particularly regarding the magnitude and duration of the increase as well as its specificity. We used both a sensitive 2-site ELISA and a bioassay to monitor hf NGF-LI following septal lesions. The ELISA uses a monoclonal and a polyclonal antibody raised against mouse NGF (Saffran et al., '89). The bioassay employs E9 chick sympathetic ganglia grown for 18 hours in the presence of: medium (Ham's F12) alone, mouse NGF, hippocampal extract (from control or septal-lesioned rats) or extract plus anti-NGF antibodies (the same polyclonal antiserum used in the ELISA). No increase in hf NGF-LI was detected with the ELISA at either 1 or 2 weeks following a septal lesion. In contrast, bioassay measurements demonstrated a significant increase in NGF-like biological activity at both survival times. This discrepancy between the ELISA and bioassay could be due to differences in the sensitivity of the two assays. However, both procedures reliably detect concentrations of purified mouse NGF as low as 30 pg/ml. A more likely explanation is that the bioassay detects NGF-like biological activity not detected in the ELISA. Whatever the underlying cause, the discrepancy between the two assays emphasizes the importance of using both methods to assess alterations in tissue levels of putative neuronal growth factors. (Supported by NIH #NS17131.)

183.13

DISTRIBUTION OF [^{125}I]rhNGF FOLLOWING BOLUS INTRACEREBROVENTRICULAR (ICV) DELIVERY IN RAT BRAIN R. H. Soriano*J. Briggs [2], L. B. Jakeman, G. Bennett [1], T. Hotaling [3], P. Cossum [3], C. A. Altar and T. Nguyen [2]. Neuroscience Research, [1]Medicinal and Analytical Chem. [2]Pharm.Res. and Dev.,and [3]Safety Evaluation, Genentech, Inc. South San Francisco, CA 94080.

In order to evaluate the distribution of rhNGF delivery in the brain, iodinated rhNGF ([^{125}I]rhNGF) was delivered as a bolus injection of 1.64 pmoles/3 µl over 15 minutes into the left intracerebral ventricles (ICV) of 220 g male rats. Brains were removed after 24 hours, sectioned and exposed to film for 60 days. Positive labelling appeared in the cholinergic nuclei (medial septum, vertical and horizontal limbs of diagonal band of Broca, and substantia inominata) of all brains treated. A co-injection of approximately 100-fold excess concentration of non-labeled rhNGF blocked the labelling of these cholinergic areas suggesting specific receptor mediated uptake. Label was also localized to the left ventricular ependymal cells but was not competed by an excess of non-labeled ligand. Emulsion autoradiographs of adjacent brain sections revealed silver grains on cells in the forebrain nuclei which were counterstained with an antibody to the cholinergic marker choline acetyltransferase suggesting that label was retrogradely transported to the NGF dependent cholinergic cell bodies in the nuclei. Thus, forebrain cholinergic nuclei can specifically and bilaterally accumulate [^{125}I]rhNGF following a bolus injection.

183.14

DENERVATION OF DOPAMINERGIC NEURON WITH 6-HYDROXYDOPAMINE INCREASES NERVE GROWTH FACTOR CONTENTS IN RAT BRAIN. T. Nabeshima*[1], A. Nitta[1], T. Hasegawa[1], M. Hiramatsu[2], T. Kameyama[2], Y. Furukawa[3] and K. Hayashi[3]. [1]Dept. Neuropsychopharmacol. & Hosp. Pharm., Nagoya Univ. Sch. Med., Nagoya 466, Japan, [2]Dept. Chem. Pharmacol., Fac. Pharmac. Sci., Meijo Univ., Nagoya 468, Japan and [3]Dept. Mol. Biol., Gifu Pharm. Univ., Gifu 502, Japan.

Nerve growth factor (NGF) is a trophic protein for the magnocellular cholinergic neurons. We have reported that dopamine (DA) regulates NGF synthesis and secretion in cultured mouse astroglial cells. These results suggest that DA is one of the molecules responsible for regulation of NGF synthesis in brain. Therefore, it is important to investigate whether dopaminergic (DAergic) neurons affect to NGF contents in the various brain regions to clarify the mechanism of NGF synthesis and secretion. In this study, we measured NGF contents by EIA method in the rat brain following selective lesion of DAergic neurons by injection of 6-hydroxydopamine (23 µg/rat), neurotoxin for DA neurons, into the nigra, where cell bodies of DAergic neurons are present. DA content dramatically decreased to 3 % of the control level 0.5 day, recovered to approximate 30 % of that 1 day and kept this level until 28 days after lesion. The increase of NGF contents was found in the parietal cortex and hippocampus and it continued 0.5-28 days after the lesion. Mechanism of regulation for NGF synthesis is not clear in the present. However, from these results, it is suggested that DAergic neuronal system has some roles in NGF synthesis.

183.15

EFFECTS OF NEONATAL VISUAL CORTEX DAMAGE ON NERVE GROWTH FACTOR RECEPTOR EXPRESSION IN THE CAT RETINA.
J.-T. Xue and P. D. Spear* Department of Psychology and Center for Neuroscience, University of Wisconsin, Madison, WI 53706.

Damage to visual cortex (VC; areas 17, 18, and 19) in newborn kittens results in changes as far peripheral as the retina. Some retinal ganglion cells degenerate, and remaining ganglion cells send increased projections to the geniculate wing (retino-recipient zone of the pulvinar) and make anomalous synaptic connections in the lateral geniculate nucleus. We have begun to investigate the roles that growth factors play in these changes. In the present study, we examined the distribution of nerve growth factor receptors (NGFr) in the retina using immunohistochemical labeling with monoclonal antibodies to the NGFr. The antibodies were NGFR2 (Marano et al., 1987) and XIF1 (Clagett-Dame et al., 1990); results were similar.

Kittens received unilateral VC damage on the day of birth and survived 2 wk to >6 mo. NGFr labeling was compared in the two hemiretinae of each eye. In both hemiretinae of all animals, bands of label were present in the inner and outer nuclear layers, and diffuse labeling was present in the ganglion cell layer. This probably was Müller-cell labeling, reported by others. In all animals with up to 8-wk survival, there also was heavy pericellular labeling in the ganglion cell layer of the hemiretina projecting to the damaged hemisphere. The labeling included large cells presumed to be ganglion cells. Preliminary results suggest that the heavy pericellular labeling is not seen in animals with longer survivals (12 wk or more).

The results thus suggest that neonatal VC damage increases or maintains NGFr on retinal ganglion cells during a time when enhanced retinofugal projections develop. This raises the possibility that nerve growth factor is involved in compensatory changes after early VC damage.

183.16

NERVE GROWTH FACTOR EXPRESSION IN MOUSE SUBMANDIBULAR GLAND CELLS *IN VITRO*. M. Fahnestock* and Q. Lu, Dept. of Biomedical Sciences, McMaster University, Hamilton, Ontario L8N 3Z5, Canada.

Nerve growth factor (NGF) biosynthesis is regulated in a tissue-specific manner, and very high amounts of this neurotrophic molecule are found in the submandibular gland (SMG) of the mouse. Mouse SMG NGF is also hormonally regulated. We have developed two in vitro cell culture systems allowing us to study the mechanism and regulation of expression of NGF in SMG cells.

The first cell culture system uses primary cells from the SMG of male Swiss-Webster mice. These cells are grown in a collagen gel matrix in the presence of dihydrotestosterone, hydrocortisone, and triiodothyronine, and are primarily granular convoluted tubule cells (EM Durban, In Vitro Cell. Dev. Biol. 26:33, 1990). We show, using a mouse superior cervical ganglion bioassay, that these cells secrete NGF into the medium (7-10 ng/ml after 4 days) for at least one week.

The second cell culture system uses the mouse SMG cell line SCA-9 (T Barka et al, Lab. Invest. 42:656, 1980). We demonstrate that this cell line secretes biologically active NGF under a variety of growth conditions. In particular, SCA-9 cells do not require the addition of hormones to the medium to attain a level of NGF secretion of approximately 5-10 ng/ml.

This work is supported by NIH grant No. DE10448-01.

183.17

INFLUENCES OF NEUROTROPHINS ON DORSAL ROOT AXON BRANCHING *IN VIVO*. W.D. Snider, L. Zhang*, and I. Silos-Santiago, Dept. of Neurology, Washington University Medical School, St. Louis, MO, 63110. NGF promotes profuse neurite outgrowth from DRG explants *in vitro*. The function of this neurite-promoting activity during normal development is unknown. One way to address this issue is to assess the influence of exogenous NGF on dorsal root axonal arborizations during embryonic development *in vivo*. We have, therefore, injected embryonic rats with 10mg/kg of NGF *in utero* on days E15 and E16, and assesed dorsal root axon branching on E17 with the lipid soluble tracer DiI .

We have found that these NGF injections have pronounced effects on dorsal root axon growth in the spinal cord. Prominent axon bundles were apparent in the lateral regions of the dorsal horn which were not observed in control animals. Axons from these bundles penetrated gray matter and were longer than axons in the superficial dorsal horn in control animals. Axons in the most lateral region grew all the way across the midline and ramified extensively in the contralateral dorsal horn. These NGF-stimulated fibers, however, did not grow into the intermediate zone and ventral horn and thus were roughly constrained to their normal target fields. In contrast to axons in the lateral region, Ia fibers entering medially were less prominently if at all affected by exogenous NGF.

These results show that exogenous NGF stimulates dorsal root axon branching in the developing mammalian spinal cord *in vivo*. Furthermore, the influence of NGF may be selective for certain classes of dorsal root afferents. Influences of more recently identified neurotrophins in this same paradigm are under study.

183.18

NERVE GROWTH FACTOR AND NERVE GROWTH FACTOR RECEPTOR DISTRIBUTION IN TOUCH DOMES OF ADULT RAT SKIN - AN IMMUNOCYTOCHEMICAL STUDY. K.B. English* and N. Stayner. Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, Utah 84108

The distribution of nerve growth factor (NGF) and nerve growth factor receptor (NGFR) was examined in touch domes from adult rat hairy skin. These mechanosensitive structures are trophically dependent upon their afferent nerve supply, and likely serve as targets for regenerating type I afferent nerves (Horch, Neurosci Lett 32:281-284, 1982).

Tissue was immersed in cold isopentane, transferred to embedding medium (OCT) and snap frozen in liquid nitrogen (NGF staining), or fixed with freshly prepared 4% paraformaldehyde (PLP), prior to cryoprotection in sucrose and immersion in OCT. The primary antisera employed were rabbit anti-2.5S NGF (1:4,000; AB927,Chemicon) and mouse anti-rat NGFR (1:20; Clone 192, Boehringer Mannheim [BM]). Secondary antisera were goat anti-rabbit 1gG rhodamine conj (1:400; BM), and rabbit anti-mouse IgG fluorescein isocyanate conj (1:300, Serotec). Appropriate controls, including abolition of NGF staining following pre-incubation of NGF antiserum with 2.5S NGF (20 nM, Chemicon), were conducted to verify antisera specificity.

Immunoreactivity for NGF was intense and sharply restricted to the keratinocytes (excluding the stratum corneum) of the epidermis of touch domes. The epidermis surrounding touch domes and in tylotrich hair follicles associated with them was not stained. Merkel cells were immuno-negative for NGF but expressed positive staining for NGFR as did type I nerve endings.

The presence of NGF and NGFR in touch domes may be relevant to their proposed neurotrophic and/or neurotropic role during nerve regeneration and development. Supported by NIH grant 07983.

183.19

THE UPREGULATION OF NGF RECEPTOR mRNA EXPRESSION ASSOCIATED WITH COLLATERAL SPROUTING OF SENSORY AXONS IS BLOCKED BY IN VIVO ANTI-NGF TREATMENT. K. Mcarow*, Y. Kril and J. Diamond, Dept of Biomedical Sciences, McMaster University Medical Centre, Hamiltion, Ont. L8N 3Z5.

The collateral sprouting of intact cutaneous sensory neurons has been shown to be dependent on the presence of NGF. In our initial studies of the molecular events occuring in the DRG neurons undergoing sprouting or regeneration, NGF-R^{p75} mRNA was shown to be upregulated in the sprouting, but not the regenerating, neurons. We have now extended this work to examine both NGF-R^{p75} and NGF-R^{trk} mRNA expression in DRG neurons undergoing sprouting and in DRG neurons whose sprouting has been prevented through in vivo exposure to anti-NGF antiserum during the course of the experiment. The sprouting paradigm was set up by isolating intact dorsal cutaneous nerves (DCNs); the remaining intact DCNs will sprout into the denervated areas of skin. Animals were treated with either control serum or sheep anti-NGF antiserum, and at varying times after the initial surgery (1, 2, 4, 6, 8, 10, 12 days), animals were perfused, the appropriate DRGs were removed and frozen. Cryosections of these DRGs were taken and used for in situ hybridization (ISH) with ^{35}S-labeled riboprobes for NGF-R^{p75} and NGF-R^{trk}; hybridized slides were emulsion-coated and exposed for 1-3 weeks.

The results indicate that NGF-R^{p75} mRNA is increased by 8 days postop in DRGs from control serum-treated animals, and that this increase is restricted to the smaller neurons. In contrast, in DRGs from anti-NGF-treated animals, NGF-R^{p75} mRNA was maintained at control levels. Preliminary results for NGF-R^{trk} expression indicate an increase in expression at days 4-6 postop, followed by a decline to initial levels; there is no change in trk mRNA in DRGs from anti-NGF treated animals. It seems likely that increased NGF in the denervated skin lead to increased expression of NGF-R in neurons in which collateral sprouting is evoked. Supported by the Canadian NCE Network for Neural Regeneration and Recovery of Function, and NSERC.

183.20

NGF-DEPENDENT PERIPHERAL NERVE SPROUTING IS CORRELATED WITH INTRASPINAL SPROUTING AND FUNCTIONAL SYNAPTOGENESIS. B.A. Urschel* and J. Diamond. Department of Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, L8N3Z5.

We examined the possibility that in the adult rat, the NGF-dependent collateral sprouting of undamaged nociceptive nerves in skin will be correlated with intraspinal sprouting of primary nociceptive afferents, and that this involves a functional synaptogenesis within the ipsilateral dorsal horn. Since the immediate early gene c-fos is induced in spinal neurons post-synaptic to nociceptive afferents in response to noxious stimuli, an analysis of the pattern of c-fos induction in the spinal cord allows the functional connectivity between nociceptive afferents and spinal interneurons to be evaluated. Cutaneous nerve fields were "isolated" (by surrounding denervation) to induce peripheral nerve sprouting, but in some of the animals sprouting was prevented by daily injections of antibodies to NGF (anti-NGF). 16-21 days later the contralateral field was isolated to provide an "acute" control side. On day 22 (prior to the onset of peripheral sprouting on the acute side), nociceptive field sizes were determined, and the spinal cords processed for c-fos immunoreactivity. Increased functional synaptogenesis on the "sprouting" side was anticipated to increase the ratio of the number of c-fos positive cells/section on that side relative to the number on the acute (control) side. In unoperated animals, and in animals where peripheral sprouting was blocked by anti-NGF treatment, the ratio was not significantly different from 1.0. In the animals with peripheral sprouting the ratio was 1.8, a significant increase ($p<0.0001$). Thus intraspinal sprouting and synaptogenesis appears to correlate with peripheral nerve sprouting. The intraspinal sprouting will now be confirmed morphologically with substance P and CGRP immunoreactivity, and the possible involvement of centrally-originating NGF will be examined by intrathecal anti-NGF administration. Suppported by the Canadian Centres of Excellence for Neural Regeneration and Functional Recovery.

183.21

NGF DOES NOT REGULATE GAP 43 mRNA LEVELS IN ADULT RAT DORSAL ROOT GANGLION NEURONS. M.Hu-Tsai, C.J.Woolf[1], P.Emson[2] and J.Winter[3]* [1]Dept. of Anatomy, UCL, Gower Street, London, WC1E, 6BT. [2]MRC , Cambridge, CB2 4AT. [3] SIMR, 5 Gower Place, London, WC1E, 6BN.

GAP 43 synthesis coincides with axon outgrowth and its levels decline with the cessation of axon elongation (Skene, 1989, Ann. Rev. Neurosci. 12:127-56). Increased amounts of both GAP 43 mRNA and protein after sciatic nerve cut or crush suggest that a target-derived suppressive factor may regulate GAP 43 synthesis. Among the candidates for such a factor is NGF which controls GAP 43 expression in PC12 cells (Costello et al., 1990, J. Neurosci. 10(4):1398-1406). To determine the effects of NGF on GAP 43 gene expression on primary sensory neurons, we examined GAP 43 mRNA in adult rat dorsal root ganglion (DRG) neurons in culture in the presence of either NGF or anti-NGF antiserum using non-isotopic *in situ* hybridization with an alkaline phosphatase-linked cDNA probe. The results were quantitated at single cell level with a Seescan video-analysis imaging system. The timecourse of GAP gene expression over 1 week in culture show approximately 50% of DRG neurons are GAP 43 positive, in the presence of NGF or anti-NGF when examined after only 1h in culture. The number of GAP positive neurons increases steadily to approximately 95% at 7 days in the presence both of NGF or anti-NGF. In contrast, 95% of neurons cultured from ganglia that have been preaxotomized 4 days earlier *in vivo* are positive for GAP 43 mRNA after 1 h *in vitro*. Furthermore, the number of SP mRNA positive neurons drops from 12% (in the presence of NGF) to 0% (anti-NGF) after 1 week of growing in culture. In conclusion, our results show that GAP 43 mRNA levels in adult rat DRG neurons are independent of NGF, since the presence or absence of NGF does not affect GAP 43 mRNA levels. NGF may not be involved in initiating regeneration in sensory neurons.

MOLECULAR AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT III

184.1

THE NEUROPEPTIDE VIP: AN AUTOCRINE REGULATOR OF CELL GROWTH. A. DAVIDSON*, E.DICICCO-BLOOM, I.BLACK, M.DRAOUI, F.ZIA, G. LILING, M. FRIDKIN, D.E. BRENNEMAN, T.W. MOODY AND I. GOZES, CHEM. PATH., SACKLER MED. SCH., TEL AVIV UNIV.,ISRAEL; NEUROSCI. CELL BIOL. UMDNJ-ROBERT WOOD JOHNSON MED. SCH. PISCATAWAY, NJ08854; BIOCHEM. MOL. BIOL. GEORGE WASHINGTON UNIV. WASHINGTON DC20037; ORGANIC CHEMISTRY, WEIZMANN INST., ISRAEL; SECT. DEV.MOL.PHARMACOL., LAB. DEV. NEUROBIOL. NICHD, NIH, BETHESDA, MD20892.

THE GENE ENCODING VASOACTIVE INTESTINAL PEPTIDE (VIP) IS EXPRESSED IN THE RAT EMBRYO WITH A PEAK AT EMBRYONIC DAY 16 (E16) (GOZES ET AL., NEUROENDOCRINOLOGY 47:27;1988), SUGGESTING A ROLE IN DEVELOPMENT. PREVIOUS STUDIES IMPLIED THAT VIP CAN SERVE AS A PARACRINE GROWTH FACTOR, STIMULATING GLIAL PROLIFERATION AND CONSEQUENT NEURONAL SURVIVAL (BRENNEMAN ET AL,. PNAS 83:1159,1986; J. NEUROSCI. RES.25:386,1990). WE NOW SHOW THAT VIP FUNCTIONS AS AN AUTOCRINE GROWTH FACTOR IN NEUROGENESIS AS WELL AS IN CANCER PROMOTION.

IN THE DEVELOPING SYMPATHETIC NERVOUS SYSTEM, VIP mRNA WAS EXPRESSED AT HIGH LEVELS IN E15.5 SUPERIOR CERVICAL GANGLIA, WITH LOWER LEVELS AT BIRTH, A PATTERN CONSISTENT WITH PREVIOUS ANALYSIS OF VIP CONTENT. IMPORTANTLY, THE PEAK OF EXPRESSION CORRELATES WITH THE FUNCTION OF VIP AS A REGULATOR OF MITOSIS, DIFFERENTIATION AND SURVIVAL OF SYMPATHETIC NEUROBLASTS (PINCUS ET AL., NATURE 343:564,1990). FURTHERMORE, OUR OBSERVATIONS INDICATED THAT A NOVEL VIP ANTAGONIST (GOZES ET AL., ENDOCRINOLOGY 125:2945,1989; JPET 257:959,1991) INHIBITED VIP-INDUCED MITOGENESIS IN SYMPATHETIC NEUROBLASTS. IN ADDITION TO NEURONAL CELLS, A NUMBER OF CANCER CELL LINES EXHIBITED VIP AUTOCRINE BEHAVIOR. IN THE NON-SMALL CELL LUNG CANCER LINE NCI-H838, VIP mRNA WAS DETECTED AND VIP STIMULATED COLONY FORMATION THAT WAS INHIBITED BY THE ANTAGONIST. IN RELATED STUDIES, VIP mRNA WAS DETECTED IN ABOUT 50% OF LUNG CARCINOID CELL LINES TESTED. THE LINE NCI-H727 DISPLAYED THE HIGHEST CONTENT OF VIP mRNA, WHICH WAS INCREASED BY FORSKOLIN AS WELL AS THE PHORBOL PMA. IN CONCLUSION, VIP FUNCTIONS AS AN AUTOCRINE REGULATOR OF PROLIFERATION.

184.2

ONTOGENY OF TACHYKININ SYSTEMS OF THE RAT CAUDATE-PUTAMEN: IMMUNOCYTOCHEMISTRY AND *IN SITU* HYBRIDIZATION. L. Zhang, L. R. Lucas, J.E. Krauset, J. T. Weber* and R. E. Harlan, Department of Anatomy, Tulane Medical School, New Orleans, LA 70112 and Department of Anatomy and Neurobiology, Washington University School of Medicine., St. Louis, MO†.

The tachykinins, substance-P (SP) and neurokinin-B (NKB), are expressed in the caudate-putamen (CPu) and project to dopaminergic neurons in the substantia nigra and GABAergic neurons in the globus pallidus, respectively. To understand the independence of these two tachykinin systems, the ontogeny of SP and NKB in the rat CPu has been studied using both immunocytochemistry and *in situ* hybridization. Pups were studied at embryonic days E16, E18, E20 and E22 and postnatal days P0, P5, P10, P15 and P45. Immunocytochemistry was performed using antisera specific to SP and a NKB-related peptide, peptide 2 (PP2) on brain sections across all ages. On sections from P0, P5, and P10, *in situ* hybridization was perfomed using cRNA probes to locate neurons expressing the SP or NKB genes. At E16-E22, SP immunoreactive (ir) cell bodies were concentrated in the ventral lateral aspect of the CPu. At rostral levels, SP-ir cell bodies were not evident at E22 or later ages, while SP-ir fiber systems formed distinct patches beginning at E20. SP-ir patches became most obvious at P15, and declined in intensity at P45. At caudal levels, SP-ir cell bodies continued to be evident as late as P10. In contrast, PP2-ir, which also originated in the ventral lateral aspect of the CPu, was not detected until P10-P15. *In situ* hybridization indicated that the neurons containing SP-mRNA were concentrated in patches throughout the CPu at P0, P5, and P10. Expresion of the NKB gene was not evident in the CPu until P10 when a few labeled cells were seen in the ventrolateral region. The marked developmental differences between SP and NKB suggest independence of function of these two tachykinins in the CPu. Supported by NIH Grant # NS 24148

184.3

DEVELOPMENTAL EXPRESSION OF CORTICOTROPIN RELEASING FACTOR MESSENGER RNA IN THE OVINE BRAIN. C. Keiger [1], J.C. Rose[1], G. Brewer[2], and W.K. O'Steen[3*], Bowman Gray School of Medicine, Wake Forest University, Dept. of Physiology and Pharmacology[1], Dept. of Microbiology and Immunology[2], and Dept. of Neurobiology and Anatomy*[3].

Corticotropin Releasing Factor (CRF) is a neuroendocrine regulator of the hypothalamic-pituitary-adrenal axis. Using a quantitative RNase protection assay with an ovine antisense RNA probe, we determined the regional localization and expression of CRF mRNA at different gestational ages in the fetal sheep brain.

The amount of CRF mRNA in 75μg of total RNA was measured from the hypothalamus (HYPO), cerebral frontal cortex (CFC), hippocampus (HIPPO), amygdala (AMYG), and brainstem (BS) of 98 to 139 day gestation (DG) fetal sheep. The results are shown in the table below.

AGE	HYPO	CFC	HIPPO & AMYG	BS
98DG	0.48pg	N.D.	N.D.	N.D.
99	0.45	0.83pg	0.56pg	0.45pg
126	0.25	0.94	1.14	2.55
131	2.47	1.50	2.19	5.45
137	3.96	N.D.	N.D.	N.D.
139	1.67	N.D.	N.D.	N.D.

N.D. = Not Determined; Term = 145 ± 2 days; pg = picograms

These data show tissue-specific and developmentally regulated expression of CRF mRNA in the ovine fetal brain. The developmental distribution of CRF mRNA in extrahypothalamic areas suggests that CRF may be involved in neuromodulation/neurotransmission of functionally diverse pathways in the central nervous system during fetal life. Supported in part by NIH Grant HD 11210

184.4

DIFFERENTIAL TIMING AND SEXUAL DIMORPHISM OF VASOPRESSIN GENE EXPRESSION IN THE DEVELOPING RAT BRAIN. P. Szot* and D.M. Dorsa. GRECC, Seattle VAMC, WA 98108 and Dept. of Pharmacology, Univ. Washington, Seattle, WA 98195.

Neurons which express the vasopressin (VP) gene are located within hypothalamic nuclei including the paraventricular nucleus (PVN), supraoptic nucleus (SON) and suprachiasmatic nucleus (SCN), and are involved in the hormonal action of the peptide. Extrahypothalamic VP neurons are also present in he bed nucleus of the stria terminalis (BNST) and the medial amygdala (MA), and are thought to be involved in the neurotransmiter actions of the peptide.

In situ hybridization was performed using a 48 base oligonucleotide to the glycopeptide portion of the VP gene to measure levels of VP mRNA in 1,3,5,7,14,21,35 and 60 day old male and female Long-Evans (LE) rats in the 5 areas described above. VP mRNA in the PVN, SON and SCN was detectable in day 1 rats, but was only half the level measured in the adult. VP mRNA levels increased rapidly after day 1 and reached adult levels at 35 days. The development of the extra-hypothalamic nuclei was much slower than in the hypothalamic nuclei, and demonstrated the sexual dimorphism known to exist in these nuclei. In male rats, VP mRNA was not detected until postnatal day 3 for BNST and day 5 for MA. Both areas displayed a rise in cell number until postnatal day 7 for BNST and 14 for MA, reaching a plateau until day 21; followed by a significant increase to adulthood. VP gene expression in LE female rats developed at a significantly slower rate than males and was not detected until day 14 in the BNST and 35 for MA. We suggest that the pattern and timing of VP gene expression in BNST and MA neurons is the result of the organizational and activational effect of testosterone in the developing rat brain. (Support by the VA and NS 20311)

184.5

FUNCTIONAL EVIDENCE FOR V2 VASOPRESSIN RECEPTOR EXPRESSION IN CULTURED HIPPOCAMPAL NEURONS.

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Pharmacological studies have suggested the existence of a V_2 vasopressin (AVP) receptor in the CNS but to date there has been no direct evidence of AVP-induction of cAMP in neurons. Our experiments using cultured hippocampal nerve cells have revealed AVP-induction of cAMP via V_2 receptor activation in embryonic neurons. Hippocampal nerve cells from E18 rat pups were cultured onto PEI coated dishes in serum containing medium. Following 1-5 days in culture neurons were exposed to AVP or other peptides. AVP induced a dose dependent accumulation of cAMP in cultured hippocampal neurons that was maximal at 250 nM. The dose response revealed an inverted-U shape function in that higher concentrations of AVP showed a decline in cAMP levels. The time course for AVP-induction of cAMP formation was maximal at 15 min and was also characterized by an inverted-U shape function. Peptide specificity studies indicated that the vasopressin metabolites, AVP[4-8] and AVP[4-9] were more potent than the parent peptide in stimulating cAMP formation while oxytocin was equally as effective as AVP. The receptor linked to adenylate cyclase was, however, specific in that the V_1 agonist Phe^2,Orn^8Vasotocin did not induce cAMP formation at any of the concentrations tested whereas DAVP, a selective V2 agonist showed a clear dose dependent induction of cAMP formation. The ability of AVP to induce cAMP formation was maximal at 4 days in culture. These data are the first biochemical evidence for expression of a V_2 receptor in neurons and suggests that the V_2 receptor may play a critical role during development of the CNS.

Supported by NIH grant MH46036 to R.D.B.

184.6

INVOLVEMENT OF THE cAMP RESPONSE ELEMENT IN TISSUE AND AGE SPECIFIC REGULATION OF THE SOMATOSTATIN GENE. C. L. Szymeczek-Seay* and D.E. Millhorn, University of North Carolina , Chapel Hill, North Carolina 27599

DNA-protein binding studies from our laboratory have indicated a role for the cAMP response element (CRE) in tissue- and age- specific regulation of the somatostatin (SOM) gene. Early studies of the regulation of the SOM gene, performed in tissue culture systems, showed that the CRE bound the cAMP response element binding protein and was involved in the cAMP responsiveness of the gene. Our work, which involves the isolation of nuclear protein from tissue and measurement (by gel shift assay) of its capacity to bind to the CRE, demonstrates that protein from brain and non-brain tissues, and protein from brain regions of different ages, form varied types and levels of protein-CRE complexes. The binding of proteins to the CRE, a putative cis-acting element, suggests a role for these proteins as transcriptional activators of the SOM gene. Thus, our data suggests that the different types of protein-CRE complexes we observe with proteins from varied tissues and ages may be partly responsible for the different levels of SOM mRNA observed at different developmental ages in different tissues. Protein from liver, a non-brain, non-SOM expressing tissue, forms a complex with the CRE which is distinctively different from the complexes formed with proteins from other low-expressing tissues (i.e.adult cerebellum) or from high expressing tissues (adult diencephalon and adult pancreas). When proteins from SOM expressing tissues are extracted from rats of different ages and bound to the CRE, one observes a characteristic DNA protein complex for each particular tissue, but the levels of protein binding vary, and indeed are absent in both cerebellum and diencephalon at 28 days of age. Therefore our data suggests that the CRE may be mediating more than just the cAMP responsiveness of SOM, and perhaps more importantly,that it is possible to dissect out the mechanisms by which gene expression, hitherto studied primarily in cell culture, is operating in a native, physiological milieu.

184.7

CGRP-PHENOTYPE IS REGULATED BY RESINIFERATOXIN IN DEVELOPING DORSAL ROOT GANGLION NEURONS

D.V. Agoston[1], W.J. Nyhus[1], A. Szallasi[2*], G. Jakab[3] Lab. of Developmental Neurobiology, NICHD[1], NCI[2], Lab. of Experimental Neuropathology, NINDS[3], NIH, Bethesda MD 20892

Resiniferatoxin (RTX), an ultrapotent analog of capsaicin has been reported to release neuropeptides (calcitonin gene-related peptide (CGRP), substance P, etc.) from the central and peripheral terminals of primary afferent fibers. In order to determine the susceptibility of developing sensory neurons to this neurotoxin and to study the developmental regulation of CGRP gene expression we treated primary dissociated dorsal root ganglion (DRG) cell cultures with 0.3-30 nM of RTX. Immunocytochemical analysis revealed that RTX treatment produced rapid, dose-dependent enhancement of CGRP biosynthesis in small and medium sized neurons; however a number of large neurons also showed detectable immunoreaction. The diameter of CGRP-immunoreactive nerve fibers and their varicosities markedly raised probably due to the increased peptide transport and release. This treatment (maximum 48 hours) caused neither significant loss of neurons nor visible degenerative changes at the light microscopic level. The increase of CGRP release and the subsequent upregulation of the CGRP biosynthesis provoked by RTX may simulate physiological processes evoked by pathological metabolic changes e.g. ischemia or acidosis.

184.8

A NOVEL TRANSCRIPT HOMOLOGOUS TO SUBSTANCE P RECEPTOR mRNA IN THE DEVELOPING RAT SPINAL CORD. Paul A. St. John[1,*], Sherry L. Stephens[1], Linda Nunan[2], and Josephine Lai[2], Depts. of Anatomy[1] and Pharmacology[2], Univ. of Arizona, Tucson, AZ

Previous work from one of our laboratories [St. John and Stephens (1992) Dev. Biol. 151:XXX] showed that neurons dissociated from the E14 rat spinal cord express receptors for substance P (SPRs) within 24 hr *in vitro*, and that the number of SPRs increases steadily in culture for about 1 week. We now have measured levels of SPR mRNA in the rat spinal cord during *in vivo* development. Poly(A)$^+$ RNA prepared from the spinal cords of rats at ages E14 through birth and from adults was hybridized under stringent conditions to a ^{32}P-labeled cRNA probe derived from the C-terminal 588 bases of the rat SPR cDNA (generously provided by J.E. Krause, Washington Univ.) and analyzed by ribonuclease protection. The results showed that mRNA for the SPR is already present in the rat spinal cord at E14, before the end of neurogenesis. The protected 588-base fragment showed a 35% increase in abundance from E14 to E20, and a further 3-fold increase in the adult. A second protected fragment of approximately 470 bases was also detected at all ages examined. At E14, this band was 2.5-fold more abundant than the 588-base band, but its level decreased 6-fold by E20. In the adult rat spinal cord, this 470-base fragment constituted a minor signal. Two lines of evidence suggest that the second fragment does not arise from degradation or cross-hybridization with mRNA for other known neurokinin receptors. We are considering the possibilities that the 470-base fragment is derived from an alternatively spliced mRNA or from a slowly spliced nuclear transcript. Supported by NSF 8808506, NIH NS29657, ADCRC WC9311, and AHA IG-2-44-91.

184.9

ACTIVITY DEPENDENT ALTERATIONS IN SUBSTANCE P AND CGRP IMMUNOREACTIVITY IN NEURONS AND FIBERS IN THE EMBRYONIC CHICK SPINAL CORD. B Mendelson.* Dept. of Anatomy, U. of Ark. for Med. Sci., Little Rock, AR 72205.

In some regions of the nervous system, changes in patterns of neural activity can alter synaptic connectivity during development. To ask if patterned activity is important for the appropriate formation of neural circuitry in the spinal cord, d-tubocurarine (dtc) was administered to developing chick embryos and subsequently neuropeptide immunoreactivity was examined. In the chick, dtc directly affects spinal cord circuits, preventing the alternating bursts in motoneuronal pools. In the present study 2-3 mg/d of dtc was administered beginning at embryonic day 5 (E5). At later stages (E8-E18) the expression of the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) was assayed immunohistochemically and compared to similar immunostaining in Tyrodes-treated control embryos processed in parallel. Dtc-treatment caused a decrease in SP-like immunoreactivity (SP-LI) in a particular subset of neurons. Following the treatment, interneuronal SP-LI was dramatically reduced as was the SP-LI in most fibers in the dorsal horn. However, a specific subpopulation of fibers that project from lateral to medial just ventral to lamina 2 were much more intensely immunostained after dtc-treatment. Following dtc-treatment, motoneuronal CGRP-LI was decreased, but a subpopulation of fibers located ventral to lamina 2, projecting to the midline were more heavily immunostained in a similar fashion to the SP-LI fibers. Therefore, normal patterns of neural activity appear to be necessary for the appropriate expression of certain neuropeptides and the altered pattern of activity produced by dtc-treatment affects only a specific subpopulation of neurons. Supported by BRSG 2 S07 RR 05350 29

184.10

EFFECT OF ACTIVITY ON ENKEPHALIN AND SUBSTANCE P mRNA IN THE DEVELOPING CHICK SPINAL CORD. L.K. Garner*, B.M. Mendelson2, K.M. Albers, M. Kindy and B.M. Davis. Depts. of Pathology & Anatomy and Neurobiology, Univ. of Kentucky Med. Cntr., Lexington KY 40536; 2Dept. of Anatomy, Univ. of Arkansas Med. Sch., Little Rock, AR 72205.

Synaptic activity is thought to be a major factor in establishment of synaptic connections in the central nervous. Numerous studies have used curare to block synaptic activity to study its role in development. In our experiments, ontogeny of the mRNAs for the prohormones coding for substance P (SP) and enkephalin (ENK) was determined for the chick spinal cord. In addition, curare was applied to chick embryos during the period that primary afferents innervate the dorsal horn to study changes in expression of these mRNAs associated with decreased activity. ENK mRNA first appears in the dorsal horn at approximately stage (st) 25 and in the region of the intermediate laminae at st 27. By st 38 ENK mRNA was localized to the dorsal horn, intermediate laminae and column of Terni. SP mRNA was first seen in the dorsal root ganglion at st 30. By st 38 it was localized to the dorsal and ventral horn, and dorsal root and sympathetic ganglia. To examine the effect of decreased activity, chick embryos were treated with Tyrode's buffer, 1mg, 2mg or 3mg of curare per day from st 25 through st 39. *In situ* hybridization showed a dose dependent down- regulation for both ENK and SP mRNA in the majority of the gray matter and dorsal root ganglia (for SP). However, SP mRNA expression in the ventral horn did not appear to be affected by curare treatments. These results were confirmed by Northern analysis. Supported by NIH NS25617 to BMD.

184.11

EFFECT OF CURARE ON ENKEPHALIN mRNA IN THE DEVELOPING CHICK BRAIN . M.A. Young, L.K. Garner, B.M. Mendelson2, and B.M. Davis*. Dept. of Anatomy and Neurobiology, Univ. of Kentucky Med. Cntr., Lexington KY 40536; 2Dept. of Anatomy, Univ. of Arkansas Med. Sch., Little Rock, AR 72205.

Application of curare during development has been shown to prevent motoneuron (MN) cell death. Studies in our laboratories have shown that spinal cord mRNA production in the developing chick is down-regulated following curare application. In the study presented here, *in situ* hybridization was used to examined the ontogeny of the mRNA coding for the prohormone for enkephalin (ENK) in the chick brain. In addition, embryos were treated with Tyrode's buffer, 1mg, 2mg or 3mg of curare per day from st 25 through st 39 followed by Northern analysis of whole brains. ENK mRNA can be detected in the developing basal ganglia and in the area of the acc. occulomotor nucleus by st 25 (E5). By st 26 heavily labeled cells are also seen in the diencephalon, rostral mesencephalon and medulla. In the medulla, labeled cells form a rostral-caudal band immediately ventral to the ventricular zone. By st 30, most of the ENK positive nuclei that can be found in older embryos are present. These include all nuclei described as ENK positive by immuno studies, plus additional cell groups, e.g clusters of ENK mRNA positive cells flanking the anterior commisure and fasciculus prosencephali lateralis. Curare treatment often retarded the maturation of the embryos by 0.5- 1 stage by day 14 (the time when brains were removed). However, no difference was seen in the amount of total RNA/mg wet wt. Northern analysis showed a significant decrease in the amount of ENK mRNA in chicks treated with 2.0 or 3.0 mg curare/day. Supported by NIH NS25617 to BMD.

184.12

Developmental-Specific Changes in Opioid Peptide Gene Expression and AP-1 Proteins in Rat Hippocampi after Seizures. **K.R. Pennypacker*, M.K. McMillian, J. Douglass, and J.S. Hong. Natl. Inst. of Environ. Health Sci. Lab of Mol. and Integ. Neurosci. Research Triangle Park, NC 27709**

Administration of the seizure-inducing drugs, kainate (KA) and pentylenetetrazol (PTZ) to adult rats causes an increase in AP-1 proteins and opioid gene expression in the hippocampus. We analyzed hippocampi from postnatal day 7 (P7) and postnatal day 14 (P14) rat pups after KA and PTZ treatment for AP-1 transcription factors and opioid peptide gene expression. At both ages, the rats exhibited seizure behavior. Neither AP-1 proteins nor opioid peptide genes were affected in the hippocampus from P7 rats; however, there was a high basal level of AP-1 DNA binding. At P14, both KA and PTZ treatment increased AP-1 protein expression in the hippocampus, but only KA increased preproenkephalin and neither drug treatment affected preprodynorphin. These data suggest that the neuronal pathways involved in seizure behavior are completed early in development, while the pathways that regulate the induction of AP-1 protein and opioid peptide genes are not intact until P14 or later. Expression as well as seizure-induction of the preproenkephalin gene in the rat hippocampus occurs earlier than the preprodynorphin gene.

184.13

ONTOGENY OF THE PROENKEPHALIN SYSTEM IN THE RAT AMYGDALA: DETECTION BY IN SITU HYBRIDIZATION. D. D. Song* and R. E. Harlan. Dept. of Anatomy, Tulane Univ. School of Medicine, New Orleans, LA, 70112.

In situ hybridization was performed on coronal cryostat sections through the amygdala on embryonic day 14 (E14), E16, E18, E20, postnatal day 0 (P0), P5, P10, P15 and P20, using a random-primer labeled (^{35}S) 435 bp cDNA complementary to nucleotides encoding amino acids 56 to 200 of rat proenkephalin.

Earliest expression of PPE mRNA in the amygdala occurred at E16 in the central nucleus (Ce), immediately ventral to concurrent PPE expression in the ventrolateral caudate-putamen (CPu). Expression in the Ce was of moderate intensity. By E18, PPE expression in the Ce had increased and also occurred in the sublenticular substantia innominata (SLSI) at high levels. By E20, heavy labelling of cell bodies was also found in the posterior portion of the medial amygdala and in the intramedullary gray. By P0, weak labelling was observed in the lateral division of the bed nucleus of the stria terminalis (BSTL). By P5, an adult-like distribution was present. In addition to the already mentioned nuclei, heavy labelling of densely packed cells was also present in the intercalated nucleus, moderate labelling in the BSTL, and weak labelling in the cortical (Co), basomedial (BM), anterior portion of the medial (MeA), and ventral portion of the basolateral (BLV) amygdaloid nuclei. By P10, the intensity of labelling in the SLSI had decreased to a moderate level as seen in the adult. By P15, labelling in the BM and BLV had increased to moderate levels. By P20, PPE expression in the MeA had increased to heavy amounts and in the Co to moderate amounts. PPE expression in the amygdala was essentially adult-like both in distribution and intensity by this age. A continuum of prominent and early PPE expression in the ventrolateral CPu, Ce, medial amygdala, SLSI, and BSTL may suggest that these structures are related as parts of a common neuroanatomical system. Supported by NS24148 (REH).

184.14

G PROTEINS AND ALTERED NEURONAL PLASTICITY IN MORPHINE EXPOSED RATS. A.Gorio*, A.M.Di Giulio, M.L. Malosio, C. Finco, A.Bertelli, and P.Mantegazza. Dept. of Medical Pharmacol., Univ. of Milano, Italy.

Perinatal exposure to morphine affects neuronal plasticity in the rat brain. In morphine-exposed animals the regeneration of neurotoxin injured serotoninergic axons is markedly impaired, as well as the collateral sprouting of serotonin (5-HT), dopamine (DA) and met-enkephalin (ME) axons triggered by the neonatal lesion of noradrenergic nerve terminals. By in situ hybridization we have monitored the gene expression of specific molecules. The striatal morphine induced ME increase is correlated by a parallel increase of preproenkephalin A mRNA, while, in the same area, the reduced levels of DA are correlated by a reduced expression of D1 receptors. Morphine causes also a significant increase of Gs protein expression throughout the brain and a parallel increase of the protein amount detected by western blotting. G2i and Go subunits are apparently unaffected. Morphine exposure markedly reduces synapsin 1 expression. All these changes are normalized by neonatal 6-OHDA lesion, as if the denervation stimulus would counteract morphine effects. The data suggest a new role for G proteins in neuronal plasticity.

184.15

EFFECTS OF PRENATAL COCAINE ON THE DEVELOPMENT OF CEREBRAL CORTEX OF DUTCH BELTED RABBITS L. Jones, I. Fischer, P. Levitt* . Dept. Anatomy & Neurobiology. Medical College of PA, Philadelphia, PA, 19129.

There is clinical evidence of profound developmental behavior anomalies experienced by children who have been exposed to cocaine prenatally. This study was undertaken to establish possible structural correlates in the developing brain. Pregnant rabbits were treated with cocaine intravenously starting on the 8th day of gestation to the 29th day, using a dosage of 4mg/kg twice a day. The progeny were taken on various postnatal (P) days between birth to P14. Specific cellular changes were compared during development using a variety of immunocytochemical markers. Anti-glial fibrillary acidic protein (GFAP) was used to examine astrocyte differentiation; anti-neurofilament-H (NF-H) was used to assess axons; anti-microtubule-associated protein 2 (MAP2) to assess dendrites. At all ages examined there were no differences between cocaine-treated and normal animals in the distribution or number of GFAP or NF-H positive profiles, suggesting normal glial and axonal development. In contrast, MAP2 staining revealed short, tortuous apical dendrites of pyramidal neurons in the cerebral cortex. This was evident at birth and at all ages examined. Other structural proteins present in dendrites, such as a and b tubulin, also showed similar staining patterns with the corresponding antibodies. The data suggest a profound and specific effect of prenatal cocaine exposure on dendritic development, perhaps by altering growth patterns and/or molecular assembly of dendritic proteins. (Supported by NIDA grant DA06871-01)

184.16

LOCAL PERFUSION OF (-)SULPIRIDE VIA MICRODIALYSIS ALTERS K^+-EVOKED DOPAMINE RELEASE IN THE NEOSTRIATUM OF DEVELOPING AND ADULT RATS. S.L. Andersen* and R.A. Gazzara. Center for Developmental Psychobiology and Dept. of Psychology, SUNY-Binghamton, Binghamton, NY 13902-6000.

A within-subject dose-response analysis of (-)sulpiride was conducted in urethane-anesthetized 5, 10-11, 15-16, and 21-22 day old rat pups and adult rats. (-)Sulpiride was administered locally via a microdialysis probe located in the neostriatum. After establishment of a baseline for K^+-evoked release of dopamine (DA), increasing doses (0.1, 1, 10, 100 μM in artificial cerebrospinal fluid) of (-)sulpiride were sequentially perfused during subsequent samples of K^+-evoked DA release. Samples were collected every 15 min and DA was measured by HPLC-ED.

While presynaptic DA autoreceptors are able to modulate K^+-evoked DA release following local perfusion of the D-2 agonist quinpirole (Andersen and Gazzara, Soc. Neurosci. Abstr. 17: 364, 1991), unexpected results were obtained using the D-2 antagonist (-)sulpiride. Local perfusion of (-)sulpiride produced significant dose-dependent increases in K^+-evoked DA release in the 5, 10-11, and 15-16 day old rat pups only. In contrast, (-)sulpiride produced decreases in K^+-evoked DA release in the 21-22 day old pups and the adult rats at all doses tested.

These results suggest that locally-administered (-)sulpiride produces age-dependent effects on K^+-evoked DA release in the neostriatum.

Supported by NIH BRSG Grant S07RR07149-17.

184.17

EFFECTS OF LOCAL ADMINISTRATION OF AMPHETAMINE ON NEOSTRIATAL DOPAMINE RELEASE AS MEASURED BY IN VIVO MICRODIALYSIS IN DEVELOPING AND ADULTS RATS. R.A. Gazzara* and S.L. Andersen. Center for Developmental Psychobiology and Dept. of Psychology, SUNY-Binghamton, Binghamton, NY 13902-6000.

Amphetamine (AMPH) produces atypical responses when administered to immature organisms. For example, Trent et al. (Eur. J. Pharmacol. 204: 265, 1991) have shown that AMPH produced a increase in firing rate in nigral dopamine (DA) neurons in rat pups at postnatal days (PND) 1-6 compared with a decrease in firing rate in adult rats. In an attempt to further elucidate the neurochemical mechanisms that may underlie these AMPH effects during development, we have employed in vivo microdialysis to determine the effect of locally-administered AMPH on the extracellular level (ECL) of DA in the neostriatum of PND 5 pups and adult rats.

In urethane-anesthetized PND 5 pups and adult rats, baseline levels of DA were measured during perfusion with artificial cerebrospinal fluid (aCSF). Subsequent to establishment of baseline levels, increasing doses of AMPH (conc. in μM: 0.01, 0.1, 1, 10, 100, 1000; dissolved in aCSF) were perfused sequentially through the dialysis probe. The 0.01 μM dose produced an increase in ECL of DA in the adult rats, but had no effect in the PND 5 pups. The 0.1 μM dose produced an increase in ECL of DA in the PND 5 pups, however this increase was smaller than in the adult rats. At all doses tested, AMPH produced a greater increase in ECL of DA in adult rats than in PND 5 pups. AMPH-induced increases in ECL of DA plateaued at the 100 and 1000 μM doses in the PND 5 pups, whereas no such plateau was seen in the adult rats.

These results suggest that AMPH is much more efficacious in releasing DA from nigrostriatal terminals in adult rats than in developing rats.

Supported by NIH BRSG Grant S07RR07149-17.

184.18

EFFECTS OF DEVELOPMENTAL AGE IN RATS ON THE RELATIVE CONTRIBUTION OF LEUCINE RECYCLED FROM THE DEGRADATION OF PROTEIN TO THE PRECURSOR POOL FOR PROTEIN SYNTHESIS Y. Sun*, G. E. Deibler, & C. Beebe Smith. LCM, NIMH, Bethesda, MD 20892.

The autoradiographic determination of rates of cerebral protein synthesis with L-[1-^{14}C]leucine (Smith et al, *PNAS* **85**:9341, 1988) takes into account recycling of unlabeled leucine derived from protein degradation into the precursor pool for protein synthesis. The degree of recycling can be evaluated by measuring λ, the ratio of the steady state leucine specific activity in the precursor pool (tRNA-bound) to that of the plasma. We have shown previously that in adult rat brain recycling does occur and that the value of λ is significantly less than 1.0. As a prelude to studies of the developmental time course of regional rates of protein synthesis we have evaluated λ for the whole brain at 10, 14, 21, & 35 days of age. Rats were administered, i.v., a programmed infusion of [^{3}H]leucine designed to achieve and maintain for 60 min a constant arterial plasma [^{3}H]leucine specific activity. The [^{3}H]leucine specific activities were measured in the tRNA-bound extracts of brain and in the acid-soluble fraction of timed plasma samples; the values of λ are shown in the table. Values are lowest in the 10 day old rats and increase with developmental age until 35 days at which time the value of λ is the same as that of adult rats. These results show that early in development a greater fraction of the precursor leucine pool is derived from protein breakdown. These values of λ can now be used in the determination of rates of cerebral protein synthesis in developing rats.

Age (days)	N	Value of λ
10	2	0.41 ± 0.02
14	6	0.52 ± 0.01
21	5	0.53 ± 0.02
35	4	0.58 ± 0.01
Adult*	9	0.58 ± 0.01

Values are means ± SEM.
* Sun et al., *J Neurochem* (in press).

184.19

MOLECULAR MECHANISMS OF GERMINAL MATRIX VASCULAR MATURATION.
L.R. Ment*, W.B. Stewart, M.F. Einat, C.C. Duncan and F.H. Ruddle. Yale University Sch. of Medicine, New Haven, Ct. 06510.

Intraventricular hemorrhage (IVH) or hemorrhage into the germinal matrix (GM) occurs in over 45% of neonates of less than 34 weeks gestational age. There is evidence that the risk period for IVH is the first 4 to 5 days after birth; this risk period is independent of gestational age. We have used the newborn beagle pup as a model for this vascular disorder of the GM. The risk period for IVH in this model is also the first four days. Our previous work has shown that laminin deposition increases markedly during this period. The aim of the present study was to test the hypothesis that maturation of the vessels was regulated by activation of a number of genes during the first few days of life. We isolated microvessels from the germinal matrix of 1 and 10 day old pups. A cDNA library was prepared for each age and a differential screen was performed to look for gene expression that was present at day 10 but not at day 1. The screens were performed on 10,000 pfu using probes made from poly A plus RNA isolated from the GM microvessels of 1 and 10 day old pups. Nine positive clones were identified. A second screen was performed and the inserts of the positive clones were amplified by PCR. The cDNA insert of one positive differential band was sequenced and found to be homologous to carboxypeptidase E, a vasoactive protein known to be present in brain. These data indicate a perinatal induction of genes related to both structural and functional maturation of the vessels.

185.1

REGULATION OF ASTROCYTE DEVELOPMENT BY INTERLEUKIN-1 AND TUMOR NECROSIS FACTOR. Y. Aizenman*, D. Maciejewski, J. Holliday, F.E. Bloom and R. Milner. Department of Neuropharmacology,Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA 92037.

We have investigated the effect of Interleukin-1β (IL) and Tumor Necrosis Factor-α (TNF) on the mRNA levels of 3 proteins important in astrocyte development and function: Glial Fibrilary Acidic Protein (GFAP), Glutamine Synthetase (GS), and S100. To prevent indirect effects of cytokines via the microglia they were eliminated from the astrocytic cultures as follows: astrocytes were grown in vitro for 7 days, followed by shaking (200 RPM) for 6 hrs in serum free medium containing L-leucine methyl ester (10mM). After an overnight incubation with 10% serum and repetition of the shaking procedure, the cultures were found to be devoid of microglia as detected by florescent Low Density Lipoprotein. Treatment of these purified astrocyte cultures for 24 hrs with IL(10units/ml) caused an increase in the mRNA levels of GFAP, GS and S100. These findings taken together with the increase of IL during astrocyte development in vivo suggest a critical role of this cytokine in astrocyte differentiation. TNF(20ng/ml), on the other hand, caused a large decrease in GFAP mRNA, a moderate decrease in S100 mRNA, and no change in GS mRNA. These observations are consistent with the idea that, unlike IL, TNF is not a differentiating factor but a proliferative one for astrocytes. (Supported by MH47680)

185.2

PROLIFERATIVE EFFECT OF IGF-I ON IFAP-70/280kD-CONTAINING IMMATURE ASTROCYTIC CELLS IN CULTURE. **H.-Y. Yang*, N. Lieska, P.-Y. Chien, V. Kriho and G.D. Pappas. Department of Anatomy and Cell Biology, University of Illinois, Chicago, IL 60612.**

We have recently demonstrated that four differentiation-specific subtypes of non-stellate astrocytes can be defined in primary cultures of neonatal rat brain by use of intermediate filament (IF) cytoskeletal markers, specifically an early marker, IF-associated protein (IFAP)-70/280kD [Brain Res. 573(1992), 161]. Because of the expression of insulin-like growth factor-I (IGF-I) in fetal rat brain and its known proliferative and differentiation effects, it is hypothesized that IGF-I may be implicated in macroglial development. Due to the early developmental nature of IFAP-70/280kD, an increased proportion of IFAP-containing cells would be indicative of a proliferative effect of IGF-I in the system. To test this, primary cultures were established from neonatal rat brain and maintained in serum-containing medium for two weeks prior to transfer to: 1) serum-free medium, 2) serum-free medium plus IGF-I (100ng/ml), or 3) regular serum medium. Two days later, the phenotypic composition of the cells was determined by double-label immunofluorescence microscopy to delineate: IFAP(I)$^-$ GFAP(G)$^+$, I$^+$G$^+$, and I$^+$G$^-$ cells. The IGF-I group exhibited twice as many non-stellate cells as the serum-free control due to a two-fold increase in the number of I$^+$G$^+$ cell types and a three-fold increase in the minor number of I$^+$G$^-$ cells. It was concluded that IGF-I is mitogenic for the two IFAP$^+$ cell types. Moreover, based on the observation of mitoses among the IFAP$^+$ cells and the less differentiated state indicated by the IFAP marker, IGF-I appears not to have a maturation effect on those astrocyte stages. **Supported by NIH grant NS26395.**

185.3

GIANT TWO-DIMENSIONAL GEL ELECTROPHORESIS REVEALS POTENTIALLY NOVEL PROTEINS RELEASED BY INTERLEUKIN-1β (IL-1β) TREATED ASTROCYTES. J. W. Chang*, D. A. Young, P. D. Coleman, and M. K. O'Banion. Depts. of Neurobiology and Anatomy, Medicine, and Neurology, Univ. of Rochester Med. Cntr., Rochester, NY 14642.

Astrocytes are known to secrete factors important for normal neuronal development, survival, and recovery following injury or disease. Failure of these astrocytic responses may play a role in selected neuronal deficits noted in Alzheimer's disease. IL-1β, a cytokine that is produced by microglia may be a mitogen for astrocytes and is known to influence the release of astrocytic factors. To more fully characterize this response we have utilized high resolution giant two-dimensional (2-D) gel electrophoresis to investigate the secretion of newly synthesized proteins in primary cultures of rat astrocytes treated with recombinant human IL-1β.

Two-D gel separations of ^{35}S-labeled proteins recovered from the media at selected times after IL-1β treatment revealed at least three groups of IL-1β induced proteins: Group A, a doublet at 24-25 kDa, pI 5.6; Group B, 4 isoforms at 50-53 kDa, pI 6.2-6.5; and Group C, consisting of about 10 isoforms at 125-130 kDa, pI 6.3-7.1. Of these, groups A and C represent nearly *de novo* inductions (at least 25-fold) while group B was induced by about 5-fold. The induction of group B proteins occurred within 3 h of IL-1β treatment whereas inductions for the other groups were not observed until 8 h. Based on its location on the 2-D gels we speculate that group B is plasminogen activator inhibitor type 1, that has been previously shown to be induced by IL-1β. Studies to determine the identity of these and the other IL-1β induced proteins are underway. Their further characterization and molecular cloning may reveal novel molecules that play a role in neuronal/glial interactions. [Supported by LEAD award AG09016 and RO1 AG01121 to P.D.C.]

185.4

NUCLEAR LOCALIZATION OF CREATINE KINASE (CK) IN CULTURED ASTROCYTES (AST) BY CONVENTIONAL AND CONFOCAL MICROSCOPY: A ROLE IN PROLIFERATION. P. Manos* and J. Edmond. Dept. Biol. Chem., MRRC, UCLA Sch. of Med., Los Angeles, CA 90024

CK is an enzyme important in brain energy homeostasis. The subcellular localization of CK was examined in primary cultures of AST using immunofluorescent labeling methods and detection by both standard fluorescence and confocal laser-scanning microscopy. Using standard microscopy, the pattern of CK staining was uniform throughout the cell cytoplasm and appeared to stain the nuclear region intensely. Staining of CK in the nuclear region co-localized with the Hoechst nuclear stain. The structural details of CK in the nuclear region were examined in serial optical sections taken through the cell monolayer with a confocal microscope. CK staining was present in each section and had a granular, particulate pattern indicating a nucleoplasmic distribution. Nuclear CK was examined in AST cultured at either a low or high cell density; 92% of the low density AST contained intense CK-stained nuclei, whereas only 47% of the high density confluent AST had intense CK-stained nuclei. The rate of DNA synthesis was 8-fold higher in low density AST than in high density AST. A nuclear fraction from low density AST cultures had higher CK activity than that prepared from high density cultures. These data demonstrate the presence of high CK in the nucleus of proliferating AST and suggest a preferential localization of CK to the nucleus to meet the energy demands of cell division and/or nuclear function.

185.5

NON-NEURONAL LOCATION OF GABA$_A$ AND NMDA RECEPTORS IN EARLY POSTNATAL RAT CEREBELLUM. G.D. Pratt, J.- M. Fritschy & H. Mohler* Inst. of Pharmacology, Univ. of Zurich, CH-8006 Zurich, Switzerland.

The involvement of both GABAergic and glutamatergic receptors in the structural development of the cerebellum has been investigated by immunocytochemical receptor-mapping. Polyclonal antibodies to cDNA-derived peptides of the rat NMDA receptor and to the GABA$_A$ receptor α_1, α_3, δ sub-units plus a monoclonal antibody (bd17) recognising both β_2 and β_3 sub-units were incubated with free-floating sections and processed using the avidin-biotin immunoperoxidase technique. In Purkinje cells, at PN1, staining was evident for all five antibody categories with immunoreactivity (IR) increasing up to PN10. Thereafter, α_1 and α_3 subunit-IR in these neurones persisted until PN21 and whereas bd17 and NMDA receptor-IR was much weaker by comparison, δ IR was completely lost. Both molecular and granular cell layers were strongly immunoreactive for the α_1, δ and bd17 antisera at PN21. Conversely, NMDA receptor IR was more pronounced in the molecular layer than the granular cell layer. In the external granular layer, stained filamentous parallel processes corresponding to Bergmann glia were recognised selectively by antibodies to the α_3, δ and NMDA receptors (most strikingly up to PN10), but not to α_1 and bd17. Non-neuronal elements in white matter and the developing molecular layer were stained by bd17 up to PN10. At PN21, Bergmann glia, although faint, were still apparent for α_3 but not obvious for δ IR. In summary, during the period of cerebellar granule cell migration, Bergmann glial cells show selective expression of both excitatory and inhibitory receptors which are possibly involved in regulatory trophic neuronal/glial interactions.

185.6

REGULATION OF POTASSIUM CHANNEL mRNA'S IN RAT SCIATIC NERVE. S.Y. Chiu, A. Messing, M. Behan*, and S.S. Scherer. Dept. of Pathobiological Sci., Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI 53706; Dept. of Neurology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Recent electrophysiological studies suggest that potassium channel expression in Schwann cells, at least at the cell body, changes during myelination. However, it is unclear whether channel expression is regulated at the whole-cell level, since a major portion of the cell membrane is electrically sealed from recordings by tight myelin wrappings. We addressed this issue by examining the regulation of mRNA levels for two potassium channel genes, MK1 and MK2, in rat sciatic nerves during development and following injury. In Northern blot analysis of total RNA extracted from P2 to adult nerves, probes generated from cDNA clones for these channel genes recognize single transcripts of ≈8.6 kb for MK1 and ≈11.3 kb for MK2; the level of expression is higher for MK1 than MK2. The hybridization signal shows a developmental change that is similar for both channel genes; it increases from P2 to P14, then declines again in the adult. Permanent nerve transection in adult animals results in a dramatic reduction in mRNA levels for both potassium channel genes which lasted up to 58 days posttransection. When the nerve is crushed so that regeneration can occur, MK1 and MK2 expression initially declines, but subsequently regains the pre-crush levels. The patterns of potassium channel gene regulation resemble those of myelin-specific genes such as P_0. These results provide the first direct evidence that the expression of some potassium channel genes in Schwann cells is dependent on association with axons.

185.7

GLIAL CALCIUM SIGNALING IN INTACT MAMMALIAN WHITE MATTER. S. Kriegler and S.Y.Chiu* Graduate Program in Biophysics & Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

In slice cultures of gray matter, neuronal activities trigger $[Ca^{2+}]_i$ spiking and $[Ca^{2+}]_i$ waves in glial network, presumably via substances, like glutamate, released at synapses. Here we examine glial $[Ca^{2+}]_i$ signaling in mammalian white matter lacking neuronal cell bodies and synapses. Rat optic nerves (P2, P7, P14) were stained with fluo-3 and confocal images of $[Ca^{2+}]_i$ recorded at ~25°C or ~37°C. Glial cell bodies showed spiking or sustained $[Ca^{2+}]_i$ response to bath-applied glutamate (100-500 μM). Metabotropic glutamate agonist trans-ACPD elicited transient, sometimes spiking, $[Ca^{2+}]_i$ responses, whereas ionotropic agonists kainate and AMPA elicited a DNQX-sensitive, mostly sustained $[Ca^{2+}]_i$ response. Transient and spiking glial $[Ca^{2+}]_i$ responses were also elicited by adenosine or ATP (10-50 μM). Repetitive nerve stimulations (10-20 Hz) elicited $[Ca^{2+}]_i$ spiking in 10-20 % of glial cells in P-7 nerves; glial spiking typically occurred 20-45 sec after onset of nerve stimulations. At 37°C, the frequency for glial $[Ca^{2+}]_i$ spiking increased from 0.06 Hz to 0.11 Hz when axonal stimulation was increased from 10 to 20 Hz (n=17). This activity-dependent glial spiking was inhibited by 10 μM TTX, could not be mimicked by increasing the bath K^+ by 20 mM in unstimulated nerves alone, and occurred when nerves were stimulated in the absence of bath calcium. We suggest that glial $[Ca^{2+}]_i$ spiking in white matter is mediated by non-vesicular release of neuroactive substances, possibly through reversal of transporters when Na^+ and K^+ gradients are altered by prolonged nerve activity.

185.8

ACTIVATION OF c-FOS IN GLIA OF DEVELOPING OPTIC NERVE K.J. Mack*, S. Kriegler, S. Chang, and S.Y. Chiu. Depts. of Neurology, Biophysics, and Neurophysiology, University of Wisconsin, Madison, WI.

Recent experiments with developing optic nerves have demonstrated a stimulation-dependent calcium elevation in glia. To investigate if other glial cell events are associated with optic nerve stimulation, an immunohistochemical analysis for immediate early genes was performed in optic nerves.

In unstimulated optic nerves, an antibody to c-fos related antigens demonstrated positive cell body staining at P2, P7, and P14. Adult optic nerves showed no specific staining. The nuclear staining was most prominent at P7, and occurred in the cell bodies of presumptive glia.

Optic nerves from P7 animals were exposed for 5 to 30 min to a solution of 300 uM glutamate, latter maintained in a glutamate free solution for 2 hours, and then quickly frozen. Glutamate-treated nerves showed an increased expression of c-fos related antigens compared to control nerves.

Finally, optic nerves received 15-20 Hz electrical stimulation for 5 to 15 minutes. Two hours after this stimulation, increased immunoreactivity for c-fos related antigens was demonstrated.

These studies indicate that developing (P7) optic nerves show a baseline expression of immediate early genes, and activation through glutamate or electrical nerve stimulation results in an increased expression of these transcription factors. These results suggest that axonal activity affects early gene transcription in glia of the developing optic nerve.

185.9

EVIDENCE THAT UPREGULATION OF THE mRNA FOR GLIAL FIBRILLARY ACIDIC PROTEIN DOES NOT DEPEND ON THE SYNTHESIS OF AN IMMEDIATE EARLY GENE PRODUCT. M.R. Smith* and O. Steward. Department of Neuroscience, University of Virginia, Charlottesville, VA 22908.

Entorhinal cortex (EC) lesions lead to a dramatic upregulation of glial fibrillary acidic protein (GFAP) mRNA in the hippocampus (Steward et al., J. Neurosci., 10:2373-2384, 1990). Similar increases are also observed after hippocampal seizures (Steward et al., Proc. Natl. Acad. Sci., 88: 6819-6823, 1991). The regulation of GFAP gene expression is important in reactive astrogliosis but little is known about the mechanisms involved. The present study evaluates whether GFAP gene induction occurs as a result of activation of immediate early genes (IEGs). Gene induction via IEGs occurs as a result of the rapid synthesis of IEG proteins, which then act as transcription factors. Previous studies have shown that protein synthesis inhibitors reduce gene induction via IEGs by reducing the increases in the IEG products (Mocchetti et al., Proc. Natl. Acad. Sci., 86:3891-3895, 1989). Thus, we evaluated whether inhibition of protein synthesis blocked the increases in GFAP mRNA after EC lesion. Adult male Sprague-Dawley rats received a subcutaneous injection of aniso-mycin (100 mg/kg) in order to inhibit protein synthesis. Twenty minutes later, the EC was destroyed electrolytically. GFAP mRNA levels were assessed 4,8,12,24, and 48 hours after the EC lesion using dot blot hybridization. GFAP mRNA levels increased 2-3 fold by 12 hours post-lesion and 5 fold by 24 hours postlesion in both drug-treated and control animals. There were no significant differences in the GFAP mRNA levels between the two groups at any of the time points. These results suggest that upregulation of GFAP mRNA is not dependent on induction of immediate early genes. Supported by NIH #NS29875 to OS. MRS received predoctoral fellowship support from NIH #NS07199.

185.10

FISH GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) CLONING AND EVIDENCE OF THE PRESENCE OF GFAP TRANSCRIPT IN THE FISH OPTIC NERVE. I. Cohen, Y. Shani and M. Schwartz*. Department of Neurobiology, The Weizmann Institute of Science, 76100 Rehovot, Israel.

During development, astrocytes support axonal elongation. With maturation, concomitant changes occur in the ability of astrocytes to support axonal growth and in the type of intermediate filaments expressed by them. Controversy exists as to whether the fish optic nerve expresses GFAP and thus whether its astrocytes differ from those of the brain and spinal cord, as well as from astrocytes of optic nerves of other species. In this work we use a molecular approach to show, for the first time, the presence of a GFAP transcript in the fish optic nerve. A cDNA library originating from fish brain was constructed in λgt10 and screened with mouse GFAP probe. Two partial cDNA clones, which appeared to encode for two different forms of GFAP, were isolated and sequenced. The partial coding region of one clone (# 1) contained 214 amino acids and that of the other (# 3) contained 211 amino acids. There were several mismatched nucleotides between the two clones, but only five amino acids were found to be different: histidine/glutamine, asparagine/serine, alanine/threonine, valine/alanine, and lysine/asparagine. In the 3' noncoding regions the differences between the clones were more pronounced. In clone # 1 this region contained 573 base pairs (bp) starting from the polyA+ tail, while in clone # 3 it contained 805 bp but the polyA+ tail was not approached. Additional differences in the 3' noncoding region included nucleotide mismatches, as well as several deletions in clone # 3. The fish GFAP was found to contain approximately 2.7 bp and thus closely resembled the mammalian GFAP in size. Using the isolated fish GFAP clones, we were able to detect a GFAP transcript of 2.7 bp in the fish optic nerve.

185.11

Distribution of Myosin and Cytokeratin Immunoreactivity within the Regenerating Electric Organ of the Gymnotiform fish Sternopygus. J.M. Patterson* and H.H Zakon. Dept. of Zoology, University of Texas at Austin, Austin, Texas 78712

Weakly electric gymnotiform fish produce an electric field using an electric organ (EO) located in the tail. The EO is comprised of large multi-nucleate electrocyte cells, which have been shown to possess morphological characteristics indicative of a myogenic ontogeny. The electrocytes are surrounded by a peripheral ring of skeletal muscle fibers that lie just under the skin. We have sought to study the changes in myofibrillar and cytoskeletal proteins which occur during the differentiation of new electrocyte cells as the EO regenerates after amputation.

Seven days after removal of the terminal portion of the tail, which includes EO, muscle, nerve and other tissues, a regeneration blastema forms at the wound site. Previous work has shown that blastemal cells are progeny of myogenic satellite cells located between electrocyte cells and between skeletal muscle fibers (Patterson & Zakon, 1990). During the next 14 days, blastemal cells differentiate into electrocytes, skeletal muscle, and other tissues found within intact EO.

We have shown in the mature EO that electrocytes are labeled with the monoclonal antibody AE1, an anti-acidic cytokeratin Ab, while the peripheral muscle fibers are selectively labeled by MF20, an anti-myosin monoclonal antibody.

To look at the expression of these cell-specific epitopes over time, we have conducted a time course experiment in which regenerating EO from three fish were obtained at each of the following times post-amputation: 3 days, 6 days, 10 days, 14 days, and 21 days. At each time point, the distal regeneration blastema/stump was removed, frozen, and sectioned longitudinally for immunohistochemistry.

Changes in MF20 expression occur prior to blastema formation. At three days post-amputation, myosin, which is never expressed in mature electrocytes, is seen within intact electrocyte cells near the wound margin. MF20 staining indicates that this myosin is of a particulate nature; it is not organized into any contractile machinery. At 6 days, just as the blastema is forming, no MF20 nor AE1 labeled cells are present. At 10 days post-amputation, small, heavily-labeled MF20-positive cells predominate in the distal region of the blastema; occasional AE1-positive cells are seen in this area. Proximally, large (>200 microns in length) AE1-positive/MF20-positive cells extend from near the wound margin into the blastema. Based on their size and position, we believe these larger proximal cells represent primordial electrocytes. By 21 days, these regenerated electrocytes no longer express the myosin epitope. Thus, it appears that regenerating electrocytes transiently express a muscle-like phenotype during differentiation, and that EO amputation can cause these cells to revert to this earlier form.

Patterson, J. M. & Zakon, H. H. (1990) *Soc. for Neurosci. Abs.* Vol. 16. p. 1327.

185.12

ASTROCYTES AND THEIR CONDITIONED MEDIA REDUCE BLOOD BRAIN BARRIER LEAKAGE AND DECREASE EXPRESSION OF INHIBITORY ECM MOLECULES IN ASTROGLIAL SCARS. I. Silver*, K. Morigiwa and K. Maier. Dept. of Neurosciences, Case Western Reserve University, Cleveland, OH 44106

Precritical period astrocytes (PA) of the mammalian CNS appear to provide a supportive substrate for neurite outgrowth and to differ in their functional properties from mature astrocytes (MA). We have previously shown that PA, but not MA, suppress astroglial scarring and may suppress blood brain barrier (BBB) leakage in an *in vivo* CNS lesion model in adult rats. We have also demonstrated that the expression of putative inhibitory ECM molecules, chondroitin-6-sulfate proteoglycan (CSPG) and tenascin/cytotactin (TN), increase in CNS gray matter lesions in adult but not in neonatal rats. Here, we examined whether PA reduce CSPG and TN expression as well as BBB leakage in adult CNS lesions, and if so, whether diffusible factors or cell contact were responsible for the effect. Purified astrocyte cultures were made from neonatal rat cortex, seeded onto Millipore filters after 3 days (PA) or 30 days (MA) of aging in culture, and implanted into the forebrain of adult rats. Filters were also immersed in P3 and P30 astrocyte conditioned media pulsated in N2 media. The controls were implanted with filters immersed in DMEM/F12 or N2 media or with boiled dead astrocyte membranes (DA). After a 9 day survival period the animals were either intravenously injected with Evans Blue dye or HRP and perfused through the ascending aorta. The brains were processed to visualize dye and marker leakage or stained with antibodies to albumin and to GFAP, CSPG, TN and laminin to determine the degree and nature of the scarring. PA and PA conditioned media but not MA conditioned media significantly reduced the BBB leakage compared to control media implants, whereas MA and DA increased the leakage. The leakage was correlated with the degree of scarring and location of CSPG and TN. These results suggest that diffusible factors of PA may reinstate the BBB *in vivo* and that CSPG/TN in scars are correlated with the region of BBB leakage. Supported by Gliatech, Inc. and NS25713.

185.13
CHARACTERIZATION OF MURINE TENASCIN mRNA ISOFORMS AND ANALYSIS OF THEIR DEVELOPMENTAL EXPRESSION BY *IN SITU* HYBRIDIZATION.

U. Dörries* and M. Schachner. Dept. of Neurobiology, Swiss Federal Institute of Technology, CH-8093 Zurich, Switzerland.

The extracellular matrix molecule tenascin is, in the mouse, expressed as a group of polypeptides with molecular weights between 190 and 240 kD, depending on the tissue and the developmental stage analyzed. This heterogeneity has been shown to be, at least in part, caused by alternative splicing in the region of the fibronectin type III (FNIII) repeats. According to Northern blot analysis, the regulation of the splicing process appears to be tissue-specific and developmentally regulated. To characterize mRNA isoforms present in the mouse central nervous system, we applied PCR to cDNA from mouse brains of embryonic and postnatal stages. In addition to the isoforms already described, we could identify additional splicing variants, amongst which we found an as yet undescribed FNIII repeat which showed 97% homology to the eleventh FNIII repeat of hexabrachion, the human tenascin homologue.

In order to further analyze the spatiotemporal expression of individual isoforms, we applied *in situ* hybridization to sections of mouse embryos of different developmental stages, after amplified fragments were cloned and isoform-specific digoxigenin labelled cRNA probes were generated. Analysis of the hybridization signals revealed that the expression of all tenascin mRNA isoforms described here is dependent on the developmental stage of the embryo and regulated in a tissue-specific manner.

185.14
CHARACTERIZATION OF JANUSIN (J1-160/180) mRNA EXPRESSION IN BRAIN.

B. Fuss*, E. Wintergerst, M. Schachner. Dept. Neurobiology, Swiss Federal Institute of Technology, Hönggerberg, 8093 Zürich, Switzerland.

Janusin (formerly designated J1-160/180) has been shown to be highly related to tenascin by cDNA sequence analysis. The common structure of these extracellular matrix molecules is characterized by a cystein-rich N-terminal region, followed by EGF-like repeats, fibronectin type III (FNIII) repeats and a domain homologous to fibrinogen. In contrast to tenascin, which is expressed by astrocytes, fibroblasts and Schwann cells, janusin is described as an oligodendrocyte-derived protein. In addition to adhesive substrate properties both proteins display repulsive properties towards neurons and growth cones. For janusin, but not for tenascin, the neuronal cell recognition molecule F3/11 is involved in the initial recognition process.

The existence of alternatively spliced FNIII repeats, resulting in isoforms that are expressed in a tissue-specific and developmentally regulated manner have been previously demonstrated for tenascin. By using PCR with cDNAs from rat brain, we could identify alternative splicing in the region of the FNIII repeats which generates at least two janusin isoforms which could constitute the difference between the 160 kD and 180 kD protein components. *In situ* hybridization with janusin-specific cRNA probes confirms the expression of janusin by oligodendrocytes with a peak at around postnatal days 8 to 15, previously shown by Northern blot analysis, and suggesting a possible role during myelination. Surprisingly, there are additional signals detectable in non-oligodendroglial cells, most probably basket cells of the cerebellum. The structural homology between janusin and tenascin including the position of alternatively spliced exons could indicate that both proteins are derived from a common ancestor, although they are expressed by different cell types.

REGENERATION I

186.1
IMMUNOREACTIVITY OF REGENERATING OPTIC FIBERS OF GOLDFISH WITH MONOCLONAL ANTIBODIES AGAINST MAMMALIAN HEAVY NEUROFILAMENT.

E.I. ANDERSON and R.L. MEYER*. Developmental Biology Center, UC Irvine, Irvine, CA 92717

Two monoclonal antibodies against heavy neurofilament were found to be reactive against regenerating optic fibers in goldfish. The monoclonal antibody NE14 was found to selectively stain fibers growing from retinal explants in culture. In retinal sections of regenerating optic fibers NE14 immunoreactivity was selective for optic fibers. Sections of tectum innervated by regenerating optic fibers show enhanced staining compared to the normal optic fibers, although some staining of the tectal cells was also observed. Immunoblotting of a tectum homogenate identified a prominent band in the cytoskeletal fraction, but it was below 10 KD, which is too low to be a neurofilament protein.

In tectum, monoclonal antibody RT 97 was also found to selectively stain for regenerating optic fibers. Moreover, it selectively stained fibers that projected to, or fibers within, the stratum marginale. Immunoblotting with RT 97 identified no prominent bands in the cytoskeletal fraction, but one prominent band was seen in the cytosolic fraction at about 160 kD.

Supported by NIH EY6746.

186.2
EXPRESSION AND DISTRIBUTION OF MICROTUBULE-ASSOCIATED PROTEINS IN DRG NEURONS AFTER SCIATIC NERVE LESION IN ADULT RAT.

F. Nothias*, M. Murray, A. Tessler and I. Fischer. Dept. Anatomy and Neurobiology, Med. College of PA, Philadelphia, PA 19129.

Microtubule-associated proteins (MAPs) are major structural components of the neuronal cytoskeleton whose expression is regulated during brain development. MAP1B is present predominantly in growing axons. Tau is also present in axons and exhibits multiple isoforms that include embryonic, adult and a PNS-specific "big-tau". Sciatic nerves were transected to determine the expression and distribution of MAP1B and tau during axonal regeneration in adults. A polyclonal antibody that recognizes all MAP1B isoforms showed staining in adult DRG neurons as well as in their central and peripheral branches. A monoclonal antibody recognizing only the phosphorylated isoform of MAP1B (MAP1B-P), showed immunostaining in axons but not in perikarya. After sciatic nerve lesion, MAP1B-P immunoreactivity increased in injured sciatic nerve, but not in dorsal roots. The cell body of axotomized neurons remained devoid of immunostaining. The polyclonal antibody showed no changes in staining after axotomy. MAP1B-P is therefore restricted to axons. Its increase during axonal regeneration may reflect changes in postranslational modifications of MAP1B suggesting that MAP1B-P plays a role during regeneration of adult axons. Monoclonal antibodies that recognize the known forms of tau stained small and medium DRG neurons intensely. The large neurons were weakly or not immunostained. Axon staining was also selective. No changes were observed after sciatic nerve lesion. These results suggest that the distribution of "big-tau" is not uniform in adult DRG neurons and it is not affected by axotomy. *Supported by grants NIH-NS 24707, APA-FB1-9102, USAMRDC 51930002.*

186.3
EXPRESSION OF MAP1B AND TAU DURING REGENERATION OF NEWBORN AND ADULT RAT DRG NEURONS IN CULTURE.

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Both adult (Ad) and newborn (NB) DRG neurons are able to regenerate neurites in culture. We have compared the expression of MAP1B and tau, two prominent microtubule associated proteins, in cultured Ad DRG and NB DRG using immunocytochemical analysis. MAP1B is present in perikarya and neurites of Ad DRG neurons throughout the 7 days of culture. A monoclonal antibody directed against the phosphorylated isoform of MAP1B shows that this isoform is initially localized to cell bodies. During regeneration of neurites it accumulates in the distal end of neurites including the growth cones. The staining decreases with time in the cell bodies of neurons with long neurites. Similar patterns of staining are observed in NB DRG. These results indicate that phosphorylated MAP1B is associated with growing axons and may play a role in axonal regeneration of NB and Ad DRG neurons. The expression of tau was examined using antibodies that recognize the known forms of tau including "big-tau", an isoform that is present only in adult PNS. In Ad DRG culture, tau immunoreactivity is initially localized to cell bodies and the proximal ends of neurites. After seven days in culture some neurons show tau staining in both cell bodies and neurites, while others do not. This pattern may reflect the heterogeneity of the neuronal populations found in Ad DRG in vivo. In contrast, tau is present in all of the NB DRG cell bodies and neurites during all stages of their growth in culture. These results suggest that tau may be required for early events of regeneration in NB DRG but not in Ad DRG neurons. This may reflect changes in the expression of tau isoforms (e.g. "big tau") during development. *Supported by grants FB1-9102, NS24707, USAMRDC51930002.*

186.4
DISTRIBUTION OF MICROTUBULE ASSOCIATED PROTEINS IN ADULT AND EMBRYONIC MOUSE RETINAL EXPLANTS.

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Microtubule associated proteins (MAPs) are developmentally regulated proteins involved in microtubule polymerization and stabilization. We have asked whether MAPs in regenerating adult mouse retinal ganglion cell axons recapitulate the pattern of MAPs seen during development.

Adult and embryonic day 15 (E15) mouse retinal segments were explanted onto laminin coated coverslips and incubated in serum free media. Using a variety of monoclonal antibodies (Mabs), we stained adult and E15 explants for early (MAP5) and late (MAP1, MAP2, MAP2a+b, tau) MAPs. E15 explants were strongly immunoreactive for MAP5. Mabs to MAP2 and tau lightly stained axons and cell bodies, probably due to MAP2c and juvenile tau which are found in developing neurons. MAP1 and MAP2a+b labeling was faint or absent. In adult explants, both perikarya and axons were immunoreactive for MAP1. MAP2 and MAP2a+b mainly labeled perikarya and fibers within the explant while tau stained axons on the substrate. MAP5 immunoreactivity was variable, ranging from light labeling to labeling as intense as that seen in E15 explants.

In summary, retinal explants maintain their adult complement of MAPs, with high levels of MAP1, and tau and MAP2 segregated into axons and perikarya respectively. The exception is MAP5 which labels some adult axons at levels similar to that seen in E15 axons.

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186.5

NEUROFILAMENT PROTEINS IN REGENERATING FROG OPTIC AXONS. Y. Zhao and B.G. Szaro*. Department of Biological Sciences, S.U.N.Y., Albany, NY 12222.

Patterns of neurofilament protein (NF-P) subunit expression and the phosphorylation of NF-H and NF-M at K-S-P residues are similar in developing and regenerating PNS axons. This may be important for axonal growth, since injured mammalian CNS axons, which do not regenerate, exhibit novel patterns of cytoskeletal gene expression different from regenerating PNS axons. The optic nerve of the frog, Xenopus laevis, is one of the few vertebrate CNS projections capable of fully regenerating. To determine whether the pattern of NF-P expression and phosphorylation in regenerating frog optic axons resembles that of development, we stained optic nerves with antibodies to NF-Ps after an orbital nerve crush. NF-Ps reappeared during the second week after injury. As in developing optic axons, the appearance of XNIF- and NF-M-immunoreactivity preceded that of NF-L and NF-H; and NF-M was hypophosphorylated. By 3 weeks, staining with all antibodies, except for anti-NF-H, was as intense as in uninjured nerves. The similarity in NF-P expression and phosphorylation between regenerating and embryonal optic axons supports the idea that such changes in NF-Ps reflect a reorganization of the axonal cytoskeleton that is essential for growth.

186.6

MICROTUBULE ASSOCIATED PROTEINS IN THE REGENERATING PERIPHERAL NERVOUS SYSTEM. I.W. Fawcett, G. Ma thews*, M. Goedert and A. Matus. Physiological Laboratory, Cambridge University, England, MRC Laboratory for Molecular Biology, Cambridge, England and Friedrich Miescher Lab., Basel, Switzerland.

Some MAP proteins, particularly MAP5 and Tau, are abundant in axons and their growth cones during embryogenesis. Because of their effects on tubulin polymenrization and stabilization it is likeley that they play an important role in the process of axogenesis. Some axons, particularly those in the peripheral nervous system, are able to regenerate when cut in adulthood, although regenerative growth may be much less vigourous than embryonic growth. We have examined the disribution of MAPs in the sciatic nerve and its neurons of origin during embryogenesis and regeneration.

All normal sciatic nerve axons stain for MAP-5, and none stain for MAP-2. Tau is found in many very fine axons which do not stain for neurofilament, but is undetectable in many large diameter axons: many of these fine axons must be sensory, since neurones and axons which contain Tau and not neurofilament are plentiful in dorsal root ganglia. MAP-1 is present in all axons, but staining is more intense in the finer fibres.

MAP-5 and Tau but not MAP1 are present in axons and neurones in the hindlimb region of E14 rat embryos, including their growth cones.

In regeneration the pattern of MAP distribution does not change: MAP-5 is present in all axons, including their growth cones, MAP-1 is also present in all axons. Tau staining is intense in many regenerating axons, but fibres which stain for neurofilament but not Tau can be found. MAP staining in motoneurones does not change in regeneration.

186.7

MAP1 EXPRESSION IN DORSAL ROOT GANGLION (DRG) NEURONS DURING DEVELOPMENT AND REGENERATION. N. Taleghany* and M.M. Oblinger. Dept. Cell Biology and Anatomy and The Neuroscience Institute, Chicago Med. Sch., North Chicago, IL 60064

High molecular weight microtubule-associated proteins (MAPs) are important regulators of microtubule dynamics and have been postulated to play essential roles in regulating neuronal morphology and neurite outgrowth. We and others have previously shown that increases in tubulin gene expression accompany successful axonal regeneration in peripheral sensory neurons but little information exists about the expression of the various MAPs during this process. In the present study we examined the hypothesis that during regeneration of adult DRG neurons, MAP gene expression reverts to a pattern that reflects earlier developmental events. We examined mRNA and protein levels of two related genes, MAP1a, a "late" MAP in brain, and MAP1b, an "early" MAP in brain during postnatal development of normal DRG neurons and during axonal regeneration of adult DRG neurons. For developmental studies, DRGs were obtained from pups between 1-21 days of age; for axotomy studies, unilateral sciatic nerve crushes were done on anesthetized adult male rats and the L4 and L5 DRGs were harvested 1-28 days later. In situ hybridization, immunocytochemistry, northern blotting and western blotting analyses revealed that MAP1a and MAP1b expression during development of DRG neurons was similar to the patterns reported in brain. The axotomy experiments revealed that adult DRG neurons did not revert to earlier developmental patterns of MAP1 gene expression during axonal regeneration. For example, MAP1b expression was not augmented in regenerating DRG neurons, suggesting that the reorganization of MAP gene expression after axotomy is more complex than a simple reversion to an earlier developmental pattern.

186.8

TUBULIN AND NEUROFILAMENT EXPRESSION IN REGENERATING ADULT RAT RETINAL GANGLION CELLS ANALYZED BY IN SITU HYBRIDIZATION. L. McKerracher*, C. Essagian and A.J. Aguayo. Centre for Research in Neuroscience, Montreal General Hospital and McGill University. Montreal, Quebec, H4A 2P1.

Transected retinal ganglion cell (RGC) axons in adult rats regrow when the CNS environment of the optic nerve (ON) is replaced with a peripheral nerve (PN) graft. To investigate the expression of tubulin and NF in RGCs after ON transection in the presence or absence of a PN graft, we hybridized ß-tubulin and NF probes to cryostat sections of injured retinas at various times after axotomy or 20 days after PN-grafting. Regenerating RGC were identified by retrograde labeling from the caudal end of the graft. A reduction in signal intensity for both tubulin and NF was observed after axotomy, and quantitative autoradiography confirmed that the mRNA levels were decreased in RGCs relative to contralateral controls. In the PN-grafted retinas the overall signal intensity with both probes was also reduced; however, some RGCs hybridized intensely for ß-tubulin. Retrograde labeling revealed that such ß-tubulin "hotspots" corresponded to the somata of regenerating RGCs. Because in such retinas the ß-tubulin signal did not increase in the unlabeled RGCs we suggest that active axonal regrowth into the PN graft rather than other putative influences arising from the graft play a key role in this change in neuronal gene expression.

186.9

Acidic fibroblast growth factor prevents shrinkage of cholinergic neurons of nucleus basalis magnocellularis following cortical devascularization D. Maysinger*, B.C. Figueiredo†, P. Piccardo, P.B.S. Clarke and A.C. Cuello Dept of Pharmacology and Therapeutics, McGill University, Montreal, Canada.

The ability of acidic fibroblast growth factor (aFGF) to elicit a trophic response was first tested in rat septal cultures. Treatment with aFGF (0.1μg/ml) moderatelly increased the staining and length of glial processes immunostained for glial fibrillary acid protein (GFAP). Subsequently groups of rats (n=6) were trained in a spatial memory task (Morris water maze) and then were submitted to unilateral cortical devascularization, resulting in a 1X1 cm^2 area of neocortical infarction, and in retrograde degeneration the nucleus basalis magnocellularis (nBM) shown by ChAT immunocytochemistry (ICC) . Animals were tested 24 days post-lesion for retention of the memory tasks and were sacrificed at 30 days. Intraventricular administration of aFGF (12 μg/day for 7 days via minipump), starting just before lesion, reduced NBM cholinergic cell degeneration (assessed by morphometric ChAT ICC and radioenzymatic assay for ChAT activity) and prevented the lesion-induced impairment in the memory retention test. The morphometric analysis of ChAT immunoreactive neurons in the nBM (ventral and dorsal midportions) showed significant reduction of the cell body shrinkage and of the cholinergic fiber degeneration. The most striking difference in the memory retention test between aFGF-treated (or sham-operated) and vehicle-treated decorticated subjects was observed in the first trial compared to the following trials in the first day and in the first day compared to the trials in the following days. Supported by Center of Excellence and Medical Research Council (Canada). †CNPq and UFC (Brazil).

186.10

GAP-43 AND NGF-RECEPTOR EXPRESSION IN ADULT RAT THALAMUS AFTER INJURY OR PERIPHERAL NERVE IMPLANTATION. G.Campbell*, E.Vaudano, A.P.Davies, P.N.Anderson, A.R.Lieberman, D.J.Schreyer¶. Dept. of Anatomy, University College London, ¶Dept. of Physiology, Queen's University, Kingston, Canada.

Many neurons undergoing regeneration increase their expression of the growth associated protein GAP-43, and, in some cases, the low affinity NGF receptor (NGFr). It is not known whether the upregulation of these proteins is simply a response to axotomy or whether the upregulation is necessary for, or is a consequence of, regeneration. We have studied whether injury alone is a sufficient stimulus to increase levels of GAP-43 and NGFr protein and mRNA in thalamic neurons of adult rats. Either a stab wound was made into, or a tibial nerve was grafted into the thalamus of anesthetized adult rats. After 4-177 days brains were processed for LM and EM immunocytochemisty with monoclonal antibodies 9-1E12 (which recognizes all forms of GAP-43) and 192-IgG (for NGFr) or for in situ hybridization using radio-labelled oligonucleotide probes for GAP-43 and NGFr mRNAs.

At 7-12d large numbers of GAP-43+ axonal sprouts were present in the thalamus adjacent to the grafts and small numbers close to the lesion. GAP-43+ sprouts were present in the graft at 12-40d. A few GAP-43+ neuronal perikarya were present in the thalamus close to the stab wound at 7-12d and many were GAP-43+ near the grafts at 9-12d. Preliminary hybridization data show that GAP-43 expression was increased in the thalamus close to the lesion at 7d whereas GAP-43 mRNA levels were not elevated near the graft/brain interface at 7d but were at 12d. In the thalamus close to grafts, but not lesions, NGFr+ sprouts were clearly present at 7-12d. NGFr+ neurons were present in caudal thalamus adjacent to the lesions at 7d but were not clearly identifiable adjacent to the grafts at any survival time. However, NGFr mRNA was greatly increased around the graft tip in caudal thalamus at 7d. In conclusion, a stab wound is a sufficient stimulus to increase the expression of GAP-43 and NGFr in some thalamic neurons. However, in the presence of a substrate permissive for axonal growth the upregulation of GAP-43 is both delayed and prolonged. The increased expression of NGFr in some neurons near the lesion or graft suggests that these cells may be responsive to neurotrophic factors related to NGF.

186.11

CHANGES IN mRNA LEVELS OF NEUROTENSIN, α-AND β-CGRP, AND GAP-43 IN RAT HYPOGLOSSAL NUCLEUS AFTER THE NERVE INJURY. H. Kiyama*, N. Kobayashi, and M.Tohyama Dept. of Anatomy & Neuroscience, Osaka Univ. Med. Sch., Osaka, JAPAN.

Neurotensin(NT), α- and β-CGRP, and GAP-43 mRNA expressions were observed in the rat hypoglossal motor neurons when unilateral hypoglossal nerve was either resected or crushed. For the detection of these mRNAs an in situ hybridization histochemical method using an alkaline phosphatase labeled oligonucleotide probe was used. The decrease of NT and β-CGRP mRNA level in the operated side after the nerve injuries was observed, and the duration of the gene down - regulation was much longer in NT mRNA than in β-CGRP. The β-CGRP mRNA level observed in the resected side could recover by a month, while it took almost two months for NT mRNA to recover the normal level. Similar observation could be seen in case of crush, but the recovery of these mRNAs was quicker than in the resected case, probably because of the extent of nerve damage. GAP-43 mRNA gene expression was contrary upregulated by the injuries. The temporal profile in the upregulation of GAP-43 mRNA level was similar to that of NT. The temporal regulation of α-CGRP mRNA expression after the injuries apparently demonstrated different pattern. Nerve crush caused a few weeks upregulation of the message, however the resection showed a quick transient upregulation within a few days and later a delayed increase of mRNA after about a month. Moreover the gene expression in the contralateral side was also transiently observed for about three weeks. These findings suggest the existence of different ways of gene regulation for NT, α- and β-CGRP, and GAP - 43 mRNAs during the regeneration of the hypoglossal motor neuron, but the profound reason for these difference is subject to more investigations.

186.12

EARLY CHANGES IN FAST AXONALLY TRANSPORTED PROTEINS REFLECT DIFFERENTIAL REGULATION IN CRUSHED RAT OPTIC NERVE. L. Wodarczyk and G. W. Perry.* Program in Complex Systems and Brain Sciences, Florida Atlantic University, Boca Raton, FL. 33431.

The ability of RGCs of lower vertebrates to successfully regenerate their axons following optic nerve crush is attributed to both intrinsic factors, such as the ability to respond to damage through upregulation of fast axonally transported proteins (FTPs), and extrinsic factors, such as a growth-permissible environment provided by the glial cell population. Increases in some FTPs (e.g. GAP-43) have been reported in rat RGCs following optic nerve crush; nonetheless, axon outgrowth is limited and subsequently aborted. This suggests that the characteristic failure of mammalian RGCs to regenerate is not due to an inherent inablity to respond to damage. Moreover, an appropriate glial environment will sustain directed axonal growth. This study examines how extensive the changes in FTPs are in response to RGC damage and how comparable these changes are to those that occur in lower vertebrate RGCs.

The left optic nerves of male Sprague-Dawley rats were crushed intraorbitally (4-5 mm distal to the eye) 2 and 5 days prior to intraocular injection of ^{35}S-methionine. Following 5 hr labelling, experimental and control optic nerves were dissected and subjected to 2D-gel analysis. Quantification of labelled FTPs seen in fluorographic patterns from crushed nerves show both increased and decreased labelling in a broad range of FTPs. These changes are not unlike those seen in lower vertebrate optic nerves shortly after crush. We conclude that rat RGCs are capable of initiating the metabolic responses necessary for regeneration to begin. However, these and subsequent responses may eventually be suppressed by the presence of growth-inhibitory factors in the rat optic nerve.

Supported by NIH Grant EY06449.

186.13

ENHANCED EXPRESSION OF GAP-43 IN RETINAL GANGLION CELLS BY AN INTRAVITREOUS PERIPHERAL NERVE GRAFT AFTER INTRAORBITAL AXOTOMY IN HAMSTERS. T.F.Ng[1], K-F.So[1] and S.K.Chung*[2]. [1]Dept. of Anatomy, [2]Inst. of Molecular Biology, Univ. of Hong Kong. HONG KONG.

GAP-43 expression in retinal ganglion cells (RGCs) are studied by immunocytochemical techniques using polyclonal antibody against GAP-43 (kind gift from Dr. Benowitz). Number and soma area of GAP-43 expressing cells are measured at various time courses (1-56 days) after optic nerve (ON) is cut 2mm from optic disc intraorbitally (n=32) or after intraorbital ON cut with a vital (n=40) or a non-vital (freeze and thaw, n=24) intravitreal peripheral nerve (PN) graft. When the ON is not damaged, no immunoreactivity to GAP-43 is observed in retinas with (n=4) or without (n=25) a vital intravitreal PN graft. However, expression of GAP-43 is observed in RGCs in the ON cut animals and in the animals with ON cut and intravitreous implantation of a vital or non-vital PN graft. The time course of the immunoreactivity of GAP-43 is similar for the ON cut group (5-21 days post-axotomy) and the group receiving non-vital PN graft (5-28 days post-axotomy) but they are both different from the group receiving a vital PN graft (3-56 days post-axotomy). Thus our results show that a PN graft implanted close to RGC soma seems to influence the expression of GAP-43 so that it is expressed earlier and for a longer period of time following axotomy close to the optic disc. These changes of expression of GAP-43 may be important for enhancing axonal regeneration.

186.14

EXPRESSION OF GAP-43 IS NOT INCREASED FOLLOWING CENTRAL AXOTOMY OF ADULT RAT DORSAL ROOT GANGLION NEURONS. David J. Schreyer* and J.H. Pate Skene. Dept. of Physiology, Queen's University, Kingston, Canada K7L 3N6, and Dept. of Neurobiology, Duke University, Durham NC 27710

Peripheral nerve injury results in increased synthesis of GAP-43 and its accumulation in the injured peripheral and uninjured central axon branches of dorsal root ganglion (DRG) neurons (Schreyer & Skene, '91; J. Neurosci. 11:3738-3751). We sought to determine if the injury-associated mechanism which triggers increased GAP-43 synthesis is confined to one axon branch. Immunocytochemistry using monoclonal antibody 9-1E12 was used to monitor GAP-43 expression in uninjured adult rats, in rats two weeks after peripheral nerve crush lesions, and in rats two weeks after dorsal root crush lesions. Immunocytochemical labelling intensity was quantified from digitized images of DRG tissue sections.

Approximately half of DRG neurons were found to constitutively expresses GAP-43 in adult rats in the absence of axon injury. This constitutive GAP-43 expression ranged in magnitude from barely detectable to high, but was restricted to small DRG neurons. Following sciatic nerve lesions 1 cm. peripheral to the ganglion, virtually all DRG neurons, large and small, were found to express high levels of GAP-43. Following dorsal root lesions 1 cm. central to the ganglion, no increase in GAP-43 expression by DRG neurons above that seen in uninjured animals was detected. Thus, the injury-associated increase of GAP-43 expression in DRG neurons appears to be regulated principally by a mechanism operating through peripheral, rather than central, axon branches.

186.15

mRNA LEVELS OF PLASMINOGEN ACTIVATORS IN REGENERATING RAT SCIATIC NERVE. N.Kalderon* and D.Belin+. The Rockefeller Univ. New York, NY 10021 and +Dept. of Pathology, Geneva Univ. Sch. of Med., Geneva, Switzerland.

Schwann cell plasticity during peripheral nerve regeneration and its regulation by the regrowing severed axons are investigated on the molecular level. Plasminogen activators (PA) are key enzymes controlling a cascade of extracellular proteolysis, thus playing an important role in regulating cell and tissue plasticity. We previously determined that undifferentiated Schwann cells express high levels of PA activity, primarily of the urokinase type (u-PA). Here, the regulation of mRNA levels of both u-PA and tissue type PA (t-PA) during rat sciatic nerve regeneration were determined. Two distinct migrating cell populations, consisting mostly of de-differentiated Schwann cells that are either accompanied or unaccompanied by the regenerating axons, were analysed. These are obtained by suturing the stumps of the transected nerve to each end of an empty silicone tubing; the cells from both stumps start migrating into this chamber during the 2nd week postinjury. The severed axons regrow from the proximal stump and follow the Schwann cells reaching the distal stump cells a week later. In the adult sciatic nerve no u-PA mRNA was detected while low levels of t-PA mRNA were found. High levels of both u-PA and t-PA mRNA were found in the migrating cell populations of the two stumps (11 days postinjury). These data strongly argue that the expression of both PA genes is up-regulated by axotomy in de-differentiated, migrating Schwann cells.

186.16

NCAM EXPRESSION DURING TASTE BUD DEGENERATION AND REGENERATION. D. V. Smith*, R. Klevitsky, R. A. Akeson, and M. T. Shipley. Depts. Otolaryngology - Head Neck Surg., Pediatrics, Anatomy & Cell Biol., Univ. Cincinnati Coll. Med. and Children's Hosp., Cincinnati, OH 45267

Taste cells are continuously renewed throughout life and are trophically dependent on the peripheral gustatory nerves that innervate them. We and others have shown that the neural cell adhesion molecule (NCAM) is expressed by taste cells and their nerve fibers. In early development, NCAM molecules contain high levels of polysialic acid (PSA), which regulates the degree of cell-cell interaction mediated by NCAM. Here we have characterized the reappearance of both NCAM polypeptides and PSA in the rat vallate papilla at several points in time following bilateral crush of the glossopharyngeal (IXth) nerve using monoclonal antibodies (mAbs) to NCAM polypeptides (mAb 3F4) and to PSA (mAb 5A5).

In non-lesioned controls, NCAM is distributed on a subset of cells within the taste buds and on nerve fibers throughout the tongue, including those innervating the gustatory epithelium. By contrast, PSA is present only on nerve fibers, including a small number that enter the vallate taste buds. In the first week following bilateral IXth nerve crush, NCAM expression gradually diminishes in the gustatory epithelium; its expression returns upon reinnervation by the IXth nerve. PSA expression also diminishes in the IXth nerve after crush and reappears as the nerve regenerates. We hypothesize that PSA will be downregulated in afferent fibers as they enter the gustatory epithelium. These results suggest that NCAM is involved in the differentiation of epithelial cells into taste receptors and in the interactions between IXth nerve fibers and taste receptor cells.

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186.17

PROTEOGLYCANS OF REGENERATING NERVE, J. F. Challacombe* and J. S. Elam, Program in Neuroscience, Florida State University, Tallahassee, Florida 32306

Our previous studies demonstrated the enhanced axonal transport of $^{35}SO_4$-labeled proteoglycans in the regenerating goldfish optic system (Coughlin and Elam, Br. Res. 493, 326 (1989), Challacombe and Elam, Soc. Neurosci. Abs. 17, 49 (1991)). We have extended these studies to assess the distribution of transported proteoglycans in sequential optic tract extractions consisting of: (1) isotonic buffer followed by hypotonic buffer (soluble fraction), (2) 1M NaCl (peripheral membrane-associated), (3) 1% Triton (integral membrane), and (4) 1% Triton-1M NaCl (integral membrane-anchored). Distribution of total transported radioactivity among these extracts for unoperated controls was 35.9% soluble, 15% NaCl extracted, 20.6% Triton extracted, 21.6% Triton-NaCl extracted, and 6.9% unextracted. For 21 day regenerating tracts the values were 27.7% soluble, 13.8% NaCl extracted, 24.3% Triton extracted, 27.5% Triton-NaCl extracted, and 6.7% unextracted. As assessed by DEAE-cellulose ion-exchange chromatography, the proportion of total radioactivity in proteoglycans for each of these extracts showed a regeneration-correlated increase. However, the distribution of total transported proteoglycans among the various extracts indicated little difference between regenerating and unoperated controls. Gel filtration of transported proteoglycans on Sepharose CL-6B shows an increase in the size of proteoglycans from the soluble fraction of regenerating nerve. These results indicate that the transported proteoglycans of regenerating nerve are distributed among soluble and membranous compartments in a pattern similar to intact nerve, but with some change in the overall proteoglycan size range. (Supported by NS 20502)

186.18

ENHANCED EXPRESSION OF A PROTEOGLYCAN ON AXOTOMIZED TROCHLEAR MOTOR NEURONS. P.Iannuzzelli*,Y.Wang,A.Pimenta,J. Fischer, P.Levitt and E.H.Murphy, Department of Anatomy and Neurobiology, Medical College of Pennsylvania /EPPI, Philadelphia, PA 19129.

A monoclonal antibody, 8B3, recognizes a surface chondroitin sulfate proteoglycan associated with the soma and dendritic processes of a sub-population of neurons, including cranial and spinal motor neurons. In this study, we examined the effect of a peripheral nerve cut on 8B3 immunoreactivity in axotomized cranial motor neurons. In adult cats, the trochlear nerve was exposed and severed peripherally. Following this type of lesion, some trochlear motor neurons (TMNs) die. The surviving motor neurons regenerate and reach their target in 4 weeks (Murphy,et.al.1990 J.Comp.Neurol.292:524-536). In the normal adult cat, 8B3 labelling is observed along somata and dendritic processes throughout the trochlear nucleus. One week after axotomy, the level of 8B3 immunoreactivity in the lesioned nucleus increases compared to the normal side. At longer survival times, the difference in staining intensity between the lesioned and normal nucleus remains, with 8B3 immunoreactivity in the lesioned nucleus elevated for as long as 14 weeks post-axotomy, the longest survival time examined.

One possible explanation for this finding is that TMNs expand their dendritic arborization in response to injury. We examined this possibility using a monoclonal antibody to microtubule-associated protein 2 (MAP2) on alternate sections through the trochlear nucleus. MAP2 has been shown to be localized specifically to neuronal cell bodies and dendrites. As early as one week post-axotomy, the density of MAP2 positive dendritic processes decreases in the lesioned nucleus and this decrease is even more pronounced at longer survival times, suggesting that axotomy results in a reduced number of dendritic processes in the axotomized trochlear nucleus. These findings suggest that 8B3 immunoreactivity is rapidly increased on axotomized TMNs, and may reflect an important role for the 8B3 proteoglycan in the functional recovery of regenerated motor neurons. Supported by NIH-NS24707

186.19

ANALYSIS OF C-*JUN* mRNA AND PROTEIN IN DORSAL ROOT GANGLIA AFTER SCIATIC NERVE TRANSECTION. M. De León*, R. Nahin, S. Inagaki, B. Allen and M.A. Ruda, NAB, NIDR, NIH, Bethesda, MD 20892

The effect of nerve injury and regeneration on transcription factors in dorsal root ganglia (DRG) neurons was examined. Adult rats were anesthetized and the sciatic nerve transected. DRG at 8 hours, 1, 2, 3 and 7 days after transection were collected, their mRNA extracted and Northern blots performed using a ^{32}P-labeled 51-mer oligonucleotide probe complementary to bases 2810-2861 of rat c-*Jun*. The constitutive expression of c-*Jun* mRNA was very low in DRG. Induction of c-Jun mRNA was observed by 1 day after nerve transection, with a 6 fold peak at three days and returning to control levels by day seven. *In situ* hybridization using the same oligonucleotide probe demonstrated c-*Jun* mRNA induction in small,- medium- and large-sized DRG neurons. Immunocytochemistry demonstrated Jun protein in nuclei of DRG neurons. DNA gel mobility shift experiments showed a single DNA-protein complex that was specific to the AP-1 binding site. This complex was increased in ipsilateral as compared to contralateral DRG extracts. The amount of DNA-protein complex was reduced by Jun protein antisera, but was not altered when treated with a Fos antibody. These data suggest that c-*Jun* may play an important role in transcriptional regulation of DRG neurons in the response to neuronal injury and regeneration.

186.20

TESTOSTERONE EFFECTS ON TUBULIN GENE EXPRESSION IN REGENERATING HAMSTER FACIAL MOTOR NEURONS (HFMN). K.J. Jones[1] and M.M. Oblinger[2]*, Dept. Physical Therapy[1], Univ. Illinois at Chicago, Chicago, IL 60612 and Dept. Cell Biology and Anatomy[2], Chicago Med. Sch., North Chicago, IL 60064.

We have previously shown that testosterone propionate (TP) differentially regulates the regenerative properties of injured HFMN through an enhancement of the nerve cell body response to injury. In this study, the hypothesis that TP increases the rate of regeneration by altering cytoskeletal gene expression was tested. Adult intact male hamsters were subjected to right facial nerve axotomies. One-half of the axotomized animals received 2 subcutaneous implants of TP, and the other half were sham-implanted. At 2 and 7 days postoperative (dpo), the animals were sacrificed and either cryostat sections collected for in situ hybridization or total RNA was isolated from the facial nuclear groups for northern blot analysis. Hybridizations with cDNA probes to class II beta-tubulin and alpha1-tubulin mRNAs were done. The autoradiograms from in situ hybridizations were quantitatively analyzed using a computerized image analysis system and the northern blots were assessed by densitometry. The results from both series of experiments revealed that axotomy induced significant increases in the levels of both tubulin mRNAs by 7 dpo, and that testosterone augmented the expression of tubulin mRNAs in the axotomized neurons. Supported by NIH grants NS28238(KJJ) and NS21571 (MMO).

186.21

INSULIN-LIKE GROWTH FACTORS REGULATE THE RATE OF SCIATIC NERVE REGENERATION. G.W. Glazner, S. Lupien, J.A. Miller, and D.N. Ishii*. Physiology Depts., Colorado State Univ., Fort Collins, CO 80523; Amgen, Inc., Thousand Oaks, CA 91320.

Prolonged denervation results in atrophy and degeneration of organs, and the slow rate of mammalian nerve regeneration is a risk factor for permanent paralysis. It is, therefore, of interest to identify the endogenous factors that can influence the rate of peripheral nerve regeneration. Insulin-like growth factors (IGFs) can enhance neurite outgrowth in vitro, and IGF-II gene expression is implicated in the development and regeneration of neuromuscular synapses. We report that local infusion of IGF-II near a site of sciatic nerve crush significantly increased ($P < 0.01$) the rate of regeneration in rats. On the other hand, local infusion of an anti-IGF antiserum caused a sustained reduction in rate ($P < 0.01$) for 6 days, showing that the spontaneous rate of regeneration was continuously dependent on nerve IGF activity. IGF-II mRNA content was increased per poly(A)$^+$ RNA ($P < 0.03$) as well as per nerve ($P < 0.001$) in deafferented nerves. These results support the hypothesis that IGFs help regulate the rate of peripheral nerve regeneration. (Supported in part by Grant RO1 NS24787 from the National Institute of Neurological Disorders and Stroke)

186.22

INDUCTION OF NEUROTROPHIC FACTORS GENE EXPRESSION IN THE KAINIC ACID LESIONED THALAMUS. M.P. Junier *, M. Peschanski. CJF INSERM 9102, Fac de Médecine, 8 rue du Général Sarrail, 94010 Créteil, France.

Kainic acid injection in the ventro-basal nucleus of the thalamus (VB) triggers a complete neuronal loss while sparing afferents to the lesioned nucleus. The lesion is followed by a dramatic cellular rearrangement leading to the sequential proliferation of activated microglia, reactive astrocytes and Schwann cells. These different glial cells are putative sources of neurotrophic factors and their appearance at the lesion site is correlated with the initial maintainance of axonal projections issued from the dorsal column nucleus (DC). Along the weeks the number of projections issued from the DC decreases although some are still identified 18 months post-lesion. To unravel some of the cellular mechanisms allowing long term maintainance of axonal projections at the lesion site, we sought to determine the timing of neurotrophic factor gene expression in the lesioned VB. Messenger RNA was extracted from both lesioned VB and its intact contra-lateral counterpart. Using ribonuclease protection assays VB mRNAs were hybridized to a riboprobe complementary either to TGF alpha, or NGF, or low affinity NGF receptor (LNGFR) mRNAs. Both TGF alpha and NGF message were detectable in intact VB. TGF alpha mRNA levels did not vary as compared to the controlateral VB up to 40 days post-lesion. At this time TGF alpha mRNA levels increased over contra-lateral VB levels. On the opposite, NGF mRNA levels tended to decrease in the lesioned VB until resuming 40 and 60 days post-lesion to quantities similar to those observed in the contra-lateral intact VB. At the same time points LNGFR mRNA were detected in the lesioned VB. Previous studies suggest that at least two cell types could account for the increased expression of TGF alpha and NGF genes: reactive astrocytes (for both TGF alpha and NGF) and Schwann cells (for NGF). Cellular localization of TGF alpha and NGF mRNA are under current investigation. These results suggest that cellular rearrangement induced by kainic acid lesion of the VB is accompanied with the induction of neurotrophic factor genes expression susceptible to exert trophic effects on axonal afferents deprived of their neuronal targets.

186.23

MODULATION OF CALBINDIN EXPRESSION IN PUTATIVE RENSHAW CELL AFTER SCIATIC NERVE LESION. P.P. Sanna[1*], M. R. Celio[2], F. E. Bloom[1] and M. Rende[3]. [1]Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA, USA; [2]Institute of Histology, University of Fribourg, Fribourg, Switzerland; [3]Institute of Human Anatomy, A. Gemelli Medical School, Rome, Italy. A subpopulation of calbindin-immunoreactive (CB-IR) neurons in lamina VII of the spinal cord has been proposed to be Renshaw cells, the anatomical substrate for recurrent inhibition. We used sciatic nerve crush to disrupt the activity of motorneurons and presumably of local interneurons. One week after sciatic nerve crush, CB-IR neurons had virtually disappeared ipsilaterally, between L4-L6, but were still immunoreactive controlaterally and cranially to the lesioned levels. Similar effects were produced by intramuscular administration of botulinum toxin. The time course for the onset of these changes was consistent across animals. CB-IR neurons reappeared during reinnervation of muscle along a rough cranio-caudal gradient and appeared normal within 6 to 8 weeks after the crush. However, a significant degree of inter-animal variation was observed in the timing and pattern of their reappearance on the lesioned side. The slower conduction velocity of α-motorneurons, their reduced activity and the reduction of their input on Renshaw cells may result in reduced activity of these latter neurons and hence in reduced need for Ca^{++} buffering power. The present results suggest that the CB expression in putative Renshaw cells of the anterior horn is plastic and that its maintenance depends on motorneuron electrical activity. The fact that similar changes occurred in CB-IR neurons following intramuscular botulinum toxin emphasizes the critical nature of neuro-muscular transmission in the expression of CB.

186.24

THE INITIAL STAGES OF NEURAL REGENERATION ARE DEPENDENT UPON INTRACELLULAR CALCIUM LEVELS
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The ability of a neuron to repair a transected process through the generation of new outgrowth has been the topic of investigations dating back to Ramon y Cajal. There are, undoubtedly, several stages, from the repair of the existing axon to the reformation of appropriate connections, that have to be considered analytically in order to understand functional regeneration. We focus on the earliest stages of regeneration, namely, the formation of a new growth cone from a transected process. Motivated by the finding that the motility of neuronal growth cones can be completely inhibited by high intracellular calcium levels, we examined whether the proximal stump of a severed neurite is also influenced by potentially inhibiting levels of intracellular calcium ([Ca2+]i). To test this hypothesis, we severed individual neurites after elevating their [Ca2+]i to varying degrees and asked whether this would influence the probability of formation of a functional neuronal growth cone. The formation of a new growth cone was a rapidly occurring event in standard culture medium. The time course and absolute degree of growth cone formation, however, were highly dependent on [Ca2+]i. If transections were performed after experimentally increasing [Ca2+]i by either depolarization or via calcium ionophores, regeneration became gradually slowed and less complete, indicating that regeneration is fastest and most successful at basal levels of intracellular calcium. Likewise, when decreasing the extracellular calcium concentration, the time course of growth cone formation was also continuously slowed and the percentage of regenerating neurons decreased. These findings may bear significantly on studies attempting to optimize conditions for neuronal regeneration. Either a transient rise in the intracellular- or a decrease in the extracellular-calcium concentrations will contribute to impaired regeneration and possibly to neuronal cell death. Since both of these events can be simultaneously observed after neuronal injury and in certain types of neurological diseases, they may prove to be important factors in determining the degree to which neuronal regeneration can be expected to occur.

186.25

EXTRINSIC DENERVATION INDUCES CHANGES IN NEUROPEPTIDE AND PHOSPHORYLATED HEAVY NEUROFILAMENT IMMUNOREACTIVITY IN SUBMUCOSAL PLEXUS OF GUINEA PIG ILEUM A.M. Yunker* and J.J. Galligan Dept. Pharm. and Tox. and Neuroscience Program, Michigan State University, E. Lansing MI 48824

Following extrinsic denervation of the small intestine there are changes in surviving nerve fibers associated with submucosal arteries. The purpose of this study was to further describe the changes in neuropeptide immunoreactivity (ir) and to determine if denervation alters phosphorylated heavy neurofilament (pNF-H)-ir. Tissues were obtained 1,7,10, or 24 weeks after denervation, denervation was verified using glyoxylic acid to reveal neuronal stores of norepinephrine, and tissues were processed using immunohistochemical methods. In normal animals submucosal arterioles were innervated by extrinsic sympathetic and calcitonin gene related peptide (CGRP)-/substance P (SP)-ir sensory nerve fibers; submucosal enteric nuclei and axons contain pNF-H-ir. One week after denervation extrinsic nerves were not detected; furthermore, SP-ir or vasoactive intestinal peptide (VIP)-ir nerve fibers were found associated with arterioles. SP-ir decreased, VIP-ir increased in the submucosal plexus, and pNF-H-ir was reduced. Seven weeks after denervation, SP-ir nerve fibers had formed a sparse perivascular plexus and multiple VIP-ir nerve fibers were found with arterioles. Neuropeptide-ir of ganglia was similar to control, but pNF-H-ir in nuclei was decreased and nerve fibers were not immunoreactive. Ten weeks after denervation, meandering SP-ir fibers were associated with arterioles, sympathetic nerve fibers returned to ganglia, and nuclear pNF-H-ir was normal. Finally, twenty-four weeks after denervation extrinsic nerve fibers were associated with arterioles and nerve fibers contained pNF-H-ir. This study concludes denervation induced changes in neuropeptides and pNF-H may represent an adaptive response to allow normal intestinal function in the absence of extrinsic control. (Supported by AHA/Michigan Grant-in-Aid)

186.26

Neurotransmitter Receptor Modulation Following Unilateral L1-S2 Deafferentation. S.Croul*, A.Sverstiuk*, W.Battisti* and M.Murray*. Depts. Pathology* and Anatomy/Neurobiology,* Med.Col.Penna., Phila., Pa. 19129

Following surgical deafferentation of the spinal cord, cut dorsal roots do not regenerate, but spared projections compensate for this loss by forming collateral sprouts and thereby modifying the synaptic organization of the dorsal horn. Locomotor functions recover with a time course and pattern which parallel and may therefore in part be accounted for by these changes in synaptic organization. Light microscopic immunocytochemistry has shown sprouting by selected undamaged intraspinal projections including those that express the transmitter substance P (SP) (Wang SD et.al., J.Comp.Neurol. 304;555-568, 1991). Quantitative immunoelectron microscopy supports these results by demonstrating loss and consequent recovery of SP containing terminals (Zhang et.al.,Soc. for Neurosci. Ab., 1991). Effects on second order neurons in the dorsal horn may include modulation of neurotransmitter receptors which parallel these afferent changes. To test this hypothesis, adult rats were subjected to L1-S2 unilateral dorsal rhizotomy and sacrificed at 0.5, 1, 2, 6, or more than 20 weeks after surgery. Spinal cords from 3 rats per time point were cryosectioned and used to evaluate changes in the receptor binding density of the SP (NK1) receptor in the dorsal horn. NK1 binding density was increased in lamina II of the deafferented side by 1 week after surgery by a factor of 1.5 compared to the control side. It remained elevated at 2 and 6 weeks(2 times control) but returned to control values by 20 weeks. N -methyl-D-aspartate(NMDA) receptor binding using ^{3}H-MK801 shows clear binding to the dorsal horns in rat lumbar spinal cord. Clarification of the magnitude, time course and cellular localization of the changes will help to define the second order neuronal response to spinal deafferentation and its impact on functional recovery. Supported by USPHS Grant NS24707

CALCIUM CHANNELS: PHYSIOLOGY I

187.1

CALCIUM CHANNEL STABILITY IN LARGE NEURONAL INSIDE-OUT PATCHES. B.D. Johnson and L. Byerly.* Dept. of Biol., USC, L.A., CA 90089.

One of the most universal properties of Ca^{2+} channels is their tendency to "wash out" or "run down" during whole-cell voltage-clamp recording. Washout can be reduced or prevented by using the perforated patch technique, or by including such intracellular components as ATP, cyclic AMP, and kinases in the recording electrode. Although restorative, these additions to the recording electrode may have multiple effects within the cell. To address the issue of Ca^{2+} channel lability in a neuronal preparation, we have developed a large, inside-out patch technique similar to that described by Hilgemann (1990; *Nature* **344**, 242-5). These large patches provide an easily quantifiable macroscopic Ca^{2+} channel current of 20-100 pA and clear access to the cytoplasmic side of the channel. Patch electrodes with an inner diameter of 5-10 μm, dipped in a mixture of oils and filled with 4 mM Ba^{2+}-Tris saline, are sealed (5-50 GΩ) to acutely isolated neurons from the snail *Lymnaea stagnalis*. In the cell-attached configuration, I_{Ba} (measured in standard *Lymnaea* saline) appears stable over the period of observation (up to 30 minutes). Following excision into a low Ca^{2+} solution (2 nM) containing 1 mM Mg^{2+}, I_{Ba} declines rapidly with a τ of 75 seconds (N=11). Future work will determine if higher free Ca^{2+}, G-proteins, or agents involved in phosphorylation (ATP, cAMP, kinases, phosphatase inhibitors) will stabilize I_{Ba} in this cell-free preparation. If I_{Ba} can be stabilized sufficiently, this preparation will be used to determine the sensitivity of these Ca^{2+} channels to intracellular Ca^{2+}. This work was supported by NINDS grant NS28484.

187.2

EFFECTS OF TEMPERATURE ON Ca CHANNELS IN CHICK SENSORY NEURONES. P. Acerbo, M. Nobile and C. Marchetti*. *Ist. di Cibernetica e Biofisica, CNR, 16146 Genova, Italy.* We investigated the effects of the temperature on Ca channels in cell-attached patches, in chick dorsal root ganglion neurones. In 110mM $BaCl_2$ and at T=20°C, from Vh=-50mV, we observed three different types of HVA calcium channels: one BayK-sensitive channel, "L" (conductance 23pS);a BayK-insensitive, fast inactivating channel, "FI"(13pS); a BayK-insensitive not-inactivating channel with similar conductance but different kinetics from FI. When the temperature was changed from 15°C to 30°C, all three conductances increased with Q_{10} 1.4,1.32,1.28 respectively and the mean open time of the three channels decreased. Moreover, both FI and L channels inactivated faster at 15°C than at 30°C; FI channel activity was concentrated during the first 20ms at 30°C, while at 15°C the channel openings were distribuited on the whole trace; as a consequence, during a 150ms trace, the open probability was lower at higher temperature. In averaged traces of about 50 individual records the peak current increased with Q_{10} 1.35 (L) and 1.64 (FI).These data are in agreement with our previous results on whole-cell currents. (Pflug. Arch. 415, 1990)

187.3

MODAL GATING OF L-TYPE CALCIUM CHANNELS IN RAT CEREBELLAR NEURONS. D. Pietrobon* and L. Forti. C.N.R. Mitoc. Physiol. Center, Univ. of Padova, 35100 Padova, Italy.

Gating of single L-type calcium channels in rat cerebellar granule cells is complex and shows some peculiar properties not previously reported in other cells. The same L-type channel can have two different gating patterns. The less frequent "high- Po gating" (g=27 pS, 90 mM Ba^{++}) is similar to the previously described activity with the typical short openings (mode 1) and some rare long-openings (mode 2). The transitions between modes 1 and 2 have voltage dependent rate constants similar to those reported in cardiac cells (Pietrobon and Hess, 1990, Nature 346:651). In the presence of (+) 202-791 the mean open time is ~10 ms at 20 mV. In contrast, the more frequent "low-Po gating" (g=24 pS) has a mean open time of only 2 ms at 20 mV in the presence of agonist, and shows almost no activity in its absence (mode 1) except for some openings with mean open time ~1-2 ms (mode 2). In the range 0-30 mV the open probability of the "low-Po gating" does not increase, closed times do not become shorter and open times appear to shorten with voltage. Positive prepulses induce very long openings at negative voltages. The resulting average current shows a typical rising phase after the prepulse, which we show not to be due to recovery from inactivation, but rather to the peculiar voltage dependent properties of the open-closed transitions inside mode 2. These properties of the "low Po gating" result in minimal calcium influx during depolarization and potentiated calcium influx upon repolarization. **Supported by Fidia Research Laboratories.**

187.4

A NON T-, N- OR L-TYPE CALCIUM CHANNEL MEDIATES RELEASE OF TRANSMITTER FROM CEREBELLAR GRANULE CELLS IN TISSUE CULTURE. R.D. Randall* and W. Raabe. Depts. Neurology, Physiology and Neuroscience Graduate Program, VA Med. Ctr. and U. of Minnesota, Minneapolis, MN 55417.

The characteristics of the calcium channel responsible for transmitter release from cerebellar granule cells were investigated in rat cerebellar neurons grown in primary tissue culture. Whole cell patch voltage clamp recordings were obtained from large (≥15 μm ∅) cerebellar neurons, presumed to be Purkinje cells. Excitatory postsynaptic currents (EPSCs) were elicited by stimulation of a nearby granule cell (≤8 μm ∅) with an unpolished patch pipette.

Removal of Ca^{2+} from the extracellular solution, substitution of Ba^{2+} for Ca^{2+}, or addition of Cd^{2+} (20 μM) abolished evoked EPSCs but not action potential independent miniature EPSCs. This demonstrates the Ca^{2+}-dependence of transmitter release and that Ca^{2+} does not significantly contribute to the postsynaptic currents examined. Addition of T-, N- and L-type calcium channel antagonists Ni^{2+} (500 μM), ω-conotoxin GVIA (1 μM) or nifedipine (1 μM) failed to abolish evoked EPSCs. These observations indicate that the calcium channel mediating the release of excitatory synaptic transmitter from cerebellar granule cells is not of the T-, N- or L-type. This suggests the existence of an uncharacterized calcium channel.

(Supported by a grant from the Department of Veterans Affairs)

187.5

DIFFERENT VOLTAGE-DEPENDENT CALCIUM CHANNELS AT PRE- AND POSTSYNAPTIC STRUCTURES IN HIPPOCAMPAL AREA CA1. L.G. Wu* and P. Saggau. Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

We investigated the pre- *versus* postsynaptic distribution of different types of voltage-dependent calcium channels in the Schaffer collateral/commissural pathway (SCC) synapsing on CA1 pyramidal cells in hippocampal slices of guinea pigs. Synaptic structures were selectively loaded with the calcium indicator Fura-2 AM (Saggau et al., Biophys J 61, 1992). Pre- and postsynaptic calcium transients (pre-Ca^{2+}, post-Ca^{2+}) were optically recorded from the region of apical dendrites of area CA1, by single-pulse stimulating either orthodromically via SCC or antidromically via *alveus* (alv). The effects of different types of calcium channel blockers on pre-Ca^{2+}, post-Ca^{2+}, field EPSP (fEPSP), and population spike (pop) were investigated and preliminary results are summarized in the following table (=: no significant change; ---: no data).

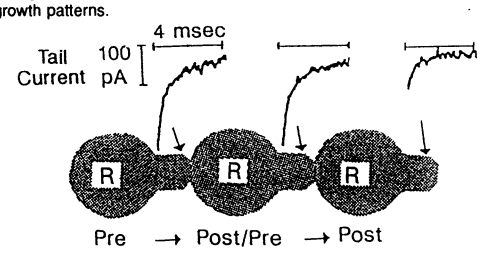
	orthodromic stim of SCC		antidromic stim of alv		
	pre-Ca^{2+}	fEPSP	post-Ca^{2+}	post-Ca^{2+}	pop
ω-conotoxin (1 μM)	-45%	-85%	-98%	-20%	=
Nifedipine (10 μM)	=	=	=	---	---
Nimodipine (40 μM)	=	+20%	+15%	+12%	=
Phenytoin (100 μM)	-10%	-30%	---	---	---
$NiCl_2$ (50 μM)	=	=	---	---	---

Our results suggest the existence of N-type (ωCTX-sensitive), but not L-type (dihydropyridine-sensitive) calcium channels in presynaptic terminals. The dose-response curve of ωCTX showed with 1 μM a saturating reduction of about 45% of pre-Ca^{2+}, which suggested the existence of another type of calcium channel in the presynaptic terminals. Phenytoin, a T-type channel blocker, decreased pre-Ca^{2+} reversibly, and delayed the orthodromic volley. The decreasing effect of ωCTX on post-Ca^{2+} induced by antidromic stimulation was reversible, suggesting the existence of either N-type or T-type calcium channel in the apical dendrites of CA1 pyramidal cells. Supported by grants of the Cain Foundation and the Whitaker Foundation to P. Saggau.

187.6

ANTEROGRADE AND RETROGRADE EFFECTS OF SYNAPSE FORMATION ON CALCIUM CURRENT DISTRIBUTION IN PRE- AND POST-SYNAPTIC CELLS. F. Fernandez-de-Miguel* R.L. Cooper, W.B. Adams and J.G. Nicholls Biocenter, CH-4056 Basel, Switzerland

Leech Retzius cells were plated as triads, with the tip of one Retzius cell touching the soma of another. Ca^{++} current distribution was measured by loose-patch clamp recording. With three cells arranged in a row, the last of the cells was purely postsynaptic. It showed a dramatic reduction in Ca^{++} tail currents in the initial segment compared to tail currents in either the first cell, (purely presynaptic) or the second cell of the chain (postsynaptic to the first cell and presynaptic to the third). These results show that neurons upon which chemical synapses have formed express different Ca^{++} currents depending on whether they themselves have formed presynaptic terminals on other cells. This change in current distribution is accompanied by marked differences in growth patterns.



187.7

CHARACTERIZATION OF CALCIUM CHANNELS IN RAT BRAIN SYNAPTOSOMES USING STOPPED-FLOW FLUORIMETRY AND FURA 2. M.M.Thomas* and S.M.J.Dunn. Dept. of Pharmacology, Univ. of Alberta, Edmonton, Canada T6G 2H7

The functional properties of voltage-dependent calcium channels have been measured in synaptosomal preparations from rat brain. Synaptosomes were equilibrated in low potassium (5 mM) buffer and loaded with the fluorescent calcium chelating dye, Fura 2, by incubation with the membrane permeant acetoxymethyl ester derivative. Depolarization-dependent uptake of calcium was measured in stopped-flow experiments by following the fluorescence changes of the entrapped dye. Rapid mixing with a high potassium (30 mM final) buffer resulted in an increase in intrasynaptosomal calcium concentration as measured by a quench in Fura 2 fluorescence at 390 nm. The magnitude of the quench was dependent on extrasynaptosomal potassium concentration and was completely blocked by the inorganic calcium channel blockers, cadmium, cobalt and lanthanum with EC_{50} values of 7.1 μM, 11.6 μM and 0.8 μM respectively. The observed quench was unaffected by the neuronal N-type calcium channel blocker, ω-conotoxin GVIA (1 μM). The dihydropyridine antagonist nitrendipine did not affect the observed quench over the concentration range, 10 nM to 10 μM. However, another dihydropyridine derivative, nimodipine blocked approximately 25% of the flux response. Supported by AHFMR and NIH GM-42375.

187.8

UNITARY CALCIUM CHANNEL CURRENTS RECORDED FROM SYNAPTIC VARICOSITIES OF EMBRYONIC XENOPUS MOTONEURONS IN VITRO. S.D. Meriney*, B. Yazejian and A.D. Grinnell. Jerry Lewis Neuromuscular Res. Ctr., UCLA School of Med., Los Angeles, CA 90024.

Presynaptic calcium channels are of critical importance in the regulation and modulation of chemical transmitter release. However, due to their relatively inaccessible distribution in the presynaptic membrane, with rare exception, they have been difficult to study directly. We have studied unitary calcium channel currents in varicose synaptic regions of *Xenopus* motoneuron neurites using the cell-attached patch-clamp configuration. Primary nerve-muscle cultures were prepared from stage 19-21 embryos. For isolation of single calcium channels, cultures were bathed in a normal frog Ringer and the patch pipette was filled with 100 mM $BaCl_2$, 10 mM Hepes, 2 μM TTX, and 100 μM 3,4 DAP. In two-day old cultures, we have measured two voltage-gated conductances (10-12 pS and 17-19 pS), both of which were resistant to dihydropyridine agonist modulation. In addition, we have evidence for a third conductance smaller than 10 pS.

Supported by grants from the NIH (NS06232) NSF (BNS8919841) and The Council For Tobacco Research.

187.9

KINETICS AND VOLTAGE DEPENDENCE OF THE HIGH THRESHOLD CALCIUM CURRENT IN RAT NEOCORTICAL NEURONS. A.M.Brown, P.C. Schwindt* and W.E.Crill Dept. of Physiology & Biophysics, Univ. of Washington Sch. of Med., Seattle, WA 98195.

High threshold (HT) calcium currents were recorded in pyramidal neurons acutely isolated from sensorimotor cortex of 14-28 day old rats using the whole cell patch clamp configuration. The HT current consists of dihydropyridine-sensitive, ω-conotoxin-sensitive and residual components (Sayer et al, Neurosci. Letts. 120, 1991). Kinetics were derived from the time course of current onset and offset (tails); the voltage dependence of activation (m_∞) was derived from tail current amplitude. While nifedipine (5 μM) and ω-conotoxin (10 μM by puffer pipette) partially suppressed the peak current, these agents altered neither time constants nor voltage dependence. Hence, the HT current of these neurons is composed of kinetically homogeneous subcomponents. Current onset was best fit by m^2 kinetics. Tail time constants increased with depolarization and fit m^2 kinetics only negative to -30 mV. The measured instantaneous current-voltage relation (IIV) was steeper than predicted by the constant field equation. The former but not the latter predicted a constant permeability over the range of potentials measured (-20 to +25 mV). Using m^2 kinetics and constant permeability, measured peak currents could be reconstructed from independently measured parameters (m_∞, time constants, IIV). Supported by ONR N0014-90-5-1627, NS 16792, and W.M.Keck Foundation.

187.10

LOW VOLTAGE-ACTIVATED (LVA) CALCIUM CURRENT IN BULLFROG DORSAL ROOT GANGLION (DRG) CELLS USING INTRACELLULAR L-ARGININE. J.C. Hackman*, C.A. Rodriquez, A.M. Holohean and R.A. Davidoff, VAMC and Dept Neurology, Univ of Miami School of Medicine, Miami, FL 33101.

Acutely dissociated *R. catesbiana* DRG cells were used for whole cell Ca^{2+} current recordings. Measurements of cell size and capacitance indicated at least a bimodal distribution. ≈ 50% were small-medium in size; mean diameter: 36.3 ± 1.0 μ; mean capacitance: 48 ± 1 pF. Larger cells; mean diameter: 44.4 ± 1.7 μ; mean capacitance: 95 ± 2 pF.

The following solutions were used: *Intracellular* (mM): 110 CsCl *or* 110 L-arginine, 10 EGTA, 5 $MgCl_2$, 40 HEPES, 2 ATP, 0.3 GTP; pH 7.4 adjusted with CsOH; *Extracellular*: 135 TEA-Cl, 10 Glucose, 10 HEPES, 0.0007 TTX, 5 $CaCl_2$; pH 7.4 adjusted with TEA-OH. Depolarizing step pulses of 5-10 mV from holding potentials (Hp) of -40 to -100 were used to examine high voltage (HVA) and LVA currents. Inward currents evoked using this protocol readily distinguished HVA currents at Hp of -50 mV and LVA currents at Hp of -80 mV. A ratio of the current evoked at -40 mV to the peak current was obtained from the I-V curve from Hp of -80 mV and used as a measure of LVA current.

When Cs was used, the amount of LVA Ca^{2+} current was not significantly different for different cell sizes if Ca^{2+} was used as the current carrier. Because Cs has been found to produce an outward current through Ca^{2+} channels, L-arginine was substituted (Xu, 1991). With L-arginine, the amount of LVA current was significantly greater in small-medium cells compared to larger cells (21% vs 12%). This may be explained by the larger Ca^{2+} currents seen in L-arginine as compared with Cs. (Supported by VAMC Funds MRIS #1769 and #3369 and USPHS grant #17577).

187.11

WHOLE CELL PATCH CLAMP RECORDING OF CALCIUM CHANNELS IN BULLFROG DORSAL ROOT GANGLION CELLS USING INTRACELLULAR ARGININE. Alice M. Holohean, Carlos A. Rodriquez, John C. Hackman and Robert A. Davidoff*, VA Medical Center and Department of Neurology, University of Miami School of Medicine, Miami, FL 33101.

Whole cell current recordings were made from acutely dissociated *R. catesbiana* dorsal root ganglion cells using the following solutions: *Intracellular* (mM): 110 CsCl *or* 110 L-arginine, 10 EGTA, 5 $MgCl_2$, 40 HEPES, 2 ATP, 0.3 GTP; pH 7.4 adjusted with CsOH; *Extracellular*: 135 TEA-Cl, 10 Glucose, 10 HEPES, 0.0007 TTX, 5 $CaCl_2$; pH 7.4 with TEA-OH. Depolarizing step pulses of 5-10 mV from holding potentials (Hp) of -40 to -100 mV were used to examine Ca^{2+} currents. Two populations of cells with high or low/medium capacitances (Cm) were studied.

Using Cs in the intracellular solution, at Hp of -80 mV, there were no significant differences between the high Cm (90±3 pF) and low/medium Cm (53.53±3.60 pF) cells for either current density (0.018±0.004 nA/pF and 0.017±.004 nA/pF, respectively) or reversal potential (38±4 mV and 37±4 mV, respectively). Small outward currents, presumably produced by Cs were observed at higher depolarizing voltages and were blocked by Cadmium.

When Cs was replaced by L-arginine (Xu, 1991), both the current density (0.037±.006 nA/pF for high Cm cells and 0.031±0.007 nA/pF for low/med Cm cells) and the reversal potential (49±5 mV and 53±6, high and low/med Cm cells, respectively) increased significantly. Outward currents were minute and rarely observed. (Supported by VAMC Funds MRIS #1769 and #3369 and USPHS grant #17577).

187.12

VOLTAGE CLAMP ANALYSIS OF CALCIUM CURRENTS IN ACUTELY-ISOLATED ADULT RAT NEOSTRIATAL NEURONS D.J. Surmeier*, J. Bargas and A. Howe, Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis, TN 38163.

Calcium currents are of importance to a variety of cellular functions including short- and long-term regulation of excitability. Recently, we have shown that cultured neostriatal neurons express both low-voltage activated (LVA) and high-voltage activated (HVA) currents (Bargas et al., *Brain Res.*, 541 (1991) 70-74). We have found that adult neurons differ from cultured neurons in that adult neurons rarely express LVA currents and have heterogeneous HVA currents.

Neurons were acutely isolated from juvenile and adult rats using conventional procedures. In brief, 400 μm frontal sections were enzymatically treated (pronase E, 1.5 mg/ml) for 30 minutes at 35 C in oxygenated HEPES-buffered salt solution (pH=7.3). After washing, striata were mechanically dissociated in a low Ca (200 μM) medium and plated for recording. Recording used conventional whole cell techniques.

In the majority of adult neurons (>90%, n=130), only HVA calcium currents were observed. In 5 mM calcium or barium, currents activated near -40 mV and peaked near 10 mV. These currents could be separated pharmacologically into two components. One component was blocked by the dihydropyridine antagonist nifedipine (Ki=126 pM, n=15) or ω-conotoxin (1-10 μM, n=10). The other component of the current was resistent to block by either dihydropyridines or ω-conotoxin. This work was supported by USPHS grant NS 28889.

187.13

HETEROGENEITY OF CALCIUM CHANNELS OF DIFFERENT RAT SYMPATHETIC NEURONS. Chu Chen and Geoffrey G. Schofield.* Department of Physiology, Tulane University School of Medicine, New Orleans, LA 70112.

It has been speculated that the electrophysiological and pharmacological characteristics of ion channels of sympathetic neurons may be dependent upon the end organ innervated by the neurons. In the present study we tested this possibility by investigating the properties of Ca^{2+} currents of superior cervical ganglion (SCG) and paravertebral ganglion (PVG) neurons removed from the same rats by using the whole-cell patch clamp technique. SCG and PVG (T_{10}-L_2) neurons from adult male Sprague-Dawley rats were isolated by an enzymatic dispersion method and Ca^{2+} currents were recorded using external and internal (pipette) solutions designed to isolate Ca^{2+} currents. Ca^{2+} current inhibition induced by norepinephrine (NE, 5 μM) was larger in SCG than that in PVG ($P<0.01$). Normalized tail current activation curves demonstrated that both SCG and PVG neurons could be fitted to a double Boltzmann equation. The activation curve of Ca^{2+} currents from SCG neurons was shifted more hyperpolarized compared with the curve of PVG neurons. In addition, Ca^{2+} channel density, expressed as maximal tail current normalized to cell capacitance, was higher in PVG than that in SCG. The differences in the biophysical properties of Ca^{2+} currents and sensitivity to NE in different populations of sympathetic neurons may reflect the heterogeneity of Ca^{2+} channels in neurons that innervate different tissues.

187.14

ROLE OF NERVE-MUSCLE CONTACT IN EXCITATION-SECRETION COUPLING. D. B. Gray*, C. Rossi, S. Korn, and G. Pilar. Department of Physiology and Neurobiology, Univ. of Connecticut, Storrs, CT 06269.

In chick ciliary ganglion (CG) neurons, K+ evoked secretion of acetylcholine (ACh) is mediated by Ca++ influx at terminals in the iris and choroid coat of the eye and in cocultures with muscle. The calcium pharmacology of ACh release has been shown to vary with presence of target and developmental age (Gray et al., Neuron 8: 715, 1992). At embryonic day 14 evoked ACh release from CG neurons cocultured with muscle is mediated by calcium influx through channels which are inhibited by nifedipine. In cultures of CG neurons cultured without muscle, this release is not sensitive to nifedipine. This dependence on the presence of muscle does not appear to be mediated by soluble trophic factors released by the muscle. Neurons cultured alone were either exposed to media conditioned by muscle or grown on aclar cover slips to prevent contact between neurons and muscle, and placed in muscle cultures for 24 hrs. Nifedipine was unable to inhibit evoked release in either condition. However, evoked release from st. 40 CG neurons grown on lysed myotube membranes for 24 hrs was reduced by 50% ($p< .05$) by 10 uM nifedipine. These results suggest that contact of neurons with muscle cell membrane can induce the coupling of L-like channels to ACh secretion. We are currently examining the pharmacology of whole cell calcium currents to determine if changes in the coupling of channels to secretion observed above may be mediated by differential expression of calcium channels in these cells. Supported by NIH grant to G. Pilar #5R01 NS 10338.

187.15

TWO COMPONENTS IN VOLTAGE AND TIME DEPENDENT INACTIVATION OF HVA CALCIUM CURRENTS IN PITUITARY CELLS. I. Nussinovitch*, E. Keller and O. Matzner. Dept. of Anatomy, Hebrew Univ. Sch. of Med., Jerusalem, Israel.

Influx of calcium through voltage sensitive channels is an important step in the regulation of hormone secretion from pituitary cells. Two populations of voltage sensitive calcium channels have been described in these cells; low voltage-activated (LVA) and high voltage-activated (HVA) channels. It is known from studies on pituitary cells and neurons that HVA calcium currents are partially inactivated when holding potential (Vh) is changed from -80 mV to -40 mV. In the present study we examined the kinetics of this inactivation. Experiments were performed with the whole-cell mode of the patch-clamp technique on two types of rat pituitary primary cultures: anterior cells (somatotrophs) and intermediate cells (melanotrophs). HVA calcium currents were recorded from pituitary cells in response to voltage steps (200 ms) to 0 mV first from Vh= -80 mV and then from Vh=-40mV (frequency 0.1Hz). The change in Vh from -80 mV to -40 mV completely and immediately blocked LVA currents and elicited a slow time dependent inactivation of the HVA calcium currents. We found that this inactivation of the currents can be described as the sum of two exponential decays with the average time constants of 38 ± 16 and 248 ± 102 seconds (mean ± S.D., n=8) for the sustained currents(measured at the end of the voltage step). Similar values were obtained for the inactivation of peak calcium currents.

These two time constants of inactivation may reflect inactivation of two different HVA channel subtypes or alternatively, inactivation of two different states of the same HVA channel in pituitary cells.

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187.16

HIGH- AND LOW-THRESHOLD CALCIUM CURRENTS COEXIST IN MAGNOCELLULAR NEUROSECRETORY CELLS (MNCs). T.E. Fisher*, S.H.R. Oliet, and C.W. Bourque. *Center for Research in Neuroscience, Montreal General Hospital and McGill University, Montreal, Canada.*

MNCs release oxytocin and vasopressin from axon terminals in the neurohypophysis. While calcium influx is known to be important for neurosecretion, as well as the regulation of electrical activity in these cells, somal voltage-gated calcium currents have not been characterized in MNCs. Whole cell voltage-clamp recordings from the somata of acutely dissociated MNCs from adult rats have revealed multiple subtypes of voltage-gated calcium current. In media containing 2mM Ca^{2+}, 40mM TEA, 4mM 4-AP, and 0.2 μM TTX, voltage steps from -100mV to voltages positive to -70mV elicited a rapidly activating (τ≈5-10ms) and inactivating (τ≈20-40ms) inward current. At higher potentials (above -35mV) additional inward currents appeared consisting of both an inactivating component and a non-inactivating nifedipine-sensitive component. Addition of 20-100 μM Ni^{2+} caused a selective and reversible inhibition of the low-threshold component. These data suggest that MNC somata express "T"-, "N"-, and "L"-like calcium currents. Interestingly, previous voltage clamp studies of the isolated nerve terminals of these cells found no evidence for "T"-type currents (Lemos & Nowycky, 1989). Taken together, these results suggest that somata and terminals can differentially regulate the expression of calcium channels. *Supported by FCAR and MRC.*

187.17

FURTHER DIVERSITY OF LVA Ca CHANNELS WITHIN BURST FIRING NEURONS OF THE CNS: LONG LASTING CURRENTS IN RAT LATERAL HABENULAR CELLS. J.R. Huguenard*, M.J. Gutnick, and D.A. Prince. Dept. of Neurology & Neurological Sciences, Stanford Univ. Medical Center, Stanford, CA 94305.

We previously demonstrated differences in transient, low-voltage-activated (LVA) Ca currents between two different burst-firing cell types in the thalamus, namely thalamocortical relay (TCs) cells and neurons of the reticular nucleus (nRt). These differences lead to distinct, cell-specific burst-firing patterns: TCs have relatively short bursts, while nRt bursts are prolonged. *In vitro* voltage recordings from lateral habenular (lHB) cells have demonstrated Ca-dependent bursts comparable to those in nRt. Another similarity to nRt is the propensity of lHB cells to fire an oscillatory series of bursts in response to appropriate stimuli. We hypothesized that the LVA current in lHB cells would be similar to or equivalent to that in nRt, and different from that in TCs. We obtained whole-cell voltage clamp recordings (n=29) from acutely isolated rat (age 6 - 34 days) lHB cells, and found they possess Ca-dependent LVA currents that differ from those in both nRt and TC cells. Important differences from previously described LVA currents included an inactivation process with prominent slow and fast components, equivalent permeability to Ba and Ca, and activation rates intermediate between those in nRt and TC cells. The LVA current in lHB cells was similar to that of other cell types in terms of sensitivity to divalent cations, succinimide anticonvulsants, and amiloride. We conclude that there is a remarkable diversity in LVA currents within the CNS. Furthermore, we hypothesize that slow activation and inactivation of LVA currents may be important for the generation of rhythmic Ca-burst oscillations. Supported by NIH grants NS06477 and NS12151.

187.18

N-TYPE CALCIUM CHANNEL INACTIVATION IS ENHANCED BY PHOSPHORYLATION. M. A. Werz*, K. S. Elmslie and S. W. Jones. Department of Physiology & Biophysics, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

Effects of phosphatase and protein kinase inhibitors on the predominantly N-type calcium current of bullfrog sympathetic ganglion neurons were investigated using the whole cell configuration of the patch clamp technique. Calcium currents of cells dialyzed with 1 μM okadaic acid, a phosphatase inhibitor, initially showed inactivation similar to controls but over time the majority (15 of 17) developed a rapidly inactivating component. After 50 to 70 minutes of cell dialysis, 5 to 11% (mean 8%) of current inactivated over 50 msec in controls, compared to 5 to 60% (mean 29%) for cells treated with okadaic acid. The difference is predominantly attributable to the development of a component inactivating with a time constant of 15 msec in cells dialyzed with okadaic acid, 10 times faster than the most rapidly inactivating component in controls. To determine whether okadaic acid was affecting phosphorylation, additional agents were studied. Nor-okadone, an inactive analog, did not alter the rate of inactivation compared with controls. Calyculin-A, a phosphatase inhibitor structurally dissimilar to okadaic acid, increased the rate of inactivation though less potently. Staurosporine, a protein kinase inhibitor, completely blocked the increase of inactivation by okadaic acid. Thus, the observed enhanced inactivation is likely to result from phosphorylation of the calcium channel or of a nearby site. The phosphorylation state of N-channels may underly the spontaneous shifting between inactivating and non-inactivating modes of gating (Plummer & Hess, *Nature*, **351**, 657-659, 1991) and the variability among published reports of N-channel inactivation rates.

187.19

PROTEIN KINASE INHIBITORS BLOCK THE PHORBOL ESTER INDUCED INCREASE IN CALCIUM CURRENTS EXPRESSED BY RAT BRAIN RNA IN XENOPUS OOCYTES. J.L. Costantin, M.D. Mauk*, and M.N. Waxham. Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston TX 77225

RNA from five-week old rat forebrain expresses voltage-activated, cadmium-sensitive, nifedipine (DHP), ω-Conotoxin and BAY K-8644 insensitive Ca^{2+}-currents in Xenopus oocytes. Two electrode voltage clamp was employed and current sweeps were elicited by a voltage step from -90mV (holding potential) to +10mV. Using a Cl^--free, 40mM Ba^{2+} recording solution after 24 hours in Cl^--free ringers, we recorded a 56% increase in peak Ba^{2+} current 14 minutes after the addition of 100nM Phorbol 12,13-Dibutyrate (PDBu). 100-1000nM of the inactive analog to PDBu, 4α-Phorbol 12,13-Dibutyrate (4α-PDBu) did not increase these voltage-activated Ba^{2+}-currents. DHP and ω-Conotoxin also remained ineffective after PDBu treatment. Kinase inhibitors K-252a and staurosporine reduced the increase in Ba^{2+}-currents elicited by PDBu. K-252a (2.1 and 1.1uM) inhibited the PDBu induced increase by 55% and 43%, respectively, normalized to PDBu. Staurosporine (10uM) completely blocked the increase caused by PDBu, 1uM staurosporine reduced the PDBu increase by 59%. Thus, we conclude that the action of PDBu is mediated through the action of protein kinases, most likely protein kinase C (PKC), and not through non-specific membrane effects. We are currently exploring the action of more specific peptide inhibitors of PKC on this PDBu effect and are investigating the effect of injecting purified PKC directly. The DHP and ω-conotoxin-insensitive currents expressed here are similar to those that have been shown to be largely P-type, due to their sensitivity to funnel web spider toxin (Lin et. al., PNAS 87, 4538-4542,(1990). It is unclear to us why oocytes would preferentially express a P-type channel over L- or N- channel types. Oocytes are a good preparation for injection of purified kinases or peptide inhibitors of kinases to look at phosphorylation regulation of a largely P-type current.

188.1

NYSTATIN PATCH MEASUREMENT OF CALCIUM INFLUX ACTIVATED BY MUSCARINIC AGONIST IN NEUROBLASTOMA CELLS. C. Mathes* and S. H. Thompson. Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950 and *Interdepartmental Program in Neurosciences, BRI, UCLA, LA CA 90024.

Production of cGMP following muscarinic receptor activation in N1E-115 cells requires calcium influx. We measured this calcium influx during voltage and current clamp experiments with the nystatin whole-cell patch configuration. Bath administration of carbachol activated a 3-10 pA current in cells held at -60 mV. The current was unaffected by the nicotinic AChR blocker curare (10 μM). The current increased during hyperpolarizing voltage steps. This indicates that it results from an increase in conductance and therefore is different from block of M-current. In current clamp experiments carbachol activated a 3-5 mV depolarization and broadened calcium spikes elicited by anode break. The external saline contained (in mM): 25 $CaCl_2$, 90 Tris-HCl, 10 TEA-Cl, 0.8 $MgCl_2$, 5 glucose, 25 HEPES and pH 7.4 - 7.5 at 30° C. The internal saline contained 100-200 μg/ml nystatin dissolved in (mM): 16 CsCl, 70 Cs_2SO_4, 5 $MgSO_4$, 10 HEPES, 100 sucrose and pH 7.2. [supported by NIH NS14519 to SHT]

188.2

CHOLINERGIC ENHANCEMENT OF LOW VOLTAGE-ACTIVATED (LVA) CALCIUM CHANNELS IN DISSOCIATED MEDIAL SEPTUM/DIAGONAL BAND (MS/nDB) NEURONS. W.H. Griffith*, J.D. Hood and M.J. Davis. Depts. of Medical Pharmacol. & Toxicol. and Medical Physiology. College of Medicine, Texas A&M University, College Station, TX 77843.

Cholinergic regulation of voltage-dependent Ca^{2+} channels may result in either enhancement or inhibition depending upon cell type, species of Ca^{2+} channel and/or second messenger systems involved. We have previously shown that cholinergic cells in the MS/nDB have LVA channels. We extend that work to show that LVA channels in some cells are specifically enhanced by carbachol (10 μM). LVA currents were recorded using the cell-attached patch clamp recording configuration from acutely dissociated MS/nDB neurons of adult guinea pigs. Isotonic K-aspartate was used to zero the cell's resting potential and barium (100 mM) inside the pipette was used as the charge carrier across the Ca^{2+} channel. Holding potentials were -100 or -80 mV with test potentials to -40 or -30 mV. The open probability (NP_o) of LVA channels was increased in a population of cells (36.9 $\pm$ 2.2%, n=3); whereas no increase was seen in two other cells. Ensemble averages of LVA channels were also enhanced by carbachol (6 of 10 cells). These results show that in a population of MS/nDB cells, LVA channels are enhanced by carbachol (10 μM). Supported AG07805 (WHG) and HL46502 (MJD)

188.3

GABA B RECEPTOR MODULATION OF HIGH VOLTAGE ACTIVATED BARIUM CURRENTS IN ACUTELY DISSOCIATED RAT HIPPOCAMPAL NEURONES. I.M. Stanford*, S.J. Caddick, H.V. Wheal and J.E. Chad. Dept. of Physiology and Pharmacology, Univ. of Southampton, SO9 3TU, UK.

Postsynaptic activation of GABA B receptors in the central nervous system (CNS) activates a potassium conductance responsible for the late inhibitory post synaptic potential. In peripheral neurones the GABA B receptor agonist baclofen also inhibits Ca^{++} currents (I_{Ca}) (Dolphin A.C. and Scott R.H., 1987, J.Physiol. 386, 1-17). In cultured CNS neurones it has been reported that baclofen reduces an 'L' type I_{Ca} in cerebellar granule cells (Wojcik W.J.*et al*, 1990, Neuropharm. 10, 969-972) and 'N' and 'L' type I_{Ca} in hippocampal pyramidal neurones (Scholz K.P. and Miller R.J., 1991, J. Physiol 444, 669-686).

Whole cell voltage clamp of acutely dissociated hippocampal neurones from 12 day old rats, allows the study of voltage activated currents without the space clamp problems associated with other preparations. The neurones retain their morphology so that CA1 pyramidal cells may be easily identified. After pharmacological blockade of typical sodium and potassium conductances, Ba^{++} currents (I_{Ba}) were activated from a holding potential (HP) of -80mV. There was little evidence of low voltage activated, T type currents, even when stepping from a HP of -100mV.

The I_{Ba} exhibited conventional activation and inactivation kinetics. Application of 20μM $\pm$ baclofen reduced peak current, for a maximal response, by 26% (n=5) without apparent effect on the voltage dependence of activation. The activation kinetics were slowed, increasing the latency to peak current by 11% (n=5). There was no apparent effect on inactivation. This preliminary data indicates GABA B receptors may directly inhibit post synaptic Ca^{++} currents, thus reducing pyramidal cell excitability by increasing spike threshold. A similar mechanism may also be involved in presynaptic inhibition of transmitter release.

Supported by MRC studentships to I.S and S.J.C and S.E.R.C. project grant (J.C.)

188.4

DOPAMINE INCREASES VOLTAGE ACTIVATED CALCIUM CHANNEL CURRENTS IN CULTURED WHITE BASS HORIZONTAL CELLS. C. Pfeiffer-Linn* and E.M. Lasater. Dep'ts. Physiology and Ophthamology, Univ. of Utah, Salt Lake City, UT. 84108.

H_2 cone horizontal cells in the white bass retina contain D1 receptors linked to adenylate cyclase and are innervated by dopaminergic plexiform cells. We have examined the effect of dopamine on voltage sensitive L-type sustained calcium currents on the H_2 cone horizontal cells using the whole cell patch clamp technique on cultured cells.

Isolated identified H_2 horizontal cells were held at -70mV and stepped in 10mV increments from -70 to +70mV or ramped over this range. Calcium currents were enhanced using 10mM extracellular calcium or barium in saline which pharmacologically suppressed Na^+ and K^+ currents. Pressure ejected dopamine increased the sustained voltage dependent calcium currents in these cells by as much as 200% (EC_{50}=7μM) without affecting its voltage dependence. This increase in calcium current is mimicked by the D1 receptor agonist, SKF 38393 (1μM). The D1 receptor antagonist, SCH 23390 (10μM), blocked the effect of 10μM dopamine completely.

The effect of dopamine on horizontal cells has been linked to increased adenylate cyclase activity. In this study, the membrane permeant cyclic AMP derivative, 8-(4-chlorophenylthio)-cyclic AMP, mimicked the effect of dopamine on the voltage sensitive calcium current. These results indicate that dopamine is acting through the D1 receptor on H_2 horizontal cells to activate a cyclic AMP mediated pathway which modulates voltage sensitive calcium channels to increase calcium influx. (Supported by NRSA EY06246, NEI EY05972 and grants from Research to Prevent Blindness, Inc.).

188.5

NMDA-INDUCED CALCIUM TRANSIENTS ARE ATTENUATED BY VOLTAGE-SENSITIVE CALCIUM CHANNEL ANTAGONISTS G.A. Skeen* and H.S. White Anticonvulsant Drug Development Program Dept. of Pharmacol. & Toxicol., Univ. of Utah, S.L.C., UT, 84108

The N-methyl-D-aspartate (NMDA)-preferring glutamate receptor is thought to be associated with an ion channel permeable to calcium (Ca2+), but distinct from voltage-sensitive Ca2+ channels (VSCC). The present investigation indicates that NMDA-preferring channels and VSCC's may be associated. Specifically, T- and L-type VSCC antagonists nickel (Ni2+) and nitrendipine (NT) respectively, were examined for their ability to block NMDA-evoked Ca2+ transients of mouse cerebellar granule cells. Primary cultures of granule cells were grown on 25 mm cover slips and maintained for 7-9 days. Mature cover slips were loaded with the Ca2+-sensitive fluorescent probe indo-1 (5 μM) for 20 min. Intracellular Ca2+ transients were evoked by the co-perfusion of 100 μM NMDA and 100 μM glycine, or by perfusion of 1 mM L-glutamate (GLU). Agonist solutions (NMDA, GLU) were prepared in magnesium-free buffer, and were perfused over the cells for 3 min to evoke intracellular calcium transients. Drug effects were studied by application of drug solutions with an agonist for 2 min following 1 min of agonist alone. Hence, each cover slip served as its own pre-drug control. NMDA-evoked transients were blocked completely by 10 nM MK-801. Ni2+ (100 μM) and NT (10 μM) individually attenuated NMDA-induced responses by 40-60%. In combination, Ni2+ (100 μM) and NT (10 μM) attenuated the NMDA transient by >80%. GLU-evoked transients were decreased by 60% with MK-801 (10 nM) alone. The subsequent combination of Ni2+ (100 μM) and NT (10 μM) with MK-801 (10nM) produced no additional attenuation. These data suggest that Ni2+ and NT may exert their effects on GLU and NMDA-evoked calcium transients in a manner similar to MK-801. Furthermore, these results suggest that VSCC's and NMDA-associated ion channels may be coupled. (Supported by NIH grants 2-RO1-NS22200 and GM07579)

188.6

INCREASE OF L-TYPE CALCIUM CURRENT BY BASIC FIBROBLAST GROWTH FACTOR IN ACUTELY ISOLATED NEURONAL CELLS FROM RAT VENTREOMEDIAL HYPOTHALAMUS. N. Matsuki*, H.Koike and H.Saito. Dept. of Chem. Pharmacol., Faculty of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo 113, Japan.

Whole-cell calcium currents were recorded from acutely isolated neuronal cells of the ventromedial hypothalamus from neonatal rat. Three types of Ca currents were recorded. Low threshold current (T-type) was activated at -40 to 0 mV when holding potential (HP) was -90 mV and inactivated rapidly. But only 10 % of the cells had this current. One of high threshold currents was slowly inactivated (L-type) and suppressed by nicardipine (10^{-5}M). The third current had intermediate inactivation rate and was activated when HP was more negative than -60 mV. This current was thought to be N-type, but ω-conotoxin (10^{-5}M) did not specifically suppress it. Effects of basic fibroblast growth factor (bFGF) on the Ca currents were investigated. At 10 ng/ml bFGF increased the L-type current in about 10 % of the cells studied. But other two currents were not affected. Although there was a great heterogeneity among the cells, neurotrophic and neuromodulatory effects of bFGF can be partly attributed to the augmentation of the L-type Ca current.

188.7

CONCENTRATION DEPENDENCE OF TRANSMITTER EFFECTS ON CALCIUM CURRENT KINETICS IN FROG SYMPATHETIC NEURONS. K. S. Elmslie* & S. W. Jones. Dept. Physiol. & Biophys., Case Western Reserve Univ., Cleveland, OH 44106.

Several neurotransmitters act through G proteins to slow the activation kinetics of N-type calcium currents. If the shift in kinetics reflects binding and unbinding of a G protein to the calcium channel, the time constant for slow activation might depend on the concentration of activated G protein. Lopez & Brown (*Neuron* 7:1061-1068, 1991) found such an effect, but Kasai (*J. Physiol.* **448**:189-209, 1992) did not. We have reexamined this by comparing large and small effects of transmitters (or GTP-γ-S). No clear changes in time constants have been found (see Figure). Either the slow activation process does not involve G protein binding, or the binding step is not rate limiting in our conditions.

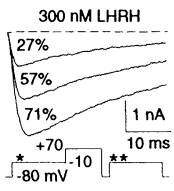

Figure: Difference currents, between records taken before (*) and after (**) the effect of chicken II luteinizing hormone-releasing hormone was reversed by a step to +70 mV. The % inhibition of current by LHRH during the prepulse to -10 mV is indicated. The largest effect was from the first application of LHRH to this cell; the others were at different times during a second application of LHRH.

188.8

ACTION OF GTP-γ-S ON CALCIUM TAIL CURRENTS IN RAT DORSAL RAPHE NEURONES. J.S. Kelly* and R.H. McAllister-Williams. Dept. of Pharmacology, Univ. of Edinburgh, 1 George Square, Edinburgh, Scotland, EH8 9JZ.

Serotonin has been shown to cause an inhibition of high-threshold calcium currents in dorsal raphe neurones (Penington et al, 1991, J. Neurosci, 11, 3594). In common with other vertebrate neurones, intracellular application of the non-hydrolysable GTP analogue, GTP-γ-S, is able to mimic the action of the transmitter. We have investigated a possible further action of GTP-γ-S on calcium tail currents.

Whole cell patch clamp current recordings were made from acutely isolated adult rat dorsal raphe neurones, with barium as the charge carrier. Currents were elicited by voltage jumps from -100 to -10 mV for 150 ms, and tail currents examined as the voltage was returned to -100 mV. All measurements were made at 20°C.

The time constant of single exponential curves fitted to the tail currents was 3.03 +/- 0.33 ms, n=38 (mean +/- standard error) in the control situation. When 200 μM GTP-γ-S was included in the patch pipette solution this increased significantly to 3.85 +/- 0.27 ms, n=23 ($p < 0.005$, Mann-Whitney-Wilcox test). To help examine which component of the calcium current was responsible for this increase, cadmium was applied to the bath solution. While 200 μM cadmium produced an 87 +/- 3% reduction in the current amplitude, the magnitude and rate of the tail current were not diminished (n=6). However, 2 mM cadmium produced a 95% reduction in current amplitude, and completely abolished the tail currents (n=2).

In addition to inhibiting high threshold current, it appears that GTP-γ-S also produces a retardation of calcium tail current kinetics. The type of current underlying this action would appear to be relatively cadmium insensitive. This would normally be explained as low threshold or 'T' type current (Fox et al, 1987, J. Physiol. 394, 149), however, one would expect such current to have fully inactivated during the 150 ms voltage jump. It is therefore not clear exactly which type of calcium current underlies the prolonged tail current produced by GTP-γ-S.

R.H.McA.-W. is a Wellcome Clinical Research Fellow.

188.9

INTERLEUKIN-1ß INHIBITS CALCIUM CHANNEL CURRENTS IN ISOLATED HIPPOCAMPAL CA1 NEURONS. C. R. Plata-Salamán[1]* and J. M. H. ffrench-Mullen[2], [1]Univ. of Delaware, Newark, DE 19716 and [2]Dept. Pharmacology, ICI Pharmaceuticals, ICI Americas Inc., Wilmington, DE 19897.

The effect of recombinant human interleukin-1ß (rhIL-1ß) on voltage-gated Ca^{2+} channel currents was examined in enzymatically dissociated CA1 neurons from adult guinea pig hippocampi using the whole-cell patch clamp technique. The external solution contained 3 mM Ba^{2+} (I_{Ba}) and the internal solution NMG-Cl, CS^+-BAPTA and Mg^{2+}-ATP. I_{Ba} was examined with 10 ms to 200 ms depolarizing command steps from a holding potential of -80 mV to a test potential of -10 mV. Compounds were applied by rapid superfusion. RhIL-1ß rapidly depressed only a fraction of both peak and late Ca^{2+} currents (measured 5 ms prior to the end of step) in a concentration-dependent manner. Depression of the peak current was (mean % depression): 2.4 (n=5), 9.4 (n=13), 19.4 (n=9), 19.8 (n=12), 21.9 (n=9) and 22.5% (n=10) at 0.98, 1.97, 7.9, 15.8 and 31.2 pg/10 μl rhIL-1β. Depression of the late current was: 4.2, 15.9, 28.5, 31.2, 32.4 and 32.7%. The apparent K_D's were 2 pg/10 μl and an nH of 2.8 for both peak and late current. At concentrations >1.97 pg/10 μl, the block was irreversible but with no further effect on the remaining current. Application of greater concentrations (up to 2 ng/10 μl) did not result in further depression of the inward Ca^{2+} current (n=10). The depressive effect of rhIL-1ß was completely blocked by the concomitant application of a specific rhIL-1 receptor antagonist (R & D Systems, MN) in concentrations 25-fold higher than rhIL-1β. The antagonist alone (49.3-197 pg/10 μl) had no effect. This demonstrates a specific and direct action of rhIL-1ß. These results show that IL-1β depresses the voltage-gated Ca^{2+} channel currents at pathophysiological concentrations (≥1.97 pg/10 μl). This may play a role in: 1) the regulation of neuronal excitability; 2) the induction of neurological manifestations during disease; and 3) in the induction and/or progression of neurodegenerative processes.

188.10

INTERLEUKIN-1ß INHIBITION OF CALCIUM CHANNEL CURRENTS IN ISOLATED HIPPOCAMPAL CA1 NEURONS: PHARMACOLOGY AND MODE OF ACTION. J. M. H. ffrench-Mullen[1]* and C. R. Plata-Salamán[2], [1]Dept. Pharmacology, ICI Pharmaceuticals, ICI Americas Inc., Wilmington, DE 19897 and [2]Univ. of Delaware, Newark, DE 19716.

The mode of action of recombinant human interleukin-1ß (rhIL-1ß)-induced inhibition of the voltage-gated calcium (Ca^{2+}) channel currents was examined in enzymatically dissociated hippocampal CA1 neurons from adult guinea-pig using the whole-cell patch clamp technique. RhIL-1ß showed no change in the peak I-V relationship at 1.97, 7.9 and 31.2 pg/10 μl (n=4). The time course of activation was speeded up (n=3) but there was no change in voltage-dependent activation by rhIL-1ß (n=2). The rate of deactivation of the current was unaffected (n=3). Peak current remaining in presence of ω-conotoxin (CgTX, GVIA; 10 μM) block was potentiated 11.9, 19.9 and 26.9%, while the late current (measured 5 ms prior to the end of step) was potentiated 8.9, 13.6 and 19.9% at 1.97, 7.9 and 31.2 pg/10 μl rhIL-1β (n=4, each) respectively. Peak and late currents in the presence of nifedipine (10 μM) were minimally affected. In the presence of CgTX plus nifedipine, the peak current was potentiated 17 (n=3), 17.6 (n=9) and 19% (n=4) at 1.97, 7.9 and 31.2 pg/10 μl rhIL-1β. Similar potentiation was observed in the late current; (±)BAY K 8644 (1 μM) potentiation of the Ca^{2+} current was blocked by rhIL-1ß (7.9 pg/10 μl). With GTP-γ-S (100-500 μM) in the recording pipette, rhIL-1ß had minimal effect on the peak current, whereas it suppressed the late current 12.1, 25.3 and only 9.3% at 1.97, 7.9 and 31.2 pg/10 μl (n=5) respectively. In the presence of CgTX plus nifedipine and GTP-γ-S (100 μM) in the electrode, rhIL-1β potentiated the peak current by 9.3, 12 and 9.9% at 1.97, 7.9 and 31.2 pg/10 μl rhIL-1β (n=5), whereas the late current was not affected (n=5). These results suggest that at pathophysiological concentrations (≥1.97 pg/10 μl), rhIL-1ß depresses a fraction (L-type) of the Ca^{2+} current in hippocampal CA1 neurons via G-protein(s).

188.11

A DIHYDROPYRIDINE-SENSITIVE T-TYPE CALCIUM CURRENT REQUIRED FOR ADRENOCORTICOTROPHIC HORMONE (ACTH)-STIMULATED CORTISOL SECRETION J.J. Enyeart*@, B. Mlinar@, J.A. Enyeart, and B.A. Biagi#. Depts. of Physiology#, Pharmacology and the Neuroscience Program@, The Ohio State University, Columbus, OH 43210-1239.

Many neurons and endocrine cells express multiple Ca^{2+} channels which couple membrane depolarization to secretion. Cortisol secretion by adrenal zona fasciculata (AZF) cells is stimulated by the pituitary peptide ACTH in a Ca^{2+}-dependent manner. Ca^{2+} channels in these cells have not been described, and their role in cortisol secretion is not clear. The whole cell version of the patch clamp technique was used to identify and characterize Ca^{2+} channels in enzymatically-dissociated bovine AZF cells. Nearly every cell (84 of 86) expressed only a low voltage activated, rapidly inactivating T-type Ca^{2+} current. Kinetics of T current activation and inactivation were described by a Hodgkin-Huxley model of m^4h form. The rates of T current activation, inactivation, deactivation, and reactivation approached maximum voltage independent values at extreme potentials.

Ca^{2+} current in AZF cells was blocked by the T channel selective antagonist penfluridol and by the dihydropyridine antagonist nimodipine with IC_{50}s of 300 nM and 3 μM respectively. At the same concentrations, these antagonists also inhibited ACTH-stimulated cortisol secretion measured over periods ranging from 3 to 48 hours. Cortisol-secreting cells of the adrenal gland are unusual among secretory cells in expressing only T-type Ca^{2+} channels which are sensitive to dihydropyridines and required for ACTH-stimulated cortisol production.

188.12

NIMODIPINE BLOCK OF L-TYPE CALCIUM CHANNELS IN A PRIMARY CULTURE OF RAT HIPPOCAMPAL NEURONS. P.E. TanPiengco and R.T. McCarthy*, Miles, Inc., West Haven, CT 06516.

Nimodipine inhibits L-type calcium channel current with high affinity in rabbit peripheral neurons but comparable high-potency block has not yet been demonstrated in central neurons. Whole-cell and cell-attached patch recordings were used to characterize voltage-dependent calcium channels in a primary culture of hippocampal neurons from neonatal rat pups. Whole-cell and cell-attached patch recording after 2-4 days in culture failed to detect dihydropyridine-sensitive current. However after 6-14 days in culture, these cells expressed a 22 pS [110 Ba^{+2}] conductance L-type channel which was resistant to inactivation by depolarized holding potentials (V_h= -30 mV) and enhanced by the calcium channel agonist BAY k 8644 (30-90 nM). Nimodipine inhibition of these calcium channels was dose-dependent. At 100 pM, nimodipine reduced the activity of L-type channels while at 10 nM the drug completely inhibited sustained L-type current (T_t= 150-160 msec; n=5). Direct effects of nimodipine on L-type calcium channels in central neurons may contribute to the therapeutic effects of nimodipine in central neurons.

188.13

IDENTIFICATION OF DIHYDROPYRIDINE BINDING REGIONS WITHIN THE $\alpha 1$ SUBUNIT OF CARDIAC CALCIUM CHANNELS.
H. Nakayama*, A.Kuniyasu, M.Taki and Y.Kanaoka, Faculty of Pharm. Sci., Hokkaido Univ., Sapporo, 060 Japan.

To identify regions which are involved in the formation of the dihydropyridine (DHP) receptor site of cardiac L-type calcium channels, the alpha 1 subunit of the channel complex was specifically labeled with a novel DHP-type photoaffinity probe, [^{3}H]diazipine which binds to the receptor with higher affinity than to the skeletal muscle receptor. Photolabeled regions were identified by probing labeled proteolytic fragments with several anti-peptide antibodies recognizing different segments of the alpha 1 sequence. A part of the alpha1-associated [^{3}H]diazipine label was contained in the 3 kDa tryptic fragment derived from the loop between segments S5 and S6 in domain III. This homologous region to the skeletal muscle channel which we have proposed to contribute to formation of the transmembrane pore (PNAS, **88**, 9203-7, 1991), is also considered to play such a role. Another part of the labeling occurred in a fragment that is not highly homologous to the skeletal muscle counterpart and contains a transmembrane segment of domain IV. Our data suggest that the cardiac DHP receptor is also formed by close apposition of two discontinues regions of the alpha1 subunit sequence in domain III and IV. It is proposed that amino acid residues at the putative extracellular surface in the loop connecting segments IIIS5 and IIIS6 fold into the extracellular end of transmembrane segment in domain IV to contrib-ute to formation of the DHP receptor site.

188.14

L-TYPE CA^{+2} CHANNEL ANTAGONISTS ENHANCE NEUROTRANSMITTER RELEASE.
H.K. Kramer*, E.P. Chiopelas, H.M. Akbari, and E.C. Azmitia. Dept. of Biology, New York University, NY, NY 10003.

Recent studies have indicated the presence of unique N and L Ca^{+2} channel subtypes to be found in heterogenous distribution throughout the brain. It has been suggested that while the N channel regulates Ca^{+2} influx that is coupled to neurotransmitter release, it appears that the L-subtype may play an important role in regulating internal Ca^{+2} levels during and after depolarization. This study examined the effects of the L-channel antagonist nimodipine (NIM) on the release of ^{3}H-5HT induced by K^+, phorbol 12-myristate 13-acetate (PMA), and 3,4-methylenedioxymethamphetamine (MDMA).

Rat forebrain synaptosomes were loaded with ^{3}H-5HT (50nM) and incubated in normal or Ca^{+2}-free buffer. Release of ^{3}H-5HT was tested with KCL (30mM), PMA (10^{-6}M), MDMA (10^{-6}M), and NIM (10^{-9}M) either alone or in combination. In normal buffer, NIM had no effect on PMA or MDMA-induced release, however, it did significantly ($p<0.01$) potentiate 30mM KCL induced-release. In Ca^{+2}-free buffer, NIM did not alter KCL or MDMA-induced release, but did significantly augment ($p<0.05$) PMA-induced release.

These results indicate that NIM elicits its effects by blocking the efflux of Ca^{+2} through the L-channel. Consequently, L-channels appear dually sensitive to both terminal Ca^{+2} concentration and electromotive force. In summary, our data shows that a function of the L-type channel in synaptosomes is to fine tune internal Ca^{+2} levels in order to promote a brief release of neurotransmitter, and to allow the efflux of Ca^{+2} after the membrane potential has become positive. (Supported by Miles Inc. to E.C.A and NIDA contract # 271-90-7403)

188.15

NIMODIPINE INHIBITS EFFULX OF $^{45}Ca^{2+}$ FROM RAT BRAIN SYNAPTOSOMES.
Wan K. Kim-Park* and Efrain C. Azmitia, Department of Biology New York University, New York, New York 10003

A known L-type Ca^{2+} channel blocker, nimodipine has been shown to modulate neuronal development, plasticity and aging. The possible role of nimodipine was tested on $^{45}Ca^{2+}$-uptake using rat brain synaptosomes. A micro-assay system has been developed to measure $^{45}Ca^{2+}$-uptake into synaptosomes. Using 96-well plate and a total volume of 150ul, preincubated (at 30°C for 15 min) synaptosomal allquot was added to prewarmed buffer containing ^{45}Ca for 1-30 sec. The uptake is stopped by quenching buffer containing 12 mM EGTA. The uptake was linear over 1-30 sec in resting and stimulating buffer. Nimodipine at a dose of 1 nM, stimulated $^{45}Ca^{2+}$ uptake in resting buffer (5mM K^+) and stimulating buffer (68.5mM K^+) significantly ($p<0.05$). The stimulation was the greatest at 1 sec and by 30 sec the stimulation was not seen.This paradoxical stimulation of ^{45}Ca-uptake by nimodipine was either due to an increase in uptake or a decrease in release. At a dose of 1nM, nimodipine inhibited ^{45}Ca efflux from the preloaded synaptosomes. The inhibition was more pronounced during 1 sec incubation in stimulating buffer than resting buffer. These results suggested that the L-type Ca^{2+}-channels can permit the reverse flow of Ca^{2+} depending on the membrane potential and Ca^{2+} equllibrium. The study is supported by NIDA contract (271-90-7403).

188.16

THE EFFECT OF POLYAMINES ON VOLTAGE-ACTIVATED CALCIUM CHANNELS.
M. D. Herman*, E. Reuveny, and T. Narahashi. Dept. Pharmacol.&Neurosurg., Northwestern Univ. Med. Sch., Chicago, IL 60611

The polyamines have been implicated to modulate cytoplasmic calcium concentration and are correlated with selective neuronal vulnerability in cerebral ischemia. In order to determine whether the polyamines modulate voltage-activated calcium channels, whole-cell and single-channel patch clamp experiments were performed with N1E-115 mouse neuroblastoma cells. L-type calcium channel currents showed a 34 ± 21% increase (n=6 cells) during external application of 1 mM putrescine. There was no change in the kinetics of the current and no shift in the current-voltage relation along the voltage axis. T-type calcium channel currents were not affected by 1 mM putrescine. External application of 1 mM spermine and 1 mM spermidine had no effect on T- and L-type calcium channels.

In order to test whether the effect of putrescine is mediated by a second messenger, specific protein kinase C and cyclic AMP-dependent protein kinase inhibitors, staurosporine and KT5720, respectively, were applied prior to putrescine. Staurosporine 200 nM prevented the increase of the L-type calcium current by 1 mM putrescine, whereas a 200 nM KT5720 did not inhibit the putrescine effect. The increase of L-type channel currents by putrescine may be mediated by protein kinase C but not the cyclic AMP-dependent protein kinase.

The effect of putrescine on single L-type calcium channels was studied using the cell-attached configuration of the patch clamp technique. Putrescine 5 mM applied to the external solution caused an increase in open time of the single channel current without changing the conductance of the channel. The number of channel openings longer than 3 ms increased from 6 to 76, and the number of channel openings longer than 9 ms increased from 0 to 27 in 345 depolarizing steps compiled from three cells. This single channel study supports the idea that putrescine acts on the cytoplasmic side of the membrane. Putrescine is the only polyamine elevated in ischemia, and does not affect the NMDA-receptor mediated channel. This suggests that the putrescine-induced enhancement of the L-type calcium channel activity may play an important role in calcium related neurotoxicity.

188.17

LIPOPHILIC AMINO ALCOHOLS WITH CALCIUM CHANNEL BLOCKING ACTIVITY.
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A series of novel lipophilic amino alcohols, analogs of the anticholinergic drug vesamicol, were evaluated for Ca^{2+} channel blocking activity. The effects of these drugs on depolarization-induced intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) transients were examined in single NG108-15 cells and dorsal root ganglion (DRG) neurons in culture, using Indo-1 based microfluorimetry. Structure-activity studies indicated that features required for Ca^{2+} channel blocking activity were distinct from those required for anticholinergic activity. In particular, the anticholinergic activity, but not the Ca^{2+} channel blocking activity, was enantioselective. One of the most active compounds 3-(3-bromophenyl)-2-hydroxy-1-[1-(4-phenylpiperidinyl)]propane was characterized in more detail. This compound inhibited the dihydropyridine-sensitive Ca^{2+} channel response in NG108-15 cells, evoked by depolarization with 50 mM K^+, with an IC_{50} of 5 μM. In DRG neurons, it inhibited the $[Ca^{2+}]_i$ transients elicited by field potential stimulation, which are mediated by all three Ca^{2+} channel subtypes. A key element required for Ca^{2+} channel blocking activity was the presence of an electron withdrawing substituent on the pendant phenyl ring. These lipophilic amino alcohols may provide a chemical starting point for the development of more potent compounds with broad-spectrum Ca^{2+} channel blocking activity.

188.18

DM-9384, A NOOTROPIC AGENT, INTERFERES WITH THE INHIBITORY ACTION OF OPIATES ON N-TYPE CALCIUM CHANNELS IN NG108-15 CELLS.
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We have previously reported that DM-9384 (nefiracetam), a newly-developed cognitive enhancer, facilitates a long-lasting (type II or N/L-type) Ca channel current in NG108-15 cells. DM-9384 also diminishes an inhibitory effect of enkephalin on the type II current. These DM-9384 actions can be mimicked by elevating the intracellular level of cAMP (Yoshii et al., Soc. Neurosci. Abstr. 17, 774, 1991). In the present study, we have further examined the effect of DM-9384 on the type II channel by isolating N-type component from L-type. The membrane was held at -50 mV to inactivate a transient (type I or T-type) current. Nifedipine (5-10 μM), an L-type channel blocker, reduced type II currents more than 50% and prevented the action of DM-9384 (1 μM). When Leu-enkephalin (50 nM) was applied in the presence of nifedipine, the type II currents were further reduced. The inhibitory effect of enkephalin was reversed by DM-9384 (1 μM). This DM-9384 action was mimicked by forskolin (10 μM) and dibutyryl cAMP (1 mM). The results suggest that DM-9384 interferes with the inhibitory action of opiates on N-type Ca channels via cAMP system.

188.19

SITE OF ACTION OF COGNITIVE ENHANCER DM-9384 THAT ENHANCES LONG-LASTING CALCIUM CHANNEL CURRENTS IN NG108-15 CELLS. M. Yoshii[1]*, S. Watabe[2], Y.L. Murashima[1], T. Shiotani[3] and M. Tanaka[4]. [1]Dept. of Neurophysiology, Tokyo Inst. of Psychiatry, Tokyo 156, Japan and [2]Explor. Res. Lab. II, [3]Medical Devel. Dept., [4]Devel. Res. Lab., Daiichi Pharmaceutical Co.,Ltd., Tokyo 134, Japan.

We have reported recently that DM-9384 (nefiracetam), a nootropic agent, can facilitate long-lasting (type II or N/L-type) Ca channel currents in NG108-15 cells (Yoshii et al., Soc. Neurosci. Abstr. 17, 774, 1991), and that the effect results from an increase in the intracellular level of cAMP (Murashima et al., Neurosci. Res. Suppl. 16, S61, 1991). In the present study, we have further investigated the site of action of DM-9384 using the whole-cell patch-clamp technique. DM-9384 (1 μM), when applied intracellularly through the patch electrode, enhanced type II currents as usually seen when applied externally. Then, proconvulsant agent pentylenetetrazole (PTZ) and Ro 5-4864 were applied in order to alter intracellular Ca^{2+}. PTZ (10 μM) did not affect the DM-9384 action, whereas Ro 5-4864 (10 μM), which reduced type II currents, diminished the effect of DM-9384. When inhibitory G-proteins were activated by Leu-enkephalin (50 nM) or intracellularly applied GTPγS (50 μM), the action of DM-9384 appeared more consistently. The results suggest that the site of action of DM-9384 is inside the cell, and regulated by G-protein systems rather than intracellular Ca^{2+}.

188.20

PGE1-ACTIVATED CALCIUM CHANNELS IN NIH 3T3 CELLS. Y. Shin, E. Ito, K. Oka, C. Collin and F. Gusovsky*, LBC, NIDDK and LMCN, NINDS, NIH Bethesda, MD 20892.

Calcium mobilization was studied in single NIH 3T3 cells preloaded with the fluorescent probe fura-2. Dose-response relationships were obtained for the ability of different PGs in generating increases in intracellular calcium. The dose-response relationships were in good agreement with the potencies of these compounds in stimulating phosphoinositide breakdown. PGE1 was remarkable since it was completely ineffective in inducing phosphoinositide breakdown even at 10 μM. However, PGE1 induced maximal increase in intracellular calcium at a concentration of 1 μM. A time course indicated that PGE1-induced calcium increase has a faster onset of action than PGF2α-induced response. In the absence of extracellular calcium, PGF2α elicits maximal calcium increase through the release of IP3 and subsequent release of calcium from intracellular stores. In a remarkable contrast, PGE1-induced calcium response is completely eliminated in the absence of extracellular calcium, suggesting the involvement of a calcium channel in PGE1-mediated action. PGE1 also induces a large increase in cyclic AMP accumulation in NIH 3T3 cells. However, neither forskolin, which activates adenylate cyclase directly, nor IBMX, a phosphodiesterase inhibitor, induce any change in intracellular calcium. PGE1-induced calcium influx was inhibited with divalent cations (cobalt, cadmium, nickel) at 100 μM, with the L-type calcium channel blocker nifedipine at 100 nM and with methoxyverapamil at 1 μM. However, neither divalent cations nor nifedipine affected PGE1-induced cyclic AMP accumulation. The results suggest that PGE1 induces increases in intracellular calcium through the activation of calcium channels that share the pharmacology of L-type calcium channels.

188.21

THE EFFECT OF HOLOLENA CURTA VENOM CONSTITUENTS ON Ca^{2+} INFLUX IN MAMMALIAN AND AVIAN SYNAPTOSOMES. Paul M. Lundy*, Anita Hong[+] and Robert Frew, Defence Research Establishment Suffield, Box 4000, Medicine Hat, Alberta, Canada, T1A 8K6: [+]NUROS Corp, 2020 Lundy Ave., San Jose, CA, USA, 95131.

Hololena curta venom (HC) potently inhibits Ca^{2+} influx and neurotransmitter release from rat synaptosomes. The constituents of the venom responsible for this activity and the channel on which they act were investigated. Crude venom was separated into 3 approximate molecular weight ranges by use of a Centricon micro-concentrator followed by reversed phase HPLC purification using a Vydac C_{18} column. The eluent was fractionated at 1 min intervals. Sixty fractions were collected for each mol. wt. range. The biological activity of the eluted fractions was measured by their ability to inhibit K^+ evoked synaptosomal Ca^{2+} influx. Two separate biologically active areas were found, one containing material in the range of 4,000 daltons and another of about 8,000 daltons. Ca^{2+} influx in rat brain synaptosomes was inhibited by crude venom (1:2,500), both fractions and $CdCl_2$ (0.1 mM) but was unaffected by the N channel inhibitor ω-CgTX. In chicken brain synaptosomes, Ca^{2+} influx was inhibited by both fractions, crude venom (1:2,500), $CdCl_2$ (0.1 mM) and also ω-CgTX (1 μM). The results provide pharmacological evidence for the existance of a high voltage activated Ca^{2+} channel in rat brain which does not correspond to the N or L designation. Synthetic FTX (0.1 mM) failed to affect Ca^{2+} influx in either tissue. HC appears to contain constituents which block the N channel as well as the ω-CgTX resistant channel.

188.22

CHARACTERIZATION OF ANTIBODIES AGAINST A CANDIDATE, PRE - SYNAPTIC CALCIUM CHANNEL SUBUNIT OF TORPEDO. A. Mastrogiacomo and C.B. Gundersen*, Department of Pharmacology and Brain Research Institute, UCLA School of Medicine, Los Angeles, California 90024.

The omega-conotoxin-sensitive, N-type Ca^{2+} channel has been implicated in the regulation of neurotransmitter release at a number of vertebrate synapses (see: Miller, Science 235; 46-52). This laboratory has recently cloned the cDNA encoding a candidate, N-type, Ca^{2+} channel subunit from Torpedo nervous tissue(C.B. Gundersen and J.A. Umbach, submitted $^{2+}$ Neuron). This protein is designated CCCS, for candidate Ca^{2+} channel subunit.To facilitate the biochemical isolation and tissue localization of CCCS, antisera against synthetic peptides(from the deduced primary structure of CCCS)have been produced in rabbits. Antiserum against the C-terminal 12 residues specifically immunoprecipitate CCCS produced by in vitro translation in rabbit reticulocyte lysate. On Western blots of the in vitro translation of CCCS, a protein of about 27 KD is recognized by immune serum but not by pre-immune serum. Binding of antibodies to this 27 KD band is abolished by excess C-terminal peptide of CCCS. When this antiserum is used to probe Western blots of a microsomal fraction of Torpedo electroplax a protein of about 34 KD is specifically identified. We are examining in more detail the tissue and subcellular distribution of this antigen. Moreover, we are investigating the basis of electrophoretic mobility shift of the antigen that is detected in in vitro translation versus tissues.

188.23

Lambert-Eaton Syndrome IgG Inhibits Calcium Channels Involved in Exocytosis in Small-Cell Lung Cancer Cells. Michael P. Viglione*, Thomas J. O'Shaughnessy and Yong I. Kim. *Departments of Biomedical Engineering, Neuroscience & Neurology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.*

The Lambert-Eaton syndrome (LES) is an autoimmune neuromuscular disorder. Patient weakness is a consequence of the blockade of presynaptic calcium channels resulting in a deficient release of ACh at the neuromuscular junction (NMJ). Frequently, LES patients also develop small-cell lung cancer (SCLC). It is currently thought that SCLC is the antigenic stimulus for the production of the autoantibodies which cross-react with Ca^{2+} channels at the NMJ. We have previously shown that LES IgG can inhibit both I_{Ca} (Kim & Neher, *Science* 239:405, 1988) and exocytosis (Kim *et al., J. Physiol.*, 446:245P) in bovine adrenal chromaffin cells.

SCLC cells (NCI-H146) were incubated with serum from an LES patient or from a healthy individual for 24 hours (1 mg/ml IgG). After achieving the whole-cell configuration, cells were given a 500 msec depolarizing stimulus to +10 mV (V_h=-80 mV). Five pulses were applied to all cells at a frequency of 0.2 Hz. We simultaneously recorded I_{Ca} and the resulting changes in membrane capacitance due to each depolarization with 10 mM $[Ca^{2+}]_o$.

LES serum was able to reduce both the peak I_{Ca} and the I_{Ca} measured at the end of the stimulation by ~40% ($p<0.05$). Exocytosis, as measured by increases in membrane capacitance, was reduced by slightly >50% ($p<0.05$) in the LES-treated cells. Thus, LES autoantibodies are capable of reducing I_{Ca} in SCLC cells which participate in exocytosis. As the release of ACh is reduced at the NMJ in patients with the syndrome, it may be that the Abs which reduce secretion from SCLC cells are the ones which block the presynaptic Ca^{2+} channels at the NMJ. (Supported by NIH grant NS18607 and the Muscular Dystrophy Association).

188.24

Dihydropyridine-sensitive Ca^{2+} Channels Participate in Exocytosis in Small-Cell Lung Cancer Cells. Thomas J. O'Shaughnessy, Michael P. Viglione and Yong I. Kim*. *Departments of Biomedical Engineering, Neuroscience & Neurology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.*

Small-cell lung cancer (SCLC) is an aggressive cancer frequently associated with the paraneoplastic disorder, Lambert-Eaton Syndrome (LES). The presence of SCLC is thought to be the antigenic stimulus for the production of autoantibodies which inhibit presynaptic Ca^{2+} channels at the neuromuscular junction. Therefore, it is of interest which type(s) of Ca^{2+} channels underlie secretion in these cells.

The effects of 10 μM nicardipine, 1 μM ω-conotoxin (CgTx) or vehicle were studied on the calcium currents (I_{Ca}) and exocytosis in SCLC cells (NCI-H146), as measured by increases in membrane capacitance, using the patch-clamp technique. A series of 5 depolarizations 500 msec in duration was applied to each cell from V_h=-80 mV to +10 mV.

Nicardipine was able to reduce I_{Ca} at the end of the stimulation by more than 50% ($p<0.005$) while inhibiting the peak by less than 10%. Exocytosis, however, was reduced by 60% ($p<0.05$). ω-conotoxin reduced both the peak and plateau I_{Ca} by approximately 25%. While a clear effect on secretion was not obvious, about half of the CgTx-treated cells displayed reduced I_{Ca} and exocytosis compared to controls. Therefore, it appears that calcium ions mediating exocytosis in SCLC cells enter through DHP-sensitive and possibly ω-CgTx-sensitive Ca^{2+} channels. It is not certain, however, whether LES antibodies are interacting with DHP-sensitive or ω-CgTx-sensitive Ca^{2+} channels to reduce exocytosis in SCLC cells. (Supported by NIH grant NS18607 and the Muscular Dystrophy Association).

188.25

PROFILE OF DUAL Ca^{2+} AND Na^+ CHANNEL BLOCKERS ON KCl- AND VERATRIDINE-INDUCED SYNAPTOSOMAL $[Ca^{2+}]_i$ INCREASE.

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A common feature of many synthetic compounds developed as blockers of neuronal Ca^{2+} channels or Na^+ channels is that they are active on both channel types. The determination of structural properties that favor specificity for one channel type requires the assessment of relative channel selectivities using uniform experimental conditions. We have investigated rat neocortical synaptosomes loaded with Fura 2 for this purpose, and tested the effects of channel blockers on increases in synaptosomal $[Ca^{2+}]_i$ induced by veratridine or depolarizing concentrations of KCl. The increase in $[Ca^{2+}]_i$ caused by veratridine consisted of a sustained plateau after a relatively slow rise. K^+-depolarizations also resulted in a sustained plateau, although concentrations of 16.5 mM and greater elicited a transient initial peak. The increase in $[Ca^{2+}]_i$ due to veratridine was almost entirely blocked by tetrodotoxin, whereas the response to K^+ was unchanged. Thus, the response to K^+-depolarization appears devoid of Na^+ channel activity, whereas that of veratridine reflects both a Na^+ and a Ca^{2+} channel component. A determination of IC_{50}'s of several dual Na^+ and Ca^{2+} channel blockers (e.g. flunarizine, RS 87476, R 58735) revealed that they were 3- to 9-fold more potent on the plateau induced by 10 μM veratridine than on the peak evoked by 33 mM KCl. They also inhibited the delayed plateau in K^+-depolarization by ~ 85%. The latter result indicates that the sustained $[Ca^{2+}]_i$ increase in K^+-depolarization may be caused by Ca^{2+} channel activity; alternatively, if the increase is due to the depolarization-driven electrogenic Na^+/Ca^{2+} exchanger as is often assumed, then this exchanger must be considered to be susceptible to inhibition by the dual channel blockers.

188.26

LAN-1 HUMAN NEUROBLASTOMA CELLS BECOME RESPONSIVE TO MEMBRANE DEPOLARIZATION AND MAITOTOXIN INDUCED $[Ca^{++}]_i$ INCREASE AFTER RETINOIC ACID-INDUCED DIFFERENTIATION.

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LAN-1 is a neuroblastoma cell line devoid of Ca^{++} channels activated by membrane depolarization or by Ca^{++} channels activators like maitotoxin (MTX) or BAY K8644. Since cell differentiation can be accompanied by functional expression of ion channels, LAN-1 cells were exposed for 1-7 days to 10μM retinoic acid (RA) and $[Ca^{++}]_i$ was monitored by fura-2 single-cell microfluorimetry. Morphological differentiation already became evident at the 3th day of RA exposure. By contrast, already after 1 day of RA treatment, LAN-1 cells showed $[Ca^{++}]_i$ elevation in response to depolarizing concentrations of K^+. This effect was still present after 3,5,7 days of RA-induced differentiation. Interestingly, even maitotoxin, a toxin described as an activator of Voltage Sensitive Calcium Channels (VSCC) in different neuronal cell types, became effective in stimulating $[Ca^{++}]_i$ increase in single LAN-1 cells. These results seem to suggest that: 1.Differentiation of LAN-1 cells induced by RA can induce the appearance of Ca^{++} channels activated by depolarizing stimuli 2. MTX beaves as a rather selective activator of VSCC since it is inactive in undifferentiated cells. (Supported by 40% and 60% MURST founds and CNR grants to L.A. and G.F.D.R.)

188.27

A NOVEL SUBSTITUTED GUANIDINE BLOCKER OF GLUTAMATE RELEASE.

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In ischemic brain tissue, blockade of presynaptic calcium channels in glutamatergic nerve terminals should attenuate the excessive release of glutamate leading to nerve cell death. A novel rapid kinetic method was used to resolve kinetic components of Ca-dependent, K^+-stimulated ^{3}H-glutamate release from rat brain nerve terminal preparations (synaptosomes) *in vitro:* a "phasic" component (decay const. <200 msec) and a more persistent component. A family of substituted guanidines were evaluated for their ability to block K^+-stimulated ^{3}H-glutamate release and ^{45}Ca uptake into rat brain synaptosomes. The ability to block glutamate release of compounds initially tested (at [10 μM]) correlated well with their ability to block the persistent but not the phasic component of Ca dependent glutamate release. A synthetic effort targetted to develop more potent and selective blockers of glutamate release led to CNS 1145. CNS 1145, at [3-10 μM], selectively blocked the persistent vs. the phasic component of glutamate release; it was more potent in its ability to block glutamate release than predicted from its ability to block synaptosomal ^{45}Ca uptake. In whole cell recordings of frog dorsal root ganglia and N1E-115 neuroblastoma cells, respectively, CNS 1145 blocked N-type Ca channels and TTX-sensitive Na channels at [2-20 μM]. CNS 1145 reversibly blocked excitatory synaptic transmission in hippocampal slice preparations. A dual action on presynaptic Ca channels and additional sites is one possible explanation for the relative potency of CNS 1145 as a glutamate release blocker. Selective block of the persistent vs. phasic Ca-dependent glutamate release may be a desirable characteristic for a neuroprotective agent.

188.28

MAMMALIAN VOLTAGE-GATED CALCIUM CHANNEL CURRENTS ARE BLOCKED BY Al^{3+}.

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Aluminum is the third most abundant element in nature. Aluminum in dialysis patients can cause dementia and has been suggested to play a role in Alzheimer's disease. Neurons from dorsal-root ganglia (DRG) of 2 to 4-day-old rat pups were cultured. Voltage activated calcium channel currents, carried by Ba^{2+}, were recorded using the whole cell patch clamp configuration. Patch pipette electrodes had resistances between 2 to 5 MΩ. Electrodes were filled with (in mM): CsCl 140; HEPES 10; EGTA 10; $MgCl_2$*6 H_2O 4 and Na-ATP 2. The external solution had the following composition (mM): TEA Cl 135; HEPES 10; glucose 10; $BaCl_2$ 10; $MgCl_2$ 1; TTX 0.002; pH was adjusted to 7.1-7.4. Neurons were routinely clamped at -80 mV. Voltage activated calcium channel currents were elicited every 5 to 15s by stepping from the holding potential of -80 mV to different potentials between -45 mV to +35 mV for 75 ms.

Al^{3+} blocked sustained and transient components of voltage activated calcium channel currents of DRG cells. Although solutions surrounding the neuron completely exchanged within 10-30s, a steady state blockade of calcium channel currents was reached within 1-5 min. Upon wash there was little or no recovery. Threshold concentration for blockade was 5-10 μM Al^{3+} and a total blockade (>80%) of the calcium channel current was reached with concentrations of 200 μM Al^{3+} or higher. The IC_{50} was 83 μM Al^{3+} and the Hill slope was around 3. The degree of blockade was very pH dependent and was greater at higher concentrations of H^+. Frequently, we observed a depolarizing shift in the peak of the current-voltage relation after applying Al^{3+}. The degree of the shift depended on the concentration of Al^{3+} but differed from cell to cell. We conclude that Al^{3+} is a potent and irreversible blocker of voltage activated calcium channel currents.

ACETYLCHOLINE: MUSCLE NICOTINIC RECEPTORS

189.1

cAMP and Calcium Regulate Nicotinc Acetylcholine Receptor Subunit RNA Levels

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During development of the neuromuscular junction there is a switch in expression of nAChR genes from an embryonic-($\alpha_2\beta\gamma\delta$) to an adult-type ($\alpha_2\beta\varepsilon\delta$). Embryonic-type genes are expressed throughout the muscle fiber prior to innervation. However, after innervation, extrajunctional expression of these genes is suppressed by nerve induced muscle activity and the adult-type receptors are expressed beneath the neuromuscular junction. The mechanisms by which this regulation is transduced to the genome is not known. We have developed a rat primary muscle culture system that suppresses the expression of the embryonic-type receptors when stimulated to contract with extracellular electrodes. The expression of the adult-type ε-subunit and the muscle creatine kinase genes were unchanged. We are using this *in vitro* culture system to characterize the signal transduction systems mediating the effects of nerve induced electrical activity on embryonic-type nAChR gene expression. We report here that agents that increase intracellular levels of cAMP can reverse the suppressive effect electrical activity has on the α–, β–, γ– and δ– subunit RNAs. The adult-type ε–subunit and the muscle creatine kinase RNAs were unchanged under the same conditions. Furthermore, increasing intracellular levels of calcium with the calcium ionophore A23187 decreases the levels of the α–, β–, γ–, δ– and ε–subunit, but not muscle creatine kinase, RNAs in cultures made inactive with tetrodotoxin. Currently, we are investigating the possible roles of other second messenger systems, as well as, continuing to study the pathways by which cAMP and calcium mediate their effects on the expression of the nAChR subunit RNAs. This work was supported by grants from NIH and MDA.

189.2

SODIUM BUTYRATE- AND CYCLIC AMP-DEPENDENT REGULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR EXPRESSION IN RMO AND BC3H-1 MUSCLE CELL LINES.

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Sodium butyrate (NaBu) or dibutyryl cyclic AMP (dbcAMP) treatments induce rapid, 90% downregulation of muscle-type nicotinic acetylcholine receptor (nAChR) expression in total particulate fractions of BC3H-1 mouse non-fusing muscle cells in concert with substantial (alphal, betal, or delta) or complete (gamma) inhibition of nAChR subunit gene transcription. Here, we demonstrate that these effects are accompanied by downregulation (≥ 60%) of cell surface nAChR expression and loss (≥ 90%) of nAChR-mediated function. Comparable effects are observed on functional nAChR expression by the rat fusing muscle cell line, RMO. Whereas downregulatory effects of NaBu or dbcAMP are evident within 6 hr of drug treatment and persist for at least 5 days, treatment of BC3H-1 cells with forskolin or with 8-(4-chlorophenylthio)-adenosine 3':5'-cyclic monophosphate induces functional downregulation and losses of cell surface nAChR that persist for only 1-2 days and reverse to control values 5 days after drug exposure. Continuing studies using the BC3H-1 and RMO systems are designed to test the hypothesis that motor neuronal influences on muscle cell development involve modulation (perhaps via co-released calcitonin gene-related peptide) of muscle cell cyclic AMP levels.

189.3

TRANSIENT CO-EXPRESSION OF ACETYLCHOLINE RECEPTOR SUBUNIT AND MYOD1 mRNA IN CULTURED MUSCLES. X. Su, S. Berman*, T. Sullivan and S. Bursztajn. Mailman Research Center, McLean Hospital and Harvard Medical School, Belmont, MA 02178.

We have quantified mRNA levels of acetylcholine receptor (AChR) subunits and the myogenic transcription factor MyoD1 in chicken muscle primary cultures by a nonradioactive, PCR-based method. We first prepared total RNA from cells and cRNA standards from cDNA clones. As controls, known amounts of cRNA of a foreign gene were added to both the cellular RNA and cRNA. After cDNA synthesis and PCR, signals were detected by the chemiluminescence method. The amount of mRNA in a sample was deduced from the cRNA standards after correction for its system efficiency determined from the control cRNA. With this method, we measured mRNA levels of α, γ and δ subunits of AChR and MyoD1 in chicken primary muscle cultures. We found peaks for all the mRNAs studied on Day 2 when myoblasts began to fuse. The RNA returned to nearly basal levels afterwards. After myotube formation, α subunit mRNA had a second peak on Day 6 but γ and δ subunits' mRNA had second peaks on Day 7. Among the three subunits, δ subunit mRNA was expressed the least. On the other hand, MyoD1 mRNA remained at basal level during Day 6 and Day 7, suggesting that MyoD1 was not required for the AChR gene expression after cell fusion. The data indicate that gene expression for each AChR subunit during muscle development is differentially regulated by mechanisms which are being investigated.

189.4

FUNCTIONAL NICOTINIC RECEPTORS ARE EXPRESSED IN C3H 10T1/2 CELLS TRANSFECTED WITH THE cDNA FOR MyoD, A MYOGENIC REGULATORY FACTOR. R. Odeh*, J. Philie, M. Szyf and M. Quik. Dept. Pharmacol., McGill U., Montreal, Que., Canada, H3G 1Y6.

Evidence now indicates that MyoD represents a transcriptional activator for muscle-specific genes including those for nicotinic acetylcholine receptor (nAChR) subunits. MyoD binding sites are present in the 5' flanking regions of DNA of the α and γ subunits of the nAChR and their presence is required for the expression of these subunits in muscle cells. The present studies were designed to investigate whether transfection of a non-muscle cell line with MyoD could induce the expression of a functional muscle nAChR. A mesodermal stem cell line C3H 10T1/2 was stably transfected with MyoD cDNA. Myogenic clones were isolated which formed myotubes in fusion medium. The presence of a nicotinic α-bungarotoxin (α-BGT) receptor population was demonstrated by saturable ^{125}I-α-BGT binding which was of high affinity (K_d of 1.5 nM). Time course studies showed that the receptors increased to a plateau level by 8 days in culture. Subsequent studies demonstrated the presence of carbachol-stimulated ^{22}Na uptake suggesting that the receptor was functional. Moreover, function correlated well with the levels of ^{125}I-α-BGT binding. Both ^{125}I-α-BGT binding and carbachol-stimulated ^{22}Na uptake were potently inhibited by α-BGT and d-tubocurarine (IC_{50}s of 2 nM and 250 nM, respectively). Thus, this receptor has a pharmacological profile characteristic of a muscle nAChR. To conclude, MyoD not only binds to 5' flanking regions of specific nAChR subunit genes (α and γ), but as shown in the present studies results in the expression of nAChRs which are functional. (Supported by MRC Canada.)

189.5

CYTOSKELETAL MECHANISMS IN THE REGULATION OF MUSCLE-TYPE NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) EXPRESSION BY TE671/RD CELLS. Merouane Bencherif* and Ronald J. Lukas, Barrow Neurological Institute, Phoenix, Arizona 85013

Treatment of TE671/RD human cells with inhibitors of actin polymerization (cytochalasins A, B, C, D, E, H, or J at concentrations of 5 ug/ml) produces a two- to five-fold increase in nAChR per unit of total membrane protein (B_{max} for ^{125}I-labeled o-bungarotoxin binding) with no change in nAChR affinity (K_d) or muscarinic receptor density. These effects are apparent between 1 to 2 days of drug treatment, increase to plateau between 3 to 6 days, and are only slowly reversible with a time constant of 2 days. These results reflect an up to 10-fold increase in intracellular nAChR whereas cell surface receptor density is unchanged or slightly decreased. Functional studies using a $^{86}Rb^+$ efflux assay indicate that cell surface receptors are fully responsive to agonist, and Northern blot analysis shows a 4 to 5-fold coordinate increase in the expression of mRNA encoding alphal, betal, gamma or delta subunits. When cells are co-treated with non-labeled alpha bungarotoxin to block surface nAChR, cytochalasin up-regulation was still observed suggesting that it does not require transit of nAChR through a cell surface pool. The magnitude of nAChR up-regulation following chronic exposure to nicotine (1mM) is similar to but not additive with that of cytochalasin treatment. These data support a role for microfilaments in the regulation of nAChR expression.

189.6

DIFFERENTIAL EFFECT OF SPONTANEOUS ACTIVITY ON ACETYLCHOLINE RECEPTOR STABILITY IN PRIMARY RAT MUSCLE CELL CULTURES. J.P. O'MALLEY & M.M. SALPETER* Section of Neurobiology & Behavior, Cornell University, Ithaca NY 14853

Cultured rat myotubes express acetylcholine receptors (AChRs) that decay with a double exponential indicating the existence of two populations of receptor. Approximately 80 % have a $t_{1/2}$ of ~ 1 d and 20 % have a $t_{1/2}$ of ~ 3 d. These half-lives are similar to those reported for rapidly degrading (R_f) and slowly degrading (R_s) AChRs at denervated neuromuscular junctions (Shyng & Salpeter J. Neurosci. 10:3905-3915). Like the AChRs at the denervated neuromuscular junction, the myotube R_S AChRs are stabilized to a degradation $t_{1/2}$ of 8 d by cAMP analogues, while the degradation $t_{1/2}$ of the R_f AChRs are unaffected.

In this study AChRs were labeled with [^{125}I]-α-bungarotoxin on day 4 or day 10 of culture (i.e. before or after the myotubes become spontaneously active on day 6) and the loss of labeled receptors measured for the next 13 days. We found that the R_S AChRs inserted in quiescent 4 day old myotubes had a $t_{1/2}$ of 3 days which did not change throughout the 13 day sampling period. However the R_S AChRs inserted in active 10 day old myotubes decayed with a $t_{1/2}$ of 8 days. In both cases the R_f retained their $t_{1/2}$ of 1 day. Because our results show that myotubes can simultaneously have some Rs which degrade with a $t_{1/2}$ of 3 d and others with a $t_{1/2}$ of 8 d, we suggest that the degradation rate of the Rs AChR depends on the time at which they were inserted, i.e before or after the initiation of activity, and does not simply slow down as the myotube ages.

We conclude that spontaneous muscle activity can maintain the stability of newly inserted R_S AChRs but does not stabilize them once they are in an accelerated state. In that respect spontaneous activity differs from cAMP which does stabilize the accelerated Rs AChR.

Supported by NSF grant NS09315 (MMS) and MDA postdoctoral fellowship (JOM)

189.7

DEGRADATION RATE OF MUSCLE ACHR EXPRESSED IN *XENOPUS* OOCYTES. E. Liu, O. Hamill, T. Podleski* and M.M. Salpeter. Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

The *Xenopus* oocyte system has proven very useful in studying properties of proteins expressed from injected mRNAs, and much has been learned about the physiological role played by the different subunits of the acetylcholine receptor (AChR). We were interested in using the oocyte system to study the regulation of turnover of muscle AChRs. Using culturing conditions for prolonged maintenance of oocytes (developed by Quick, Naeve, Davidson, and Lester, Biotechniques in press), functional AChRs were expressed from mouse and Torpedo α β γ and δ subunit mRNAs, and their degradation rates determined. Resting potentials were used as a criterion for oocyte viability.

We found that when the oocytes were maintained at 16°C, degradation $t_{1/2}$ values of 4.7 ± 0.5 (n=7) and 5.6 ± 0.6 (n=3) days were obtained for the mouse and Torpedo AChRs respectively. These values are slower than the ~ 1 day half life seen for embryonic AChRs in mouse myotubes at 37°C. and closer to the value of ~ 2 1/2 days previously reported for AChR in *Xenopus* myotomal muscles at 20°C (Brehm et al., J. Neuroscience 3:101-107, 1983). We suggest that when properly maintained, the *Xenopus* oocyte system is usable to study conditions which may regulate AChR degradation. The role of temperature, subunit composition, and associated proteins will be examined.

Supported by NSF grant NS09315.

189.8

N-GLYCOSYLATION OF NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNITS IS REQUIRED FOR EFFICIENT INTRACELLULAR TRANSPORT OF ASSEMBLED RECEPTORS. Vaughn M. Gehle* and Katumi Sumikawa. Dept. of Psychobiology, Univ. of California, Irvine CA 92717-4550.

We previously demonstrated that removal of the conserved N-glycosylation site on the four subunits of the *Torpedo* nicotinic AChR does not prevent subunit assembly, but rather causes changes in both degradation rates of mutant subunits and transport of assembled receptors (Mol. Brain Res. 11: 17-25, 1991). These results were obtained by directed mutagenesis of the Asn residue in the N-glycosylation consensus sequence (Asn-X-Ser/Thr) to an Asp residue. Additional experiments to determine how the negative charge associated with the Asp affected our results are reported here. For these experiments, we created additional mutant subunits by changing the Ser or Thr residues to Ala. These second mutant subunits were tested for assembly and degradation rate by immunoprecipitation of the subunits expressed in *Xenopus* oocytes. Functional studies were also performed using voltage-clamp techniques and α-bungarotoxin binding assays. Our results demonstrate that the degradation rate changes seen previously were mainly due to the addition of the negative charge while the decrease in transport of receptors to the surface membrane was caused by the loss of the carbohydrate moiety. Thus, for the four subunits of the *Torpedo* AChR, glycosylation of the nascent peptides serves mainly to aid in efficient transport of assembled receptors to the cell surface membrane.

189.9

TUNICAMYCIN INCREASES DESENSITIZATION OF JUNCTIONAL AND EXTRAJUNCTIONAL ACH RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES BY A MECHANISM INDEPENDENT OF BLOCKING N-GLYCOSYLATION. Tomoyuki Nishizaki and Katumi Sumikawa*. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717-4550.

In muscle cells, it was shown that tunicamycin (TU), an inhibitor of protein N-glycosylation, reduced the appearance of functional AChRs in the plasma membrane either by blocking the assembly of AChR subunits or increasing the rate of degradation of AChR molecules. In *Xenopus* oocytes injected with AChR mRNAs and treated with external TU alone, N-glycosylation was only partially blocked. Therefore, in these oocytes relatively large membrane current responses to ACh still could be observed. Interestingly, extrajunctional and junctional mouse muscle AChRs expressed in oocytes in the presence of TU desensitized more rapidly than the corresponding AChRs synthesized in the absence of TU. The two types of AChR expressed in non-TU-treated oocytes could be distinguished by their different rates of desensitization, but TU diminished this difference. The effect of TU on the AChR desensitization appeared to be reversible and co-application of TU with ACh also caused a similar effect on desensitization of these AChRs, suggesting the effect of TU was mediated by a mechanism independent of blocking N-glycosylation.

189.10

SECOND MESSENGER MODULATION OF ACETYLCHOLINE RECEPTOR CHANNELS IN MYOTUBES FROM *dy* MICE. Alfredo Franco-Obrégon* and Jeffry B. Lansman. Department of Pharmacology, UCSF, San Francisco, CA. 94143.

The activity of nicotinic acetylcholine receptor channels recorded from cell-attached patches from *dy* myotubes differs from that recorded from wild type myotubes in two ways. First, spontaneous openings of acetylcholine receptor channels are observed at a much greater frequently in *dy* myotubes. Secondly, the sensitivity of acetylcholine receptor channels to acetylcholine appears to be reduced in *dy* myotubes when compared to wild type myotubes.

Excising patches of myotube membrane from the surface of *dy* myotubes results in a steady drop in spontaneous activity, suggesting that diffusible cytosolic second messengers are responsible for maintaining spontaneous openings. Adding bromo-cAMP or Forskolin to cultures of wild type myotubes results in an increase of spontaneous channel openings and a concomitant reduction in the responsiveness of acetylcholine receptor channels to acetylcholine, mimicking the *dy* dystrophic condition. We propose that the *dy* phenotype could arise as a result of a defect of the adenylate cyclase signalling pathway.

189.11

A SMALL CONDUCTANCE ACh CHANNEL IS PRESENT FOLLOWING RECOVERY FROM DESENSITIZATION AT STAUROSPORINE-TREATED SNAKE ENDPLATES. J.C. Hardwick* and R.L. Parsons. Dept. of Anatomy & Neurobiology, The University of Vermont, Burlington, VT 05405.

Pretreatment of snake twitch muscle fibers with staurosporine, a protein kinase inhibitor, decreased the extent of AChR recovery from carbachol-induced desensitization (Hardwick et al., Brit. J. Pharm. 104:879, 1991). Here we show that the decrease of recovery by 0.5μM staurosporine, determined by a decrease in MEPC amplitude, was dependent on the duration of carbachol exposure. Following a 1 min. exposure to 540μM carbachol, staurosporine-treatment did not alter AChR recovery from desensitization. However, with a 5-10 min. exposure to carbachol, staurosporine pretreatment significantly decreased MEPC amplitude during recovery for periods up to 1 hr. The decrease in MEPC amplitude was not associated with a shift in the reversal potential. Single channel measurements of ACh-activated currents from cell-attached patches demonstrated that a single class of ACh-activated currents with a slope conductance of 45-49 pS was present at both control and staurosporine-treated endplates. Following recovery from a 5 min. exposure to 540μM carbachol, small amplitude ACh-activated channels were recorded at staurosporine-treated preparations in addition to the large conductance channels. The reversal potential of the small conductance channels was similar to that of the large conductance channels. The small conductance channels were not present following recovery from desensitization at control endplates. We propose that the staurosproine induced decrease in extent of recovery is due to the presence of small conductance channels which in control preparations are converted to large conductance channels by a staurosporine-sensitive protein kinase. Supported by NIH grants NS 08580 and NS 25973 and a MDA grant.

189.12

SPERMIDINE AND PUTRESCINE MODULATE THE FUNCTION OF THE PERIPHERAL NICOTINIC ACETYLCHOLINE RECEPTOR. M.M. Froes-Ferrao*[1], M.C. Heluy-Dantas[1], E.X. Albuquerque[1,2] & R. Rozental[1]. [1]Lab. Mol. Pharmacol., IBCCF, UFRJ, Rio de Janeiro, Brazil 21944; [2]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Spermidine and putrescine are ubiquitous polyamines and much experimental evidence indicates their role in cell development. Their possible actions on membrane receptors were indicated by the findings of Anis *et al.* (*J. Pharmacol. Exp. Ther.*, 254:764, 1990) showing that spermidine displaces α-bungarotoxin and histrionicotoxin from their binding sites on the nicotinic acetylcholine receptor (nAChR). Here, we further evaluated the interactions of these polyamines on single channel currents generated by the activation of the nAChR applying the cell-attached patch-clamp technique to single fibers isolated from the interosseal muscle of the frog *Leptodactylus ocellatus*. Neither spermidine nor putrescine applied to the single muscle fibers at concentrations up to 10 μM induced any single channel current. However, spermidine and putrescine (1-100 μM) both increased the number of flickers of single channel currents elicited by 0.4 μM acetylcholine (ACh). In addition, these polyamines decreased the mean open time and prolonged the burst duration of the ACh-activated single channel currents, in a concentration- and voltage-dependent manner. Our findings taken altogether suggest that putrescine and spermidine interact with a site most likely located within the ion channel of the nAChR. Therefore, these data support the idea that these polyamines play a key role in the regulation of the activation of the nAChRs. Support: FINEP, UFRJ/UMAB Mol. Pharmacol. Training Program and CNPq.

189.13

$GABA_A$ ANTAGONISTS PICROTOXIN AND BICUCULLINE ALTER NICOTINIC ACETYLCHOLINE CHANNEL KINETICS IN CULTURED EMBRYONIC RAT MYOTUBES Q.Y.Liu*, V.Smallwood and J.L.Barker Lab of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

The effects of $GABA_A$ receptor antagonists picrotoxin (PTX) and bicuculline (BIC) on nicotinic acetylcholine receptor (AChR) in cultured embryonic rat myotubes were first recorded in the cell-attached patch-clamp mode. This embryonic AChR current was of low conductance (slope conductance: 35.4 $\pm$ 0.6 pS), long lasting (open time constant, τ_o: 6.3 $\pm$ 0.5 ms) type (with 2 μM ACh in the pipette) as reported previously. Its reversal potential (RP) was at +60.8 $\pm$ 3.3 mV pipette potential (Vp). In our experimental conditions, the ratio of the total time the channels stayed in the open state over the total recording time of 25 seconds ($T_{open}/T_{recording}$) was 31.3 $\pm$ 4.1%. 50 μM PTX significantly decreased τ_o of the channel to 1.7 $\pm$ 0.1 ms (-73.0% as compared with control, $p < 0.01$) and $T_{open}/T_{recording}$ to 19.3 $\pm$ 3.0% (-38.3%, $p < 0.05$) without significantly affecting its conductance (34.3 $\pm$ 0.6 pS) or RP (+55.1 $\pm$ 4.3 mV Vp). BIC in the same concentration was less powerful in decreasing τ_o but more powerful in decreasing $T_{open}/T_{recording}$. τ_o and $T_{open}/T_{recording}$ in BIC were 4.2 $\pm$ 0.6 ms (-33.3%, $p < 0.05$) and 11.4 $\pm$ 3.0% (-63.6%, $p < 0.01$), respectively. Channel conductance and RP in BIC (33.7 $\pm$ 0.7 pS and +63.3 $\pm$ 2.5 mV Vp, respectively) were not significantly different from control. The effects of PTX and BIC were also recorded with the whole-cell voltage clamp technique. 50 μM PTX and BIC significantly decreased whole-cell embryonic AChR currents to 77.4 $\pm$ 6.9% ($p < 0.05$) and 63.1 $\pm$ 4.6% ($p < 0.01$) of control values, respectively. We conclude that $GABA_A$ receptor antagonists PTX and BIC can alter embryonic AChR channel kinetics in rat skeletal myotude without changing their unit conductance or ion selectivity.

189.14

CHARACTERIZATION OF A NON-MOTILE ZEBRAFISH MUTANT, *nic*, THAT LACKS FUNCTIONAL ACETYLCHOLINE RECEPTORS. Diane Sepich, Robert Ho, and Monte Westerfield*, Institute of Neuroscience, University of Oregon, Eugene, Oregon.

One of the first signs of neuromuscular synapse formation is the clustering of acetylcholine receptors (AChRs) beneath the nerve terminal. To study steps in synapse assembly, we are characterizing a mutation in zebrafish, *nic* [b107] which blocks the expression of clustered and functional AChRs in muscle cells.

During normal development, zebrafish muscle requires contact with a neuron to cluster AChRs. The *nic* defect could block either the neuron's ability to signal the muscle or the muscle's ability to respond. We tested in which cell the mutation acts by transplanting precursor cells from labeled wild-type donor embryos into mutant embryos. After axon outgrowth and synaptogenesis, we visualized the AChRs with rhodamine-αbungarotoxin. Wild-type muscle cells transplanted into mutant embryos clustered AChRs. In contrast, mutant muscles failed to cluster AChRs even when innervated by wild-type motoneurons.

To test whether the mutation alters the AChR alpha subunit gene or its expression, we isolated the corresponding zebrafish cDNA and performed Southern analysis. A restriction fragment length polymorphism in the alpha subunit cDNA is linked to the mutation.

We conclude that the *nic* defect acts solely within the muscle cell to block AChR synthesis or clustering and may be due to a mutation within the alpha subunit gene. Supported by NIH NS21132 and HD22486.

189.15

ANTIBODIES AGAINST THE LEVAMISOLE ACETYLCHOLINE RECEPTOR OF THE NEMATODE C. ELEGANS. J.R. Romeu[1], K.S. Helmer[1], J.T. Fleming[2] and J.A. Lewis[1].* [1]Division of Life Sciences, University of Texas at San Antonio, San Antonio, TX 78249 and [2]MRC Laboratory of Molecular Biology, Cambridge, England CB2 2QH

Mutations conferring resistance to the nicotine analog levamisole identify acetylcholine receptor (AChR) mutants of the nematode C. elegans. Mutation of the AChR blocks the toxic muscle-hypercontracting effects of levamisole. By transposon tagging and by crosshybridization with the Drosophila AChR subunit gene ard, several genes associated with levamisole resistance have been cloned and sequenced. By homology to Drosophila and vertebrate subunits, unc-38 encodes an alpha subunit and unc-29 encodes a non-alpha subunit. Mutations have been isolated in each of these genes that block levamisole agonism with little effect on motor behavior, suggesting the binding site of the nicotine analog may be between subunits. Efforts are underway to identify the amino acid residues altered in the mutants. A monoclonal antibody that we raised against highly purified receptor shows punctate, synapse-like staining in the dorsal and ventral nerve cords where muscle arms receive innervation from motorneurons (G. Ruvkun, pers. comm.). Similar staining occurs in the central neuropil. Because the staining is weak, polyclonal antibodies are being raised against unc-38 and unc-29 fusion proteins. Our goal is to identify mutants affecting synapse formation.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY II

190.1

EXCITATORY AMINO ACIDS IN GLAUCOMA: A POTENTIALLY NOVEL ETIOLOGY OF NEURONAL LOSS IN THIS OPTIC NEUROPATHY. Evan B. Dreyer* & Stuart A. Lipton. Departments of Ophthalmology and Neurology, Harvard Medical School, Children's Hospital, Boston, MA 02115

The optic neuropathy secondary to glaucoma has generally been ascribed to an intraocular pressure higher than the nerve can tolerate. Traditional wisdom has held that glaucomatous damage occurs at the lamina cribrosa, by either a mechanical or vascular etiology. Glaucoma does, however, injure retinal ganglion cells, which are bona fide neurons of the CNS. This suggests that other neurotoxic etiologies -- in particular excitatory amino acids -- known to play a role in CNS damage, may contribute to the neuronal loss seen in glaucoma. We therefore analyzed amino acid levels in vitreous specimens from patients with open angle glaucoma, as well as controls. Vitreous specimens were selected because of the proximity of the vitreous to the retinal ganglion cell layer. Vitreous was obtained from fifteen patients and twelve controls at the time of cataract surgery. The mean glutamate level in the vitreous of patients with a greater than three year history of open angle glaucoma was 22.3±2.8 µmols/litre, as compared to 12.6±1.8 µmols/litre in controls. No significant difference was found in other amino acids between these two groups. Given the known neurotoxic effects of glutamate, this suggests that this excitatory amino acid may play a role in retinal ganglion cell death in glaucoma. Furthermore, this raises the possibility of alternative therapeutic modalities for limiting ganglion cell loss.

190.2

ASSESSMENT OF INTRASTRIATAL INJECTIONS OF QUINOLINIC ACID. M. Levivier*[1], S. Holemans[2], D. M. Gash[3], S. Przedborski[4], J-M. Maloteaux[2], and J. Brotchi[1]. [1]Dpt of Neurosurgery, Univ. Libre de Bruxelles - Hôp. Erasme, B-1070 Brussels; [2]Dpt of Neurochemistry, Univ. Catholique de Louvain, B-1200 Brussels, [3]Dpt of Neurobiology & Anatomy, Univ. of Rochester Med. Center, Rochester, NY 14642; [4]Dpt of Neurology, Columbia Univ., New York, NY 10023.

Quinolinic acid (QA) lesions of the striatum are used as a rat model of Huntington's disease. In order to evaluate mechanisms by which transplants protect against excitotoxicity [Pearlman et al., *Exp. Brain Res.*, 84:303-310, 1991], we analyzed the protective effect the NMDA-glutamate receptor antagonist MK-801 against striatal QA toxicity using behavioral and neurochemical measures.

Seven days after QA injection (100 nmol in 1 µl), saline pre-treated rats showed marked apomorphine-induced rotations toward the lesioned side, while MK-801 (10 mg/kg, i.p., 30 min prior to the QA injection) pre-treated animals showed no significant rotations. In saline pre-treated rats, QA injection caused significant decreases in [^{3}H]MK-801-labeled NMDA receptors, [^{3}H]SCH23390-labeled dopamine D1 receptors, [^{3}H]spiperone-labeled dopamine D2 receptors and [^{3}H]hemicholinium-3-labeled high affinity choline uptake sites, as well as a marked increase in [^{3}H]PK11195-labeled glial cells. Except for [^{3}H]hemicholinium-3 binding, these changes were almost completely prevented by MK-801 pre-treatment. Scopolamine (0.25 mg/kg, i.p., 10 min. prior to the MK-801 injection) completely prevented QA-induced [^{3}H]hemicholinium-3 binding loss. Histological assessment confirmed the protective effect of MK-801 on striatal neurons, but all groups of animals showed major cortical damage on the side of the QA injection.

This study shows that most QA-induced striatal damage may be prevented by MK-801; however severe cortical damage is present in both saline and MK-801 pre-treated rats. This study also provides some evidence that QA toxicity on cholinergic neurons might result from interactions between NMDA and muscarinic receptors.

190.3

A HUMAN IMMUNODEFICIENCY VIRUS (HIV)-DERIVED PEPTIDE AUGMENTS NMDA-INDUCED BRAIN INJURY IN PERINATAL RATS. Faye S. Silverstein*, Madhavan Nair, Stanley Schwartz and John D.E. Barks. University of MichiganMedical School, Ann Arbor, MI, 48109

In vitro data suggest that HIV neurotoxicity may be mediated, in part, by activation of NMDA-type excitatory amino acid (EAA) receptors. We examined the impact of co-injection of an HIV-derived peptide ("env-gag") with NMDA on the severity of NMDA-mediated injury in perinatal rodents. "Env-gag", a recombinant HIV fusion peptide (derived from gp41 and p24) has potent bioactivity *in vitro* . 7 day old rats received injections of: env-gag, NMDA, or both co-injected, into right striatum [through cortex and dorsal hippocampus (HIP)]; controls received NMDA co-injected with an E. Coli-derived peptide (n=6). Neuropathology was assessed 5 days post-injection, and graded from "0"=normal to "3"=severe damage in HIP and striatum and/or thalamus; HIP cross-sectional areas were also measured. Env-gag alone caused no injury. 37 animals received 5 nmol NMDA [alone or with env-gag (1 or 50 ng, LOW DOSE or 100 ng, HIGH DOSE), and injury was detected in 22/29 survivors. In animals that received NMDA + 100 ng env-gag neuropathology scores were highest [means: 2.3 vs 1.4 at "Low dose" vs. 1.1 with NMDA alone, p=0.01 Kruskal-Wallis (KW)] and reductions in right HIP area were most pronounced (mean reduction in area -28 % vs -13 % vs -8% with NMDA alone, p=0.02, KW). Co-injection of the expression vector peptide with NMDA did not influence the severity of NMDA-mediated damage.

Thus, these data provide evidence that an HIV-derived peptide increased EAA-induced neuropathology *in vivo*. and strongly support the hypothesis that locally secreted HIV peptides may potentiate the neurotoxicity of endogenous EAA neurotransmitters in HIV-infected brain.

190.4

MK801 INCREASES BRAIN CELL CULTURE YIELDS. **D.J. Leszczyszyn*, J.S.Hong and M.K.McMillian. LMIN/NIEHS/ NIH, MD14-06, Research Triangle Park, NC 27709**

Neuroprotective effects of NMDA receptor antagonists during striatal and hippocampal cell preparations were examined in neonatal Fischer 344 rats. Viable cell yield for each tissue increased 2.5-fold after 1-2 hours of MK801 (10 mg/kg i.p.) pretreatment. Injection of MK801 was critical. Adding MK801 to dispersing solutions was only 40% as effective at increasing cell yields. PCP (10 mg/kg i.p.) was 50% as effective as MK801. The competitive NMDA receptor antagonist CPP (50 mg/kg i.p.) only slightly increased cell yield unless also added to the dispersing solution; under this condition CPP was fully as effective as MK801, confirming involvement of NMDA receptors. The protective effect of MK801 was not due to anesthesia or hypothermia. L-type Ca^{2+} channel blockade, nitric oxide synthesis inhibition, and dextromethorphan and ketamine did not produce major increases in viable cell yields. The protective effect of MK801 was restricted to the neonatal period; no pronounced increase in cell yields were observed in 7 day old rats. Our results show that NMDA antagonists greatly increase neonatal striatal and hippocampal cell yields for culture and, more importantly, suggest that a large population of NMDA-sensitive cells are lost during routine culture of these tissues.

190.5

EXCITOTOXIC NMDA RECEPTOR ACTIVATION RESULTS IN INHIBITION OF CAM KINASE II ACTIVITY IN RAT CORTICAL CELL CULTURE. S.B. Churn*, S. Sombati, and R.J DeLorenzo. Department of Neurology, Medical College of Virginia, Richmond, VA 23298.

Excitotoxic activation of glutamate receptors results in a delayed neuronal necrosis which is thought to be calcium dependent (J Neurosci 1987;7:369). In addition, the NMDA glutamate receptor subtype has been implicated as mediating the glutamate-induced delayed neuronal cell death (J Neurosci 1988;8:185). CaM kinase II, is a neuronally enriched, calcium-regulated enzyme which is inhibited in models of excitotoxicity such as stroke (Stroke 1990;21:1715)and epilepsy (Epilepsy Res 1991;9:211). Therefore, we examined whether selective excitotoxic activation of the NMDA glutamate receptor would result in inhibition of CaM kinase II activity. Cortical cells cultured for 14 days from embryonic (E15) rats were exposed to 10 μM glycine plus 100 μM NMDA or 500 μM glutamate for 10 minutes. Following 60 minutes recovery, the cells were harvested and studied for CaM kinase II activity under standard conditions. Non-excitotoxic (2 minute) exposure to glutamate did not result in inhibition of CaM kinase II. Glutamate exposure for 10 minutes resulted in an extended neuronal depolarization (END) and significant inhibition of CaM kinase II activity (66.4 $\pm$ 4.9% of control), which was blocked with the co-administration of MK-801 (20 μM). The glutamate induced inhibition was observed within 10 minutes after excitotoxic glutamate exposure and was observed at all time points measured. Furthermore, removing calcium from the culture medium resulted in almost complete protection from glutamate-induced inhibition of CaM kinase II. Excitotoxic exposure of neurons to NMDA resulted in greater than 50% inhibition of CaM kinase II activity. The results suggest that excitotoxic activation of the NMDA glutamate receptor subtype results in a calcium-dependent inhibition of CaM kinase II.

190.6

NMDA-INDUCED FODRIN TURNOVER IN VIVO: ASSOCIATION WITH NEURONAL INJURY AND DISSOCIATION FROM CONVULSIONS. M.S. Miller*, P. M. Fritz, M. Lewis and D. Luttinger, Sterling Winthrop Pharmaceutical Research Division, Rensselaer, NY 12144.

Elevations in intracellular Ca^{++} have been implicated in the etiology of neuronal injury following exposure to excitatory amino acid agonists (EAAs). Increased intracellular Ca^{++} can activate calpain, a Ca^{++}-dependent protease which is capable of hydrolyzing cytoskeletal proteins, including fodrin. Degradation of the cytoskeleton may contribute to EAA-induced neuronal injury. A previous study (Siman et al., 1989) demonstrated increases in fodrin turnover (**FTO**) in adult rat brain and prolonged convulsions following icv injection of NMDA. CPP, a competitive NMDA antagonist, blocked NMDA-induced **FTO** without affecting convulsions suggesting a dissociation. In the present study, neonatal rats were treated intrastriatally with single doses of NMDA. **FTO**, convulsions, and neuronal injury were subsequently quantified. Test agents were administered ip 30 min. prior to NMDA. MK-801 produced dose-related inhibition of **FTO**, convulsions, and neuronal injury with no selectivity (ED50 approx. 0.3 umol/kg for each). The anticonvulsant, chlordiazepoxide, produced dose-related inhibition of convulsions (MED 30 umol/kg) without reducing **FTO** or neuronal injury at doses up to 100 umol/kg. Thus, NMDA-induced **FTO** in vivo is associated with neuronal injury and not convulsions. These data extend the dissociation between convulsions and **FTO**/neuronal injury.

190.7

THE EFFECTS OF CALCIUM ANTAGONISTS AND MILD HYPOTHERMIA ON HIPPOCAMPAL GLUTAMATE CONCENTRATIONS FOLLOWING REPEATED ISCHEMIA. M. Matsumoto, M. S. Scheller, and M. H. Zornow* Dept. of Anesthesiology, Univ. of California, San Diego, CA 92093-0629

This study compared the effects of the calcium antagonists S-emopamil and nimodipine, with mild hypothermia (32°C) on extracellular concentrations of glutamate following transient cerebral ischemia. Hippocampal glutamate concentrations were measured in rabbits (n=17) using in vivo microdialysis. Administration of drugs (S-emopamil 1 mg/kg bolus, 0.1 mg/kg/min infusion or nimodipine 10 µg/kg bolus, 1 µg/kg/min infusion), or institution of hypothermia was initiated prior to the first ischemic insult. Animals were subjected to two 7.5 min episodes of global ischemia separated by a one hour interval of reperfusion. Ischemia was produced by neck tourniquet inflation (20 psi) with hypotension (< 50 mmHg). Each episode of ischemia resulted in significant increases in the hippocampal concentrations of glutamate in all groups. Peak concentrations of glutamate were significantly lower in the S-emopamil and the hypothermia group ($p < 0.05$, by 2-way ANOVA). Magnitude of the effect of S-emopamil approaches that provided by mild hypothermia. These findings suggest a possible mechanism for the reported neuroprotective properties of phenylalkylamines.

Table. Extracellular glutamate concentration Mean ± SEM (µM)

	baseline	1st ischemia	2nd ischemia
Control	7.4 ± 1.2	45.4 ± 3.7	49.1 ± 4.9
Nimodipine	5.9 ± 1.1	37.8 ± 4.6	41.8 ± 4.1
S-emopamil	4.0 ± 0.5	29.3 ± 2.8*	30.0 ± 3.0*
Hypothermia	5.4 ± 1.4	20.4 ± 2.3*	22.0 ± 1.4*

190.8

DEVELOPMENT OF GLUTAMATE-INDUCED EXCITOTOXICITY IN EXPERIMENTAL THIAMINE DEFICIENCY. A.S. Hazell* and A.M. Hakim. Cerebrovascular Research Unit, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4

Cerebral microdialysis has been used to investigate the role of extracellular glutamate (glu) in the development of the selective histological lesions characteristic of thiamine deficiency.

Male Sprague Dawley rats (300 g) were placed on a thiamine deficient diet with administration of pyrithiamine. A 1 mm dialysis probe was implanted by stereotaxic means into the ventral posterior medial thalamus, a vulnerable region of the brain, in pre-symptomatic (day 13) animals showing no evidence of behavioural changes (n=4). Samples were collected for 48 h at 12 h intervals, following which a second probe was introduced into the same region on the contralateral side with sampling in a similar manner. The dialysates were then subjected to HPLC analysis for determination of glu content.

Side to side comparison of this amino acid showed no significant difference. Progression of the disorder resulted in an increase in extracellular glu compared with pre-symptomatic values, rising to 200-300% which coincided with the onset of the acute symptomatic stage of the illness (loss of righting reflexes) 36 h later. Subsequently, levels of glu were observed to fall below basal values.

The reason for these alterations in glu is unclear at this time, although several processes may be involved including edema, a failure of glial and neuronal uptake mechanisms, and cell death. In experimental thiamine deficiency, the fate of vulnerable regions of the brain may be determined by the temporal profile of increased glu release rather than its absolute concentration.

190.9

NEURONAL CELL DEATH IN ORGANOTYPIC CULTURES OF RAT HIPPOCAMPUS IS PREVENTED BY GLUTAMATE RECEPTOR ANTAGONISTS. Lucas D. Pozzo Miller* and Dennis M.D. Landis. Depts of Neurology and Neurosciences, Case Western Reserve University, Cleveland, OH 44106

A consistent pattern of neuronal cell death occurs in organotypic slice cultures from rat hippocampus during the second and third weeks in vitro. In most slices studied after 7 days in vitro, there is little evidence of cell death. However, neuronal degeneration is present in most slices studied at 14 days, and persists in a few cultures studied at 21 days. At 14 and 21 days, slices with relatively mild cell degeneration contained degenerating neurons in the CA3 region. In slices with more extensive damage, cell death spreads along the pyramidal cell layers into CA1 and CA4. To investigate the causes of the spontaneous neuronal death in vitro, we compared the extent of cell loss in cultures exposed to 3mM kynurenic acid, 100µM D-APV, or 10.5mM Mg^{++} (In all cultures, Ca^{++} was 1.1mM). Cultures were treated from 7 to 21 days in vitro, and the medium was changed at 48 hour intervals. The extent of cell death was estimated by morphological criteria in cultures fixed with aldehydes, embedded in plastic, and sectioned to 0.5µ thickness. Both high magnesium and kynurenic acid prevented cell death at 14 days in vitro. The extent of cell death was markedly decreased by treatment with APV, but was still apparent. These observations indicate that studies of cell death in this in vitro system will have to take into account spontaneously occurring neuronal cell death. Moreover, the fact that astrocytes in these cultures share characteristics with reactive astrocytes may reflect the continuing cell destruction over the first three weeks in vitro.

190.10

NEUROTOXIN-INDUCED INTRACELLULAR CA^{2+} OSCILLATIONS ARE BLOCKED BY CA^{2+} CHANNEL AND NMDA RECEPTOR ANTAGONISTS. T. M. Piser*, and S. A. Thayer. Department of Pharmacology, University of Minnesota Medical School, Minneapolis MN, 55455.

Oscillations in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) were evoked by exposure of single rat hippocampal neurons to HIV-1 envelope protein (gp120; 200 pM). Intracellular free $[Ca^{2+}]_i$ was recorded from single cells grown in primary culture using indo-1 based microfluorimetry. gp120-induced $[Ca^{2+}]_i$ oscillations were completely and reversibly blocked by the NMDA receptor antagonist CGS19755 (10 µM), the voltage-gated Ca^{2+} channel blocker nitrendipine (1 µM), and tetrodotoxin (1 µM) (n=4). Spontaneous $[Ca^{2+}]_i$ transients observed in these cultures were also glutamatergic, as indicated by complete block with the non-NMDA glutamate receptor antagonist CNQX (10 µM). But in contrast to those induced by gp120, spontaneous oscillations were insensitive to nitrendipine. Removing Mg^{2+} from the media evoked $[Ca^{2+}]_i$ oscillations with similar pharmacology to the gp120-evoked $[Ca^{2+}]_i$ transients; Mg^{2+}-free $[Ca^{2+}]_i$ oscillations have been shown to be neurotoxic. We hypothesize that neurotoxins such as gp120 convert "normal" dihydropyridine-insensitive synaptic activity into excitotoxic dihydropyridine-sensitive activity. We have observed the recruitment of nitrendipine-sensitive, action potential-mediated Ca^{2+} influx in the presence of the K^+ channel blocker TEA (2 mM), suggesting a potential mechanism for the induction of nitrendipine sensitive glutamatergic neurotransmission.

190.11

GAMMA-ACETYLENIC GABA CAUSES EXCITOTOXIC LESIONS IN THE RAT HIPPOCAMPUS. O.G. McMaster*, H. Baran, F.Du, H.-Q. Wu, [1]E.D. French and R. Schwarcz. Maryland Psych. Res. Center, Baltimore, MD 21228 and [1]Dept. Pharmacol., Univ. Arizona Coll. Med., Tucson, AZ 85724.

Gamma-acetylenic GABA (GAG) shares many pharmacological properties with aminooxyacetic acid (AOAA). While both compounds are blockers of GABA transamination, they also inhibit kynurenine aminotransferase, the biosynthetic enzyme of the neuroprotectant kynurenic acid. Intrahippocampal injections of GAG (60-240 nmoles/1 μl), similar to AOAA injections (Exp. Neurol., 113:378, 1991), caused excitotoxic lesions in rats. GAG appeared to be approximately equally toxic to CA3/hilar neurons and CA1 pyramids while CA2 neurons and granule cells were clearly less vulnerable. Choline acetyltransferase activity, a marker of extrinsic afferents, remained unchanged in GAG-lesioned tissue, indicating the axon-sparing nature of the insult. Moreover, GAG lesions were prevented by the NMDA receptor antagonists MK-801 and AP7. However, unlike AOAA lesions, GAG lesions were independent of the anesthetic used during surgery. Upon iontophoretic application, GAG, like AOAA, did not excite CA1/CA3 cells. Taken together, GAG-induced lesions share many characteristics of AOAA lesions but subtle differences exist. GAG and AOAA seem to belong to a new family of excitotoxic agents which produce lesions indirectly by metabolic derangement and/or inhibition of kynurenate production. Supported by grant NS 16102.

190.12

EFFECTS OF CO-INJECTION OF GLUTAMATE (Glu) AND INHIBITORS OF SODIUM-DEPENDENT Glu UPTAKE (SDGU) INTO STRIATUM. B.A. McLaughlin, D.R. Lynch, and M.B. Robinson*, Children's Seashore House; Depts. of Ped., Pharm., and Neurol., U. of PA, Phila., PA, 19104.

Substantial evidence suggests that excessive activation of excitatory amino acid (EAA) receptors contributes to the neuronal degeneration observed after a variety of CNS insults. SDGU transport is presumed to prevent the accumulation of extracellular EAAs in the normal brain. Previous studies show that dihydrokainate, a weak inhibitor of transport activity, does not cause toxicity *in vivo*. DL-*threo*-hydroxyaspartate (THA), a 50-fold more potent inhibitor of SDGU, has been co-injected with Glu. Striatal toxicity was observed in one study (McBean, J. Neurochem.44:247,1985), but not in another (Mangano, Brain Res.Bull 10:47,1983). In the present study, neurotoxicity of EAAs was examined by stereotactically injecting compounds into the striatum (1 or 2μL, pH = 7.4). Four days later, choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) were measured. As previously reported, kainate (6 nmol) caused seizures, circling, barrel rolling and reductions in ChAT (37 ± 4) and GAD (35 ± 4). Glu (1 μmol) was co-injected with either THA (1 μmol) or another potent uptake inhibitor, L-*trans*-pyrrolidine-2,4-dicarboxylate (1 μmol). At these doses, Glu alone or in combination with these uptake blockers caused neither the behavioral effects observed with kainate, nor the reductions in GAD or ChAT. At these concentrations of SDGU inhibitor, synaptosomal and glial uptake should be dramatically inhibited (Bridges, Neuro.Sci.Abs.17:393, 1991 & unpubl.). These studies suggest that unlike kainate toxicity, Glu toxicity is limited in this experimental paradigm.(Pew, Sloan, GM34781)

190.13

SELECTIVE VULNERABILITY IN FOCAL ISCHEMIA DEPENDS ON EARLY CUMULATIVE EXPOSURE TO GLUTAMATE. H. Osuga, AM. Hakim*. Cerebrovascular Research, Montreal Neurological Institute and Hospital, Montreal, Quebec, Canada, H3A 2B4

<Introduction>The simultaneous permanent occlusion of the middle cerebral artery and the internal carotid artery results in infarction of the striatum first followed by the overlying cortex. The aim of this study was to evaluate the changes in extracellular glutamate concentration in this model in both striatum and cortex using microdialysis. We hypothesized that glutamate levels would rise in proportion to the ischemic vulnerability of the brain region.

<Material and Methods>Day 1: Male Sprague-Dawley rat weighing 250-300g were anesthetized with 2% halothane and mounted on Kopf stereotaxic device. After making two burr holes, two microdialysis probes(OD 0.5mm, membrane length 1.0mm, Carnegie Medicine) were implanted into left caudate nucleus and parietal cortex. Day 2:The probe-implanted rat was anesthetized and left femoral artery was cannulated to monitor blood pressure and blood gas. The proximal middle cerebral artery was then coagulated and ipsilateral carotid artery was ligated. During and after surgery, Ringer's solution was perfused at 4μl/min and dialysate was collected at 5 min intervals. The glutamate concentrations in dialysate were measured by HPLC.

<Results>The pre-occlusion glutamate level in dialysate was 1.03 ± 0.47μM in striatum and 1.00 ± 0.41μ mM in cortex. Immediately after occlusions, glutamate levels rose first in striatum then in cortex. Peak glutamate concentration in striatum (7.28 ± 3.60 μM) and cortex (5.64 ± 2.24 μM) after ischemia were not significantly different. the cumulative integral of glutamate vs. time was obtained and was significantly higher in striatum than in cortex during the first hour of ischemia($p < 0.05$). At longer durations of ischemia, this parameter was not significantly different between striatum and cortex.

<Conclusion>We conclude that in this model striatum is more vulnerable than cortex to ischemic insult because it is exposed to higher cumulative levels of glutamate in the first hour, but both regions eventually infarct because their total exposure to glutamate is identical.

190.14

SYSTEMIC NEONATAL NMDA ADMINISTRATION RESULTS IN BEHAVIOURAL DEFICITS IN THE ADULT RAT. J. Kleim, M. Saari*, S. Kish, L. Ridley, B. Fairey, G. McClenaghan and M. McIsaac. Neuroscience Research Unit, Nipissing University, 100 College Dr., North Bay, Ontario, P1B 8L7.

This study examined the effects of systemic neonatal NMDA administration on the behavioural and physiological development of the rat. On Day 7 postpartum, approximately 160 rats were randomly allocated to one of four drug treatment groups; Saline, 2mg/Kg, 3mg/Kg or 4mg/Kg of NMDA with equal numbers of male and female animals in each. Those in the NMDA groups received a single subcutaneous (s.c.) injection of either 2mg/Kg, 3mg/Kg or 4mg/Kg of NMDA in a saline vehicle. Subjects in the saline group received an equivalent volume of physiological saline (0.9%, NaCl, s.c.). On Day 12, open field activity levels were measured on half of the animals and body and brain weights were taken. Behavioural testing on the remaining animals began on Day 56 postpartum and included tail flick, rotor rod, Morris water maze and open field activity. The animals were sacrificed on Day 70 and body and organ weights were obtained. Analysis of Variance (ANOVA) revealed significant effects of the drug treatment on Day 12 body and brain weights and on adult tail flick, rotor rod and Morris water maze performance. No drug effects were observed on adult body or organ weights.

190.15

CARBAMAZEPINE-INDUCED NEUROTOXICITY IN CULTURED CEREBELLAR GRANULE CELLS: EVIDENCE FOR AN INTERACTION WITH NMDA GLUTAMATE RECEPTORS. X.-M. Gao* and D.-M. Chuang. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892

We have recently demonstrated that carbamazepine (CBZ) induces a concentration-dependent delayed neurotoxicity in cerebellar granule cells and that NMDA exposure blocks CBZ neurotoxicity by an NMDA receptor-mediated mechanism (Gao & Chuang, *Neurosci. Lett.* 135:159-162, 1992). The present study was undertaken to investigate possible mechanisms underlying CBZ-induced neurotoxic effects. Treatment of cerebellar granule cells with 100 μM CBZ for 3 days was found to selectively increase NMDA-induced phosphoinositide (PI) turnover, with a concomitant decrease in PI response to carbachol, norepinephrine and 5-HT, largely because of CBZ-induced neuronal death. ^{3}H-MK-801 binding to NMDA receptors in intact cells was also increased following a 3-day treatment with CBZ with an EC_{50} of about 30μM. Moreover, NMDA-induced release of preloaded ^{3}H-D-aspartate (which labeled glutamate uptake pool) was significantly enhanced by long-term treatment with 100 μM CBZ, but basal release was reduced. CBZ displaced ^{3}H-MK-801 binding to intact cells by an IC_{50} of approximately 28 μM, but its long-term neurotoxicity was unaffected by the presence of NMDA antagonists MK-801 (10 μM) and APV (200 μM), although these two antagonists effectively prevented the neuroprotective effects of NMDA against CBZ neurotoxicity. Our results suggest that an indirect allosteric interaction of CBZ with NMDA receptors contributes to CBZ-induced neurotoxicity.

190.16

NEURONAL LOCALIZATION OF QUINOLINIC ACID IN THE RAT BRAIN. J.R. Moffett*, P. Kok, V. Paiss G. Suvannavejh, S. Gaudet, and M.A.A. Namboodiri. Dept. of Biology, Georgetown Univ. Washington D.C. 20057.

Quinolinic acid (QUIN) has attracted increased attention in recent years as an excitotoxin possibly involved in neurodegenerative disorders such as Huntington's disease. Recent findings also indicate that many chronic infections , including AIDS, are associated with increased concentrations of QUIN in selected areas of the brain. To understand the mechanism of action and role of QUIN in the nervous system, a knowledge of its cellular localization is essential. In the present study, we have accomplished this by an immunohistochemical approach using highly specific antibodies against QUIN. Polyclonal antibodies against QUIN were raised by immunizing a rabbit with QUIN conjugated to protein or adsorbed to colloidal gold. Immunohistochemistry of carbodiimide fixed tissue from rat brain demonstrated that neurons are the most immunoreactive elements for QUIN. Among the most immunoreactive neurons were pyramidal cells of neocortex, pyriform cortex and hippocampus. This direct immunohistochemical approach provides a new tool for studying QUIN-mediated neurotoxicity. Further, the present results strengthen the candidacy of QUIN as an endogenous agonist of the NMDA receptor system. (Supported by NIH grant DK37024 and program project NS 28130).

190.17

CHRONIC INTRASTRIATAL DIALYTIC ADMINISTRATION OF QUINOLINIC ACID REVEALS SELECTIVE SPARING OF NADPH-DIAPHORASE NEURONS.

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Huntington's disease (HD) is marked by striatal atrophy resulting from loss of medium spiny neurons. Striatal neurons that contain NADPH-diaphorase are selectively spared in HD. One theory of HD suggests that selective neuronal loss results from excitotoxic effects of the excititory amino acid quinolinic acid (QUIN) in the striatum. Using a microdialysis probe mated to an Alzet 2002 mini-osmotic pump (Bazzett et al, 1991, J. Neurosci. Meth. 40:1), we administered 220 µl of phosphate buffered saline containing 0, 880 nmoles, 3.3 µmoles or 8.8 µmoles QUIN to the striata of rats over a 3 week period. Animals that received 3.3 µmoles QUIN had significant striatal atrophy that could be attributed to two distinct areas of neuronal loss. First, an area of necrosis surrounded the probe area, and was marked by a lack of neurons and dense gliosis. The region surrounding the necrotic area was a marked by a significant reduction in nissl stained cells, with no change in the density of NADPH-diaphorase staining neurons compared to the intact striatum. In addition, there was a reduction in cytochrome oxidase staining that closely matched these two areas of cell loss. The striata of animals that received 880 nmoles QUIN appeared identical to those that received vehicle. The striata of animals that received 8.8 µmoles QUIN showed severe nonselective atrophy of the striatum and some surrounding structures. Dialytic delivery of QUIN produces an area of selective neuronal destruction of that resembles selective neuronal loss seen in HD. This selective neurodegeneration produced by chronic exposure to QUIN more closely models the course of HD.

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190.18

THE NEUROTOXIN QUINOLINIC ACID IS SELECTIVELY ELEVATED IN SPINAL CORDS OF RATS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE).

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EAE is an animal model of multiple sclerosis (MS) in which demyelination and paralysis are evident. Quinolinic acid (QA), an NMDA receptor agonist, is an endogenous neurotoxin formed from tryptophan. The role of endogenous neurotoxins in EAE, or MS, is unknown.

Lewis rats were immunized with guinea pig myelin basic protein, in Freund's adjuvant, intradermally. The rats were killed 12-15 days later, when hindlimb paralysis was evident, and their tissues assayed for QA by GC/MS, using [^{13}C-U]quinolinate as the internal standard.

In five separate experiments, basal levels of QA in spinal cord (8.03 ± 0.93 fmol/mg tissue) were markedly elevated (to 94.70 ± 11.08 fmol/mg tissue). Brain tissue (cortex and corpus callosum), plasma, and liver contents of QA were not altered in EAE. Thus, the alterations in QA appear to be specific for the affected tissue. It is not currently known whether QA contributes to the demyelination found in EAE. However, it is entirely possible that the increase in QA in the spinal cord may contribute to the disability by altering NMDA receptor function. If QA is pathogenic in EAE, this finding could have therapeutic implications for MS.

190.19

THE NEUROTOXIN QUINOLINIC ACID: DIFFERENTIAL MODULATION OF BRAIN AND BLOOD LEVELS.

J.B. Erickson*, E.M. Flanagan, S.Y. Chang, M. Salter, and J.F. Reinhard, Jr. The Wellcome Research Laboratories, Research Triangle Park, NC 27709 USA, and Beckenham, Kent BR3 3BS U.K.

Quinolinic acid (QA) is an endogenous NMDA receptor agonist and a neurotoxin. It is synthesized as a product of tryptophan (Trp) metabolism, and in the liver it is a precursor for the synthesis of NAD. Much is known about the hepatic synthesis of QA, but very little is understood about the formation of this substance in the brain. We examined the differential accumulation of QA in serum and brain of both rats and mice following peripheral administration of several biosynthetic precursors to QA. Quinolinate was quantitated using GC/MS with [^{13}C-U]QA as an internal standard. In rats, the i.p. administration of Trp (0.5 to 2 mmol/kg) elevated brain QA 5 to 50 fold over that of the control levels 2 to 4 hours post injection. Serum QA was similarly increased but reached maximal levels at earlier time points than did brain QA. In contrast, anthranilate (AA) did not elevate either brain or plasma QA. In mice, Trp, kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilate, and AA (each at 0.3 mmol/kg) resulted in QA brain levels of 142, 651, 14700, 589, and 78 % of control respectively. Trp, kynurenine, and 3-hydroxyanthranilate also elevated serum QA, but in contrast to the brain, 3-hydroxykynurenine did not significantly elevate serum QA. Anthranilate raised serum QA approximately 2-fold but did not elevate brain QA. Rat liver homogenates but not rat brain homogenates formed $^{3}H_2O$ when incubated with [3,5-^{3}H]AA, suggesting that AA is hydroxylated in the periphery but not in the brain. The results suggest that brain and peripheral blood QA are differently controlled, as implied from the observed species differences, and in the brain, anthranilate is not an efficient intermediate for QA formation.

EXCITATORY AMINO ACIDS: ANATOMY AND PHYSIOLOGY I

191.1

Nitric Oxide (NO) Donor Actions on NO-Synthase Containing Neurons of the Mesopontine Tegmentum *in vitro*.

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The effects of the nitric oxide (NO) generating compounds, sodium nitroprusside (SNP) and isosorbide dinitrate (ISDN), on responses to NMDA were studied in the nitric oxide synthase (NOS) -containing neurons of the pedunculopontine (PPT) and lateral dorsal tegmental (LDT) nuclei using intracellular recording methods in a guinea pig brain slice preparation. NOS-containing neurons were identified by combined intracellular injection of biocytin and NADPH-diaphorase staining, which identifies brain NOS (Dawson et al, '91, PNAS:88). Focal application of NMDA produced an increase in firing rate and membrane depolarization which was strongly inhibited by SNP (300µM), while ISDN (up to 600 µM) had no systematic effect. Control experiments showed the SNP action to be specific for NMDA receptor-mediated responses since SNP had no effect on the responses to focal glutamate application, nor on the synaptic potentials evoked by local electrical stimulation in control Ringer containing 3 mM Mg^{++}. Also, SNP had no effect on the Ca^{++}-spike evoked by brief current injection in the presence of TTX, TEA, and 4-AP, thus excluding a nonspecific block of Ca^{++}-channels as a possible mechanism of action. Since ISDN did not have a similar effect, it appeared that the response blockade by SNP was not mediated by NO. Further experiments showed that the SNP effect was mimicked by potassium ferrocyanide, thus suggesting it was largely an action of the liberated ferrocyanide. Thus, the multiple actions of SNP must be considered in interpretations of studies which use this agent strictly as an NO-donor. Supported by NS27881.

191.2

cGMP AND NITRIC OXIDE SYNTHESIS IN THE STRIATUM OF THE RAT: AN IMMUNOCYTOCHEMICAL APPROACH.

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We studied the relationship between the cellular components in the striatum synthesizing nitric oxide (NO) and the cellular structures responsive to NO by synthesizing cyclic GMP.

Striatal tissue slices (300 µm) were prepared and incubated in vitro. Drugs were added to the slices and incubations were terminated by adding the formaldehyde fixative. Slices were processed for immunocytochemistry as described (De Vente et al, Eur. J. Neurosci 2:845 (1990). NOS was demonstrated using an antibody against rat brain NOS (courtesy Dr. S. Moncada, Wellcome Research Institute). cGMP was visualized using an antibody directed against formaldehyde fixed cGMP.

NOS in the striatum was found in relatively few cell somata with abundant processes containing numerous varicosities. cGMP ICC after stimulation of the soluble guanylate cyclase with nitroprusside (0.1 mM, 10 min) showed cGMP in a dense fiber network with numerous varicosities. Very few cell bodies were found that showed some cGMP-immunoreactivity. After stimulation with NMDA (0.1 mM, 2 min) cGMP was found accumulated in a dense varicose fiber network; this was sensitive to inhibition by 0.1 mM N^{G}-nitro-arginine methyl ester and by 50 µM AP-5. No cell somata were positive for cGMP after stimulation with NMDA. It is concluded that in the striatum there is a close correlation between the localization of NOS and the soluble guanylate cyclase, and between the activation of NMDA receptors, the activation of NOS and the production of cGMP.

191.3

NITRIC OXIDE IN RETINA:RELATION TO EXCITATORY AMINO ACIDS & EXCITOTOXICITY. W.J.Nicklas* & G.D.Zeevalk. Neurology Dept. UMDNJ-RWJ Med.Sch., Piscataway, N.J.08854.

The excitatory amino acids (EAAs), glutamate (glu), NMDA and kainate(KA) increase cGMP in chick retina. Guanylate cyclase can be stimulated by several mechanisms, one of which is through activation of NO synthase and formation of nitric oxide(NO). This study was done to determine if NO pathways exist in retina and are linked to EAA-induced increases in cGMP. Exposure of embryonic day 15 retina from chick for 10min to either 1mM glu, 100μM NMDA or 100μM KA increased cGMP content 2-3fold. The environmental neurotoxins domoic acid (DO, 20μM), ß-oxalyl-amino-L-alanine (BOAA,200μM), but not ß-methyl-amino-L-alanine (BMAA,3mM), also increased cGMP. The NO synthase inhibitor L-nitro-N-arginine(L-NNA,100μM) completely blocked the increases in cGMP induced by the above glu-agonists. Glu agonist induced increases in cGMP were receptor mediated. MK-801, a NMDA receptor antagonist blocked NMDA and glu-stimulated cGMP formation, whereas, CNQX, a KA/AMPA receptor antagonist blocked cGMP increases produced by KA, BOAA and DO. To examine the involvement of NO pathways in NMDA-mediated toxicity, the ability of L-NNA to protect against both acute and delayed excitotoxic damage, from a 60min exposure to NMDA, was assessed. Acute excitotoxicity, monitored by swelling-induced GABA release at 60min and delayed cell death, determined by LDH release following a 24hr recovery period, were unchanged by the presence of L-NNA. These studies show that NO pathways exist in retina and link EAA receptor activation to cGMP formation, however, this pathway may not play a role in NMDA-mediated excitotoxicity.

191.4

REGULATION OF TETRAHYDROBIOPTERIN BIOSYNTHESIS IN CULTURED NEURONS BY cAMP-DEPENDENT GENE EXPRESSION. K. Hirayama,* and M. Zhu and G. Kapatos. Cellular and Clinical Neurobiology Program, Dept. of Psychiatry, Wayne State University School of Med., Detroit, MI. 48201

Tetrahydrobiopterin (BH4) is the essential cofactor for the biosynthesis of monoamines and nitric oxide. To investigate the possible regulation of BH4 biosynthesis by cAMP-dependent mechanisms we have used monolayer cultures of hypothalamic (HYP) and mesencephalic (MES) neurons derived from day 15 rat embryos and maintained in the absence of glia. Fourteen-day MES and HYP cultures were treated with different concentrations of 8-bromo-cAMP (8BrcAMP, 0-5 mM) for 24 hours. BH4 levels were increased in a concentration-dependent manner in both cultures although the increase was greater in HYP than MES. Incubation with forskolin or IBMX produced a similar increase in BH4. The time course of the 8BrcAMP effect was maximum at 24 hours. Analysis of BH4 turnover indicated that 8BrcAMP increased BH4 levels by stimulating BH4 synthesis without altering degradation. The increase in BH4 levels was completely prevented by inhibitors of gene transcription and translation. GTP cyclohydroxylase 1 (GTPCH1) is the first and rate-limiting enzyme in the BH4 biosynthetic pathway. GTPCH1 mRNA was increased by 8BrcAMP in both culture systems. These data demonstrate that cAMP increases BH4 levels by increasing BH4 biosynthesis. This increase in BH4 biosynthesis appears to be due to an increase in GTPCH1 gene expression. (Supported by NIH NS26081)

191.5

LOCAL ADMINISTRATION OF QUINOLINIC ACID IN THE RAT HIPPOCAMPUS INDUCES EXPRESSION OF c-FOS AND NGFI-A H.W.G.M. H. Boddeke[1], L. Massieu[2], P.H. Kelly[1]* and J.M. Palacios[3] 1 Preclinical Research Sandoz Pharma LTD., Basle, Switzerland, 2 Instituto de Fisiologia Celular, UNAM, 04510- Mexico D.F., México, 3 Laboratorios Almirall, Barcelona, Spain.

We have studied the effect of intrahippocampal administration of quinolinic acid on the temporal expression of mRNAs encoding the immediate early genes c-*fos* and NGFI-A, by *in situ* hybridization histochemistry.

After administration of quinolinic acid (120 nmol) to the left hippocampus, expression of mRNA of both IEGs was transiently stimulated. Maximal expression was found between 1 and 3 hours. mRNA of both early genes was simultaneously expressed in the ipsilateral and contralateral sides in the granule cell layer of the dentate gyrus, the pyramidal cell layer of the CA1 and CA3 fields as well as in the cortex. After pretreatment with the non-competitive NMDA antagonist MK-801 (2 mg/kg i.p. -30 min) the increased expression of both IEGs was partially prevented in the hippocampus and completely in the cortex. No inhibition was observed after treatment with the AMPA antagonist NBQX (30 mg/kg i.p. -15, -5 and +10 min). The present results show that the effect of quinolinic acid is mediated by NMDA and not by AMPA receptors.

191.6

THE NMDA RECEPTOR ANTAGONISTS CPP AND MK-801 EXERT DOSE AND GENOTYPE-DEPENDENT DIFFERENTIAL EFFECTS ON LONG-TERM HABITUATION TO NOVELTY IN TWO RAT STRAINS. M.P. Pellicano, F.Siciliano and A.G. Sadile (SPON: European Brain and Behaviour Society). Dip. Fisiologia Umana & Funzioni Biologiche Integrate "F. Bottazzi", Univ. Naples "Federico II", I-80138 Naples, Italy.

A series of experiments was designed to investigate the role N-methyl-D-aspartate (NMDA) receptors play in behavioral plasticity. Adult male rats of the *Naples High-Excitability (NHE)* and *Naples Low-Excitability (NLE)* lines, selectively bred for arousal to spatial novelty, were given i.p. the NMDA receptor antagonist CPP (0.01 - 5mg/Kg) or MK-801 (0.01 - 2.5 mg/Kg), or vehicle soon after a 10min-test in a Làt-maze. Retention was run one week later. Habituation of activity and defecation score was monitored by the between-test decrement (LTH) in the frequency of corner-crossings and rearings (with prevailing cognitive and non-cognitive meaning, respectively) and of fecal boli. The results indicate that (i) in the NLE-rats, both isosteric and allosteric blockers facilitate LTH of cognitive components at low and high doses, and inhibit LTH of emotional components at low doses of the isosteric blocker; and, conversely, that (ii) in the NHE-rats, isosteric blockade at low doses facilitates LTH of cognitive components in the entire exposure, whereas allosteric blockade by high doses of MK-801 impair LTH of cognitive components, and facilitate LTH of emotional components. The dose and genotype-dependent differential effects of NMDA receptor antagonists lend support to their hypothesized *modulatory* role in behavioral plasticity. Moreover, the dissociation between LTH of cognitive and non cognitive components suggests a role of NMDA receptors in their parallel processing, as suggested earlier (Behav.Brain Res., 39:187, 1990).

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191.7

NMDA RECEPTOR MEDIATED PLASTICITY IN THE DORSAL HORN AFTER PERIPHERAL NERVE INJURY. Stephen B. McMahon* & *Gary R. Lewin Dept. Physiology, UMDS (St.Thomas'), London SE1 7EH. *Neurobiology & Behavior, SUNY, Stony Brook, New York, NY 11794.

In adult rats one sciatic nerve was sectioned and the cut ends entubated leaving a 1 mm gap to maximally disrupt the spatial organization of regenerating afferents. The receptive field (RF) properties of dorsal horn cells in medial L5 were studied up to 10 weeks later, under urethane anaesthesia. When cutaneous reinnervation was incomplete (4-5 weeks), RFs were diffuse and relatively large (mean size 210 $\pm$ 23 mm^2) significantly larger than on the contralateral control intact side (118 $\pm$ 17 mm^2, p<0.005). After 10 weeks (complete reinnervation) RFs had reduced in size to 139 $\pm$ 17 mm^2 (p<0.001 *cf* 4/5 week data, and p>0.8 *cf* intact data), and usually consisted of one contiguous area. Thus, with time, dorsal horn cells only a subset of afferents, those with adjacent peripheral RFs.

We also studied RF properties in animals that had additionally received the NMDA receptor antagonist MK801 continuously for a four week period before the terminal experiment (0.015 mg/kg/hr, via an implanted osmotic pump). In intact animals, MK801 treatment resulted in a small, but non-significant, increase in RF size (162 $\pm$ 17 mm^2). In contrast, 8-9 week lesioned animals treated with MK801 had extremely large RFs (456 $\pm$ 41 mm^2, p<0.001 *cf* lesioned, untreated animals).

We suggest that when the peripheral topography of primary sensory neurons is disrupted (following regeneration), coincident activity in afferents that newly innervate adjacent peripheral territory strengthens the central connectivity of these afferents (acting via NMDA receptors) at the expense of other inputs, and so results in the emergence of restricted contiguous RFs. Supported by a NATO twinning grant.

191.8

LATERAL VENTRICULAR (LV) N-METHYL-DL-ASPARTATE (NMA) ADMINISTRATION ELICITS ACTH SECRETION IN FETAL SHEEP AT 125-130 DAYS GESTATION (dGA). P.W. Nathanielsz* & T.J. McDonald Laboratory for Pregnancy and Newborn Research, Dept. Physiology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853.

The fetal paraventricular nucleus (PVN) plays a central role in control of increased ACTH secretion in fetal sheep in response to hypoxemia and hypotension as well as preceding parturition (McDonald & Nathanielsz, Am.J.Ob.Gyn.165:764). The transmitter(s) that activates PVN control of ACTH secretion via CRH and AVP is not known. Aspartate is an excitatory central nervous system neurotransmitter.

Hypothesis: NMA, the mixed aspartate analog given to the fetus via the LV would stimulate ACTH release.

Methods: A LV catheter (LVC) was placed in 4 fetal sheep at 119-121 dGA. At 125-130 dGA either saline (S) or NMA (0.3 - 30 μg in S) was administered as a single injection via the LVC, one dose per day only. Fig 1. shows cumulative fetal ACTH responses over 30 min.

Results: 30 μg NMA via the fetal LVC stimulated ACTH secretion p<0.05vs S.

Conclusions: NMA stimulates ACTH release from the fetal pituitary at site(s) of action to be determined.

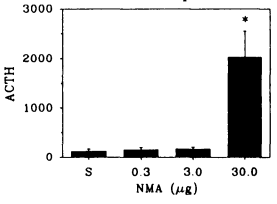


Fig. 1 Percent change of ACTH from baseline in 4 fetal sheep at 125-130 dGA given NMA via a LVC as a single injection. *p<0.05vs S.

191.9

N-METHYL-D,L-ASPARTATE (NMA) MODULATES LUTEINIZING HORMONE (LH) BUT NOT GROWTH HORMONE (GH) SECRETION FROM PIG PITUITARY CELLS. C.R. Barb*[1], J.B. Barrett[1], G.B. Rampacek[2] and R.R. Kraeling[1]. [1]USDA-ARS, and [2]University of Georgia, Athens, GA 30613.

Administration of NMA to pigs increases GH and suppresses LH secretion. Cultures of anterior pituitary cells from luteal (LUT; n=5), follicular phase (FOL; n=3) and OVX (n=6) pigs were treated with NMA (10^{-4}, 10^{-6} or 10^{-8} M) or 2-amino-5-phosphonopentanoic acid (AP5; 10^{-4}, 10^{-6} or 10^{-8} M) to determine if NMA affects the pituitary directly. Secreted LH and GH were measured at 4 h after treatment. Basal LH and GH secretion (control; C) were 1.1 ± 0.6, 4.4 ± 2.1 and 5.6 ± 1.3 ng/well and 5.2 ± 1.2, 7.5 ± 1.2 and 5.2 ± 1.7 ng/well for FOL, LUT and OVX, respectively. Relative to C, 10^{-4} M NMA increased (P<.05) LH secretion 3.2- and 3.9-fold in FOL and OVX cultures, respectively. The NMA-antagonist AP5 10^{-4} M, inhibited (P<.06) the effect of 10^{-4} M NMA in FOL cultures, but not in OVX cultures. GnRH increased (P<.05) LH levels 2.2-, 1.7- and 2.6- fold in FOL, LUT and OVX cultures, respectively. Neither NMA nor AP5 altered basal GH secretion. However, GHRH increased GH secretion 1.8-fold in all cultures. Therefore, excitatory amino acids stimulate LH secretion, but not GH directly at the pituitary.

191.10

THE LH RESPONSE TO NMDA DECLINES DURING NMDA-INDUCED PRECOCIOUS PUBERTY. **C. Smyth*, M. Wilkinson. Depts. of Obst. and Gynecol., Physiol. and Biophys., Dalhousie University, Halifax, N.S. B3H 4H7 Canada.**

Daily injections of the glutamate agonist NMDA reliably induce precocious puberty in female rats (Neurosci. Abstr.106.7 (1991). A restricted injection schedule of NMDA (20 mg/kg/day; age 24 -28 days) similarly advances the day of first ovulation (V.O.). (CON: 34.2 ± 0.6 (n=10); NMDA (24 to VO): 29.8 ± 0.4 (n=10); NMDA (24 to 28): 29.4 ± 0.5 (n=10)). The pituitary-ovarian (P-O) axis is also stimulated by daily NMDA injections as shown by an estrogen-dependent rise in uterine weights between day 24 and 30. Stimulation of the P-O axis by daily injections of GnRH (5 ng/100 g), which gives an LH response equivalent to NMDA (20 mg/kg), does not advance puberty. Therefore, the stimulation of the P-O axis by NMDA is secondary to its effects on the maturation of the hypothalamus.

We have shown that there is a decline in the LH response to NMDA as female rats approach puberty (J. Neuroendocr. (1992). Using the restricted NMDA model of precocious sexual maturation we now show that the normal decline in the LH response to NMDA occurs sooner in the treated than in the control group ($p<0.001$). An LH dose-response curve for NMDA at age 28 days demonstrates that the NMDA-treated group is less responsive to NMDA than the control group ($p<0.001$). The source of NMDA desensitization is not located in the pituitary as an LH dose response curve for GnRH at age 28 days is identical in the NMDA-treated and control groups. In conclusion, since NMDA induces precocious puberty it is possible that the decline in hypothalamic response to NMDA is a necessary event in sexual maturation.

This work was supported by the Canadian MRC

191.11

RELEASE OF AMINO ACIDS IN THE DORSAL HORN OF RAT SPINAL CORD INDUCED BY ELECTRICAL STIMULATION OF SCIATIC NERVE **V. Paleckova*, J. Palecek, D. J. McAdoo, and W. D. Willis Marine Biomedical Institute, UTMB, Galveston TX-77555 - 0843**

Extracellular concentrations of nine amino acids (AA's - aspartate, glutamate, asparagine, serine, glycine, threonine, alanine, taurine and glutamine) in the dorsal horn of 17 Sprague Dawley rats were determined using in vivo microdialysis in acute experiments under urethane anesthesia. Basal levels of AA's were determined from samples of the perfusing solution - artificial cerebrospinal fluid (ACSF) collected 2 hours after the fiber insertion. Then samples during and after the stimulation of both sciatic nerves at C-fiber strength (20-30 V, 1 Hz, 0.5 ms) were obtained. CNQX (non-NMDA antagonist; 2 mM) and tetrodotoxin (TTX; 10μM) were added to the perfusion ACSF (approximate gradient across the membrane was 10 : 1) in 6 and 5 experiments respectively. The level of AA's in ACSF samples was determined using high pressure liquid chromatography (HPLC). Differences in the AA levels were statistically evaluated using Kruskal-Wallis analysis and the Wilcoxon paired test.

The basal extracellular concentrations of AA's corrected for fiber efficiency were (μM ± S.E., n=17) : Asp 2.9±0.4; Glu 3.8±1.1; Asn 1.66±0.4; Ser 3.2±0.6; Gly 6.5±1.3; Thr 9.1±1.8; Ala 5.0±1.1; Tau 9.6±3.2; Gln 132.5±55.2. During the control stimulation while perfusing with normal ACSF the concentration of all the AA's but glutamine significantly increased from the prestimulation level. All the AA's but taurine returned to the baseline level after the stimulation. We did not find a significant increase in the concentration of AA's during the nerve stimulation when CNQX or TTX were present in the ACSF.

Our data support a role for AA's in neurotransmision in the spinal cord dorsal horn. This work was supported by NS 11255, NS 09743 and a grant from Bristol-Myers Squibb Corp.

191.12

SEXUAL DIFFERENCES IN N-METHYL-D-ASPARTATE RECEPTOR-MEDIATED REGULATION OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS IN THE RAT. E.J. Wagner*, K.E. Moore and K.J. Lookingland. Dept. of Pharm. & Tox., Michigan State Univ., E. Lansing, MI 48824.

Dopamine released from tuberoinfundibular nerve terminals in the median eminence inhibits the secretion of prolactin from the anterior pituitary. The purpose of the present study was to examine the effects of N-methyl-D-aspartate (NMDA) receptor blockade on prolactin secretion and on the activity of tuberoinfundibular dopaminergic (TIDA) neurons [estimated by either the accumulation of 3,4-dihydroxyphenylalanine (DOPA) 30 min after the administration of the decarboxylase inhibitor NSD-1015 or the concentration of the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the median eminence] in male and female rats. The NMDA receptor antagonist MK-801 (1.0 mg/kg; s.c.) markedly reduced plasma levels of prolactin in both male and female rats. MK-801 also produced a dose-dependent decrease in DOPA accumulation, and a time-dependent decrease in DOPAC concentrations in the median eminence of female rats. In contrast, MK-801 had no effect on DOPA accumulation or DOPAC concentrations in the median eminence of male rats. Removal of the tonic stimulatory effects of prolactin on TIDA neurons in female rats by immunoneutralization of endogenous prolactin did not prevent MK-801 from decreasing DOPA accumulation. In the median eminence of ovariectomized female rats, however, MK-801 was unable to decrease DOPA accumulation . These results suggest that NMDA receptor activation tonically stimulates TIDA neurons in female but not in male rats by a prolactin-independent mechanism, and that ovarian hormones modulate this NMDA receptor-mediated regulation of TIDA neuronal activity. (Supported by ADAMHA Grant MH 42802.)

191.13

TIME COURSE OF SEIZURE-INDUCED INHIBITION OF ENZYMATIC HYDROLYSIS OF NAAG. J.L. Meyerhoff[1]*, D.L. Yourick[1], R.E. Carter[2], and J.T. Coyle[3]. [1]Dept. Medical Neurosciences, Walter Reed Army Inst. Res., Washington, DC 20307; [2]Dept. Pharmacology and Molecular Sci., Johns Hopkins Univ. School of Medicine, Baltimore, MD, 21205; [3]Dept. Psychiat., Harvard Medical School, Belmont, MA 02178.

N-acetylated-α-linked acidic dipeptidase (NAALADase), is a membrane-bound peptidase which hydrolyzes the endogenous neuropeptide N-acetyl-aspartyl-glutamate (NAAG) to N-acetyl-aspartate (NAA) and the excitatory amino acid, glutamate. We have previously reported that, 48 hours after the last of a series of 5 daily electrically stimulated convulsions, NAALADase activity is significantly suppressed in the entorhinal cortex (Meyerhoff et al., Brain Res. 505:130-134, 1989). In an effort to determine the time course of this suppression, we measured brain regional NAALADase activity at 1, 6, 24, 48 and 96 hours after a series of 5 electrically stimulated convulsions. Suppression was seen in entorhinal cortex at all time points examined. Although there is evidence that NAAG might be a neurotransmitter, this dipeptide could also function as a precursor form of glutamate, which is liberated by the dipeptidase. The suppression of NAALADase activity might decrease availability of glutamate in certain CNS synapses in these rats, and might represent a homeostatic mechanism to reduce excitability. This finding might be relevant to understanding the mechanisms of the therapeutic and/or amnestic effects of electrically stimulated seizures.

191.14

MK-801-INDUCED HYPERMOTILITY IS DEPENDENT ON DOPAMINERGIC NEUROTRANSMISSION IN THE NUCLEUS ACCUMBENS (NA). L.J. Wallace*, D.L. Willins, and N.J. Uretsky. College of Pharmacy, The Ohio State University, Columbus, Ohio 43210.

Systemic administration of the non-competitive NMDA receptor antagonist, MK-801, in rats stimulates locomotion. The present experiments investigate the role of central dopamine (DA) transmission in this response. Depletion of catecholamine stores with reserpine (5 mg/kg, 18 hrs) or α-methyl-p-tyrosine (250 mg/kg, 4 and 1 hour) markedly inhibited the locomotor response to MK-801 (0.1 mg/kg, sc). While the D-1 DA receptor antagonist, SCH23390 (0.03 mg/kg, sc) did not reverse the locomotor response to MK-801, the D-2 receptor antagonist, eticlopride (0.03 mg/kg, sc) decreased this response. In combination these antagonists reduced the locomotor response to a greater extent than did eticlopride alone. The hypermotilty response to MK-801 was also inhibited by the administration of either SCH23390 or eticlopride into the nucleus accumbens (NA). The administration of MK-801 (10 μg) into either the ventral tegmental area (VTA) or the NA stimulated a marked locomotor response, which was attenuated by the systemic administration of DA receptor antagonists. These results demonstrate the importance of central DA transmission in the hypermotility response to MK-801 and suggest a role for endogenous DA in the NA in this response. These results further suggest that MK-801 may act either at the level of the VTA or NA to stimulate locomotion.

191.15

pros-METHYLIMIDAZOLEACETIC ACID EFFECTS ON GLUTAMATE AND ASPARTATE EXTRACELLULAR LEVELS IN BRAIN REGIONS OF FREELY MOVING RATS. P. Blandina*[1,2], G. Cherici[1], F. Moroni[1] and J. P. Green[2]. [1]Dip. di Farmacologia Preclinica e Clinica, Universitá di Firenze (I) and [2]Dept. of Pharmacology, Mount Sinai School of Medicine, C.U.N.Y., New York, NY 10029.

The severity of Parkinson's disease was correlated with the cerebrospinal fluid levels of pros-methylimidazoleacetic acid (p-MIAA), an endogenous substance in brain and other tissues (Prell et al., J. Neural Transm. 3:109-125, 1991). However, no causal relationship has been established. The possible contribution of the excitatory amino acids to the neural toxicity in Parkinson's disease prompted us to investigate p-MIAA effects on glutamate (GLU) and aspartate (ASP) release, using a microdialysis technique. Perfusion flow rate was 2 µl/min. Twenty four hours after implantation with dialysis fiber, striatum, hippocampus or cortex of male Wistar rats (200g) released spontaneously in the micromolar range GLU and ASP, measured with HPLC coupled with fluorescent detection. p-MIAA (1-0.01 mM), given through the dialisys fiber, increased concentration-dependently GLU baseline up to three-fold in striatum, but not in hippocampus and cortex. In none of these regions p-MIAA increased ASP baseline. p-MIAA effect was not mimicked by its isomer tele-methylimidazoleacetic acid (1 mM). p-MIAA (1 mM) did not influence the uptake of GLU by striatal slices. The presence of stereo-, region-specific and direct releasing mechanism(s) is suggested.

191.16

PHTHALLIC ACID LESIONS OF RAT BASAL FOREBRAIN-DIFFERENTIAL EFFECTS ON CHOLINE ACETYLTRANSFERASE IN CORTEX AND AMYGDALA. R.J. Boegman*, J. Cockhill, K. Jhamandas, R.J. Beninger.† Pharmacology & Toxicology and Psychology,† Queen's University, Kingston, Ontario, Canada K7L 3N6.

Phthallic acid excites cells in rat cerebral cortex and provokes acetylcholine release from striatal slices in vitro (Stone, Br. J. Pharmacol. 81 175, 1984; Lehmann et al. J. Pharmacol. Exper. Therap. 232 873 1985). We have previously observed that cholinergic neurons in the nucleus basalis (nb) projecting to the cortex or amygdala show characteristic excitotoxic responses depending on the neurotoxin infused. We demonstrate here the effects of phthallic acid on cholinergic neurons of the nb. Phthallic acid (50-750 nmoles/0.5 µl) was unilaterally infused into the nb of male Sprague Dawley rats (275-300 g). After seven days the cortex and amygdala were assayed for choline acetyltransferase (ChAT) activity. A 60% reduction in ChAT activity of the amygdala was obtained at doses between 100 and 300 nmoles. In contrast the ChAT activity of the cortex remained unaltered until a dose of 750 nmoles was infused; at this dose a 55% reduction was obtained. Phthallic acid-induced neurotoxicity was antagonized by kynurenic acid and APV suggesting an NMDA receptor mediated mechanism. In addition, phthallic acid was not neurotoxic in 12 day old rat pups, indicating that a presynaptic input is required for its neurotoxic effect.

Supported by NCE Network for Neural Regeneration & Recovery.

EXCITATORY AMINO ACIDS: PHARMACOLOGY III

192.1

THE COMPETITIVE NMDA ANTAGONIST, LY274614, ALTERS MORPHINE TOLERANCE BUT NOT OPIOID RECEPTORS OR MORPHINE'S DISTRIBUTION. P.J. Tiseo*, J. Cheng, G.W. Pasternak and C.E. Inturrisi. Depts. of Pharmacology and Neuroscience, Cornell U. Med. College, New York, N.Y. 10021.

Co-administration of LY274614 (LY) with morphine significantly attenuates the development of morphine tolerance in rats. Furthermore, morphine tolerant animals infused with LY (sc at 24 mg/kg/24 hrs) regained their analgesic sensitivity to morphine whether co-administration of morphine was continued or discontinued. The tolerance produced by morphine and prevented by LY results in a 10-fold shift of the morphine dose-response curve and appears to be primarily pharmacological and not behavioral. Steady-state plasma morphine levels were not significantly different after 7 days of co-administration of LY compared to saline. Infusion of LY for 7 days did not increase the affinity or density of mu, delta, kappa$_1$ or kappa$_3$ opioid receptors in rat brain homogenates as assessed by ligand binding assays. Furthermore, the IC_{50}s for LY in mu$_1$, mu$_2$, delta, kappa$_1$ or kappa$_3$ ligand binding assays were greater than 10 uM. These results indicate that LY can prevent or reverse the development of morphine tolerance without reducing the analgesic response, altering morphine's systemic distribution or upregulated opioid receptors. The ability of LY to alter the development and maintenance of tolerance is presumed to be mediated at NMDA receptors. (Supported in part by DA01457 and DA02615.)

192.2

DEXTROMETHORPHAN AND DEXTRORPHAN BUT NOT DTG PREVENT MOTION SICKNESS IN CATS. J.B. Lucot*, Dept. Pharmacol., Wright State Univ., Dayton, OH 45435.

Ten to twelve female cats susceptible to motion sickness were tested biweekly in a motion device modelled after a Ferris wheel. Tests lasted for 30 min followed by one min of observation at rest. The sigma ligand DTG had no significant effect on motion sickness over the dose range of 0.03 to 3.0 mg/kg given SC. Dextromethorphan significantly decreased motion sickness over the same dose range. Dextrorphan, a metabolite of dextromethorphan, was roughly 10X more potent than dextromethorphan at suppressing motion sickness. High doses of dextromethorphan and dextrorphan but not DTG produced slight ataxia of the hindlimbs without significantly impairing the righting reflex or the vestibulo-ocular reflex. The relative effects of these drugs on different receptors leads to the suggestion that blockade of NMDA/PCP but not sigma receptors can suppress motion sickness.

192.3

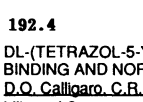
EFFECTS OF BTS 54 505, A METABOLITE OF SIBUTRAMINE ON EXCITATORY AMINO ACID AND MONOAMINE EVOKED RESPONSES IN THE RAT DORSOLATERAL GENICULATE NUCLEUS (dLGN). G. Scott, G.P. Luscombe[1] & R. Mason*. *Dept. of Physiol. & Pharmacol., Queens Medical Centre, Nottingham NG7 2UH, UK. [1]Boots Pharmaceuticals Research Dept., Nottingham NG2 3AA, UK.*

Tricyclic antidepressants have been shown to produce an open channel block of NMDA receptors similar to MK-801 and ketamine. Here we report the effects of BTS 54 505, the primary amine metabolite of the non-tricyclic putative antidepressant sibutramine, on NMDA-, 5-HT- and NA-evoked responses in the rat dLGN *in vivo*. Male Lister hooded rats were anaesthetised with urethane (1.3-1.5g.kg^{-1}, i.p.) and seven-barrelled micropipettes were used to record extracellular neuronal activity and for ionophoresis. BTS 54 505 inhibited NMDA-evoked activity in the dLGN [mean ED_{50} ± S.E.M: 17 ± 7nA, n=8] but had no effect on AMPA-evoked activity. BTS 54 505 was ejected at a current (20nA; 120s) that had no effect on basal firing rate and the effect of ionophoresed 5-HT (10mM, 0-30nA) and NA (0-40nA) were compared before and during BTS 54 505 application. The recovery time, i.e. the period required by the neurone to recover by 50% (RT_{50}) from termination of the ionophoretic ejection, was used as an index of the efficacy of the transmitter uptake process. BTS 54 505 prolonged the suppression of firing by 5-HT [mean RT_{50} ± S.E.M:(control) 13.0 ± 2.0s;(BTS 54 505) 65.7 ± 24.3s; n=6] and also prolonged the potentiation of firing by NA [mean RT_{50} ± S.E.M:(control) 29.0 ± 1.7s;(BTS 54 505) 130.8 ± 6.7s; n=6]. The magnitude of 5-HT- and NA-evoked responses was unaffected by BTS 54 505. These studies show that BTS 54 505 is a potent inhibitor of NA and 5-HT uptake and that elevation of endogenous 5-HT levels by uptake block does not contribute to the inhibition of NMDA-evoked activity by BTS 54 505.

GS is a MRC collaborative student with Boots Pharmaceuticals plc

192.4

DL-(TETRAZOL-5-YL) GLYCINE: POTENCY AND EFFICACY IN MK801 BINDING AND NOREPINEPHRINE RELEASE. W.H.W. Lunn*, D.D. Schoepp, D.O. Calligaro, C.R. Salhoff and P.J. O'Malley. Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285.



DL-(Tetrazol-5-yl) glycine is the simplest (tetrazol-5-yl) α-amino acid. It has been shown to be a highly active NMDA agonist (Schoepp, et al., Eur. J. Pharmacol. 203:237-243, 1991). With a view to better establishing potency and efficacy we have measured the stimulatory effect of DL-(tetrazol-5-yl) glycine, (TG), on [^{3}H] MK-801 binding, comparing it to responses seen with other NMDA agonists L-glutamic acid (GA), cis-methanoglutamate (CMG), and NMDA. The EC_{50} values for the four compounds were 0.07, 0.12, 0.18 and 6.86 µM, respectively. TG and GA exhibited approximately equal efficacies, in that they each caused a 60% increase in specifically bound [^{3}H] MK801 at the maximally effective concentration of 10 µM. CMG was somewhat less efficacious, with a 40% rise, whilst NMDA only led to a 25% increase at the 10 µM concentration.

Occupation of the NMDA receptor by agonists leads to the release of norepinephrine (NE) from the neurons involved. TG, CMG, and NMDA were investigated in this capacity, their relative potencies being indicated by respective EC_{50} values of 2.03, 16.5 and 75 µM. TG was the most efficacious of the three, since it resulted in a maximal release of 90% of the total [^{3}H] NE at 3 µM, whereas that evoked by CMG or by NMDA was only 50% under the same conditions. The NE release in response to TG was completely abolished by LY274614 and 5,7-dichlorokynurenate, competitive and noncompetitive NMDA antagonists respectively. NMDA receptor activation would, then, seem to be causative in the NE release.

These and previously obtained data define TG as the most potent α-amino acid selective NMDA agonist found to date. This probably accounts for its extreme neurotoxicity (see following abstract).

192.5

THE NMDA AGONIST D,L-(TETRAZOL-5-YL)GLYCINE IS AN EXTREMELY POTENT IN VIVO EXCITOTOXIN. D.D. Schoepp*, W.H.W. Lunn, C.R. Salhoff, and J.W. McDonald, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285

D,L-(Tetrazol-5-yl)glycine (TG) is a highly selective NMDA agonist with nanomolar in vitro potency (Schoepp et al., Eur. J. Pharmacol 203: 237-243, 1991). Receptor binding experiments showed that TG has 2- and 42-times greater affinity at NMDA receptors than cis-methanoglutamate (CMG) and NMDA, respectively. Electrophysiological experiments suggested that among these NMDA agonists, TG was more toxic to neurons relative to its NMDA receptor affinity. In this study, the in vivo excitotoxic potency of TG was compared to CMG and NMDA. Adult (250-300g) and neonatal (7 day-old) rats received stereotaxic unilateral intrastriatal injections of compounds. In adult rats TG produced marked loss of striatal GABA neurons (as indexed by glutamic acid decarboxylase activity (GAD)) and ACh neurons (as indexed choline acetyltransferase (ChAT) activity). Dose-response showed that TG was about 100- and 500- times more potent than CMG and NMDA, respectively. In neonatal rats, TG produced significant brain damage as indicated by brain weight losses 5 days later. TG was about 50- and 150-times more potent than CMG and NMDA, respectively, in the neonate.

	Calculated Dose (nmoles)		
	TG	CMG	NMDA
Adult Rat			
GAD (50% loss)	0.433	61.3	197
ChAT (50% loss)	0.458	17.1	205
Neonatal Rat			
20% Damage	0.12	7.0	18

The in vivo excitotoxic potency of TG relative to other NMDA agonists indicates that this compound has high efficacy and potency at the NMDA receptor. TG may be a very useful agent to probe further the in vivo role of NMDA receptors in brain pathology .

192.6

ELECTROCHEMICAL INTERACTIONS OF NMDA AND NOREPINEPHRINE IN THE CEREBELLUM OF RATS. Y. Wang* and J.C. Lin. Department of Pharmacology, National Defense Medical Center, Taipei, Taiwan, R.O.C.

In the present study, we used high-speed (5Hz) chronoamperometric recording techniques using Nafion-coated carbon fiber electrode coupled with pressure ejection of NMDA (n-methyl-d-aspartate) to investigate the effects of NMDA on noradrenergic (NE) nerve terminals. Adult male rats were anesthetized with urethane and placed on a stereotaxic frame. NMDA or NE were applied directly to the cerebellum through the multibarrel pipette. We found that local application of NMDA (1 mM, <200 nl) produced an increase of NE overflow in the cerebellum. In addition, the clearance/ diffusion of locally-applied NE was also enhanced by NMDA. This effect was antagonized by systemic injection of desipramine (25 mg/kg, i.p.). Taken together, these data suggest that NMDA enhances NE turnover in the NE-containing nerve endings in the cerebellum.

192.7

EFFECT OF GLYCINE AND NMDA RECEPTOR LIGANDS ON RAPID TOLERANCE TO ETHANOL. J.M. Khanna*, H. Kalant, A. Chau and G. Shah. Department of Pharmacology, University of Toronto, and Addiction Research Foundation, Toronto, Canada M5S 1A8.

We recently reported that NMDA receptor antagonists [(+)MK-801 and ketamine] prevent rapid tolerance to EtOH-induced hypothermia and motor impairment (tilt-plane test) in rats tested repeatedly on the first EtOH exposure ("intoxicated practice"). The role of the NMDA system in rapid tolerance was now defined further. (1) On day 1, rats received either saline (S) or D-cycloserine (CS; 3 mg/kg), an agonist at the glycine receptor, 30 min before EtOH (2.3 g/kg IP) or S. At the end of tilt-plane testing they were given EtOH (0.7 g/kg) or S, together with CS (3 mg/kg) or S. On day 2, only the EtOH + CS group showed rapid tolerance to EtOH alone (2.3 g/kg). (2) (+)MK-801 (0.25 mg/kg) pretreatment totally blocked the facilitation of rapid tolerance by CS. (3) To examine whether the effect of the NMDA system on tolerance was paradigm-specific, two groups of rats were given S or EtOH on day 1 but one group received no testing, while the other was handled at the times corresponding to testing in the original experiments (dummy tests). EtOH dosage was 4 g/kg IP (2 or 2.3 g/kg before testing, and 2 or 1.7 g/kg respectively at the end of testing) on day 1. On day 2, half of each group was tested for hypothermia and half on the tilt-plane. The dummy testing group showed rapid tolerance, but the non-tested group did not. Ketamine pretreatment (1 mg/kg), which was previously found to block rapid tolerance in the "intoxicated practice" paradigm, did not block tolerance in the dummy test group. These results confirm the role of the glycine-NMDA system in rapid tolerance, and suggest that it is specific for some adaptation that occurs during intoxicated practice.

192.8

BLOCKADE OF THE DEVELOPMENT OF CHRONIC TOLERANCE TO ETHANOL BY (+)MK-801. J. Francis*, P.H. Wu, J-F. Liu, S.J. Mihic, A.D. Lê and H. Kalant, Department of Pharmacology, University of Toronto, Toronto, Ontario Canada M5S 1A8.

Previous studies indicated that learning and memory play important roles in the development of tolerance to ethanol (EtOH). (+)MK-801 has been shown to impair learning and might thus also block the development of tolerance to EtOH. Rats were trained to criterion on the moving belt, a complex motor coordination test. Acute injection of (+)MK-801 (an NMDA channel blocker) in doses of 0.05-0.25 mg/kg i.p. produced dose-related impairment on this test. A dose of 0.1 mg/kg, that had negligible effect by itself, potentiated the acute effects of EtOH, shifting the ED_{50} from 1.75 to 1.0 g/kg. In a chronic experiment, half of the rats then received (+)MK-801 (0.1 or 0.25 mg/kg i.p.) or saline daily, followed 30 min later by EtOH (1.8 g/kg, i.p.) and 3 practice runs on the belt, and 1 hr later a second dose of (+)MK-801 (0.1 increasing to 0.2 mg/kg by increments of 0.05 mg/kg every 4th day) or saline. The other half received the same drugs but EtOH followed the practice. (+)MK-801 (0.25 mg/kg plus a second dose of the drug) blocked the functional tolerance to EtOH in both groups, but (+)MK-801 (0.1 mg/kg) did not. Tolerance to MK-801 did not occur over 2 wks of treatment. Results indicate that NMDA receptors are involved in development of chronic tolerance to EtOH, as shown previously with rapid tolerance. The interpretation of these data is not wholly clear, and an important question is unresolved. Both EtOH and (+)MK-801 inhibit the NMDA receptor-ion channel complex and produce motor impairment, but chronic EtOH treatment produces functional tolerance whereas chronic (+)MK-801 treatment prevents the development of tolerance.
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192.9

FURTHER STUDIES ON THE INTERACTION OF PENTAMIDINE WITH THE NMDA RECEPTOR. L.J. Reynolds*, K.D. Rothermund, D.M. Zeleski, K.A. Hartnett and E. Aizenman, Depts. Pharmacol. and Physiol., Univ. of Pittsburgh, Pittsburgh PA 15261.

We have previously shown that pentamidine is a non-competitive antagonist of the NMDA receptor *in vitro* (J.Neurosci. 12:970, 1992). The goal of these studies was to investigate the mechanism of action of pentamidine and several of its analogues. Using [^{3}H] dizocilpine binding to rat brain membranes we compared pentamidine to Zn^{2+}. Spermidine had marked allosteric effects on the inhibition of [^{3}H] dizocilpine binding by Zn^{2+}, while having minor effects on pentamidine concentration response curves. We also found that the affinity of Zn^{2+} could be significantly reduced by the histidine modifying reagent diethylpyrocarbonate. DEPC also prevented Zn^{2+} from slowing the dissociation of [^{3}H] dizocilpine. As DEPC had no effect on the action of pentamidine we conclude that pentamidine and Zn^{2+} bind to distinct sites on the NMDA receptor complex.

While pentamidine is neuroprotective at 5μM it becomes directly toxic above 30μM after prolonged exposure. We examined several analogues of pentamidine (Tidwell et al. J.Med.Chem. 33:1252, 1990) for their protective and toxic properties. Of the six drugs tested two were equally toxic to pentamidine, while one was slightly toxic and three showed no toxicity at 30μM. However all compounds inhibited [^{3}H] dizocilpine binding and NMDA-induced $[Ca^{2+}]_i$ changes, and two drugs tested were also neuroprotective. This shows that the effects of pentamidine on the NMDA receptor and its toxicity are unrelated, and that it may be possible to find pentamidine-related drugs that have a wider margin of safety.

192.10

THE PRESENCE OF FREE D-SERINE IN RAT BRAIN. A.Hashimoto*, T.Nishikawa, T.Hayashi, N.Fujii, K.Harada, T.Oka and K. Takahashi. Div. of Mental Disorder Res., Natl. Inst. of Neurosci., NCNP, 4-1-1, Ogawa-Higashi, Kodaira, Tokyo 187 Japan and Group of Cell and Information, Precursory Res. for Embryonic Science and Technology, Research Development Corporation of Japan, 2-5-2, Nagata-cho, Chiyoda-ku, Tokyo 100, Japan.

Free amino acid enantiomers in rat brain extracts were analyzed as their N,O-pentafluoropropionyl isopropyl derivatives by gas chromatography. A peak X, which exhibited the same retention time as the derivative of authentic D-serine, was detected in the brain extracts. Electron impact and positive chemical ionization mass spectra of the peak X of the brain extracts were identical to those of authentic D-serine. The content of free D-serine and the ratio of D-serine/total serine in the brain were estimated to be 0.27 μmol/g and 0.23, respectively. These data provide the first evidence that substantial quantities of free D-serine are present in mammalian brain tissues and suggest that free D-serine in rat brain may be a novel candidate of an endogenous ligand for the strychnine-insensitive glycine binding site for the N-methyl-D-aspartate receptor.

192.11

CYCLOHEXYLADENOSINE ATTENUATES N-METHYL-D-ASPARTATE-INDUCED PURINE RELEASE. V.M. Sciotti* and D.G.L. Van Wylen. Departments of Pathology and Physiology, SUNY-Buffalo School of Med. and Bio, Sci., Buffalo, NY 14215.

Activation of N-methyl-D-aspartate (NMDA) receptors leads to increased neuronal activity. Adenosine depresses the neuronal activity associated with excitatory amino acids. We investigated the effects of NMDA on cerebral interstitial fluid (ISF) adenosine (ADO), inosine (INO), and hypoxanthine (HYP) in the presence and absence of N^6-cyclohexyladenosine (CHA), an ADO A_1 receptor agonist. Microdialysis probes were implanted bilaterally in the caudate nucleus of halothane-anesthetized rats (n=4) and perfused with artificial cerebrospinal fluid (CSF). Following baseline collections, the probe on one side of the brain was perfused with CSF containing 10^{-3}M NMDA, while the probe on the other side of the brain was perfused with 10^{-3}M NMDA + 10^{-7}M CHA. Dialysate levels are shown below (mean±SEM). *p<0.05 vs baseline, #p<0.05 vs opposite side.

	ADO(μM)	INO(μM)	HYP(μM)
BASELINE	0.42±0.07	0.63±0.11	3.91±0.66
NMDA	0.63±0.14	1.30±0.31*	7.44±1.27*
BASELINE	0.50±0.08	0.94±0.16	5.38±0.53
NMDA+CHA	0.57±0.16	0.64±0.22#	6.33±0.39#

CHA prevents the NMDA-induced increase in ISF purines, possibly by acting presynaptically to inhibit neurotransmitter release or postsynaptically to interfere with membrane excitability. Supported by NIH HL-40878.

192.12

DIFFERENTIAL EFFECTS OF COMPETITIVE AND NON-COMPETITIVE NMDA ANTAGONISTS ON THE cAMP ACCUMULATION, DOPAMINE RELEASE IN VIVO AND PREPULSE INDUCED INHIBITION OF ACOUSTIC STARTLE RESPONSE

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Therapeutic potentials of NMDA receptors antagonists recently evidenced by experiments with MK-801 are limited by its phencyclidine like effects. Therefore, in spite of the heuristic value of analysis based on MK-801 a need arises for further studies reevaluating the therapeutic potentials with compounds which are devoided of PCP like properties. In the present study we analyzed, the impact of competitive and non-competitive antagonists CGP37849 and MK-801 in three experimental paradigms: release of dopamine in the rat prefrontal cortex, prepulse induced inhibition of acoustic startle response (PPI) and forskolin stimulated accumulation of cAMP. It was found that MK-801 induced a pronounced, dose dependent elevation of dopamine release in the rat prefrontal cortex, while the effects of CGP37849 in this respect were week and inconsistent. MK-801 opposite to CGP37849 abolished the PPI, but in contrast, CGP37849 attenuated the amplitude of acoustic startle response. The forskolin-induced cAMP accumulation was enhanced in a dose dependent manner by MK-801 whereas CGP37849 was devoided of any significant effect on forskolin-induced cAMP accumulation. Therefore, CGP37849 as a new putative therapeutic agent markedly differs form MK-801 concerning its influence on dopaminergic and noradrenergic systems of rat brain.

192.13

COMPETITIVE NMDA RECEPTOR ANTAGONISTS, CGP 39551 AND CGP 37849, DO NOT IMPAIR SPATIAL MEMORY AT ANTIEPILEPTIC DOSES IN RATS. A. Ylinen*, J. Sirviö, T. Hartikainen and P.J. RIEKKINEN. Dept. of Neurology, Univ. of Kuopio, Kuopio, Finland.

Several NMDA receptor antagonists have been shown to have anticonvulsive properties in experimental models of epilepsy. However, in most of these experiments the animals have exhibited ataxia and other side effects at anticonvulsive doses, especially the non-competitive NMDA receptor antagonists have been shown to cause impairment of memory. In the present experiments we studied the effects of two competitive NMDA receptor antagonists, CGP 39551 and CGP 37489 administered systemically at anticonvulsive doses, on the acquisition of water maze task. In contrast to MK 801 which was used a reference compound, CGP 39551 and CGP 37849 did not impair spatial memory at an ED_{50} dose in the maximal electroshock test (MES) ($p > 0.1$). CGP 39551, but not CGP 37849, slightly impaired spatial memory at double this dose ($p=0.001$) and, both of drugs caused impairment at four times higher dose than the ED_{50} in MES ($p < 0.001$). The present results suggest that there is a larger therapeutic window of these new competitive NMDA antagonists compared to MK 801 and emphasize the possibility to develop NMDA receptor-antagonizing antiepileptic drugs.

192.14

4-CHLORO-3-HYDROXYANTHRANILIC ACID IS A RELATIVELY POOR INHIBITOR OF QUINOLINIC ACID PRODUCTION IN THE RAT BRAIN IN VIVO. J.L. Walsh*, H.-Q. Wu and R. Schwarcz. Maryland Psych. Res. Center, Baltimore, MD 21228.

Quinolinic acid (QUIN) has been speculatively linked to the pathogenesis of several neurological, psychiatric and neuroviral diseases. In the mammalian brain, QUIN appears to be synthesized exclusively by enzymatic conversion of 3-hydroxyanthranilic acid (Adv. Exp. Med. Biol., 294:185, 1991). 3-Hydroxyanthranilic acid oxygenase blockers are therefore of potential therapeutic value. 4-Chloro-3-hydroxyanthranilic acid (4-Cl-3HANA) is known to be a highly potent, reversible inhibitor of brain QUIN synthesis in vitro (K_i: 6 nM; Biochem. Pharmac., 42:985, 1991). We have now assessed its potency in unanesthetized rats in vivo. 3-Hydroxyanthranilic acid (30 μM) was introduced to the hippocampus by microdialysis, and extracellular QUIN was determined hourly in the dialysate. Various concentrations of 4-Cl-3HANA (3 μM-3 mM) were introduced by the same route after attainment of steady-state QUIN levels, and the drug was discontinued after 3 hours. Total inhibition of de novo QUIN synthesis was seen only at the highest dose used (IC_{50} ~300 μM), and QUIN concentrations returned to steady-state levels within 3 hours after drug discontinuation. Using the same experimental paradigm, i.v. injection of 14 mg/kg 4-Cl-3HANA caused a 44 ± 9% decrease in extracellular QUIN levels within 3 hours (maximal effect). Supported by USPHS grant NS 16102.

192.15

POTENTIATION OF NEUROLEPTIC ACTIVITY BY POSITIVE MODULATORS OF NMDA FUNCTION. O. Gandolfi*, R. Dall'Olio and A. Poli. Department of Pharmacology, University of Bologna, Via Irnerio n. 48, I-40126 Bologna, Italy.

According to the view that N-methyl-D-aspartate (NMDA) agonists could be seen as putative therapeutic agents in schizophrenia, the present study was aimed at investigating whether the NMDA positive modulator D-cycloserine (DCS) could show neuroleptic activity. When given alone, DCS (3, 6, 12 mg/kg i.p.) failed to affect the stereotyped behaviour induced by 0.5 mg/kg. s.c. apomorphine. Nevertheless, when DCS (3 mg/kg) was administered in combination with the D1 or the D2 antagonists SCH 23390 or YM 09151-2 respectively at doses which by themselves were ineffective in blocking apomorphine induced behaviour, a neuroleptic effect occurred. Furthermore, the positive NMDA modulator allowed (-)-sulpiride, that "per se" never antagonized the apomorphine-induced stereotypy, to exhibit a full neuroleptic activity. The dose of DCS which potentiated antipsychotic effect of dopaminergic blockers also counteracted hypermotility induced by the NMDA negative modulator MK-801 (0.25 mg/kg), indicating the specificity of DCS effect. The results suggest that DCS could be a useful supplement in the therapy of psychotic disorders.

192.16

EFFECTS OF STRYCHNINE-INSENSITIVE GLYCINE RECEPTOR LIGANDS ON DISCRIMINATIVE STIMULUS EFFECTS OF N-METHYL-D-ASPARTATE (NMDA) CHANNEL ANTAGONISTS. J. M. Witkin* and T. D. Steele. Drug Development Group, Psychobiology Lab, NIDA Addiction Res. Center, Box 5180, Balt. MD 21224.

Glycine acts as a co-agonist for activation of the NMDA receptor ion channel through a strychnine-insensitive receptor, which is a potential target for novel therapeutic agents (eg. anticonvulsants, antidepressants). We evaluated the behavioral effects of glycine receptor ligands in rats trained to discriminate either (+)-MK-801 or phencyclidine (PCP) from saline, to predict whether they might induce PCP-like subjective effects in humans. The partial agonist, 1-aminocyclopropane-carboxylic acid (ACPC), did not mimic the discriminative stimulus effects of either (+)-MK-801 or PCP. Antagonists of the strychnine-insensitive glycine receptor, 7-chlorokynurenic acid or (+)-3-amino-1-hydroxypyrrolid-2-one [(+)-HA-966] also did not substitute for the discriminative stimulus effects of MK or PCP. These data suggest that functional antagonists of the strychnine-insensitive glycine receptor will be devoid of the subjective side-effects characteristic of NMDA channel ligands.

192.17

NEUROPROTECTIVE EFFECTS OF CARVEDILOL, A NEW ANTIHYPERTENSIVE, AT THE N-METHYL-D-ASPARTATE RECEPTOR. P.G. Lysko*, K.A. Lysko, and G. Feuerstein. Dept. of Pharmacology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406.

Carvedilol was found to be neuroprotective (IC_{50}=1.1μM) against glutamate-induced excitotoxicity in our model system of energy-stressed rat cerebellar granule cell neurons which rapidly succumb to glutamate via N-methyl-D-aspartate (NMDA) receptor-mediated events. Carvedilol was also a potent inhibitor of neuronal lipid peroxidation (LPO) induced by Fe^{2+}/vitamin C-generated hydroxyl radical (73% inhibition at 10μM). However, this protective action did not seem to apply to glutamate-induced excitotoxicity in the granule cells, since glutamate neurotoxicity was not associated with the formation of LPO products (thiobarbituric acid-reactive substances). Rather, carvedilol inhibited NMDA/glycine-induced increases in intracellular calcium ($[Ca^{2+}]_i$), as measured in Fura-2/AM-loaded neurons, diminishing $[Ca^{2+}]_i$ by a maximum of 66 ± 5% (n=8) with a 50% inhibitory concentration of 0.8μM. Prior addition of 5μM dihydropyridines did not shift the dose-response of carvedilol, but did significantly lower the NMDA/glycine-stimulated response to 64% of untreated (n=8, p=0.014). Furthermore, carvedilol displaced ^{3}[H]MK-801 binding to rat cortical membranes with a K_d of 29.4 ± 2.2μM (n=6, LIGAND analysis). These data therefore suggest that, in addition to its antihypertensive and anti-lipid peroxidative functions, carvedilol has neuroprotective activity as a non-competitive inhibitor at the NMDA receptor and may have therapeutic value in cerebral ischemia and stroke.

192.18

P-9939, A NOVEL NEUROPROTECTIVE AGENT. A.M. Szczepanik, R. Kosley, Jr., M. Li, R. Dunn, F. Camacho, R. Fishkin, K.M. Brooks, F. Wirtz-Brugger, L. Leonard, L.L. Martin, C.A. Wilmot*. Neuroscience Strategic Business Unit, Hoechst-Roussel Pharmaceuticals, Inc., Somerville NJ 08876-1258.

Competitive and noncompetitive NMDA antagonists protect against neuronal loss due to ischemia or excitotoxin-induced lesions in animal models. Glycine antagonists, via an allosteric site associated with the NMDA receptor, are also neuroprotective. P-9939 [2-amino-N-(1H-indol-1-yl)acetamide HCl] was characterized as a low efficacy glycine partial agonist. P-9939 inhibited [^{3}H]glycine binding (IC_{50} 36 μM) and exhibited biphasic effects on [^{3}H]TCP binding, with stimulation at low concentrations (EC_{50} 3.6 μM, producing 40% of the maximal effect of glycine at 100 μM) and inhibition at high concentrations (IC_{50} 780 μM). Unlike the prototypical partial glycine agonist D-cycloserine (DCS), P-9939 was not a cognitive enhancer in passive avoidance (rat, 10-40 mg/kg, ip) and impaired performance at doses ≥ 100 mg/kg. Biological testing of P-9939 also included behavioral studies, cerebellar cGMP in mice and electrophysiology in the *Xenopus* oocyte expression system. P-9939 partially protected against NMDA-induced rat striatal lesions in single or multiple doses, as measured with the markers ChAT and GAD, whereas DCS had no effect. These data suggest that P-9939 may have therapeutic potential in pathological conditions associated with excitotoxic mechanisms.

PEPTIDES: RECEPTORS II

193.1

CLONING OF A CANDIDATE PEPTIDE RECEPTOR FROM HYPOTHALAMUS HOMOLOGOUS TO NEUROMEDIN RECEPTORS. S.K. Welch, B.F. O'Hara, E.L. Sutin, L. Bitting, D. Edgar*, and T.S. Kilduff. Sleep Research Ctr., Stanford Univ., Stanford, CA 94305.

As part of our study of the hypothalamic suprachiasmatic nuclei (SCN), we are using PCR with degenerate primers to clone and identify members of the G-coupled neurotransmitter receptor family expressed in this nucleus. One of our novel clones is a candidate peptide receptor most similar to receptors for the tachykinins, neuromedin K, substance P, and substance K. RNA was isolated from rat SCN, reverse transcribed, and PCR amplified with degenerate primers homologous to conserved transmembrane domains (TMD) of the receptor family. PCR products were subcloned and partially sequenced. Clone UHR-1 (Unknown Hypothalamic Receptor-1) encodes TMD 3-6 of a candidate receptor which is 28% identical to the tachykinin receptors. We obtained a 3.7 kb cDNA from a hypothalamic library. Hydropathy analysis of the inferred amino acid sequence suggests seven TMDs, 2 potential glycosylation sites, and two consensus sites for protein kinase phosphorylation. The cDNA has a 1.5 kb 3' untranslated region, 2 polyadenylation sites, and a (GA)-rich repeat. Hybridization to a Southern blot of rat, human, and mouse DNAs suggests a single UHR-1 gene and a small number of related sequences. The tissue distribution of UHR-1 is being examined by Northern blotting. A 4.2 kb mRNA is expressed in rat hypothalmus, pituitary, cerebellum, cortex and midbrain, and a 2.5 kb message is apparently expressed in several peripheral tissues. We are also using in situ hybridization to examine expression of this receptor in the brain. (Research supported by the UpJohn Company)

193.2

MULTIPLE GENE TRANSCRIPTS OF THE SOMATOSTATIN RECEPTOR (SSTR): TISSUE-SELECTIVE DISTRIBUTION AND cAMP REGULATION. Y.C. Patel*, G. Kent, C.B. Srikant, and R. Panetta. Fraser Laboratories, McGill University, Royal Victoria Hospital, Montreal, Quebec, Canada H3A 1A1.

mRNA transcripts of 2 recently cloned SSTR subtypes have been reported to be selectively expressed in the human intestine (SSTR1:mRNA of 4.8 kb) or brain and kidney (SSTR2:mRNAs of 8.5, 2.5 kb) (PNAS 89:251, 1992). The presence of mRNAs encoding for these receptors in normal pituitary and adrenal cortex or in pituitary tumor cell lines, all of which represent major SSTR positive tissues, has not been reported. In the present study we have characterized the expression of gene transcripts homologous to the SSTR2 sequence in rat pituitary, adrenal, and other tissues, and in AtT-20 mouse pituitary tumor cells. Total RNA was analysed by Northern blot under moderate stringency with a full length human SSTR2 cRNA probe. Expression of a 2.3 kb mRNA was detected in cerebral cortex, hypothalamus, anterior pituitary, adrenal cortex, stomach, pancreas, and colon. This message was found in low abundance in kidney, but was undetectable in liver, lung, testes, and muscle. The presence of an additional 2.8 kb mRNA species was evident only in pituitary, hypothalamus, and adrenal cortex. Furthermore, selective abundance of the 2 mRNAs was seen in pituitary (2.8 kb > 2.3 kb), and hypothalamus (2.8 kb < 2.3 kb). AtT-20 cells expressed a single 2.3 kb transcript which showed a significant 2 fold increase with forskolin stimulation. The reported 4.8 kb mRNA selective for SSTR1 was not observed in any tissue in these studies.

CONCLUSIONS: (i) Two closely related transcripts of SSTR2 of 2.3, 2.8 kb are expressed either singly or together in a tissue-selective manner in the rat. (ii) The 2.3 kb mouse pituitary mRNA is regulated by cAMP.

193.3

PROGRESS TOWARDS CLONING OF A NOVEL SOMATOSTATIN RECEPTOR. H. Kong*, M. Theveniau, C.M. Spencer, C.W. Murray, J.H. Eberwine and T. Reisine. Dept. of Pharmacology, Univ. of PA Sch. of Med., Philadelphia, PA 19104-6084.

Previous work from our group supports the hypothesis that multiple somatostatin (SRIF) receptors are expressed in mammals. Recently, three SRIF receptor genes have been cloned from human and mouse libraries. However, biochemical and pharmacological studies indicate that at least one more SRIF receptor subtype has yet to be cloned. We had previously developed an antibody (Ab) directed against a rat brain SRIF receptor. This Ab detects a 60 kDa protein in rat brain and the mouse cell line AtT-20. It does *not* cross-react with any of the recently cloned SRIF receptors transiently expressed in COS cells. Therefore, this anti-SRIF receptor Ab was used to screen an AtT-20 cell expression library. Clones identified by the Ab were analyzed and the inserts sequenced. A 2 kb insert has been isolated that has several hydrophobic regions. No significant similarity to other sequences in the GenBank database has been observed. Northern analysis revealed that this clone hybridizes with RNA from AtT-20 cells but not RNA from COS cells (which do not normally express SRIF-R). In order to obtain full length coding region, this clone is currently being used to screen other cDNA libraries. [Supported by grants NIMH MH 48518 and MH 45533 (T.R.) and training grant MH 14654 (C.W.M.).]

193.4

CHARACTERIZATION OF TWO CLONED SOMATOSTATIN RECEPTORS. S. Rens-Domiano*, S. Law, Y. Yamada, S. Seino, G. Bell and T. Reisine. Dept. Pharmacology, Univ. of Pennsylvania, Phila. PA 19104 and HHMI, Univ. Chicago.

Somatostatin is a neuropeptide that induces a variety of physiological responses by interacting with specific cell surface receptors. Several lines of evidence have suggested that multiple SRIF receptors exist. Two of these, called $SRIF_1$ and $SRIF_2$, can be differentiated by their ability to bind MK 678 and CGP 23996-like compounds with high affinity, respectively. Recently, two SRIF receptor genes (SSTR1 and SSTR2) were cloned from human and mouse genomic libraries. We have characterized the pharmacology of these two cloned receptors expressed in CHO cells. Both cloned receptors could be labeled by [^{125}I]Tyr^{11}-SRIF and bound SRIF with high affinity. SSTR1 was also able to bind CGP 23996-like compounds, but not MK 678. In contrast, SSTR2 bound MK 678 with high affinity, but was insensitive to CGP 23996-like compounds. High affinity agonist binding to SSTR2 was abolished after agonist pretreatment, whereas SSTR1 did not desensitize. GTPγS and pertussis toxin treatment decreased high affinity agonist binding to SSTR2, while agonist binding to SSTR1 was not altered, suggesting that only SSTR2 couples to pertussis-toxin sensitive GTP-binding proteins. These results demonstrate that these two cloned receptors are pharmacologically distinct and can be differentially regulated. The data also suggests that the cloned SSTR1 receptor is similar to the previously characterized $SRIF_2$ receptor, and the cloned SSTR2 receptor is similar to $SRIF_1$. Currently, the function of these two cloned receptors is unknown, since forskolin-stimulated adenylyl cyclase activity was not inhibited by SRIF in cells either stably (CHO) or transiently (COS-7) expressing the two cloned receptors. Both of these cloned receptors are glycoproteins, and like brain SRIF receptors, high affinity agonist binding was decreased after removal of sialic acids by neuraminidase indicating a role for sialic acids in high affinity agonist binding. Supported by NIMH grants 45533 and 48518 and NAMI (TR) and NRSA postdoctoral fellowship (NS 09002) and NARSAD (SRD).

193.5

PROPERTIES OF THE CLONED MOUSE SSTR3 SOMATOSTATIN RECEPTOR . S.F. Law*, K. Yasuda, S. Rens-Domiano, C.D. Breder, C.B. Saper, T. Reisine and G.I. Bell. Graduate Group in Cell Biology, Dept. of Pharmacology, Univ. of PA Philadelphia, PA 19104, HHMI and Depts. of Biochem., Mol. Biol., Pharm., Physiol. Sci., and Neurol., Univ. Chicago , Il. 60637.

Recently, two somatostatin (SRIF) receptor genes have been cloned (Yamada et al. PNAS 89:251, 1992) and have been referred to as SSTR1 and SSTR2. We have cloned a novel third member of the SRIF receptor family and have referred to it as SSTR3. The receptor has approximately 50% amino acid identity with the other two receptors. It binds SRIF, SRIF-28 and the SRIF analog CGP 23996 with high affinity but expresses low affinity for the SRIF agonists MK 678 and SMS-201-995. The receptor can be desensitized when continuously exposed to agonist. Furthermore, SSTR3 couples to pertussis toxin sensitive G proteins and mediates SRIF inhibition of adenylyl cyclase activity. These findings indicate that both the pharmacological and functional properties of SSTR3 differ from the other cloned SRIF receptors. Interestingly, both SSTR3 and SSTR2 couple to pertussis toxin sensitive G proteins but only SSTR3 functionally couples to adenylyl cyclase. Studies are underway to determine the regions of the receptors needed for G protein association and coupling to adenylyl cyclase. Supported by MH-45533 and 48518.

193.6

ONTOGENY OF SOMATOSTATIN RECEPTOR SUBTYPES IN RAT BRAIN. K. Raynor*, M. Theveniau, and T. Reisine. Dept. of Pharmacology, Univ. of PA, Phila., PA, 19104.

Adult rat brain expresses at least two receptor subtypes for the neuropeptide somatostatin (SRIF). $SRIF_1$ receptors are sensitive to hexapeptide SRIF analogues such as MK 678, whereas $SRIF_2$ receptors are insensitive to hexapeptide analogues. To determine whether expression of SRIF receptors subtypes is developmentally regulated in the rat brain and cerebellum, we labelled $SRIF_1$ and $SRIF_2$ receptors with [125I]MK 678 and [125I]CGP 23996, respectively, in membranes of rat brain from embryonic day 13 (E13) to postnatal day 18 (P18). Embryonic rat brain (E13, E16) express exclusively $SRIF_1$ receptors which show a 3-fold increase in levels from E13 to E16. The level of $SRIF_1$ receptors continues to rise with a peak at P2 (representing a 10-fold increase relative to E13) after which the level remains relatively constant. In contrast, $SRIF_2$ receptors are not present in embryonic brain, but appear by P2. The level of $SRIF_2$ receptors rises steadily to P8 after which the level is maintained. Pharmacological characterization of $SRIF_1$ and $SRIF_2$ receptors in membranes from P13 shows that these receptors are indistinguishable from those expressed in adult rat brain. $SRIF_1$ and $SRIF_2$ receptors are also expressed in the postnatal rat cerebellum and show parallel increases in levels to P5 followed by a decrease to lower levels by P16. The pattern of expression of $SRIF_1$ receptors identified by radioligand binding parallels results found by immunoblotting with antibodies to a 60 kDa rat brain SRIF receptor. Autoradiographic studies are underway to delineate the localization of SRIF receptor subtypes during development. [Supported by grants NIMH MH 48518 and MH 45533.]

193.7

DEVELOPMENTAL STUDIES OF THE 60 KDa SOMATOSTATIN RECEPTOR IN THE RAT BRAIN

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The neuropeptide somatostatin (SRIF) is present in various tissues and widely distributed throughout the nervous system. A number of studies have shown that SRIF is developmentally regulated. The purpose of our work is to determine using specific antibodies for the 60 kDa brain SRIF receptor whether the SRIF receptor is also developmentally regulated. Our preliminary results using an immunoblotting procedure demonstrate that the level of the 60 KDa SRIF receptor is not changed during the development in the whole brain as well as in subregions such as the hypothalamus, cerebral cortex, hippocampus and striatum. However, regulation of the expression of the SRIF receptor is observed in the cerebellum after birth. From P3 to P8 the SRIF receptor has reached maximal level of expression and then dramatically disappears. These results are particularly interesting since major modifications of the cell distribution in the cerebellum take place at this time of the development. Studies are in progress to determine which cells in the cerebellum as well as in the other brain regions (such as the cerebral cortex where cell migration also takes place during development) express the SRIF R during development. Using pharmacological and immunological approaches we will attempt to determine which subtypes of the SRIF R are regulated.
Supported by NIMH grants MH45533 & 48518 and the Human Frontiers Scientific Program grant LT-475/90.

193.8

PROPERTIES OF SOMATOSTATIN$_1$ RECEPTOR SUBTYPES IN AtT-20 CELLS. M. Tallent*, K. Raynor, M.A. Dichter, and T. Reisine. Depts. of Pharmacology and Neurol. and Inst. of Neurol. Sci., Univ. of PA, Philadelphia, 19104.

We have recently identified two subtypes of somatostatin (SRIF) receptors in the rat brain which can be distinguished by their affinity for the agonist MK 678. SRIF1 receptors bind MK 678 with high affinity whereas the SRIF2 receptor is insensitive to this peptide. Characterization of SRIF receptors in AtT-20 cells now show that two subclasses of the SRIF1 receptor are expressed in this cell line. Both receptors have a high affinity for MK 678. However SRIF1a receptors do not bind a putative somatostatin antagonist (SA), and high affinity agonist binding in these receptors is abolished upon continuous exposure to MK 678. SRIF1b receptors bind SA and are less sensitive to regulation by exposure to MK 678. Both MK 678 and SA inhibit forskolin stimulated cAMP formation and potentiate an inward rectifier K+ current in AtT-20 cells, suggesting that the SRIF1b receptor is coupled to adenylyl cyclase and a K+ channel. MK 678, but not SA, can inhibit a high voltage activated Ca++ current in AtT-20 cells suggesting that SRIF1a receptors may selectively couple to Ca++ channels in these cells. These preliminary results suggest that the subtypes of SRIF1 receptors couple to different cellular effector systems. Studies are underway to further investigate the pharmacology and functional properties of these two receptors and to determine whether they couple to different G proteins. Supported by MH 45533, 48518, and GM 34781.

193.9

CLONING AND EXPRESSION OF A RAT SOMATOSTATIN RECEPTOR ENRICHED IN BRAIN. *X.-J. Li[1], M. Forte[2], R.A. North[2], C.A. Ross[1] and S.H. Snyder[1]*. [1]Johns Hopkins University School of Medicine, Department of Neuroscience, 725 N. Wolfe Street, Baltimore, MD 21205; [2]Vollum Institute, The Oregon Health Sciences University, Portland, OR 97201.*

A cDNA encoding a rat somatostatin (SRIF) receptor was identified by use of degenerate oligonucleotide primers and polymerase chain reaction (PCR) amplification of cDNA prepared from transcripts expressed in rat brain. The complete cDNA encodes a protein of 391 amino acids with seven potential transmembrane domains. Expression of the cDNA product in transfected COS-7 cell lines provides the same high affinity of binding to [^{125}I-Tyr^{11}]-SRIF-14 as that of rat cerebral cortex tissues. However, the binding of [^{125}I-Tyr^{11}]-SRIF-14 to cloned rat SRIF receptor is not displaced by MK678, a SRIF analog that partially displaces [^{125}I-Tyr^{11}]-SRIF-14 binding sites in membranes of rat cerebral cortex. Northern analysis and in situ hybridization indicate that mRNA (4.0 kb) for cloned rat SRIF receptor is preferentially expressed in rat brain regions such as cerebral cortex and hippocampus with no detectable expression in most peripheral organs. This pattern contrasts with the exclusive peripheral expression of a recently cloned human SRIF receptor. The cDNA probe of rat receptor detects mRNA from mouse brain but not from human cerebral cortex and cerebellum.

193.10

MOLECULAR CLONING AND CHARACTERIZATION OF FOUR MEMBERS OF THE SOMATOSTATIN RECEPTOR GENE FAMILY. L.L. Demchyshyn, J. Comess, R.K. Sunahara, P. Seeman, H.H.M. Van Tol, C.B. Srikant^, Y.C. Patel^, and H.B. Niznik.* Dept. of Pharmacology and Psychiatry, University of Toronto; ^Fraser Laboratories, Royal Victoria Hospital, Montreal, Canada, H3A 1A1 and the Mol. Neurobiol Lab., Clarke Institute of Psychiatry, Toronto, Canada, M5T 1R8.

Somatostatin receptors are members of a superfamily of G-linked receptors which act as biochemical modulators in the release of hormones and other secretory proteins. Pharmacological and biochemical evidence suggest the existence of various receptor subtypes selective for somatostatin-14 and somatostatin-28. PCR amplification of human genomic DNA with degenerate oligonucleotide primers constructed to conserved segments of transmembrane domains 3 and 6 followed by high stringency hybridization screening yielded four genomic clones, HG2-3, HGA1-22, HGA2-22 and HG9-2. Nucleotide sequence analysis indicates two intronless genes encoding 391 and 369 amino acid proteins displaying affinity for somatostatin-14 which have recently been characterized as SSTR1 and SSTR2. These have been functionally expressed in mammalian fibroblast cell cultures. A third gene, SSTR3, encodes for a 407 amino acid protein displaying considerable homology within the seven putative transmembrane domains to SSTR1 (72%) and SSTR2 (57%). Northern blot analysis of various brain regions reveals a transcript size of approximately 4.0 kb. The fourth gene displays transmembrane homology of 50%, 75%, and 55% to SSTR1, SSTR2 and SSTR3 respectively. Northern blot analysis has revealed no discernable message within brain regions for this gene.

193.11

CHARACTERIZATION OF A cDNA ENCODING A BOVINE SOMATOSTATIN RECEPTOR AND REGIONAL DISTRIBUTION IN BRAIN. W. Xin*, M.-L. Wong, J. Rimland, E.J. Nestler and R.S. Duman. Laboratory of Molecular Psychiatry, Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Somatostatin, a tetradecapeptide, is widely distributed in the central nervous system and peripheral tissues where it has a number of actions most notable being regulation of neuroendocrine function. Using degenerate primers for the third and sixth transmembrane domains of G protein-coupled receptors and the polymerase chain reaction, we have cloned a somatostatin receptor from bovine locus coeruleus. DNA sequencing of this receptor cDNA revealed greater than 93% homology at the nucleotide level and greater than 99% identity between the predicted amino acid sequences of the bovine receptor and the human and mouse somatostatin receptor subtype 2 (SSTR2).

Northern blot analysis showed that the bovine SSTR2 cDNA hybridizes to a unique 2.2kb transcript in different bovine brain regions with highest density in cerebral cortex, cerebellum, and thalamus and lower levels in neostriatum, locus coeruleus, dorsal raphe, and in peripheral tissues. In addition, bovine SSTR2 also hybridizes to a rat 2.2kb transcript as well as a larger transcript (>7kb) with levels of expression similar to that in bovine brain. To further characterize expression of SSTR2, *in situ* hybridization on rat brain slices is being carried out using bovine SSTR2 cDNA as the probe. The full-length cDNA of bovine SSTR2 is being subcloned and expressed in mammalian cell lines to study the functional and ligand binding properties of this receptor.

193.12

ISOLATION OF NOVEL G-PROTEIN COUPLED RECEPTOR cDNAs FROM LOCUS COERULEUS AND VENTRAL TEGMENTUM BY PCR. J.D. Alvaro', E.J. Nestler, and R.S. Duman. Interdepartmental Neuroscience Program, Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale Univ. School Med.,New Haven, CT 06508.

Studies in our laboratory have focused on the function of the locus coeruleus (LC) and ventral tegmentum (VT), two brain regions which have been implicated in drug addiction and a number of other psychiatric disorders. In continuing our efforts to identify G-protein coupled receptors localized in these regions, we have used degenerate oligonucleotide primers corresponding to highly conserved regions within the third and sixth transmembrane domains of the receptor superfamily to PCR amplify both bovine LC and rat VT double stranded cDNA. As seen on an agarose gel, the subsequent PCR reactions yielded greater than 9 cDNA bands for the LC and more than 5 bands for the VT. The reaction mixture for the LC was blunt-end cloned into pBluescript, and the VT reaction mixture was subcloned into the pCR1000 TA vector. Preliminary sequence data on individual clones indicate that both previously identified as well as putatively novel receptor fragments have been amplified in each brain region. The novel cDNAs display hydropathicity plots indicative of G-protein coupled receptors and retain a number of highly conserved residues found in other members of the receptor superfamily. Northern blot analysis of these cDNAs is under way to determine the regional distribution of each clone, and cDNA libraries will be screened to isolate full length clones for each putative receptor.

193.13

SEQUENCE AND LOCALIZATION OF A RAT HOMOLOGUE OF A NEUROPEPTIDE Y RECEPTOR cDNA. M-L. Wong*, W. Xin, E. J. Nestler, R. S. Duman. Lab. of Molecular Psychiatry, Depts of Psychiatry and Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06508.

Northern blot analysis of a NPY receptor mRNA sequenced by our laboratory (Rimland et al. Mol. Pharmacol. 40:869, 1991) from bovine locus coeruleus, referred to as LCR1, is regionally distributed in both brain and peripheral tissues. Spleen is a peripheral organ particularly enriched in LCR1 mRNA and for that reason a spleen tissue library was obtained for this study.

We used the full length LCR1 cDNA as a probe to screen a poly dT/random primed rat spleen library to isolate the rat homologue of that NPY receptor. The rat LCR1 has a sequence homology of approximately 85% when compared to the bovine clone. NPY has been implicated in a variety of physiological and pharmacological actions including cardiovascular, gastrointestinal, and neuroendocrine modulation. The rat LCR1 cDNA clone will be particularly useful for studies of the regulation and function of NPY receptors.

193.14

NEURON-SPECIFIC CLONING OF G PROTEIN-COUPLED RECEPTORS IN THE POND SNAIL *LYMNAEA STAGNALIS*. C.P. Tensen*, C.C. Gerhardt, E.R. van Kesteren, R.J. Planta, E.Vreugdenhil and H. van Heerikhuizen. Department of Biochemistry and Molecular Biology, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.

Signal transduction pathways mediated by G protein-coupled receptors, play an important role in several neuronal processes (information processing, learning, behaviour, etc). The cloning of cDNAs that encode vertebrate neuronal signal transduction components has revealed the existence of several large superfamilies of polypeptides which share primary sequence homology and which have similar secondary structures. Despite these extensive molecular studies, however, little is known about the precise *in vivo* interaction of specific members of the different superfamilies.

Aiming at the elucidation of such interactions in well-characterized neurons that are amenable to experimental manipulation, we have focussed our attention on the central nervous system (CNS) of the pond snail, *Lymnaea stagnalis*. The limited complexity of the CNS, the large size of its neurons (50-200 µm) and the multidisciplinary expertise on a number of identified neurons make the *Lymnaea* CNS a well-suited model system.

We have carried out cell-specific cDNA cloning by using RNA isolated from (groups of) individual well-characterized neurons from the *Lymnaea* CNS. Thus, we have cloned and characterized a number of different cDNAs encoding signal transduction components, including G protein-coupled receptors (both "classical" neurotransmitter receptors as well as putative neuropeptide receptors). The predicted structural properties of some of these proteins, the expression of the corresponding genes and their potential role in neuronal signal transduction will be discussed.

193.15

Isolation and characterization of human cDNA clones related to the NPY/PYY Y1 receptor.

I. Lundell, A. G. Blomqvist, F. Yee#, H. Yoo#, C. Söderberg*, C. Wahlestedt# and D. Larhammar. Dept. of Medical Genetics, Uppsala University, S-75123 Uppsala, Sweden. # Dept. of Neurology and Neuroscience, Cornell University Medical College, New York, N. Y.

We have recently described the isolation and characterization of a human NPY/PYY Y1 receptor cDNA clone (Larhammar et al, J. Biol. Chem., 1992). The NPY/PYY receptor family includes at least two additional pharmacologically distinct receptors called Y2 and Y3. In order to isolate Y1-related receptors we screened a human fetal brain cDNA library at low stringency conditions. Several clones were isolated. Sequencing revealed that at least three clones correspond to partially and/or aberrantly processed Y1-receptor transcripts.

We have identified two introns in the Y1 gene, one in the 5'-untranslated region and one immediately after the segment encoding transmembrane region 5. One of the cDNA clones has an L1 repeated element fused to the splice acceptor of exon 2. The other two clones also begin with Y1-unrelated DNA, but joined to different positions in the coding region, presumably corresponding to cryptic splice acceptor sites. One of the latter clones also still contains intron 2. This is in agreement with our RT-PCR analyses on mRNA from several cell lines, some of which displayed a larger Y1-related product, as sequencing revealed this to correspond to Y1 mRNA retaining intron 2. Although intron 2 is only approximately 100 nucleotides, it cannot be translated as a termination codon precedes exon 3. Based upon cDNA clone frequency, the aberrantly processed Y1 transcripts may correspond to more than 5% of the total Y1 mRNA in human fetal brain.

193.16

Cloning and functional expression of a human neuropeptide Y receptor of the Y1 subtype

A. G. Blomqvist*, F. Yee#, H. Yoo#, E. E. Jazin, C. Wahlestedt# and D. Larhammar. Dept. of Medical Genetics, Uppsala University, S-75123 Uppsala, Sweden. #Department of Neurology and Neuroscience, Cornell University Medical College, New York, NY

Neuropeptide Y (NPY) and peptide YY (PYY) are structurally related peptides that function as a neurotransmitter and gastrointestinal hormone, respectively. At least three distinct NPY and/or PYY receptors, called Y1, Y2, and Y3 have been identified pharmacologically. We describe here a human Y1 cDNA clone isolated from a fetal brain library. The Y1 receptor consists of 384 amino acids and has the characteristic seven transmembrane regions of G-protein-linked receptors. In the region spanning the transmembrane regions, it displays 30% sequence identity to the tachykinin receptors but only 22% identity to the recently described bovine NPY receptor *Mol. Pharmacol.* 40, 869-875

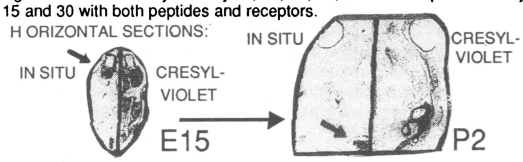
Northern blot analysis revealed a mRNA of 3.5 kb in the human neuroblastoma cell line SK-N-MC. Southern blot analysis indicated that the Y1 gene is present in the genome as a single copy. When expressed in COS1 kidney cells the Y1 receptor, HY1-5, binds ^{125}I-labelled NPY and PYY with displacement patterns typical for a Y1-receptor, i.e. PYY≥NPY≥[Leu^{31},Pro^{34}]NPY>>NPY2-36>NPY13-36. NPY and PYY stimulation of HY1-5 accelerated $^{45}Ca^{2+}$ release and inhibited forskolin stimulated adenylate cyclase activity.

The results are in agreement with the expected characteristics of a NPY/PYY-receptor of the Y1 subtype.

193.17

DISTRIBUTION OF NPY/PYY AND THEIR G-PROTEIN COUPLED RECEPTORS IN THE DEVELOPING RAT BRAIN. E.E.Jazin, S.Söderström#, C. Wahlestedt†, T. Ebendal#, and D. Larhammar*. Dept. of Medical Genetics, and #Dept. of Dev.Biol., Uppsala University,S-751 23 Uppsala, Sweden. †Dept.of Neurology and Neuroscience, Cornell University Medical College, N.Y., N.Y., U.S.A.

Neuropeptide Y (NPY) is involved in a variety of functions including eating behavior, blood pressure control, memory retention, and sexual behavior. While NPY is expressed through-out the brain, its homolog, peptide YY (PYY), which has similar biological functions as NPY, is only found in selected brainstem neurons in addition to gut endocrine cells. At least three different receptors named Y1, Y2 and Y3 have been characterized pharmacologically. We have cloned the rat and human Y1 receptor (J.Biol.Chem., in press) and the human and rat homologs of a published suggested bovine NPY receptor of the Y3 subtype (Mol. Pharmacol. 40:869-875). We are studying the distributional correlation of the two peptides and the receptors in different parts of the brain during rat development by in situ hybridization. The figure shows the change in distribution of the NPY-Y3-receptor candidate reactivity from embryonic day 15 (E15) when the labeling is more evident in the developing striatum (arrow), to postnatal day 2 (P2) when labeling was observed in the hindbrain (arrow) and retina. Similar experiments are now being conducted at embryonic days 9, 11, 13, 16, and 20 and postnatal days 2, 15 and 30 with both peptides and receptors.



193.18

VARIANT TRANSCRIPTS OF THE MOUSE GONADOTROPIN RELEASING HORMONE RECEPTOR GENE W. Zhou*[1], M. Tsutsumi[1], S.C.Sealfon[1,2] [1]Fishberg Center for Reserch in Neurobiology and [2] Dept. of Neurology, Mount Sinai School of Medicine, New York, NY 10029.

We have isolated a cDNA from a gonadotrope cell line which encodes a functional mouse GnRH receptor (clone WZ25). The predicted receptor structure has seven transmembrane (TM) domains, characteristic of G-protein coupled receptors, but lacks a typical intracellular C-terminus. Library screening has identified three additional transcripts which contain deletions in comparison with the functional receptor. Clone WZ34 contains a deletion at +909 eliminating TM VII and the C-terminus. The deletion in clone WZ15 encompasses most of TM IV-VI (+ 523 to +739) and alters the reading frame, leading to a premature stop codon. Clone WZ16 is identical to the cDNA for the functional receptor until TMVI (+739), at which point a mouse repetitive sequence is encountered. Synthetic RNA transcripts from the deletion containing clones, unlike clone WZ25, did not induce a response to GnRH when injected into *Xenopus* oocytes. The identification of these transcripts suggests that the mouse GnRH receptor is a complex gene. The mechanism of generating these transcripts, their tissue specificity and their functional significance remain to be determined. (supported by NSF 91-06877).

193.19

CLONING AND CHARACTERIZATION OF ET-3 SPECIFIC RECEPTOR FROM XENOPUS MELANOPHORES. S. Karne*, C. K. Jayawickreme, and M. R. Lerner. Howard Hughes Medical Inst., Depts. of Internal Medicine, Phar. & Neuroscience, Yale Univ. School of Med, New Haven, CT 06510

Endothelins belong to a family of potent vasoactive peptides consisting of three isopeptides, ET-1, ET-2, and ET-3. Vasopressor activity, radioligand binding and cross-linking affinity studies have suggested the existence of multiple, possibly three, receptor subtypes. Recently, two subtypes of endothelin receptors, which belong to the G-protein coupled receptor family, have been cloned: (1) ET_A, which is specific for ET-1 and (2) ET_B, which is non-selective.

We report here the cloning of an endothelin receptor which is specific for ET-3. The existence of such a subtype, termed ET_C, has been demonstrated in cultured bovine endothelial cells and in cultured rat anterior pituitary cells. While studying the endogeneous G-protein coupled receptor pathways in Xenopus dermal melanophores, we observed that these cells contained a receptor highly specific to ET-3. Using the polymerase chain reaction and degenerate oligonucleotides to conserved regions of other G-protein coupled receptors, we isolated and sequenced a unique fragment from the melanophores. This fragment was used to screen and isolate a clone of 2.2 kb in length, which has the characteristic seven transmembrane domains of other G-protein coupled receptors. Sequence comparison of this clone with those in GenBank revealed that this clone is highly homologous to the endothelin receptors only.

193.20

PHARMACOLOGY OF THE CLONED RAT NEUROTENSIN RECEPTOR EXPRESSED IN 293 CELLS. Barbara S. Slusher*, Anna E. Zacco, John A. Maslanski, Tyrrell E. Norris, and Michael W. McLane. Department of Pharmacology, ICI Americas, Wilmington, DE 19897.

A cDNA clone for the rat neurotensin receptor (NTR) was isolated, sequenced, functionally expressed in 293 cells and pharmacological and second messenger studies were conducted. Poly(A)+ RNA from rat ventral tegmental area was reverse transcribed and subjected to nested PCR using specific NTR primers (Tanaka et al., 1990). The resulting 1.3 kb cDNA was isolated, cloned into a phagemid vector and sequenced to ensure that it was devoid of mutations. Subsequently, the NTR cDNA was subcloned into the eukaryotic expression vector pRC-CMV and the resulting plasmid was transiently transfected into 293 cells. The binding of [^{3}H]neurotensin to membranes prepared from the NTR cDNA-transfected cells displayed specificity and saturability, with an apparent Kd of 0.54 nM. We compared several neurotensin analogs for their ability to inhibit 0.5 nM [^{3}H]neurotensin binding. The apparent half-maximal concentrations for the inhibition (IC_{50}) of binding for neurotensin 8-13, [gln4]-neurotensin, neurotensin pseudopeptide H-[Ψ8,9] (Lugrin, 1991), neurotensin, acetylated neurotensin 8-13, xenopsin, neuromedin N, LANT-6, and [D-Tyr]neurotensin was 0.19 nM, 0.30 nM, 0.70 nM, 1.6 nM, 1.7 nM, 3.2 nM, 15.9 nM, 18.5 nM, 9.3 μM, respectively. Neurotensin 1-8, neurotensin 1-11, and [D-Typ11]neurotensin (1 μM) had no effect on receptor binding, consistent with the observation that the C-terminal portion of neurotensin contains the structural requirements for receptor binding. Levocabastine (1 μM) also had no effect, suggesting that the cloned receptor was the high affinity NTR. We also tested the cloned NTR for second messenger associations. Neurotensin (100nM) stimulated an increase in [^{3}H]inositol phosphates in the NTR cDNA-transfected cells to approximately 3-fold over basal; no stimulation was observed in non-transfected cells. Pharmacological characterization of this response is ongoing.

PEPTIDES: RECEPTORS III

194.1

BINDING PROFILE OF CGRP$_1$ SELECTIVE ANTAGONIST, [^{125}I] Tyr^0 hCGRPα_{8-37}, IN RAT BRAIN. D. van Rossum[1*], D. Ménard[1], A. Fournier[2], S. St-Pierre[2] and R. Quirion[1]. (1) Douglas Hospital Research Centre and Department of Pharmacology & Therapeutics, McGill University, Montréal, Québec, Canada (2) INRS-Santé, Pointe-Claire, Québec, Canada.

Based on the differential potencies of the fragment hCGRPα_{8-37} and the analog [Cys(ACM)^{2,7}]hCGRPα in peripheral tissues, we have proposed the existence of two classes of Calcitonin Gene-Related Peptide (CGRP) receptors, the CGRP$_1$ and CGRP$_2$ subtypes (Dennis *et al.*, JPET, 1989,1990). The binding characteristics of [^{125}I] Tyr^0 hCGRPα_{8-37}, a CGRP antagonist with preferential affinity for CGRP$_1$ receptor subtype, were studied in an attempt to visualize the distribution of CGRP$_1$ sites in rat brain, using an autoradiographic method. Specific [^{125}I] Tyr^0 hCGRPα_{8-37} binding (70%) was regionally distributed in rat brain, with the highest level of binding present in the cingulate cortex, nucleus accumbens, amygdaloid body, caudate putamen (tail), habenular nuclei, temporal cortex, pontine nuclei, superior and inferior colliculus, cerebellum (molecular layer), inferior olive and spinal vestibular nuclei. Competition assay in rat brain membrane preparations revealed one high affinity binding component with a K_i of 15 nM and a total receptor population of 1.2 pmol/mg protein. These results demonstrate the potential usefulness of [^{125}I] Tyr^0 hCGRPα_{8-37} as a radioligand to label rat brain CGRP sites. Supported by the MRC of Canada.

194.2

ONTOGENY OF CHOLECYSTOKININ RECEPTORS IN THE BRAZILIAN OPOSSUM BRAIN. M.C. Kuehl-Kovarik*, L.R. Ross, S. Spencer, and C.D. Jacobson. Department of Veterinary Anatomy and Neuroscience Program, Iowa State University, Ames IA 50011

We have studied the distribution of cholecystokinin (CCK) receptors in the brain of the developing marsupial, *Monodelphis domestica*. Receptors were localized in tissue sections using Bolton-Hunter I^{125}-labelled CCK, as we have described previously (Neurosci, Abst. 17:807, 1991). Silver grains were evident in the trigeminal ganglion and nuclei, facial motor nucleus (VII), vagus nerve, and presumptive nucleus of the solitary tract by postnatal day 5 (5PN). By 10PN, the hypothalamus, presumptive interpeduncular nucleus, and specific amygdaloid nuclei labelled as well. As evidenced by specific binding, receptors in the cortex, cerebellum, and cerebellar nuclei were also identified. Receptors in portions of the limbic system, ventromedial hypothalamic nucleus, and spinal trigeminal nucleus were found by 15PN. The forming reticular thalamic nucleus demonstrated dense labelling in the 15PN animal that decreased with age and was absent in the adult. By 25PN, the caudate-putamen and ventral tegmental nucleus contained CCK receptors. In general, labelling in the 35PN animal closely resembled that of the adult. It is interesting that VII contains high levels of I^{125}-labelled CCK (as confirmed by tract tracing studies) and that the density of labelling is even greater in younger (10 and 15PN) animals. This is the first report of CCK binding in the facial motor nucleus. These results indicate that CCK may play a unique role in the nursing neonatal marsupial. Studies are underway to determine if this labelling pattern is unique to this marsupial.

194.3

CHOLECYSTOKININ AFFECTS MULTIPLE CURRENTS IN SYMPATHETIC NEURONS OF CELIAC GANGLION H. Xian, J. S. Coggan, S. R. Knoper and D. L. Kreulen* Dept. of Pharmacol. and Inter. Med., Coll. of Medicine, Univ. of AZ, Tucson, AZ 85724

Cholecystokinin (CCK), a peptide neurotransmitter in mammalian prevertebral sympathetic ganglia, is involved in regulation of gastrointestinal activities. Previous studies have demonstrated that CCK octapeptide (26-33) sulfated (CCK8-S) induces a slow membrane depolarization via activation of CCK-A receptors in guinea-pig celiac ganglion neurons in primary culture. We further studied the mechanism of CCK-induced depolarization in these cells with whole-cell voltage clamp technique. CCK8-S (250 nM - 1 mM) by pulse application (1.5 sec) induced a concentration- and voltage-dependent inward shift in holding current in voltage-clamped neurons (Vh: -30 mV to -90 mV). In some cells, the inward shift was biphasic. The reversal potential of CCK-mediated inward shift in holding current was near E_K (-80 mV). The inward shift in holding current was blocked in the presence of 2 mM barium in the bath, indicating that CCK-induced inward shift in holding current may be mediated by change of potassium conductance, i.e., decreasing an outward potassium current. However, in some cells, CCK8-S increased an inward current at hyperpolarizing potentials (-50 mV to -120 mV). These studies suggest that CCK-induced slow membrane depolarization may involve the regulation of multiple currents. HL-27781, DK-36289 and Lilly Research Labs.

194.4

RELATIONSHIP BETWEEN RADIOLIGAND ANTAGONIST/AGONIST BINDING RATIOS AND FUNCTIONAL EFFICACY OF CHOLECYSTOKININ RECEPTORS IN RAT PANCREAS. **S. Patel, K.L. Chapman, A.J. Smith and S.B. Freedman* Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex, CM20. 2QR, U.K.**

We have examined whether the ability of agonists to distinguish between high and low affinity states of the cholecystokinin (CCK-A) receptor in rat pancreas can be predictive of relative efficacy. Radioligand binding studies were performed using the agonist [^{125}I]Bolton Hunter CCK-8S (50pM) and the antagonist [^{3}H]devazepide (0.2nM) in rat pancreatic membranes. Phosphatidyl-inositol (PI) turnover and amylase secretion was measured in rat pancreatic acini. CCK-8S and caerulein exhibited high antagonist/agonist binding ratios (340 and 160 respectively) and were full agonists in the PI and amylase assays, whereas devazepide and the CCK-B antagonist L-365,260 displayed ratios close to unity (1 and 2.4 respectively) and were without agonist efficacy in either functional assay. The peptoid CCK-B antagonist CI-988 had a higher binding ratio of 15 and was a full agonist in the amylase assay, an effect reversed by devazepide (100nM). Thus, antagonist/agonist binding ratios for compounds acting at CCK-A receptors are predictive of functional activity. Furthermore, it is possible for an agent to be an antagonist at CCK-B receptors yet an agonist at CCK-A receptors.

194.5

CCK_A RECEPTORS MEDIATE PHOSPHATIDYL-INOSITOL TURNOVER IN AR 4-2J CELLS. **K. G. Pratt, D. Bryce*, and S. H. Zorn. Central Research Div., Department of Neuroscience, Pfizer Inc, Groton, CT 06340.**

AR 4-2J rat pancreatoma cells reportedly express both CCK_A and CCK_B receptors. In these cells, the octapeptide CCK-8 and other CCK peptide fragments have been previously shown to induce PI turnover, and it has been suggested from these studies that this response may be mediated through a stimulation of CCK_A receptors. The present study was undertaken to characterize the effect of CCK-8 on PI turnover in AR 4-2J cells by studying the effects of selective CCK_A and CCK_B receptor antagonists on CCK-8 stimulated inositol phosphate (IP) accumulation. AR 4-2J cells, prelabelled with [^{3}H]-*myo*-inositol, were exposed to various concentrations of CCK-8 and 10 mM lithium in the presence and absence of selective CCK_A and CCK_B receptor antagonists. CCK-8 produced a dose-dependent stimulation of IP accumulation (EC_{50} ≈1nM). The response to 10nM CCK-8 was completely blocked by the selective CCK_A receptor antagonist L-364,718 with an IC_{50} < 100 nM. In contrast, the selective CCK_B antagonist L-365,260 was less potent in this regard producing only a 40% reduction of the CCK-8 response at a concentration of 1 μM. These data are consistent with the notion that CCK stimulation of PI turnover in AR 4-2J cells is mediated by a selective interaction with CCK_A and not CCK_B receptors.

194.6

CHARACTERIZATION OF NOVEL CCK ANALOGS USING THE SMALL CELL LUNG CANCER CELL LINE, H345. D.G. Witte, A. M. Nadzan, M. Rodriguez†, J. Martinez†, and C. W. Lin*. Neuroscience Research Division, Abbott Laboratories, Abbott Park, IL 60064. †CCIPE-Faculté de Pharmacie, Montpellier, France.

The interaction of the novel CCK analogs JMV-180, JMV-320, JMV-332 and A-63387 with CCK-B/gastrin receptors on small cell lung cancer (SCLC) cells was investigated. JMV-320 and JMV-332, conformationally restrained cyclic analogs of the C-terminal hepta- and hexapeptides of CCK, respectively, and A-63387, a conformationally restrained analog of the C-terminal tetrapeptide of CCK, are CCK-B/gastrin selective ligands. JMV-180, Boc-Tyr(SO_3-)-Nle-Gly-Trp-Nle-Asp-2-phenylethyl ester, is a CCK-7 analog which is non-selective for CCK-A and B receptors. JMV-180, JMV-320 and JMV-332 potently inhibited specific binding of ^{125}I-CCK-8 to CCKB/gastrin receptors expressed on the SCLC cell line NCI-H345 (H345) with IC50 values of 4.9, 1.8 and 7.0 nM, respectively. JMV-320 and JMV-332 stimulated intracellular calcium ($[Ca^{2+}]_i$) release in a dose-dependent manner in cells pre-loaded with Indo-1. A-63387 also stimulated $[Ca^{2+}]_i$ release in a dose-dependent manner. In contrast, JMV-180 did not stimulate $[Ca^{2+}]_i$ but inhibited the $[Ca^{2+}]_i$ release elicited by 10 nM CCK-8 in a dose-dependent manner. These data indicate that JMV-320, JMV-332 and A-63387 function as CCK-B/gastrin receptor agonists while JMV-180 functions as a CCK-B/gastrin receptor antagonist in H345 cells.

194.7

EXCITATORY EFFECTS OF CCK-8 IN THE RAT NUCLEUS TRACTUS SOLITARIUS (NTS). H. Rhim*, S.R. Glaum & R.J. Miller, Dept. of Pharm. and Phys. Sci., University of Chicago, Chicago, IL. 60637.

Cholecystokinin (CCK) is a major intestinal hormone which plays an important role in satiety and gastric motility. It is also known that this neuropeptide is involved in the central control of anxiety, feeding behavior and nociception. Two major CCK receptor types, CCK_A and CCK_B , have been found in the brain. Both CCK receptors coexist in the rat NTS, which is an important relay center for the integration of peripheral and central neuronal activities related to gastrointestinal, cardiovascular and respiratory functions.

In order to study actions of CCK in the NTS, we conducted experiments using whole cell patch clamp recording in rat brain stem slices. Application of CCK-8(S) (100nM-10μM) produced excitatory effects including depolarization and increased spontaneous excitatory synaptic activity. Under voltage clamp conditions, CCK-8(S) induced an inward current and increased spontaneous synaptic activity in a dose related manner between 10 nM and 10μM. These excitatory effects were partially blocked by the selective CCK_B receptor antagonist, LY-288513. Excitatory postsynaptic currents (EPSC), evoked by the stimulation in the tractus solitarius, were not affected by CCK-8(S) (100nM, n=4). These data suggest that CCK receptor activation may be involved in regulating gastrointestinal and other reflex systems in the NTS.

194.8

PURIFICATION AND CHARACTERIZATION OF A MEMBRANE-BOUND CHOLECYSTOKININ-BINDING PROTEIN FROM HUMAN BRAIN. R.Lu*, M-C Liu, Y.Gu, Q.Shen and Y.Yu. Department of Neurobiology, Shanghai Medical University, Shanghai 200032, P.R. China

A membrane-bound cholecystokinin octapeptide(CCK-8)-binding protein was purified from human brain cortex using column affinity chromatography on immobilized sulfated tyrosine and CCK-8. The purified protein was electrophoresis homogenity with an apparent molecular weight of 35,000 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions. Using immobilized CCK-8 fragment as ligands, the 35-KDa protein was found to bind CCK-8 (sulfated and nonsulfated form) and Asn-Tyr(SO_3)-Met, but not to bind Asn-Tyr-Met-Gly and Met-Gly-Trp-Met-Asp-Phe, suggested that either sulfated tyrosine or enough length of the peptides was necessary for the binding. The binding between CCK-8 and CCK-binding protein was PH-dependent, being strong from PH 5.5 to 7.5, beyond this range becoming weaker. Divalent cations Ca^{2+} ,Mg^{2+} and Mn^{2+} with 5 mM of concentration added in the assay mixture significantly promoted the binding. The seventeen amino acid sequence of N-terminal of the CCK-binding protein was almost same as mitochondrial malate hydrogenase of pocine heart.

194.9

AUTORADIOGRAPHIC ANALYSIS OF GASTRIC INHIBITORY POLYPEPTIDE BINDING SITES IN THE RAT BRAIN, Andrew M. Kaplan and Steven R. Vigna*, Depts. of Cell Biology and Medicine, Duke University Medical Center, Durham, NC 27710.

Synthetic porcine gastric inhibitory polypeptide (GIP) was iodinated and purified by HPLC and used to localize GIP binding sites in frozen sections of rat brain. Saturable binding was present in several brain regions including cortex, anterior olfactory nucleus, lateral septal nucleus, subiculum, inferior colliculus, and inferior olive. Saturable ^{125}I-GIP binding was time-dependent, reversible, high affinity, and specific for GIP. Peptides with amino acid sequences similar to GIP such as secretin, vasoactive intestinal polypeptide, glucagon, and peptide histidine isoleucine did not inhibit saturable ^{125}I-GIP binding. Saturable ^{125}I-GIP binding was not observed in other rat organs surveyed such as spinal cord, pituitary, stomach, small intestine, colon, pancreas, liver, heart, and skeletal muscle. We conclude that a saturable ^{125}I-GIP binding site with the pharmacological properties of a GIP receptor is expressed in certain regions of the rat brain.

194.10

STRUCTURAL CHARACTERIZATION OF VARIOUS FRAGMENTS AND ANALOGS OF CALCITONIN GENE-RELATED PEPTIDE. A. Fournier*[1], M. Mimeault[1], S. St-Pierre[1], Y. Dumont[2] and R. Quirion[2]. [1]INRS-Santé, Université du Québec, Pointe-Claire (QC), Canada and [2]Douglas Hospital Research Centre, McGill University, Verdun (QC), Canada.

Biological studies using the fragment hCGRP$_{8\text{-}37}$ and the linear analog [Cys-(Acm)^{2,7}]hCGRPα suggested a heterogeneity of CGRP receptors. Moreover, conformational analysis of hCGRPα and its related fragments and analogs, proposed the presence of a helical stretch in the molecule, involving amino acid residues 8 to 18. Therefore, we evaluated the biological effects of hCGRPα and homolog peptides in relation to their structural characteristics. Thus, hCGRPα exhibited potent agonistic properties in the guinea pig atrium and rat *vas deferens* bioassays. However, the opening of its disulfide bridge gave an analog ([Cys(Acm)^{2,7}]hCGRPα) almost inactive in the guinea pig atria but still showing substantial agonistic effects in the rat *vas deferens*. On the other hand, hCGRP$_{8\text{-}37}$ displayed potent antagonistic properties in the guinea pig atrial preparation while it was much less potent in inhibiting the rat *vas deferens* twitch response. Structural analyses showed that the removal of valine-8 in the fragment hCGRP$_{8\text{-}37}$ did not alter the α-helix stability nor the antagonistic properties of the fragment. However, subsequent deletion of N-terminal amino acids resulted in an important decrease of antagonistic potency as well as in a substantial reduction of the α-helix content. Thus, it suggests that the α-helix content might play a predominant role in the interaction between the so-called CGRP$_1$ receptor and its ligand. On the other hand, helical stability does not appear as a highly critical parameter for the CGRP$_2$ receptor. Indeed, although [Cys-(Acm)^{2,7}]hCGRPα exhibited 77% of the helical content of hCGRPα, it displayed a relative potency of only 2% in the *vas deferens* preparation. Supported by the MRCC.

194.11

DISTRIBUTION OF ^{125}I-GALANIN BINDING SITES IN THE ADULT AND DEVELOPING BRAZILIAN OPOSSUM BRAIN. J. K. Elmquist*, A. Kao, M. C. Kuehl-Kovarik, and C.D. Jacobson. Department of Veterinary Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011.

We have previously described the distribution of galanin (GAL)-like immunoreactivity in the adult and developing Brazilian opossum brain. In this study immunoreactivity was shown to be present in several areas of the brain as early as postnatal day 1 (1PN), when neurogenesis is actively taking place in *Monodelphis domestica*. To understand the functionality of these results, the elucidation of GAL binding sites in the brain of adult and developing brains was accomplished using ^{125}I labelled GAL as described previously for cholecystokinin (Neurosci. Abst. 17:807). The distribution of GAL receptors in the adult brains was very similar to that which has been reported in other species including the rat. In the 1 PN brains, light binding was detected in the forming brainstem in the presumptive solitary and spinal trigeminal nuclei. Outside the brain moderate binding was seen in the vagus nerve. By 5 PN, light to moderate binding was detected in the forming ventral hypothalamus and amygdala. Moderate to dense binding was seen in the forming midbrain and brainstem at this age. By 11 PN, dense binding was observed in the basal hypothalamus, septum, amygdala, tectum, and medulla. The pattern seen at 25 and 60 PN was comparable to that seen in the adult. In summary, we have found that GAL receptors as evidenced by receptor autoradiography, are detectable as early as 1-5 PN. Coupled with the presence of immunoreactivity at this stage of development, our results indicate that GAL may be playing a role in development and differentiation of the opossum.

194.12

SR 48692: A POTENT NON-PEPTIDE ANTAGONIST OF NEUROTENSIN RECEPTORS IN MURINE BRAIN. P. Kitabgi*[1], C. Labbé-Jullié[1], J. Mazella[1], D. Gully[2], M. Poncelet[2], P. Soubrié[2], G. Le Fur[2], A. Brouard[3], D. Pelaprat[3] and W. Rostène[3]. [1]IPMC-CNRS, 06560 Valbonne, France; [2]SANOFI RECHERCHE, 31036 Toulouse, France; [3]INSERM U339, Hôpital St-Antoine, 75012 Paris, France.

The CNS actions of the tridecapeptide neurotensin (NT) have been extensively studied in the rat and mouse. However, the lack of NT antagonist has hampered the elucidation of its central physiological roles. Here we demonstrate that SR 48692 is a potent and selective non-peptide NT antagonist in murine brain. SR 48692 inhibited the specific binding of ^{125}I-NT to adult rat and mouse brain homogenates with IC$_{50}$ values of 35-80 nM. In the presence of 10 μM levocabastine (LC) which selectively and totally blocks NT binding to its low affinity binding sites in adult rat and mouse brain, IC$_{50}$'s were decreased to about 5 nM. In cultured rat mesencephalic neurons, new-born mouse brain homogenates, and membranes from COS 7 cells transfected with the cloned high affinity rat brain NT receptor, systems all devoid of LC-sensitive NT binding sites, IC$_{50}$'s for SR 48692 binding ranged from 4 to 13 nM. Altogether, these data show that in murine brain SR 48692 binds preferentially and with high potency to the functional, high affinity, LC-insensitive NT receptor. Unilateral intrastriatal injections of 10 pg NT in mice induced contralateral rotations which were dose-dependently inhibited by prior p.o. or i.p. administration of SR 48692. Time-course studies with a maximally effective dose of 80 μg/kg (p.o.) showed that SR 48692 antagonism was significant as soon as 30 min post-injection, maximal (85%) 1 to 2 h post-injection and still significant after 8 h. These data demonstrate that SR 48692 has a good oral bioavailability, crosses the blood brain barrier and exerts long-lasting NT antagonism in vivo.

194.13

A POTENT NON-PEPTIDE ANTAGONIST OF NEUROTENSIN RECEPTORS: EVIDENCE IN THE GUINEA-PIG BRAIN. **D.Gully[1], M.Heaulme[1], P.Kitabgi[2], P.Soubrié[1], W.Rostène[3] and G.Le Fur*[1] [1]SANOFI RECHERCHE 31036 Toulouse, and 34082 Montpellier, France; [2] IPMC-CNRS 06560 Valbonne, France; [3] INSERM U.339 Hôpital St Antoine 75012 Paris, France.**

Neurotensin (NT) has been shown to possess several neuromodulatory roles on brain functions, mediated by an interaction of NT with specific membrane receptors. However, the lack of specific NT receptor antagonist has prevented the study of the specificity of these physiological actions. The present data describe the properties of one such potent and selective non-peptide antagonist in the guinea-pig brain. SR 48692 competitively inhibited 125INT specific binding to homogenates from adult guinea-pig brain with an IC50 of 1nM. Autoradiographic data obtained on sections at both striatal and midbrain levels confirmed that increasing concentrations of SR 48692 produced a dose-dependent decrease of 125INT labeling. 1μM SR 48692 totally inhibited 125INT binding in both ventral tegmentum and substantia nigra and was found to be more potent than unlabeled NT itself. Stimulation of 3H-dopamine release from striatal sections induced by 10 nM NT was dose-dependently counteracted by SR 48692 with an efficacy (IC50 0.5nM) quite similar to its binding affinity. In contrast, up to 100 nM, SR 48692 did not affect the spontaneous and K+-evoked release of 3H-dopamine indicating a lack of agonistic effect of the compound. SR 48692 is the first non-peptide antagonist of NT receptors described so far, which offers the possibility to study the physiological roles of NT.

194.14

THE NONPEPTIDE NK-1 ANTAGONIST CP-96,345 MODULATES [3H]NITRENDIPINE BINDING TO L-TYPE CALCIUM CHANNELS IN RAT CARDIAC AND CORTICAL MEMBRANES. A.W. Schmidt*, S. McLean and J. Heym. Central Research Division, Pfizer Inc., Groton, CT. 06340-1596.

Recently, we reported that the nonpeptide NK-1 antagonist CP-96,345 and its 2R,3R enantiomer CP-96,344 interact with L-type calcium channels (Eur. J. Pharmacol., in press). CP-96,345 and CP-96,344 inhibit [3H]desmethoxyverapamil binding to rat cardiac membranes with Ki values of 25 ± 2 and 28 ± 4, respectively. In addition, these compounds produced a dose-dependent enhancement of [3H]nitrendipine ([3H]NITR) binding in rat cortex with a 3-fold increase in specifically bound [3H]NITR obtained at a concentration of 10 μM CP-96,345 or CP-96,344. The benzothiazepine calcium channel antagonist diltiazem (DLT) is reported to enhance [3H]NITR binding by allosteric modulation of the dihydropyridine (DHP) binding site (Janis et al., 1987). Both CP-96,345 and CP-96,344 inhibit [3H]DLT binding to rat cardiac membranes with Ki values of 43 ± 10 and 31 ± 10, respectively. To further investigate the interaction of CP-96,345 and CP-96,344 with the DHP-sensitive calcium channel site, saturation studies were carried out in rat cardiac and cortical membranes to determine if the Kd or Bmax of [3H]NITR changed in the presence of 10 μM CP-96,345, 10 μM CP-96,344 or 10 μM DLT. In rat cortical membranes, the Kd for [3H]NITR increased 4-fold in the presence of 10 μM CP-96,345 or CP-96,344. In the presence of 10 μM DLT, the Kd for [3H]NITR increased 2-fold. No change in Bmax was observed in experiments with either CP compound or DLT. Similar results were obtained using rat cardiac tissue. These results are in agreement with previously reported data for DLT.

194.15

USE OF SELECTIVE ANTAGONISTS TO DISSOCIATE THE CENTRAL CARDIOVASCULAR AND BEHAVIORAL EFFECTS OF NEUROKININS ON NK_2 and NK_3 RECEPTORS IN THE RAT. P. Picard[1], D. Regoli[2] and R. Couture*[1]. Dept. Physiology, Faculty of Medicine, Université de Montréal, Montréal, Qué., Canada H3C 3J7 and [2]Dept. Pharmacology, Sherbrooke University, Sherbrooke, Qué., Canada, JIH 5N4.

The effects of intracerebroventricular (i.c.v.) injection of highly selective and potent NK_2 (SR-48968) and NK_3 (R-487, [Phe^7,β-Ala^8]NKA(4-10)) receptor antagonists were assessed on the cardiovascular and behavioral responses elicited by the i.c.v. injection of substance P (SP), neurokinin A (NKA) and [MePhe^7]-neurokinin B (NKB) in the conscious freely moving rat. SP, NKA and [MePhe^7]-NKB (25 pmol) induced mean arterial pressure and heart rate increases of the same magnitude that reached a maximum at 2-3 min post-injection. The cardiovascular responses lasted for about 30 min and were associated with typical behaviors (face washing, grooming, digging, sniffing, rearing and wet dog shakes). The cardiovascular and behavioral responses (except for wet dog shakes) elicited by NKA were significantly and dose-dependently reduced by 0.65 - 6.5 nmol SR-48968. However, the latter antagonist had no effect on the SP or [MePhe^7]-NKB mediated responses. R-487 (0.65 pmol) inhibited the cardiovascular response and wet dog shakes produced by [MePhe^7]-NKB only. SR-48968 and R-487 were devoid of cardiovascular and behavioral activity and blocked in a reversible manner. These results suggest that the central effects of NKA and [MePhe^7]-NKB are mediated by specific receptors, different from those activated by SP, and which appear to belong to NK_2 and NK_3 receptor subtypes, respectively. [Supported by the MRC of Canada].

194.16

NK-3 TACHYKININ RECEPTORS LOCALIZED ON MIDBRAIN DOPAMINE (DA) NEURONS. **A. Jon Stoessl*, Clinical Neurological Sciences, Univ. of Western Ontario, London, Ontario, Canada N6A 5A5.**

Although there is a prominent SP-containing projection from the striatum to the substantia nigra (SN), the SN is devoid of SP receptors. Studies using non-selective tachykinin ligands have suggested the presence of NK-3 binding sites in this region. We have demonstrated preferential stimulation of DA-mediated behaviours following stereotactic infusion of the NK-3 agonist senktide into the SN or ventral tegmental area (VTA). In the current study, we have sought more direct evidence for DA neuronal localization of NK-3 receptors. Unilateral destruction of ascending DA pathways was accomplished by stereotactic infusion of 6-OHDA (8 ug/2 ul) into the medial forebrain bundle of male Sprague-Dawley rats (n = 4). 2 weeks after surgery, the animals were sacrificed. 10 um coronal cryostat sections through the striatum and SN were incubated with [^{3}H]senktide to delineate NK-3 sites. In order to verify the efficacy of the lesion, adjacent sections were incubated with [^{3}H]mazindol and the sections were opposed to ^{3}H-sensitive film. Following development, the film was analyzed using quantitative densitometry and side-to-side comparisons were made using paired t-tests. 6-OHDA lesions resulted in profound loss of [^{3}H]mazindol binding in both midbrain and striatum (STR). There was a 42% decrease of [^{3}H]senktide binding in the lesioned SN ($p<0.03$), but only a 12% decrease in the VTA ($p=n.s.$). There were non-significant decreases of [^{3}H]senktide binding ipsilateral to the lesion in the dorsomedial (20%), ventromedial (15%) and ventrolateral STR (20%), but a more substantial decrease (40%, $p<0.05$) in the dorsolateral STR. These findings suggest that NK-3 receptors are located on DA neurons of the SN, and possibly on their terminals in the STR. Binding sites in the VTA may be on non-DA neurons.

194.17

TACHYKININ INDUCED PHOSPHATIDYLINOSITOL HYDROLYSIS IN TRANSFECTED CHINESE HAMSTER OVARY CELLS R. Raddatz*, P. Blount, R.M. Snider, J.E. Krause. Dept. Anatomy and Neurobiology Washington University School of Medicine, St.Louis, MO 63110 and Central Research Division, Pfizer Inc Groton, CN 06340

We are interested in the functions of Substance P (SP) in target tissues and in the cellular mechanisms by which SP mediates its diverse biological roles. Chinese hamster ovary (CHO) cells transfected with the cDNA for either rat NK-1 or rat NK-2 receptors expressing approximately 250,000 high affinity binding sites for ^{125}I-Y^1-SP per cell or 280,000 high affinity binding sites for ^{125}I-NPγ per cell, respectively, have been established as model cell systems to examine the kinetics of ligand binding and tachykinin-induced second messenger responses (Takeda et al., 1992). In this study we further characterize tachykinin-induced second messenger responses. In NK-1 receptor transfected CHO cells SP induces a rapid and transient rise in IP_3 concentration as measured by a mass assay. However, flux through the inositol phosphate pathway continues for at least 30 minutes, and then plateaus, as evidenced by the accumulation of ^{3}H-inositol phosphates (IPs) in cells prelabelled with ^{3}H-myo-inositol. The addition of the SP antagonist CP-96,345 at 15 minutes after SP stimulation results in a rapid cessation of further ^{3}H-IPs accumulation indicating that the receptor is repeatedly activated during this time. In cell lines expressing various densities of NK-1 receptor the amount of ^{3}H-IPs produced is correlated with receptor number. In the CHO cells expressing NK-1 receptors NKA is a full agonist with similar kinetics of response as compared to SP induced ^{3}H-IPs. NKA also elicits a similar ^{3}H-IPs response from CHO cells expressing the NK-2 receptor type. A cell line expressing both NK-1 and NK-2 receptors at similar numbers of high affinity sites has been used to characterize responses in cross-stimulation experiments. These studies indicate that following maximal stimulation with SP, NKA can cause additional IPs accumulation while readdition of SP elicits no further response. We conclude that these transfected cell lines represent useful model systems to examine tachykinin receptor functions.

194.18

THE EXTRACELLULAR DOMAIN OF THE NEUROKININ-1 RECEPTOR IS REQUIRED FOR HIGH AFFINITY BINDING OF PEPTIDES. Tung Ming Fong*, Hong Yu, Ruey-Ruey C. Huang & Catherine D. Strader. Dept. of Mol. Pharmacol., Merck Research Laboratories, PO Box 2000, Rahway, NJ, 07065

The neurokinin-1 receptor binds neurokinin peptides with the potency order of substance P > substance K > neurokinin B. Elucidating the molecular basis of differential peptide selectivity will require the localization of the binding domain on the receptor. In the present report, mutagenesis and heterologous expression experiments reveal that a segment of the extracellular N-terminal sequence of the neurokinin-1 receptor is required for the high affinity binding of substance P and related peptide agonists. Substitution of amino acid sequence at the N-terminal region affects the binding affinity of both intact peptides and a C-terminal substance P fragment. Glycosylation of the receptor does not change the peptide binding affinity. In addition, substitution of the valine-97 residue in the rat neurokinin-1 receptor by a glutamate residue increases the binding affinity of neurokinin B but not substance P or substance K, suggesting that the second extracellular segment is involved in peptide selectivity. These results indicate that the extracellular domain of neurokinin-1 receptor plays a critical role in peptide binding.

194.19

DISTRIBUTION OF TRH-RECEPTOR mRNA IN THE RAT BRAIN: AN *IN SITU* HYBRIDIZATION STUDY

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Using *in situ* hybridization histochemistry we have investigated the distribution of thyrotropin releasing hormone receptor (TRH-r) mRNA in the central nervous sytem of adult male rat. Coronal sections were hybridized using synthetic oligonucleotide probes complementary to mRNA for TRH-r. Among the investigated areas of the forebrain, a strong TRH-r mRNA labeling was seen in the medial portion of the nucleus accumbens, septal nuclei, bed nucleus of the stria terminalis, nucleus of the horizontal limb of the diagonal band, primary olfactory cortex, paraventricular thalamic nucleus, medial anterior cortical, central nuclei of the amygdala and ventral hippocampus. A moderate TRH-r mRNA labeling was seen in the medial preoptic areas and in the hypothalamus. In the medulla oblongata, a dense TRH-r mRNA labeling was found in the hypoglossal and in the ambiguus nuclei. A moderate labeling was seen in the vagal complex, particularly around the area postrema. The results show that the TRH-r, just as the TRH peptide, has a wide distribution in the central nervous system.

194.20

QUANITATION OF NEUROTENSIN RECEPTOR mRNA USING POLYMERASE CHAIN REACTION. B. Myers*, A. Levey and C.B. Nemeroff, Depts. Pharmacology, Neurology and Psychiatry, Emory Univ. Sch. Med., Atlanta, GA 30322.

Neurotensin (NT) is an endogenous neuropeptide hypothesized to be involved in the pathophysiology of schizophrenia and the mechanism of action of antipsychotic drugs. NT concentrations are specifically increased in the nucleus accumbens and caudate following antipsychotic drug treatment. Recently, a neurotensin receptor cDNA (NT-R) was cloned from rat brain by Nakanishi et al (1991). In order to study the regulation of NT-R gene expression, we have developed a method to quanitate NT-R mRNA with the polymerase chain reaction (PCR). Using as little as 100 pg of NT-R cDNA inserted in the plasmid pBluescript II KS+, we were able to amplify, using PCR, segments of the NT-R cDNA encoding the putative C-terminus (141 base pairs), the third cytoplasmic loop (132 base pairs) and a 500 base pair segment encoding the third cytoplasmic loop to the C terminus. Amplification of 100pg-40ng of NT-R cDNA demonstrated a proportional increase in the amount of ethidium bromide staining after electrophoresis on agarose gels. These same NT-R segments were amplified from total rat brain mRNA and poly-A mRNA by reverse PCR. This assay will allow us to quantitate changes in NT-R mRNA levels following treatment with antipsychotic drugs, and potentially, in humans with neuropsychiatric disease. (NIMH MH-39415)

195.1

ISOLATION OF A MEMBRANE PROTEIN FROM RAT LIVER WITH PROPERTIES OF THE PUTATIVE SIGMA RECEPTOR/BINDING SITE. G.K. Ehrlich[1]*, G. Singh[1], G. Lem[1], D.I. Schuster[1], H. Tiedge[2], J. Brosius[2], and R.B. Murphy[1]. [1]Department of Chemistry and Center for Neural Science, New York University, N.Y., NY 10003 and [2]Fishberg Research Center for Neurobiology, Mt. Sinai School of Medicine, Gustave Levy Place, N.Y., NY 10029.

The sigma receptor/binding site, a putative receptor protein which is found in brain and liver, has been purified from a detergent-solubilized rat liver membrane preparation by affinity chromatography. An affinity column was constructed using an oximino derivative (OB 101) of the neuroleptic haloperidol, which is known to have high affinity for the sigma site. The ligand was linked through a spacer arm to agarose. The affinity column selectively retained a single 28 kDa polypeptide, which could be eluted from the column with 1 μM haloperidol. A control affinity column, was prepared from an oximino derivative of a ketone which did not associate with the sigma receptor/binding site. The control column did not selectively occlude this 28 kDa component. The affinity purification, followed by transfer to wheat germ agglutinin-agarose and elution with N-acetyl-D-glucosamine, resulted in ca. 3,000-fold purification of the 28 kDa component. The enantiomeric benzomorphans, levallorphan (50 nM) and dextrallorphan (50 nM), were used to elute the affinity column; only dextrallorphan, which potently associates with the sigma receptor/binding site, removed the 28 kDa component, while levallorphan did not. The purified 28 kDa component was homogeneous on SDS-PAGE and is, as isolated, N-terminal blocked. The observed molecular mass is in accord with that of the sigma receptor/binding site observed by others using photoaffinity labeling. (Supported by NIDA DA 05728 (RBM) and New York University.)

195.2

LACK OF CHANGES IN *SIGMA* RECEPTORS IN THE BRAINS OF GENETICALLY DYSTONIC RATS. A. D. Weissman*[1] D. J. McCann[1] J. F. Lorden[2] and T.-P. Su[1]. [1]Neuroscience Branch, NIDA Addiction Research Center, P. O. Box 5180, Baltimore, MD 21224; [2]Dept. Psychol., Univ. Alabama, Birmingham, AL 35294.

Sigma receptors have been proposed to play a role in either the etiology or expression of dystonic symptoms in rats and this effect may be mediated at specific brain loci (Walker *et al.*, Neurology 38:961). Consonant with this hypothesis is a report of alterations in *sigma* receptors ($\uparrow B_{max}$ and $\uparrow K_d$ for [^{3}H]DTG) in brains of genetically dystonic rats (Bowen *et al.*, Eur. J. Pharmacol., 147:153). [^{3}H]DTG is now known to label two distinct binding sites in rat brain, termed "*sigma*-1" and "*sigma*-2." The present study was initiated to evaluate the relative contribution of these two sites to the reported differences in brains of dystonic rats. *Sigma*-1 sites were defined by 5 nM (+)[^{3}H]SKF-10,047 in the presence of 300 nM MK-801 (included to block binding to PCP receptors). *Sigma*-2 sites were labeled with 5 nM [^{3}H]DTG in the presence of 1 μM (+)SKF-10,047 (included to block binding to *sigma*-1 sites). Preliminary results did not reveal compelling differences between dystonic and normal brains under either of our selective assay conditions.

In an attempt to replicate the earlier report of alterations of *sigma* binding in this strain of dystonic rats, we performed [^{3}H]DTG saturation experiments. The results failed to confirm a difference between normal and dystonic littermates. These data question the relative importance of *sigma* sites in this animal model of dystonia.

195.3

Sigma Binding in Brain and Liver from Rats Deficient In Cytochrome P450-2D1. A.L. Jewell*, P.J. Wedlund and L.P. Dwoskin. College of Pharmacy, University of Kentucky, Lexington, KY 40536-0082.

Substrates for cytochrome P450-2D1 exhibit a high affinity for sigma binding sites suggesting that sigma sites may be associated with the cytochrome P450-2D1 isozyme. In contrast to male and female Sprague Dawley (SD) rats, both male and female Dark Agouti (DA) rats do not express the P450-2D1 gene product. In the present study, binding of the selective sigma ligand (+)[^{3}H]3-PPP, in whole brain and liver homogenates from female DA and control female SD rats was examined. If a subpopulation of sigma sites are associated with the P450-2D1 enzyme, then the number (B_{max}) of sigma binding sites is predicted to decrease in female DA rat brain and liver compared to control female SD rat tissues. The present study demonstrates a significant decrease (63 and 74% of control female SD values) in B_{max} with no alteration in affinity (K_D) of (+)[^{3}H]3-PPP binding in female DA rat brain and liver, respectively. These results suggest that a portion of sigma sites in brain and liver may be associated with cytochrome P450-2D1. (Supported by University of Kentucky Medical Center Small Research Grant.)

195.4

CHARACTERIZATION OF SIGMA SUBTYPE BINDING IN GUINEA PIG BRAIN MEMBRANES. J.B. Fischer*, K.J. Burke Howie, J.A. Dunn, S.Y. Ablordeppey[1] and R.A. Glennon[1]. Cambridge NeuroScience, Cambridge, MA 02139 and [1]Dept. Med. Chem., Med. Coll. Virginia, VCU, Richmond VA 23298.

The literature contains a number of suggestions of subtypes of the Sigma receptor. Hellewell and Bowen (Brain Res. 527:244, 1990) proposed characteristics for two putative subtypes, named $Sigma_1$ and $Sigma_2$. The primary distinction between these two sites is the affinity of the (+) isomers of the benzomorphan opiates for the binding sites. We have developed separate assays for these two sites using [^{3}H](+)pentazocine (courtesy of Dr. S. Hurt, DuPont/NEN) for the $Sigma_1$ site, and [^{3}H]DTG (which labels both sites with about equal affinity) in the presence of unlabeled (+)pentazocine for the $Sigma_2$ site. (+)Pentazocine showed the highest $Sigma_1$ selectivity (500-fold). Haloperidol and (+)3-PPP were marginally $Sigma_1$ selective (10-20 fold). No compounds were found to have high $Sigma_2$ selectivity; however several compounds had slightly higher affinity for the $Sigma_2$ site (BMY 14802, rimcazole, ifenprodil). In a series of related, novel Sigma ligands a broad range of selectivities was also found, allowing preliminary structure-activity analysis for the two sites. The development and use of selective ligands for these Sigma receptor subtypes should allow a better understanding of the functional roles of these receptors.

195.5

IDENTIFICATION AND CHARACTERIZATION OF SIGMA RECOGNITION SITES ON ESTABLISHED TUMORS AND TUMOR CELL LINES. J. E. Taylor* and S. R. Keyes, Biomeasure Inc., 9-15 Ave E., Hopkinton, MA 01748

Although sigma recognition sites are widely expressed in neural, endocrine, and immune tissues, the functional significance, except for a possible psychotropic action, and the cellular transduction mechanisms associated with these binding sites are unclear. Recently, Bem *et al.* (Cancer Res. 51:6558,1991) observed that fresh surgical specimens of various human tumors were enriched in sigma binding sites compared to control nonmalignant tissue, which indicates a possible role in neoplastic cell proliferation and tumor growth. While examining the therapeutic potential of various neuropeptides, opioids, and opioid-related compounds as anti-tumor agents, we also observed that established *in vivo* tumors and clonal tumor cell lines were highly enriched in sigma recognition sites. For the five tumors or tumor cell lines examined thus far for [^{3}H]DTG binding, the following receptor concentrations (fmol/mg protein) were observed:

C6 rat glioma	9483 ± 426
A549 human non-small cell lung	6556 ± 2811
NCI-H69 human small cell lung	4879 ± 103
B16 mouse melanoma	1816 ± 154
AR42J rat pancreas	178 ± 13

The rank order of potency for the ability of various sigma ligands to compete with [^{3}H]DTG binding was similar for each tumor: haloperidol > DTG > BMY 14802 > 3-PPP > PCP. However, the absolute potencies for some of the ligands were less than that observed in membranes prepared from guinea-pig brain. These results may indicate the possibility of receptor heterogeneity. Preliminary evidence obtained from growth experiments also showed that haloperidol and DTG inhibited the proliferation of B16 cells *in vitro*.

195.6

ZINC IS A POTENTIAL ENDOGENOUS LIGAND FOR ONE FORM OF THE HALOPERIDOL-SENSITIVE SIGMA RECEPTOR. Mark Connor* Terrell Patterson and Charles Chavkin, Dept of Pharmacology, Univ of Washington, Seattle WA 98195.

We have identified two components in brain extracts able to displace radioligand binding to the haloperidol-sensitive σ receptors. Ionic zinc displaces the non-selective σ ligand 1,3-di(2-[5-^{3}H]tolyl)guanidine ([^{3}H]-DTG) from two binding sites in rat brain membranes. The Ki of Zn^{2+} for the cation sensitive (σ_2) site was 48 ± 14 μM, and for the cation-insensitive (σ_1) site, the Ki was 2.4 ± 0.5 mM. Ionic zinc is present in synaptic vesicles in the brain and may function to regulate binding at the σ_2 site. To test this hypothesis, we measured the effects of metallothionein peptide 1, a specific zinc chelator, on the radioligand displacement caused by the stimulated release of putative endogenous σ ligands in the rat hippocampus. High frequency, focal electrical stimulation of the hilar region of the hippocampus caused a decrease in the specific binding of [^{3}H]-DTG and [^{3}H]-(+)-pentazocine to σ sites. The decrease in [^{3}H]-DTG binding was largely blocked by metallothionein, whereas the decrease in [^{3}H-(+)-pentazocine binding was unaffected. These results suggest that Zn^{2+} may act as an endogenous ligand at σ_2 sites in the rat hippocampal slice, and suggest that there are other endogenous ligands for σ sites that can be released by focal electrical stimulation. In addition, we have isolated a trypsin-sensitive substance from rat brain that displaces both [^{3}H]-DTG and [^{3}H]-(+)pentazocine from haloperidol-sensitive σ binding sites. The substance was recovered from acid extracts of brain by ion exchange chromatography and further purified by C18 HPLC. The functional significance of these two endogenous factors remains to be established. Supported by MH46501.

195.7

MULTIPLE BINDING SITES FOR *SIGMA* LIGANDS IN RAT SPLEEN. B.B. Whitlock and S.A. Wolfe, Jr.* Department of Medical Microbiology and Immunol., Ohio State Univ., Columbus, OH 43210.

In previous studies we detected high concentrations of σ receptors in rat spleen, and found that σ agonists suppressed *in vitro* immune activities such as mitogen- and antigen-driven lymphocyte proliferation. However, the rank order of drug immunosuppressive potency did not correlate well with the pharmacology of the σ receptor. Therefore, we hypothesized that additional binding sites for σ agonists may be active in immune cells. In the present investigation we have examined rat spleen for such additional binding sites. In competition studies, a major fraction of *d*-[^{3}H]pentazocine and *d*-3-(3-hydroxyphenyl)N-(1-propyl-2,3-[^{3}H])piperidine (*d*-[^{3}H]-3-PPP) binding was not sensitive to 10^{-4} M haloperidol, but was blocked by 10^{-5} M *d*-N-allylnormetazocine (*d*-NANM, or $SKF_{10,047}$). In contrast, specific binding of the σ ligand [^{3}H]-1,3-di-(2-tolyl)guanidine ([^{3}H]DTG) was completely blocked by either 10^{-5} M haloperidol or 10^{-4} M *d*-NANM. DTG competed for [^{3}H]haloperidol-labeled σ receptors, but [^{3}H]DTG also labeled additional sites in rat spleen that had lower affinity for *d*-benzomorphans than do σ receptors. From these observations, it appears there are at least three binding sites for σ ligands in rat spleen: 1) the well-described σ receptor, 2) a haloperidol-sensitive site that binds *d*-pentazocine, *d*-NANM and DTG, and 3) a relatively haloperidol-insensitive site that binds *d*-3-PPP and *d*-NANM. The non-σ binding sites were more prevalent in rat spleen than in cerebellum. The role of these additional binding sites in immune modulation by σ agonists is yet to be determined.

195.8

SIGMA-1 AND SIGMA-2 BINDING SITES OF RAT KIDNEY. W.D. Bowen*, G. Feinstein, and J.S. Orringer. Unit on Receptor Biochemistry and Pharmacology, Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892.

There is now evidence from several laboratories for the existence of two subtypes of sigma sites, termed sigma-1 and sigma-2 (*Trends Pharmacol. Sci.* 13: 85-86, 1992). We have previously shown using photoaffinity labeling and ligand binding that rat liver contains both of these sites (*Soc. Neurosci. Abstr.* 16: 370, 1990). Here, we characterize sigma binding sites in rat kidney. [^{3}H]DTG (which labels sigma-1 + sigma-2 sites) labeled sites with Kd = 45.8 nM and Bmax = 1,190 fmol/mg protein. [^{3}H](+)-Pentazocine (selective for sigma-1) labeled sites with Kd = 23.3 and Bmax = 229. Both ligands gave linear Scatchard plots. Competition studies revealed that sites labeled by [^{3}H]DTG and [^{3}H](+)-pentazocine had profiles which differed mainly in affinity for (+)-benzomorphans. For [^{3}H](+)-pentazocine-labeled sites: haloperidol > (+)-pentazocine > (-)-pentazocine > (+)-3-PPP > (+)-SKF 10,047 > (-)-SKF 10,047. When [^{3}H]DTG binding was carried out in the presence of 1 uM dextrallorphan (a condition which masks labeling of sigma-1 receptors): haloperidol = (-)-pentazocine > (+)-3-PPP > (+)-pentazocine = (-)-SKF 10,047 >> (+)-SKF 10,047. All curves were monophasic. The ratio of Bmax values for [^{3}H](+)-pentazocine and [^{3}H]DTG would suggest that rat kidney contains only 20% sigma-1 sites and 80% sigma-2 sites. Thus, without masking, [^{3}H]DTG gave an overall sigma-2 profile and (+)-benzomorphans exhibited biphasic curves. The ratio of sigma-1 to sigma-2 in rat kidney is similar to the ratio in rat liver, which is 25% sigma-1 and 75% sigma-2. However, the density of each site in kidney is 10-fold lower compared to liver, and is more comparable to the densities found in rat brain. Also, the relative density of sigma-2 in rat brain is lower than in liver and kidney. The wide distribution of sigma-1 and sigma-2 sites in brain, liver, kidney, and other peripheral tissues suggests important cellular functions for these sites.

195.9

MULTIPLE SIGMA BINDING SITES IN GUINEA-PIG BRAINS. C.D. Nicholson, J.H. Connick*, L. France, G. Hanlon and P.K. Fox. Organon Labs., Newhouse, Lanarkshire ML1 5SH, U.K.

Evidence is accumulating for multiple sigma sites in the mammalian CNS. Mussachio et al. (Eur. J. Pharmacol., 1991, 206: 261-264) have suggested that 4 sites (R1-R4) are present. We have addressed this problem and have examined sigma site - G protein coupling in guinea-pig brain membranes. DTG, (+)3PPP and dextromethorphan displaced [3H]-DTG (3.4 nM) with low Hill slopes of 0.5, 0.6 and 0.6, respectively. In the presence of Gpp(NH)p (100 μM), the [3H]-DTG specific binding was reduced by 31.7%, the Hill slope of (+)3PPP was increased to near unity, the ability of dextromethorphan to displace DTG was virtually abolished and the Hill slope for DTG remained low (0.7), indicating the presence of at least two binding sites. Gpp(NH)p apparently removes a dextromethorphan/DTG high affinity site, corresponding to R1 of Mussachio et al. (1991). The two binding sites for DTG remaining in the presence of Gpp(NH)p may be R3 and R4, dextromethorphan has a low affinity for these sites. R2, a dextromethorphan high affinity site, does not appear to be labelled by [3H]-DTG as this compound has a low affinity and dextromethorphan is ineffective in the presence of Gpp(NH)p. The present study suggests that DTG binds to at least three sites in guinea-pig brain at least one of which is G protein linked. If the presence of R2 is assumed, the present study is in agreement with the model of Mussachio et al. (1991).

195.10

ALLOSTERIC MODULATION OF THE BINDING OF [^{3}H](+)PENTAZOCINE TO σ SITES BY PHENYTOIN. D.L. DeHaven-Hudkins*,[1], F.Y. Ford-Rice[1], J.T. Allen[1] and R.L. Hudkins[2]. [1]Dept. Enzymology & Receptor Biochemistry, Sterling Winthrop Pharmaceuticals Research Division, Malvern, PA 19355 and [2]Albany Molecular Research, Watervliet, NY 12189.

The allosteric modulation of σ recognition sites by phenytoin (diphenylhydantoin) has been proposed based on the ability of phenytoin to stimulate binding of various σ ligands, as well as to slow dissociation from σ sites and to shift σ sites from a low- to a high-affinity state (Craviso & Musacchio, *Mol. Pharmacol.* 23: 629, 1983; Karbon et al., *Eur. J. Pharmacol.* 193: 21, 1991; McCann & Su, *J. Pharmacol. Exp. Ther.* 257: 547, 1991).

Phenytoin stimulated the binding of the σ-selective ligand [^{3}H](+)pentazocine in a dose-dependent manner, with maximal stimulation of binding observed at a final concentration of 250 μM phenytoin. The affinities of eleven structurally diverse σ reference compounds were determined in the presence or absence of 250 μM phenytoin. The affinities of caramiphen, dextromethorphan, dextrorphan, (+)3-PPP and (+)SKF-10,047 were three- to eight-fold higher in the presence of phenytoin. In contrast, the affinities of benzetimide, BMY 14802, carbetapentane, DTG, haloperidol and (+)pentazocine were unchanged in the presence of phenytoin. The relative sensitivity of σ compounds to allosteric modulation by phenytoin is not a property of all σ compounds, and may provide an *in vitro* basis for distinguishing σ subtypes and for predicting σ effects *in vivo*.

195.11

DESIGN OF DIVALENT LIGANDS AS PROBES FOR σ RECEPTOR TOPOGRAPHY. 4-PHENYLPIPERIDINYL AND 4-PHENYLPIPERAZINYL ALKYL-SPACED 5,5-DIPHENYLHYDANTOINS (PHENYTOIN). R.L. Hudkins*,[1] and D.L. DeHaven-Hudkins[2]. [1]Albany Molecular Research, Watervliet, NY 12189 and [2]Dept. Enzymology & Receptor Biochemistry, Sterling Winthrop Pharmaceuticals Research Division, Malvern, PA 19355.

Divalent ligands are defined as molecules which incorporate two discrete pharmacophores linked through a spacer. Recently we have demonstrated that the anticonvulsant phenytoin (5,5-diphenylhydantoin) allosterically modulates σ binding to the [^{3}H](+)pentazocine-labeled σ site, which suggests that phenytoin may bind to a distinct site near the competitive ligand site. Since the 4-phenylpiperidine moiety has been proposed to be the σ site pharmacophore, the rationale for the design of this series was to probe the spatial relationship of the allosteric or lipophilic phenytoin site with the competitive σ binding site. A series of 4-phenylpiperidines and 4-phenylpiperazines linked via a methylene chain to the N-3 nitrogen of 5,5-diphenylhydantoin was prepared and evaluated for affinity at [^{3}H](+)pentazocine- and [^{3}H]DTG-labeled σ sites. The compounds with a two carbon chain had weak affinity for both sites. However, as the distance was increased to six carbons a progressive increase in binding affinity was observed. The 4-phenylpiperidine derivative spaced by six carbons showed potent affinity for both [^{3}H](+)pentazocine- (K_i = 0.59 nM) and [^{3}H]DTG- (K_i = 3.02 nM) labeled sites. An hypothesis, the synthesis and structure-affinity relationships will be presented.

195.12

SIGMA LIGANDS HAVE REDUCED ABILITY TO INHIBIT THE MUSCARINIC PHOSPHOINOSITIDE RESPONSE IN CELLS DEFICIENT IN SIGMA-1 RECEPTORS. J.M. Cutts* and W.D. Bowen. Unit on Rec. Biochem. Pharmacol., Lab. of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892.

We have previously shown that micromolar concentrations of sigma ligands inhibit the ability of muscarinic cholinergic agonists to stimulate phosphoinositide (PPI) turnover in rat brain synaptoneurosomes, with a rank order of potency suggesting mediation through sigma-1 receptors (*Pharmacol. Rev.* 42: 355-402, 1990). However, sigma ligands bind to muscarinic receptors with micromolar affinity, and this has clouded the issue of sigma-1 receptor involvement in this effect. A way to test the requirement for sigma-1 receptors is to examine the effect of sigma ligands in cells which possess a muscarinic PPI response, but which do not possess a high density of sigma-1 receptors. PC12 pheochromocytoma and N1E-115 and SK-N-SH neuroblastomas contain a high density of sigma-2 sites [as well as a novel binding site for (+)-pentazocine; see Vilner et al., this meeting], but low to negligible density of sigma-1 sites (*Brain Res.* 527: 244-253, 1990; *Soc. Neurosci. Abstr.* 17: 593, #236.15, 1991). In PC12 and N1E-115 cells, DTG and (+)-pentazocine inhibited 10 uM oxo-M-stimulated PPI turnover with similar potency as in rat brain synaptoneurosomes. However, BD737, BD1008, and dextrallorphan had considerably reduced potency compared to brain synaptoneurosomes, and reduced haloperidol was essentially inactive. In SK-N-SH cells, all of the above sigma ligands showed reduced potency. Furthermore, while the inhibition seen in synaptoneurosomes is non-competitive, the inhibition observed in SK-N-SH and N1E-115 cells was competitive. Thus, unless sigma-1 receptors are present, only a weak competitive inhibition is observed. Implications for inhibition seen in the brain are: 1) at low concentrations of sigma ligand, heterologous, non-competitive inhibition is mediated by sigma-1 receptors, 2) at higher concentration, a component of the inhibition may be due to direct interaction of sigma ligands with muscarinic receptors, 3) sigma-2 sites do not appear to be involved.

196.1

α_1-ADRENERGIC ACTIVATION OF PHOSPHOLIPASE C IN XENOPUS OOCYTES IS MEDIATED VIA THE NATIVE GTP-BINDING PROTEIN, G_o E.M.Landau*, R. Iyengar, G.Omri, C.L.Ma, R.Premont and R.D.Blitzer. Department of Psychiatry, Bronx Medical Center and Departments of Psychiatry and Pharmacology , Mount Sinai Medical Center, New York, NY 10029.

The cloned α_1 receptor was expressed in Xenopus oocytes as previously described (Omri et al. NS abstracts 16,539;1990). The coupling of the receptor to the Xenopus phospholipase C was monitored by measuring the amplitude of the norepinephrine-induced, Ca^{2+}-dependent chloride current. The coupling of the receptor to phospholipase C was sensitive to pertussis toxin (PTX), but large concentrations of PTX and long exposure times were required to see an effect. Thus, to get a reduction of the response to norepinephrine by 70-80%, the oocytes needed to be exposed to 2 ug PTX for 72 hours or to 10 ug for 24 hours. The response to norepinephrine was enhanced by injecting the oocytes with 50 nl of a 50 nM solution of holo-G_o, but not by injection of G_i or vehicle. Finally, in oocytes form 5 different donors, the response to norepinephrine was reduced by 33-64% by injecting the oocytes with antisense nucleotides designed to inhibit the synthesis of Xenopus $G_{\alpha o}$. The responses were compared to those seen in oocytes injected with the corresponding sense nucleotides and were measured 6 to 24 hours after the nucleotide injection. We conclude that the coupling of the cloned α_1 adrenergic receptor to phospholipase C in the oocyte is mediated via G_o. The low sensitivity of the response to treatment with PTX may be an intrinsic property of Xenopus $G_{\alpha o}$.

196.2

DEVELOPMENTAL EXPRESSION OF THE ALPHA-1A, ALPHA-1B AND ALPHA-1C ADRENERGIC RECEPTOR SUBTYPE mRNAs IN THE RAT BRAIN. S. K. McCune*, M. M. Voigt and J. M. Hill. Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

The ontogenic expression of three alpha-1 adrenergic receptor subtypes, in E14, E16, E19, P0, P8, P14, P21 and adult brains, was examined by *in situ* hybridization using oligonucleotide probes derived from the non-homologous regions of the receptors. The alpha-1A subtype mRNA was expressed at low levels in the prenatal and P0 cortex. Specific expression in the olfactory bulb, cortex, hippocampus and reticular nucleus of the thalamus was detected in the P8 animal and persisted through adulthood. Detectable levels of mRNA were present in other thalamic nuclei only at P8 and P14. The alpha-1B receptor subtype mRNA was present in the E19 cortex, diagonal band, amygdala, pineal, mammillary nuclei, dorsal raphe and intensely in brainstem nuclei. By P0, the thalamus and globus pallidus were also visualized. With aging, the thalamic signal intensified while the expression in the globus pallidus and brainstem nuclei diminished. The alpha-1C subtype had limited expression. Low levels of mRNA were detected in E14 cortex and P0 olfactory bulb, but significant expression in the olfactory bulb, cortex and hippocampus was not seen until P8. Expression increased in olfactory bulb and hippocampus with age. The widespread nature of the expression of these alpha-1 adrenergic receptor subtypes in cortical, thalamic and limbic regions supports a role for adrenergic receptors in cognitive and affective functions. The ontogenic variations in distribution and intensity of expression may dictate changing physiologic and pharmacologic responses to catecholamines.

196.3

PHARMACOLOGIC CHARACTERIZATION OF ALPHA-2-ADRENERGIC RECEPTOR HETEROGENEITY IN RAT BRAIN. **M.F. O'Rourke*, R.C. Pleus, L.J. Iversen, and D.B. Bylund. Dept. of Pharmacology, University of Nebraska Medical Center, Omaha, NE 68198-6260.**

Four pharmacological alpha-2 adrenergic receptor subtypes have been identified using tissues and cell lines which express only one receptor subtype. In the rat, three alpha-2 adrenergic receptors have been cloned: RG20; RG10; and RNG. These clones correspond pharmacologically with the D, C, and B alpha-2 adrenergic receptor subtypes, respectively (Bylund, FASEB J. 6:832, 1992). In this study we characterized the alpha-2 adrenergic receptors in rat brain. We used [^{3}H]RX821002 and [^{3}H]rauwolscine to label receptors in membranes of rat cerebral cortex and cerebellum. Inhibition curves were generated using rauwolscine, BAM 1303, oxymetazoline, spiroxatrine, prazosin, ARC-239, and SKF 104078. The data are consistent with the conclusion that two alpha-2 adrenergic receptors, the D and C subtypes, are expressed in these areas of the brain. Approximately 80% of the alpha-2 receptors are the D subtype and 20% the C subtype. (Supported in part by NIH Grant GM 40784.)

196.4

IMMUNOCYTOCHEMICAL LOCALIZATION OF α_{2A}-, α_{2B}- AND α_{2C}-ADRENERGIC RECEPTORS IN BRAINS OF RAT AND MONKEY. C-G. Go[1]*, C. Aoki[1], O. Cartano[1], H. Kurose[2] & R. Lefkowitz[2]. [1]Center for Neural Science, New York Univ., NY, NY, 10003 & [2]Howard Hughes Med. Inst., Duke Univ. Med. Ctr, Durham, NC, 27710.

Three human genes, C-10, C-2 and C-4, with pharmacologic properties of α_2-adrenergic receptors (α_2AR) have been isolated in recent years (rev. by Lomasney et al., 1991, Biochim. Biochphys. Acta 1095: 127). Based on their rank orders of potency for adrenergic ligands and their tissue distribution, these genes have been assigned to the α_{2A}, α_{2B} and α_{2C} subtypes, respectively. Using rabbit antisera that were raised against GST-fusion proteins corresponding to the intracellular third loop of these receptor molecules (Kurose et al., ms in preparation), we show that the three subtypes of α_2AR are differentially distributed across brain regions and are differentially conserved between primates and rodents. Immunoreactivity (ir) to the C-10 (α_{2A}) gene product is readily detectable as punctate profiles within neuronal perikarya of the cerebral cortex, hypothalamus and locus coeruleus of the monkey (n=7). Ir is detectable but weaker within the corresponding regions of rat brain (n=10). Electron microscopy revealed that punctate profiles correspond to saccules that are removed from synaptic regions of the perikaryal plasma membrane. Ir to C-2 (α_{2B}) is pronounced within perikarya and dendrites of cerebellar Purkinje cells of monkey but not of the rat. Rat brain tissue is most immunoreactive to anti-C-4 (α_{2C}). Nuclei with pronounced C-4-ir include the olfactory nerve, amygdala, piriform cortex, hypothalamus and area postrema. Supported by EY08055 and 2-SO7-RR07062-26 to CA.

196.5

***IN SITU* HYBRIDIZATION STUDY OF ALPHA AND BETA ADRENERGIC RECEPTORS IN THE RAT HYPOTHALAMUS.** S. Ceccatelli* A.P. Nicholas and T. Hökfelt. Dept. of Histology and Neurobiology, Karolinska Institutet, Stockholm, Sweden, 104 01.

Noradrenergic and adrenergic input to the hypothalamus have been shown to influence the activity of neurons in this area of the brain via activation of alpha and beta adrenoceptors. In the present study, we have designed specific 48-mer oligoprobes to the recently-described DNA sequences of the rat alpha-1A, alpha-1B, alpha-2A, alpha-2B, alpha-2C, beta-1 and beta-2 adrenoceptors to localize hypothalamic cells that manufacture the mRNA for these receptor subtypes using the *in situ* hybridization technique. Complementary DNA probes were made on a DNA synthesizer to the following nucleotide sequences: 1167-1241 of rat alpha-1A, 1420-1467 of rat alpha-1B, 949-996 of rat alpha-2A (RG20), 1196-1243 of rat alpha-2B (RNG), 868-915 of rat alpha-2C (RG10), 1401-1448 of beta-1 and 2999-3046 of beta-2 receptors. All probes then were labelled at the 3'-end using terminal deoxynucleotidyltransferase and [^{35}S] dATP. The hypothalamus was rapidly removed, frozen, sectioned on a cryostat (14 µm), air dried and transferred to humidified boxes at 42° C for 18 hrs with 1 X 10^6 cpm of an end-labeled DNA oligonucleoide probe diluted in a hybridization solution consisting of 0.02% Ficoll, 0.02% polyvinyl pyrrolidone, 1% sarcosyl, 0.2 M sodium phosphate (pH 7), 10% dextran sulfate, 200 mM dithiothreitol and 500 µg/ml heat-denatured salmon sperm DNA. The sections then were washed 4 X 15 min in SSC at 55° C, dehydrated, dipped in Kodak NTB2 photographic emulsion and exposed for 6-12 weeks before development. In the hypothalamus, the paraventricular nucleus labeled strongest with the alpha-2A probe, followed in intensity by labeling with the alpha-1B, alpha-2C and beta-2 probes. Adrenalectomized and thyroidectomized animals were also examined for differences in hypothalamic labeling intensity compared to controls.

196.6

BEHAVIORAL AND NEUROPHARMACOLOGICAL ASSESSMENT OF α_2-ADRENOCEPTOR DENSITY AND FUNCTION FOLLOWING EEDQ TREATMENT. M.J. Durcan*, P.F. Morgan, M. Van Etten, and M. Linnoila. Laboratory of Neurogenetics, NIAAA, Bethesda, MD 20892.

N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) is a potent and irreversible antagonist of α_2-adrenoceptors. The repopulation of these receptors following EEDQ treatment to male NIH Swiss mice was investigated both behaviorally and neuropharmacologically. α_2-adrenoceptor turnover was assessed by investigating the binding of the selective α_2-adrenoceptor antagonist, (2-[-2methoxy-1,4-benzodioxanyl)] imidazoline (^{3}H-RX821002) to mouse whole brain tissue 1, 2, 4, 8, 12 and 16 days following treatment with EEDQ (2 mg/kg, i.p.). One day following EEDQ treatment ^{3}H-RX821002 binding is reduced to 25% of control levels and this returns to approximately 75% by day 16. The time taken for the binding sites to return to 50% of control levels was calculated to be 5.25 days. In a separate experiment the behavioral response to the α_2-adrenoceptor agonist medetomidine was investigated at the same time points following EEDQ (2 mg/kg, i.p.) treatment using the holeboard test of directed exploration and locomotor activity. Medetomidine (0.1 mg/kg) caused a highly significant ($p < .01$) reduction in both exploratory head-dipping and locomotor activity. The reduction in both head-dipping and locomotor activity was significantly ($p < .05$) attenuated in animals pretreated with EEDQ 1, 2 or 4 days earlier; longer durations of EEDQ pretreatment did not result in significant attenuation of medetomidine-induced sedation. EEDQ treatment alone did not significantly affect either of the holeboard measures. The loss of α_2-adrenoceptors can be dose dependently prevented by the administration of RX821002 prior to EEDQ treatment. This protection can be observed both in the binding of ^{3}H-RX821002 and in the behavioral responses to medetomidine. These results suggest that there is a significant receptor reserve for the sedative effects of the α_2-adrenoceptor agonist medetomidine in mice.

196.7

[^{3}H]DEXMEDETOMIDINE, AN ALPHA-2 ADRENERGIC RECEPTOR AGONIST, BINDS ALSO TO A NON-ADRENERGIC IMIDAZOLE-PREFERRING SITE IN RAT CNS. M.K.T. Savola, J.-M. Savola[1] and A. Marjamäki*. Dept. of Pharmacology, Univ. of Turku, and [1]R&D, Pharmaceuticals, Orion Corp. Farmos, Turku, Finland.

The imidazole derivative dexmedetomidine (DMD) is a potent and selective α_2-adrenergic receptor (AR) agonist. This compound has been tritiated to high specific activity (about 70 Ci/mmol) and we characterised binding of [^{3}H]DMD in the cerebral cortex and spinal cord of the rat. Saturation analysis of binding showed two affinity sites when DMD or detomidine were used to define non-specific binding in these tissues. Compared to values when epinephrine was used to define non-specific binding, presence of a non-adrenergic binding site (NAIBS) was evident in both tissues, the proportion of NAIBS being greater in the rat spinal cord. Interestingly, saturation analysis in presence of Gpp(NH)p showed only one affinity site in both tissues indicating binding to a G-protein coupled receptor. Inhibition experiments with a number of α-AR agonists and antagonists and other neurotransmitter receptor ligands indicated specific binding of [^{3}H]DMD to α_2-ARs in rat cerebral cortex. However, epinephrine could inhibit binding of [^{3}H]DMD to cerebral cortex by 80% and in spinal cord maximally by 50%. Furthermore, clonidine, rauwolscine, and yohimbine could inhibit binding by 50% but the imidazole derivatives DMD, detomidine, atipamezole, and *l*-medetomidine, and the imidazoline derivative idazoxan (at high concentration) inhibited totally binding in the spinal cord.

We conclude that [^{3}H]DMD has very complex binding characteristics in rat CNS. It binds to α_2-ARs without substantial affinity to other classical neurotransmitter receptors. However, [^{3}H]DMD binds also to a NAIBS which seems to be different from those identified with [^{3}H]*p*-aminoclonidine or [^{3}H]idazoxan but resembling that identified with [^{3}H]atipamezole.

196.8

ALPHA-2 ADRENERGIC RECEPTORS AND TYROSINE HYDROXYLASE IN THE LOCUS COERULEUS OF P VS NP AND HAD VS LAD RATS. B.H. Hwang*, G.-M. Wang, L. Lumeng and T.-K. Li. Indiana University School of Medicine, and VAMC, Indianapolis, IN 46202.

Locus coeruleus (LC) is rich in tyrosine hydroxylase (TH) and possesses a high density of alpha-2 adrenergic (A2) receptors. Ethanol administration causes changes of central monoamine neurons, but whether LC is involving in alcohol-drinking preference (AP) is not known. To explore this question, selectively bred alcohol-preferring (P) and -nonpreferring (NP) rats, as well as high alcohol-drinking (HAD) and low alcohol-drinking (LAD) rats were studied. Cryostat sections were cut, 125I-iodoclonidine was used to label A2 receptor binding, and TH antibody and 125I-secondary antibody were applied to assess TH content in the LC. Quantitative autoradiographic analyses showed that the TH content in the LC was substantially lower in P rats (24.8% reduction) than in NP rats. There was also a significant reduction (18.7%) of A2 receptor binding in P rats as compared with NP rats. However, HAD rats exhibited a higher (17.2%) TH content in the LC than LAD rats, and A2 receptor binding was similar in HAD and LAD rats. Similar A2 receptor binding was also seen in the solitary nucleus, area postrema and dorsal motor nucleus of the vagus in the P vs NP rats, and HAD vs LAD rats. Collectively, the above results suggest that adrenergic systems in the LC are not directly associated with AP. Supported in part by grant AA07611 and NS25087.

196.9

IN VIVO ELECTROCHEMICAL STUDIES OF THE EFFECTS OF A75200 ON EXOGENOUS NE CLEARANCE IN THE CEREBELLUM OF THE ANESTHETIZED RAT. J. A. Grebb*, G.A. Gerhardt, C. van Horne, A.M.Y. Lin, P.C. Bickford, J. DeBernardis, M. Pierce and J.F. McKelvy. Depts. of Pharmacology and Psychiatry, Univ. of Colorado Health Sci. Ctr., Denver, CO and Abbott Laboratories, Abbott Park, IL.

A75200,(±)-(R'*,3R*)-3-phenyl-1-(1',2',3',4'-tetrahydro-5',6'-methylenedioxy-1- naphthalenyl)methyl)-pyrolidine, methanesulfonate is a racemic mixture which is a potent alpha-2 antagonist, and an inhibitor of the uptake of norepinephrine (NE) into catecholamine-containing neurons. These properties of this new drug support the hypothesis that this compound has potential as a novel antidepressant agent. The purpose of the present study was to investigate the effects of A75200 on NE-containing neurons in vivo in the anesthetized rat using electrochemical recording techniques. NE was locally applied, either alone or following applications of A75200 or nomifensine (50-100 nanoliters of a 400 micromolar solution), into the cerebellar cortex of the anesthetized male Fischer 344 rat by pressure ejection from double-barrel micropipettes. The clearance of NE from the extracellular space was measured using rapid chronoamperometric measurements with Nafion-coated carbon fiber electrodes which were positioned 300 microns from the micropipette tips. Both A75200 and nomifensine were seen to significantly increase the amplitude and prolong the time course of locally-applied NE. A75200 is currently being evaluated in clinical trials to investigate whether it will be useful as an antidepressant.

196.10

IMPACT OF PHENYLPROPANOLAMINE AND CIRAZOLINE ON EXTRACELLULAR NOREPINEPHRINE IN RAT PARAVENTRICULAR HYPOTHALAMUS: A MICRODIALYSIS STUDY. P.J. Wellman *, A. Morien and B.T. Davies. Dept of Psychology, Texas A&M University, College Station, TX 77843-4235, USA. Adrenergic receptors within rat paraventricular hypothalamus (PVN) are known to modulate feeding behavior. In particular, activation of α2-adrenergic receptors via drugs such as clonidine enhances feeding whereas activation of α1-adrenergic receptors via drugs such as phenylpropanolamine (PPA) and cirazoline (CZ) inhibits feeding. The neuropharmacology of PPA and CZ is incompletely known. Whereas both compounds are thought to exert direct action at α1-adrenergic receptors, their activity at the α2-adrenergic receptor is unknown. One means to determine whether a compound acts as an agonist or antagonist at the α2-adrenergic receptor involves *in vivo* microdialysis measures of extracellular norepinephrine (NE) with rat PVN. α2-adrenergic agonists suppress extracellular PVN NE whereas antagonists enhance PVN NE. Rats were implanted with a concentric microdialysis probe within PVN and then treated with either vehicle (n=5), 20 mg/kg PPA (n=8) or 0.2 mg/kg CZ (n=4) with dialysates taken for 60 min before and 60 min after drug treatment. Extracellular NE was suppressed by 55% over a 60 min period in rats treated with 20 mg/kg PPA whereas 0.2 mg/kg CZ slightly increased extracellular NE by 20% within the first 40 min after injection. These results suggest that, at systemic doses that suppress feeding, PPA acts as an α2-agonist within the PVN whereas CZ has minimal action at the α2-adrenergic receptor.

196.11

TIMING OF NORADRENERGIC DEPLETION AND OCCURRENCE OF DARK CELLS IN HIPPOCAMPUS AFTER SUBCUTANEOUS ADMINISTRATION OF DSP4 OR 6-OHDA AT NEONATE. Y.-P. Lee*, M.-Y. Min and H.-M. Hwang. Dept. of Anatomy, Chang Gung Medical College, Taoyuan, Taiwan 33333, R.O.C.

Previous study indicated that subcutaneous injection of 6-OHDA or DSP4 after birth caused noradrenergic depletion, up-regulation of beta-adrenergic receptors and occurrence of dark cells in adult rat hippocampus. To investigate possible association among these events, they were examined by immunohistochemistry, receptor binding assay and microscopy respectively at different developmental ages after treatment. During the normal development, noradrenergic innervation into hippocampus was not apparent until P15. Beta-adrenergic receptor activity also revealed similar developmental pattern. After drug treatment at P0, depletion appeared to be in effect immediately. No innervation was found in hippocampus at any age. In contrast, no increase of beta-adrenergic receptor activity was found to be increased until P15. Microscopy indicated that no dark cells occur until P60 or P90. Therefore, occurrence of dark cells may not be an immediate consequence of noradrenergic depletion. (supported by NSC81-0412-B182-01)

196.12

EFFECT OF AGENTS THAT INCREASE BRAIN NOREPINEPHRINE (NE) RELEASE ON BRAIN EXTRACELLULAR cAMP LEVELS. S.M. John and E.A. Stone*. Dept. Psychiatry, New York Univ. Sch. Med., New York, NY 10016

Previously we have shown that administration of exogenous NE produces an increase in extracellular levels of cAMP in the frontal cortex of the rat. We have also shown that some stimuli that increase endogenous brain NE release such as handling stress and infusion of amphetamine produce increases in cAMP levels. In the present experiment we examined the effects of additional stimuli that increase NE release on this response. Rats were implanted with microdialysis probes in the prefrontal cortex and 48 hrs later perfused while subjected to treatments known to enhance brain NE release. It was found that i.p. administration of yohimbine and desmethylimipramine (DMI) both tended to increase cAMP levels however the responses were highly variable. I.P. injection of saline by itself had no effect but did produce a reliable increase in cAMP level if the dialysis probe was first perfused with a phosphodiesterase inhibitor. Local administration of DMI via the probe increased cAMP levels. The results suggest that stimuli that increase endogenous NE release may be capable of producing detectable increases in brain extracellular cAMP levels but the optimal conditions for detecting this response remain to be determined. Supported in part by grants AFOSR 89-0208, MH45265 and MH08618.

196.13

AUTORADIOGRAPHIC LOCALIZATION OF BETA-ADRENERGIC RECEPTORS IN THE SONGBIRD VOCAL CONTROL SYSTEM

J. M. Casto*, P. Absil, J. Balthazart, & G.F. Ball Dept. Psychology, Johns Hopkins Univ. Baltimore, MD 21218; Univ. of Liège, Belgium

Beta-adrenergic receptors have been implicated in learning and memory processes in several species. Noradrenergic activity in the songbird vocal control system may be related to song learning and production. We therefore localized β-adrenergic receptors in three avian species: two species that exhibit vocal learning (zebra finches and European starlings) and one that does not learn its vocal behavior (Japanese quail). Receptors were labeled using [^{3}H] CGP 12177, a non-selective β receptor ligand. β1 and β2 subtypes were specified by selectively inhibiting either β2 receptors with the addition of ICI 118551 (to visualize β1 receptors) or β1 receptors with the addition of atenolol (to visualize β2 receptors). Total non-specific binding was determined by the addition of propranolol. In general, β2 receptors were present in higher densities than β1 receptors in all three species. Three vocal control nuclei: the caudal part of the ventral hyperstriatum (HVc), the robust nucleus of the archistriatum (RA) and the magnocellular nucleus of the anterior neostriatum (MAN) were well defined by the high density of both the β1 and the β2 subtype in comparison to the surrounding tissue. These areas are not discernible in the quail brain based on an examination of either Nissl stained sections or the autoradiograms. In all three species, the cerebellum, the hippocampus, the optic tectum, the nucleus rotundus, nucleus mesencephalicus lateralis, pars dorsalis and and many parts of the diencephalon exhibited high receptors densities. There was a qualitative difference between the high density of receptors in the songbird telencephalon as compared to the low density in the telencephalon of quail. Although these species differences exist, immunohistochemical visualization of tyrosine hydroxlase did not reveal major differences in the distribution of cell bodies containing catecholamines suggesting that the telencephalic receptors of songbirds receive their inputs from specialized projections orginating from catecholaminergic cell groups that are common to all bird species.

196.14

LOW AFFINITY ß-ADRENERGIC RECEPTOR IN CANINE ADRENAL MEDULLA.

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Drugs active at adrenergic receptor sites modulate adrenal medullary function. To determine beta receptor binding constants and density, a canine adrenal medulla homogenate was incubated at 37°C, pH 7.4, in 10mM Tris, 130mM NaCl, 1mM ascorbate for 1hr with increasing concentrations of ^{125}I-cyanopindolol (^{125}I-CYP), with and without 150μM l-propranolol to differentiate total and nonspecific binding. Total and bound ligand were separated by vacuum filtration. ^{125}I-CYP binding was 75-90% specific and saturable. Analysis of the data with the curve fitting program, LIGAND, was consistent with a single binding site, apparent K_D 750pM. Receptor density (Bmax) was 100 fmol/mg protein. Competition experiments using increasing concentrations of l-propranolol revealed a K_i of 900nM. These data suggest the presence of a low affinity ^{125}I-CYP binding site in canine adrenal medulla. We hypothesize that this is a ß-adrenergic receptor since ß-adrenergic agonists stimulate in vivo and in vitro catecholamine secretion from adrenal medullary tissue[1,2].

Supported in part by Grant HL02018.

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CATECHOLAMINES: RELEASE

197.1

ELECTROPHYSIOLOGICAL AND NEUROCHEMICAL EFFECTS OF (-)HA-966: EVIDENCE FOR PATTERN-DEPENDENT MODULATION OF DOPAMINE RELEASE.

P.D. Shepard* and B. Moghaddam. MD Psychiatric Res. Cntr, Baltimore, MD 21228 and VA Med. Cntr., West Haven, CT 06516.

(±)1-hydroxy-3-aminopyrrolidone-2((±)HA-966) is known to cause a rapid and selective increase in striatal dopamine (DA) levels; an effect that has been attributed to the drug's ability to block the spontaneous activity of nigrostriatal DA-containing neurons (Shepard and Lehmann, JPET 261:387, 1992). Although the racemic form of HA-966 has been used extensively as a noncompetitive NMDA antagonist, the resolved enantiomeric forms of the drug possess disparate pharmacological effects. In the present series of experiments, single unit recording and microdialysis techniques were used to assess the neurochemical and electrophysiological effects of (-)HA-966 on nigrostriatal DA-containing neurons in chloral hydrate anesthetized rat. Low i.v. doses of (-)HA-966 failed to significantly affect DA neuronal firing rate (1 mg/kg (n=8): control: 3.9 ± 0.4 Hz, post(-)HA966: 3.5 ± 0.4 Hz; 3 mg/kg (n=8): control: 5.2 ± 0.7 Hz, post(-)HA-966: 4.7 ± Hz). Higher doses of the drug (10 - 30 mg/kg) produced a rapid and dose-dependent cessation in neuronal firing in 8 of 10 cells tested (latency to 50% inhibition - 10 mg/kg: 5.9 ± 1.5 min; 30 mg/kg: 3.2 ± 1.4 min). Interestingly, doses of (-)HA-966 which failed to affect firing rate, "normalized" DA neuronal firing pattern as evidenced by a significantly decrease the variability (coefficient of variation) associated with interspike interval distributions (1 mg/kg: pre-drug: 50.1 ± 6.6 %, post-drug: 36.5 ± 6.8 %, Wilcoxan signed rank test, $P < 0.02$; 3 mg/kg: pre-drug: 42.5 ± 6.5 %, post-drug: 23.3 ± 4.5 %, $P < 0.02$). Preliminary results from *in vivo* dialysis experiments suggest that these changes are accompanied by a significant reduction in striatal DA release. Taken together, these data support the hypothesis that alterations in neuronal firing pattern can modulate DA release in forebrain areas.

197.2

COMPARISON OF A NOVEL D1 RECEPTOR ANTAGONIST, SCH 39166, WITH SCH 23390 AND HALOPERIDOL ON THE IN VIVO RELEASE OF DOPAMINE AND ITS METABOLITES FROM THE RAT STRIATUM USING MICRODIALYSIS.

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In vivo microdialysis was utilized to investigate the effects of various dopamine receptor antagonists on dopamine release in the rat striatum. Time course and dose response curves were compared between D1 and D2 receptor antagonists on dopamine, DOPAC, HVA and 5-HIAA release. SCH 39166, a novel benzonaphthazepine analog, and SCH 23390, a benzazapine derivative, were compared as selective D1 receptor antagonists. Additionally, the selective D2 receptor antagonist, haloperidol was tested.

Male Sprague Dawley rats were implanted with guide cannulae, a minimum of 24 hr prior to in vivo microdialysis. On the day of the experiment, the microdialysis probes were implanted into the left striatum at the stereotaxic coordinates from bregma of + 0.2 mm, -3.0 mm lateral and 6.0 mm ventral. Subsequently, the striatum was perfused with Kreb's buffer (2.0 µl/min) for 60 min prior to obtaining a baseline level of catecholamine release. Four 10 min samples were collected for baseline determinations and the rats were then administered drug, ip. Animals were perfused for the next 3 hours and samples were collected every 10 min.

Haloperidol, the D2 receptor antagonist produced a robust dose-dependent increase in DOPAC and HVA release. A dose of 1 mg/kg of haloperidol produced an increase of over 200% of DOPAC, that was seen throughout the collection period. Lower doses of haloperidol also produced significant increases in DOPAC and HVA. In contrast, SCH 39166 produced a smaller increase in DOPAC that was significantly different from control only at the highest dose (10 mg/kg, ip). Minimal or no effects on DOPAC and HVA levels were seen at lower doses. No effect was seen on 5-HIAA release at any of the doses used. Similar findings were seen with the other D1 receptor selective antagonist, SCH 23390.

197.3

APOMORPHINE AND QUINPIROLE INHIBIT DOPAMINE RELEASE BY DIFFERENT MECHANISMS: ROLE OF DOPAMINE SYNTHESIS

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Apomorphine and quinpirole inhibit the efflux of dopamine (DA) from striatal slices. However, whereas this inhibition usually is attributed to an action of D2 agonists on release-modulating autoreceptors, apomorphine has a catechol-like structure and has been reported to inhibit tyrosine hydroxylase (TH). Thus, we wished to compare the mechanism by which these two agonists inhibit DA efflux and to determine whether inhibition of TH plays any role in the impact of apomorphine on DA release. Striatal slices were superfused in a modified Krebs bicarbonate buffer. Electrical field stimulation (5 Hz for 4.5 min) was applied and endogenous DA release was assessed using HPLC. Both apomorphine and quinpirole reduced the overflow of DA in a concentration-dependent manner. No statistical difference was found between the maximal effects of apomorphine (1 µM, -84%) and quinpirole (1 µM, -75%). However, whereas sulpiride (10 µM) strongly antagonized the effect of 1 µM quinpirole on DA overflow (-91%), this D2 antagonist only partially antagonized the effect of 1 µM apomorphine (-21%). No further inhibition of DA overflow was noted when sulpiride was applied at a 10-fold higher concentration. To assess the impact of another drug known to inhibit TH, we examined the effect of 3-iodotyrosine (2 mM). This drug almost completely abolished the overflow of endogenous DA from striatal slices (-95%), although tissue DA content remained high. Our results suggest that apomorphine, but not quinpirole, has an inhibitory influence on DA release that is not mediated by autoreceptors but instead reflects an inhibition of DA synthesis. They also indicate that inhibition of DA synthesis can markedly reduce release *in vitro* despite the continued presence of DA in tissue. (Supported in part by USPHS grant NS19608.)

197.4

PREFRONTAL CORTICAL CONTROL OF MESOLIMBIC DOPAMINE CELL FIRING AND TRANSMITTER RELEASE.

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The putative influence of the medial prefrontal cortex (PFC) on mesolimbic dopamine (DA) activity was studied with single cell recording and in vivo voltammetry techniques in the chloral hydrate-anaesthetized male rat. Microinfusion of glutamate (25 nmol) in PFC enhanced burst firing of DA cells in the ventral tegmental area (VTA) and increased DA release in nucleus accumbens. Microinfusion of lidocaine (38 nmol) in PFC decreased burst firing in VTA-DA cells and DA release in accumbens. Firing rate and regularity of firing of VTA-DA neurons were unaffected by both treatments.

The present results indicate a tonic stimulatory input from the PFC to mesolimbic DA neurons. This input seems to specifically control burst firing and burst-related transmitter release of DA neurons, underlining the role of burst firing in DA function. PFC control of DA neurons might be involved in incentive behavior and in negative schizophrenic symptomatology associated with dysfunction of the PFC.

197.5

DIFFERENTIAL EFFECTS OF MAO INHIBITORS ON L-DOPA-INDUCED DA RELEASE IN THE STRIATUM OF INTACT AND 6-OHDA-TREATED RATS. S.R. Wachtel*, H. Ramasubramaniam, and E.D. Abercrombie. Ctr. Molec. and Behav. Neurosci., Rutgers Univ., Newark, NJ 07102.

The present study examined the roles of MAO-A and MAO-B in the metabolism of dopamine (DA) formed from exogenous L-DOPA in the striatum of intact and 6-OHDA-lesioned rats. Since MAO-A in the striatum is preferentially localized in DA nerve terminals, it was hypothesized that the metabolic action of this enzyme could account in part for the relatively small effect of L-DOPA on extracellular DA in intact striata compared to DA-depleted striata. *In vivo* microdialysis, in freely moving rats, was used to monitor the extracellular level of DA in the striatum, as well as the metabolites, DOPAC and HVA. Pretreatment of intact rats with clorgyline (2 mg/kg), an irreversible, selective inhibitor of MAO-A, increased L-DOPA-induced (50 mg/kg) DA release by 2442% and decreased DOPAC and HVA by 71% and 73%, respectively. In rats with 6-OHDA lesions, clorgyline had no significant effect on the increase of DA and its metabolites elicited by L-DOPA. Pretreatment with Ro 19-6327 (1 mg/kg), a reversible, selective inhibitor of MAO-B, did not significantly affect basal or L-DOPA-induced changes in striatal DA or its metabolites in either intact or 6-OHDA-lesioned rats. Pretreatment with the irreversible inhibitor of MAO-B, deprenyl, at a dose devoid of amphetamine-like effects on DA release (1 mg/kg), also did not significantly affect L-DOPA-induced DA release in the striatum of either intact or 6-OHDA-lesioned rats. These results indicate 1) MAO-A efficiently metabolizes DA formed from exogenous L-DOPA within DA terminals, 2) destruction of DA terminals eliminates this action of MAO-A, and 3) the loss of DA terminals does not unmask or enhance metabolism of DA by MAO-B. [Supported in part by a NRSA from NINDS (SRW), USPHS grant NS19608, and The American Parkinson's Disease Association (EDA)]

197.6

EFFECTS OF THREE NEW COMT INHIBITORS ON STRIATAL DOPAMINE METABOLISM IN VIVO. S. Kaakkola, P.J. Lindsberg* and R.J. Wurtman. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

In vivo microdialysis was used to examine the effect of three new COMT inhibitors, Ro 40-7592, CGP 28014, and OR-611 (entacapone), on extracellular levels of dopamine, DOPAC, HVA, and 5-HIAA in rat striatum. A dose of 30 mg/kg of each compound was injected i.p. to male Sprague-Dawley rats under chloralose/urethane anaesthesia. Dialysis samples were collected for 20 min periods over 260 min and assayed by HPLC. All the three inhibitors significantly increased the efflux of DOPAC and decreased that of HVA. Ro 40-7592 was the most potent followed by CGP 28014. OR-611 was the least potent. Ro 40-7592 and CGP 28014 tended to increase the extracellular levels of dopamine whereas OR-611 had no effect. None of the COMT inhibitors changed significantly the efflux of 5-HIAA.

The results show that both Ro 40-7592 and CGP 28014 are potent centrally-active COMT inhibitors whereas OR-611 is principally a peripherally-active inhibitor. COMT inhibitorscan considerably modify brain dopamine metabolism and may be of clinical significance in therapy of e.g. Parkinson's disease.

197.7

OPIOID MODULATION OF [^{3}H]DOPAMINE RELEASE FROM EMBRYONIC RAT VENTRAL MESENCEPHALIC CULTURES. Smith J.A.M., Loughlin S. E. and Leslie F. M.* Dept. of Pharmacology, University of California, Irvine, CA92717.

Endogenous opioids play an important role in the regulation of ventral mesencephalic dopaminergic neurons. However, the precise mechanism underlying opioid modulation of dopamine function has not been determined. In the present study, a dissociated cell culture model of embryonic ventral mesencephalon was established in order to define the actions of selective opioids on dopamine release at the cellular level. Ventral mesencephali were dissected from rat brains at embryonic day 15. Cells were enzymatically and mechanically dissociated and plated in serum-containing media. At 8 days in vitro, each of the major morphological types of dopaminergic cell was represented in these cultures. These cells exhibited specific uptake of [^{3}H]dopamine, which was subsequently released, in a calcium-dependent manner, in response to a double pulse of elevated extracellular potassium. There was a dose-dependent increase in [^{3}H]dopamine release with increasing concentrations of extracellular potassium. Spontaneous and potassium-evoked [^{3}H]dopamine release was inhibited by kappa, but not mu or delta, opioid agonists. The selective kappa$_1$ agonist U69593 produced a dose-dependent inhibition of spontaneous and evoked dopamine release. The inhibitory effect of U69593 was blocked by the non-selective opiate antagonist naloxone and the selective kappa opioid antagonist nor-binaltorphimine. Kappa opioid inhibition of potassium-evoked [^{3}H]dopamine release was maintained in the presence of tetrodotoxin. These results provide evidence for a presynaptic localisation of functional kappa receptors on the terminals of ventral mesencephalic dopamine neurons. Embryonic rat ventral mesencephalic cultures represent an appropriate model to further characterise kappa opioid modulation of neurotransmitter release. *Supported by NS19319, NS26761 and American Parkinson Disease Association SCC.*

197.8

THE EFFECTS OF HYDRALAZINE AND NITROPRUSSIDE ON STRIATAL MONOAMINE AND AMINO ACID NEUROTRANSMITTER SYSTEMS: SIMULTANEOUS MEASUREMENT OF DRUG, NEUROCHEMICALS, AND METABOLITES IN MICRODIALYSIS PERFUSATES. P. Gamache[1]*, E. Ryan[1], J. Yu[2], T. Maher[2], and I. Acworth[1&2] [1]ESA, Inc., 45 Wiggins Avenue, Bedford, MA 01730 and [2]Department of Pharmacology, Massachusetts College of Pharmacy, Boston, MA 02115.

We have studied the effects of peripheral administration of hypotensive agents on striatal extracellular levels of neurotransmitters and metabolites using *in vivo* microdialysis in the anesthetized rat. Animals were cannulated for arterial blood pressure monitoring and i.v. drug administration. Automated derivatization, column switching, and HPLC using coulometric array detection permitted simultaneous determination of monoamines, their metabolites, and neuroactive amino acids from each perfusate sample. Hydralazine (10mg/kg, bolus) produced a marked increase (> 10 -fold) above basal levels of aspartate (ASP), dopamine (DA), GABA, glutamate (GLU), glycine (GLY) and taurine (TAU). Smaller increases, or no change, was evident for 8 other amino acids. The acid metabolites of the monoamines decreased to ca. 1/5th of the basal levels. Interestingly, a chromatographic peak corresponding to an external hydralazine standard was evident only in the post-treatment perfusate samples. To examine whether these results were mainly associated with hydralazine's hypotensive effects, another hypotensive agent was examined. Nitroprusside was infused at a rate which maintained blood pressure between 2/3 and 1/2 of baseline. This produced relative changes in the analyte levels similar to hydralazine.

These experiments show that hydralazine can traverse the blood-brain barrier and that specific effects of these drugs may involve several transmitter systems. Furthermore, this analytical technique may be useful for direct pharmacokinetic and neuropharmacological studies.

197.9

EFFECTS OF BENZODIAZEPINE AND GABA$_A$ RECEPTOR ANTAGONISTS ON STRIATAL EXTRACELLULAR DOPAMINE LEVELS: AN IN VIVO MICRODIALYSIS STUDY. R.J.Gruen*, B.Moghaddam, A.Coale, and A.J.Friedhoff. New York Univ., NY, NY 10016, Yale Univ. School of Medicine, New Haven, CT 06510.

The present study was designed to examine whether ligands which bind at the GABA/benzodiazepine receptor complex have a tonic modulatory effect with regard to striatal dopamine (DA) transmission. We therefore examined the effects of local administration of Ro15-1788, a benzodiazepine (BZ) receptor antagonist, and SR 95531, a novel GABA$_A$ receptor antagonist, on extracellular dopamine (DA$_{[e]}$) levels in the striatum of anesthetized and awake adult rats. Administration of Ro15-1788 resulted in a dose-dependent increase in DA$_{[e]}$ in both anesthetized and awake animals. The Ro15-1788-induced increase in DA$_{[e]}$ was blocked by coadministration of the BZ diazepam, as well as GABA. Local administration of SR 95531 also resulted in a dose-dependent alteration in DA$_{[e]}$ in anesthetized and awake animals. The SR 95531-induced increase in DA$_{[e]}$ was blocked by coadministration of GABA. It has been suggested that the effects of excitatory amino acids on striatal DA activity in vivo are mediated, in part, by inhibitory neurons (Moghaddam & Gruen, 1991). The results of the present study provide support for the idea that GABA has a tonic inhibitory effect on striatal DA transmission. Supported, in part, by MH-08618 (AJF).

197.10

EFFECT OF BLOOD PRESSURE (BP) ALTERATIONS ON STRIATAL DOPAMINE (DA) RELEASE IN ANESTHETIZED RATS: A MICRODIALYSIS STUDY. J.Yu, K.C.Gariepy, I.N. Acworth, T.J. Maher*. Mass. Coll. of Pharm., Boston, MA 02115 and ESA, Inc., Bedford, MA 01730

Significant evidence supports a role for CNS monoamines in cardiovascular regulation. We measured, using microdialysis, striatal ECF levels of DA and its metabolites DOPAC and HVA. Dialysate DA, DOPAC, and HVA, as well as BP were recorded during infusion of nitroprusside (NPR), hydralazine (H), phenylephrine (PHE), or methoxamine (MX) and after mechanical hemorrhage(HEM). H and NPR increased striatal DA levels by 123 ± 52 and 56 ± 11 fold as BP decreased by 50% and 57%, respectively. DOPAC and HVA levels decreased by 60% and 50% after injection of H, however little effect was seen after NPR. PHE increased striatal DA by 2 fold as BP increased by 70%, however, another α_1 agonist MA did not increase striatal DA despite a 150% BP increase. IV infusion of MX with H to maintain BP, produced only a slight increase in DA (47%). Cessation of MX infusion increased DA by 22 fold as BP decreased by 40%. HEM induced-hypotension also increased striatal DA by 9 fold. Changes in striatal DA accompany hypotensive insults and may constitute reflex mechanisms and/or result from decreased cerebral blood flow

197.11

ENHANCED STRESS-INDUCED DOPAMINE RELEASE IN THE PREFRONTAL CORTEX IN AMPHETAMINE SENSITIZED RATS. T. Hamamura and H.C. Fibiger*. Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3

Chronic administration of amphetamine (AMPH) can produce a syndrome that strongly resembles idiopathic psychosis in humans. There is also evidence that former AMPH abusers are particularly vulnerable to psychotic relapse upon exposure to psychological stress or acute re-exposure to the drug. The present study examined the possibility that dopaminergic mechanisms in the prefrontal cortex are involved in these phenomena. Rats received injections of saline (14 days) or AMPH (2 mg/kg 7 days and 4 mg/kg 7 days i.p.). After 7 days of drug abstinence, extracellular dopamine (DA) concentrations were measured in the prefrontal cortex and nucleus accumbens using *in vivo* microdialysis in freely moving rats. Mild foot shock stress (0.4 mA 10 sec/min 20 min) produced significantly greater increases in extracellular DA concentrations in the prefrontal cortex of AMPH pretreated rats than in controls. In contrast, compared to controls an AMPH (2 mg/kg) challenge produced significantly greater increases in extracellular DA concentrations in the nucleus accumbens but not in the prefrontal cortex. These results are consistent with previous reports by demonstrating that chronically pretreated rats exhibit an augmented dopaminergic response in nucleus accumbens upon re-exposure to AMPH. They also suggest that the mesocortical dopaminergic system is involved in cross-sensitization between AMPH and stress, but not in AMPH behavioral sensitization. (Supported by the Medical Research Council (PG23) and Bristol-Myers/Squibb).

197.12

ESTROGEN MODULATION OF STIMULATED DOPAMINE RELEASE MEASURED BY IN VIVO VOLTAMMETRY. T.L. Thompson* and R.L. Moss. Dept. Physiology, UT Southwestern Medical Center, Dallas, TX 75235.

Estrogen (E) modulation of dopaminergic (DA) neurons is well documented. In vitro experiments have demonstrated that E can increase DA turnover and release, as well as alter both pre- and post-synaptic DA receptors (Levesque et al. 1989, Fernandez-Ruiz et al. 1989, Becker 1990). Particular interest exists in elucidating the role of E on the modulation of nigrostriatal and mesolimbic dopaminergic neurons. In the present study we examined the effect of E on in vivo K^+ stimulated DA release in ovariectomized rats using high speed voltammetry (5Hz, IVEC-5). Nafion coated multifiber carbon expoxy electrodes, attached to multibarrel glass pipets, were lowered into the striatum and nucleus accumbens of urethane anesthetized rats. DA release was stimulated by pressure ejection of KCl (70mM) from one barrel of the micropipet. Both genomic (E primed, 5ug, 48hrs) and non-genomic (E pressure ejected 15min. prior to K^+) mechanisms were studied. E priming: 1.) caused a slight reduction in the total amount of DA released; 2.) resulted in an increase clearance of DA; and 3.) decreased the disparity between the amount of DA released on the right and left sides of the accumbens which was observed in control animals. In contrast, there was no change in the magnitude nor time course of stimulated DA release measured over 1hr after a single ejection of E ($1x10^{-3}$g/ml) in the striatum or the accumbens. Basal DA release was not effected by E infusion. These data suggest that E modulation of nigrostriatal and mesolimbic DA neurons may be due at least in part, to an alteration in reuptake mechanisms and that this modulation requires long term exposure. (MH47418)

197.13

EFFECTS OF THE INHIBITION OF DOPAMINE UPTAKE ON SYNAPTIC TRANSMISSION AND ON EXTRACELLULAR CATECHOLAMINE CONCENTRATIONS WITHIN THE SUPERIOR CERVICAL GANGLION OF THE RAT. C.G. Acosta-Farrar*[2] P. Pantano[3] J.H. Ashe[1,2] W.G. Kuhr[3]. Departments of Neuroscience[1], Psychology[2] and Chemistry[3], University of California, Riverside, CA 92521.

The objective of this study was to investigate the effects of dopamine uptake inhibitors on synaptic transmission and on stimulation induced changes in extracellular catecholamine concentrations within the superior cervical ganglion (SCG) of the rat. This was accomplished using both electrophysiological and electrochemical recording techniques.

Following incubation of the ganglion with the dopamine uptake inhibitor, GBR-12909 (5μM), the amplitude of the slow-inhibitory postsynaptic potential was selectively reduced. The amplitudes of the fast-excitatory postsynaptic potential and the slow-excitatory postsynaptic potential were not changed following GBR-12909 incubation. During the same time, the preganglionic volley also remained unchanged.

In separate experiments fast-scan cyclic voltammetry was used to examine stimulation induced changes in extracellular catecholamine concentrations within the SCG. In the presence of GBR-12909, an increase in oxidative current was recorded during and for a short period following electrical stimulation (1 to 20 Hz for 0.5 to 1.0 s) applied to ganglionic afferents. This suggests that an increase in the extracellular concentration of an oxidizable substance(s) had occurred. Voltammetric analysis suggests that a catecholamine(s) was the principle substance generating this oxidative current. In GBR-12909-free medium, stimulation-induced changes in oxidative currents were inconsistent and could not be identified as originating from catecholamine oxidation.

These results are consistent with the possible release of catecholamines either from small intensely fluorescent cells and/or principal cells within the SCG as a result of preganglionic nerve stimulation.

197.14

PHARMACOLOGICAL CHARACTERIZATION OF MONOAMINE RELEASE IN THE HIPPOCAMPUS: AN *IN VIVO* MICRODIALYSIS STUDY. K. Garlepy[1], I. Acworth[1&2]*, J. Yu[2], and T. Maher[2]. [1]ESA Inc., Bedford, MA 01730 and [2]Massachusetts College of Pharmacy, Longwood Ave., Boston, MA 02155.

The ability to routinely and accurately measure different neurotransmitters concurrently in a single brain region using one analytical system has always been desirable. We have developed a method capable of simultaneously quantitating norepinephrine (NE), dopamine (DA), and serotonin (5HT) at subpicogram levels, in under 8 minutes using conventional HPLC coupled to coulometric electrochemical detection. We have used microdialysis perfusion to examine the effects of a variety of receptor agonists on the ECF levels of monoamines in the hippocampus. Rats were anethetized with urethane and had a precallibrated 3mm "loop" type probe stereotaxically implanted into the right hippocampus. Basal concentrations of NE, DA, and 5HT were found to be approximately 2.25, 1.05, and 4.6 pg (per collection) respectively. The peripheral administration of the α_2 agonist clonidine (0.3 mg/hg i.p.) caused a major decrease in NE levels by approximately 70% within 40 minutes. Clonidine also decreased DA levels but had little effect on those of 5HT. The subcutaneous administration of the D2 agonist, apomorphine (0.5 mg/hg in 0.1 μM ascorbate), decreased DA levels by greater than 75%. More interestingly, NE levels decreased to below detection limits. In a separate experiment the 5HT agonist (±)-8-Hydroxy-2-(di-n-proplyamino) tetralin (8-OH-DPAT) (2 mg/hg i.p.) caused a major decrease in 5HT levels by greater than 50% while levels of NE increased.

These data suggest that the measured chemicals be neuronal in origin. Using this method, it is possible to examine potential interaction between transmitter systems. By assaying for monoamines only improved sensitivity to approximately 300 fg can be obtained.

197.15

SIMULTANEOUS MONITORING OF ELECTRICALLY STIMULATED RELEASE AND REUPTAKE OF NOREPINEPHRINE FROM MULTIPLE REGIONS OF RAT BRAIN. K.M. Mitchell* and R.N. Adams. Dept. of Chemistry, University of Kansas, Lawrence, KS 66045.

The aim of the present work was to quantitatively compare the temporal characteristics of norepinephrine (NE) release and reuptake in multiple regions of rat brain innervated by locus-coeruleus noradrenergic neurons. Chronoamperometry at a carbon fiber electrode allowed real time detection of NE overflow in the extracellular fluid elicited by electrical stimulation of the dorsal bundle ascending from the locus coeruleus in the urethane anesthetized rat. The kinetic parameters determined from the experimental data were used to accurately simulate the evoked responses using a model developed previously to characterize electrically stimulated dopamine overflow (Wightman et al., Brain Res. Rev. 15: 135, 1990).

Norepineprine release and reuptake were examined in the following brain regions: anterior ventral thalamus, lateral geniculate, stria terminalis, substantia innominata and cingulate cortex. All of the brain regions showed slight differences in the amount of NE release and rates of uptake. However, cortical responses were strikingly different with significantly smaller rates of uptake than in subcortical regions.

197.16

LOCAL CONTROL OF THE RELEASE OF NOREPINEPHRINE IN THE CNS: THEORY AND RESULTS. C.R. Collazo, E.A. Witte, and R.T. Marrocco *. Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

The notion of transmitter release regulated not only by electrical activity in the parent neuron but also by mechanisms in the vicinity of the terminal bouton has received increasing support (e.g., Nelson, et al, 1980). We have recently proposed two mechanisms, levels of extracellular K+ and glutamate (glu), to account for the local control of norepinephrine release in the visual cortex of anesthetized macaque monkeys. Here, we present key elements of the theory and some recent empirical results.

The cerebral cortex receives specific inputs from the thalamus and from the locus coeruleus (LC). Local control theory asserts that activity in cortical neurons modulates the release of norepinephrine (NE) from locus ceruleus terminals. Several specific predictions may be made: a) the ocular dominance and orientation selectivity of both intrinsic activity and NE release should be similar; b) exogenous K+ or glu should enhance stimulus-evoked electrical activity and NE release for both approriate and inappropriate visual stimuli; c) focal electrical stimulation of the lateral geniculate nucleus should excite V1 neurons and evoke NE release at retinotopically matched sites but not at mismatched sites; d) pharmacological blockade of LC somata should not block the release of NE produced by activation of intrinsic neurons or by local application of K+ or glu.

Predictions (a) and (b) have been empirically verified (Marrocco et al, 1987). Recent results from our lab suggest that reversible blockade of LC does not prevent local release of NE in parietal association cortex of anesthetized albino rats, thus supporting prediction (d). Our data thus suggest that local control mechanisms may modulate local NE release in visual cortex. Supported by the McDonnell-Pew Foundation.

197.17

OMEGA CONOTOXIN (ωCT) AND α_2-ADRENOCEPTOR AGONISTS (AAA) IMPAIR SYNAPTIC TRANSMISSION IN THE PERIPHERAL SYMPATHETIC NERVOUS SYSTEM (SNS) BY N-TYPE CALCIUM CHANNEL BLOCKADE (N-CCB). H.K. Schedewie, N. Boban, J.P. Kampine* and Z.J. Bosnjak . Department of Anesthesiology, The Medical College of Wisconsin, Milwaukee, WI 53226.

We have previously shown that ωCT and AAA, like dexmedetomidine (DMT), impair synaptic transmission in the peripheral SNS by N-CCB. The present investigation was designed to further delineate the site(s) and mechanism(s) of these ωCT and DMT actions. 10 stellate ganglia (SG) were isolated from adult mongrel dogs, desheathed and superfused with Krebs' buffer at pH 7.4 and 37°C. Compound action potentials (CAP) were generated by supramaximal stimulation of the preganglionic T_3 ramus and were recorded from the ventral ansa subclaviae. The percent CAP change from control was measured after SG superfusion in the following order with DMT-ATI-**DMT**-ω**CT**-ATI in a first set of experiments versus DMT-ATI-ω**CT-DMT**-ATI in a second set. Superfusate drug concentrations of ωCT $64x10^{-9}$M and DMT 10^{-6}M were those determined in dose-response studies to cause maximal CAP depression; the concentration of the α_2-antagonist atipamezole (ATI) 10^{-6}M was selected to be equimolar to DMT. Results: 1) DMT caused rapid onset CAP depression, that did not resolve by washout alone until specifically reversed by ATI; 2) ATI reversal of CAP depression was prompt and complete; 3) ωCT caused slow onset CAP depression, that was not reversible by washout or ATI; 4) ωCT administered subsequent to DMT caused insignificant additional CAP depression suggesting that both ωCT and DMT act on the N-CC; and 5) ATI was able to reverse the CAP depression resulting from the combined effect of DMT followed by ωCT, but failed to reverse the effect of ωCT followed by DMT. This suggests that DMT and ωCT may have close interactions at the receptor complex that controls calcium transport across N-CC.

SEROTONIN RECEPTORS: MOLECULAR BIOLOGY

198.1

CLONING AND CHARACTERIZATION OF A SEROTONIN RECEPTOR FROM *LYMNAEA STAGNALIS* K.S. Sugamori, R.K. Sunahara, H.C. Guan, A.G.M. Bulloch^, P. Seeman, S.J. Kish*, H.B. Niznik and H.H.M. Van Tol. Dept. of Pharmacology and Psychiatry, University of Toronto, ^Dept. of Medical Physiology, University of Calgary, Calgary, Canada, T2N 4N1, and The Laboratory of Molecular Neurobiology, Clarke Institute of Psychiatry, Toronto, Canada, M5T 1R8.

Serotonin is a major neurotransmitter in molluscs that is involved in many behaviours such as learning and memory. We have cloned a serotonin receptor (5HTlym) from the pond snail *Lymnaea stagnalis* using a strategy based on the high homology of genes encoding members of the superfamily of G-protein coupled receptors and the polymerase chain reaction. The receptor protein is composed of 509 amino acids and displays high homology to the mammalian $5HT_1$ family of serotonin receptors and to the *Drosophila* serotonin receptors. Northern blot analysis revealed the presence of two mRNA species of 2.3 and 3.2 kb in the central nervous system of *Lymnaea*. Membranes prepared from COS-7 cells transiently expressing the receptor bound [^{3}H]-LSD in a saturable manner with high affinity (Kd~0.9 nM), and exhibited a distinct pharmacological profile. The rank order of potency for inhibition by various serotonergic agonists and antagonists is as follows: methiothepin > lisuride > LSD > clozapine > ergotamine > metergoline > 5CT > SCH23390 > 5HT > quipazine > ketanserin > noradrenaline > dopamine. The pharmacological profile displayed by 5HTlym suggests that this invertebrate serotonin receptor represents a novel 5HT receptor that cannot be classified according to the classification scheme used to define mammalian serotonin receptors.

198.2

MOLECULAR ANALYSIS OF SEROTONIN RECEPTORS IN APLYSIA C.Bessho*,T.Shirai and T.Manabe# Dept. of Physics, Kyoto Sangyo Univ. and #Dept. of Physiology, Kyoto Univ. Sch. of Med., Kyoto 603

In Aplysia gill withdrawal refrex, serotonin (5HT) evokes presynaptic facilitation of sensory to motor neuron synapses. The 5HT receptors were analyzed by their functional expression of the gill mRNAs in Xenopus oocytes and assayed by electrophysiological method. The application of 5HT to mRNAs injected oocytes elicited an oscillatory current after a delay. The reversal potential of this current corresponded with that of chrolide current.The cDNA libraries from Aplysia abdominal ganglia were probed with the cDNA coding a human 5HT-1A receptor (G21, a gift from Dr. Lefkowitz, J.) at low stringency. The isolated cDNAs were amplified by PCR with degenerate primers corresponding to consensus DNA sequences of the third and sixth transmembrane segments of G protein-coupled receptors. The amplified DNA fragments (400-600 b.p) were subcloned in the vectors. The insertion DNAs were sequenced by Sanger's method. We are screening Aplysia cDNA libraries with the DNA fragments homologous to G21 for full length clones.

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198.3

EXPRESSION OF RECOMBINANT SEROTONIN RECEPTORS IN THE BACULOVIRUS-INSECT CELL SYSTEM. Eric M. Parker*, Darcy A. Grisel and Lawrence G. Iben. Department of Biophysics and Molecular Biology, Bristol-Myers Squibb Co., Wallingford, CT 06492

Three human serotonin receptor subtypes ($5\text{-}HT_{1A}$, $5\text{-}HT_{1D\alpha}$ and $5\text{-}HT_{1D\beta}$) have been expressed in the baculovirus-insect cell system. Sf9 cell membranes containing the $5\text{-}HT_{1A}$ receptor displayed both high (K_D=1.0 nM) and low (K_D=26 nM) affinity binding sites for the agonist radioligand [^{3}H]8-OH-DPAT. GTPγS (100 μM) decreased radioligand binding to the high affinity site by 80%. The $5\text{-}HT_{1A}$ receptor produced by the insect cells had the appropriate affinity for a variety of standard ligands. Western blots of insect cell membranes containing the $5\text{-}HT_{1A}$ receptor revealed 4-5 immunoreactive bands at 40-50 kDa. In contrast, insect cell membranes containing the $5\text{-}HT_{1D\alpha}$ and $5\text{-}HT_{1D\beta}$ receptors displayed a single high affinity binding site for the radioligand [^{3}H]5-CT (K_D=0.5 nM in both cases). Binding to these receptors was relatively insensitive to the inclusion of guanine nucleotides and/or NaCl in the binding assay (20-30% inhibition). The ligand binding properties of the two $5\text{-}HT_{1D}$ receptors expressed by the insect cells is similar. Metergoline is the most selective compound identified thus far (10 fold higher affinity for the $5\text{-}HT_{1D\alpha}$ receptor). It is concluded that serotonin receptors can be successfully expressed in the baculovirus-insect cell system. These receptors apparently can interact with G proteins in the insect cell membrane and thus can be detected with available agonist radioligands. This system should be a useful for the production of high levels of receptors for purification and pharmaceutical screening.

198.4

RECEPTOR RESERVE MASKS PARTIAL AGONIST ACTIVITY OF DRUGS IN A CLONED RAT $5\text{-}HT_{1B}$ RECEPTOR EXPRESSION SYSTEM. N. Adham, B. Ellerbrock, P. Hartig, R. Weinshank and T. Branchek'. Synaptic Pharmaceutical Corporation, Paramus, N.J. 07652

We have previously cloned and characterized a rat $5\text{-}HT_{1B}$ receptor (Adham et al., 1992) and subsequently observed several divergences from the expected pharmacological actions of known drugs at these receptors. For example, the β-adrenergic antagonists propranolol and pindolol (reported to be partial agonists or antagonists at native $5\text{-}HT_{1B}$ receptors) were found to be full agonists at the cloned $5\text{-}HT_{1B}$ receptor. In addition, the EC_{50} values for agonists (determined by measuring drug-induced inhibition of forskolin-stimulated cAMP release) were lower than expected from the measured K_i values determined from [^{125}I]iodocyanopindolol binding (e.g. 5-HT: EC_{50} = 0.49 ± 0.043, n=6; Ki = 5.3 ± 0.82, n=4). To investigate the degree of receptor reserve, we used the irreversible receptor antagonist N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). EEDQ treatment shifted the dose-response curve for 5-HT to the right by 5-8 fold accompanied by a reduction (20-50%) in maximal response. Furchgott analysis revealed a very steep hyperbolic relationship between receptor occupancy and response for 5-HT with 96 ± 1.4% (n=3) receptor reserve at the 50% maximal response level. In contrast, EEDQ treatment did not significantly shift the dose-response curves for pindolol. The maximal responses were reduced and a linear relationship was found between receptor occupancy and response for this compound. According to classical receptor theory, these data indicate that pindolol acts as a partial agonist relative to 5-HT but due to the large number of receptors present, the difference in the intrinsic activities of the two drugs is masked. Therefore, one has to be cautious when interpreting functional data in transfected systems that may possess large receptor reserves.

198.5

CLONING AND CHARACTERIZATION OF A NOVEL 5-CT INSENSITIVE ($5HT_{1E}$) RECEPTOR. M. Teitler*, K. J. Miller and B.J. Hoffman+, Dept. of Pharmacology and Toxicology, Albany Medical College, 47 New Scotland Avenue, Albany, N.Y. 12208.+Lab. Cell Biol., NIMH, Bethesda, MD.

Multiple serotonin receptors have been detected, originally using radioligand binding methodologies and second messenger systems, and more recently using recombinant DNA technology. These multiple receptors have been sub-divided into four families: $5HT_1$, $5HT_2$, $5HT_3$, and $5HT_4$ receptors, based on various pharmacological criteria.The potency of the tryptamine derivative 5CT (5-carboxy-amidotryptamine) was at one time used to define receptors belonging to the $5HT_1$ receptor family. However in 1989 a receptor was detected using radioligand binding methodology that fulfilled other criteria for a $5HT_1$ receptor, but displayed low affinity for 5CT. This receptor was designated "$5HT_{1E}$" (1). Recently the sequence of a clone displaying properties typical of a G-protein coupled receptor and displaying a fairly high degree of homology with previously cloned 5HT receptors was reported (2). In order to determine the properties of the receptor coded for by this sequence primers were designed 5' and 3' to the coding region, and the human gene was amplified by PCR. After subcloning, stable transfectants were produced. Preliminary data suggests that this receptor displays high affinity for 5HT (~nM) and low affinity for 5CT (~μM). Detailed pharmacological properties of this clone will be presented. (Supported by PHS grant no. MH40716)

1. S. Leonhardt, K.H. Davis, and M. Titeler, J. Neurochem. 53, 465-471 (1989)

2. Levy, F.O., T. Gudermann, M. Birnbaumer, A.J. Kaumann, and L. Birnbaumer, FEBS letters *296*: 201-206 (1992).

198.6

Agonist Activity of Sumatriptan and Metergoline at the Serotonin $5\text{-}HT_{1D\beta}$ Receptor: Evidence for a Role of a $5\text{-}HT_{1D}$ Receptor in the Anti-Migraine Action of Sumatriptan.

K.J. Miller*, A. King#, L. Demchyshyn#, H. Niznik+$, and M. Teitler*. *Dept. of Pharm/Tox, Albany Medical College, Albany, NY 12208 and Dept. of Psychiatry+ and Pharmacology#, University of Toronto, and the Molecular Neurobiology Laboratory$, The Clarke Institute of Psychiatry, Toronto, Ontario M5T-1R8.

We have recently cloned a novel human $5\text{-}HT_{1D}$ receptor subtype termed $5\text{-}HT_{1D\beta}$. CHO K1 cells expressing the serotonin $5\text{-}HT_{1D\beta}$ receptor were assayed to determine the second messenger of this receptor. Cyclic AMP radioimmunoassays revealed that the $5\text{-}HT_{1D\beta}$ receptor is negatively coupled to adenylate cyclase in this cell system, typical of the $5\text{-}HT_1$ receptor sub-family. A maximum of 50% inhibition of forskolin stimulated cAMP production was obtained with $5\text{-}HT_1$ receptor agonists which was blocked by the non-selective 5-HT receptor antagonist methiothepin, no stimulatory activity was detected. The novel anti-migraine drug sumatriptan, a putative $5\text{-}HT_{1D}$ selective compound, acted as an agonist at the $5\text{-}HT_{1D\beta}$ receptor (EC_{50}=317±90). Most notably metergoline, a putative $5\text{-}HT_1$ receptor antagonist, did not block the effects of 5-HT and was found to be acting as an agonist at the $5\text{-}HT_{1D\beta}$ receptor (EC_{50}=98±9). The ability of metergoline to act as an agonist may explain why it does not inhibit 5-HT and sumatriptan induce contraction of dog saphenous vein and other large conducting arteries. These results suggest that the $5\text{-}HT_{1D\beta}$ receptor may be the site of action of sumatriptan in preventing migraine, and that metergoline's actions on the dog saphenous vein are not contradictory to that hypothesis, as previously reported. (Supported by MH 40716 to M.T.)

198.7

LIGAND BINDING DOMAINS OF THE RAT $5HT_{1C}$ RECEPTOR DETERMINED BY SINGLE SITE MUTATION. S. Leonhardt*, B.J. Hoffman^ and M. Teitler, Dept. of Pharmacology & Toxicology, Albany Medical College, Albany, N.Y. 12208; ^Laboratory of Cell Biology, NIMH, Bethesda, MD, 20892.

$5HT_{1C}$ and $5HT_2$ receptors appear to be closely related from a pharmacological as well as molecular viewpoint, displaying similar pharmacological profiles, identical second messenger systems and a high degree of amino acid sequence homology. However there are striking differences in the interactions of 5HT with $5HT_{1C}$ and $5HT_2$ receptors: 5HT is generally 10-fold more potent in stimulating responses mediated through $5HT_{1C}$ receptors than in stimulating responses mediated through $5HT_2$ receptors. The affinity and potency of 5HT for the $5HT_{1C}$ receptor appears to be similar to other members of the $5HT_1$-receptor family. In aligning the seven transmembrane domains of the cloned mammalian $5HT_1$ and $5HT_2$ receptor families we noticed an arginine residue identical in all 5HT receptors displaying high affinity and potency for 5HT. In the mammalian $5HT_2$ receptor and in the $5HT_{DROSOPHILA}$, receptors with affinities for 5HT in the micromolar range, the arginine has been replaced by glutamine and glycine, respectively. Using recombinant PCR we have constructed a mutant $5HT_{1C}$ receptor with a glutamine residue in transmembrane domain IV. We predict that mutating the arginine residue of the $5HT_{1C}$ receptor to a glutamine will significantly decrease the affinity and potency of 5HT for the $5HT_{1C}$ receptor. The results of these and other site-specific mutagenesis studies should reveal the molecular determinants of 5HT affinity and potency at serotonin receptors.

198.8

REGULATION OF mRNA FOR $5\text{-}HT_2$ RECEPTORS IN P11 CELLS BY AGONISTS. **R. C. Ferry*, R. Artymyshyn and P. B. Molinoff, Dept. of Pharm., Univ of Penn. School of Med., Phila., PA 19104**

In several experimental paradigms, central $5\text{-}HT_2$ receptors exhibit anomalous regulatory responses. Paradoxical changes in the number of $5\text{-}HT_2$ receptors following various drug treatments and experimental manipulations may be the result of unique regulatory mechanisms or may arise from complexities of *in vivo* studies. To investigate the anomalous regulation of the $5\text{-}HT_2$ receptor, this laboratory has isolated a cell line (P11 cells) which expresses a high density of $5\text{-}HT_2$ receptors coupled to stimulation of phosphatidylinositol hydrolysis. We have previously used this cell line as a model system to demonstrate that in contrast to reports of studies carried out *in vivo*, antagonists at this receptor, including ketanserin and mianserin, do not alter the density of $5\text{-}HT_2$ receptors. Both full (5-HT) and partial (LSD, DOI) agonists, however, down-regulate the receptor with similar efficacy, an effect that has been observed *in vivo*. The mechanisms responsible for agonist-induced regulation of $5\text{-}HT_2$ receptors are not known. Changes in $5\text{-}HT_2$ receptor messenger RNA (mRNA) could occur and may be responsible for agonist-dependent changes in the density of receptors. To quantify $5\text{-}HT_2$ receptor mRNA in P11 cells, a ribonuclease protection assay was used. A 200-bp cDNA insert encoding a portion of the i3 loop of the $5\text{-}HT_2$ receptor was amplified from full-length $5\text{-}HT_2$ receptor cDNA using the polymerase chain reaction. This insert was ligated into pGEM7Z-f(+) to generate a recombinant plasmid that was used as a template to generate radiolabeled riboprobes specific for the $5\text{-}HT_2$ receptor. Exposure of P11 cells to 5-HT led to a time- and dose-dependent increase in $5\text{-}HT_2$ receptor mRNA. A significant increase in levels of receptor mRNA was seen as early as 30 min after exposure to 10 μM 5-HT and was maximal at 1.5 hr (> 2-fold). An increase in $5\text{-}HT_2$ receptor mRNA levels was also observed following exposure to 10 nM 5-HT, although greater increases in receptor mRNA levels were observed at higher doses of 5-HT. The increase in receptor mRNA was transient, returning to basal levels by 16 hr. $5\text{-}HT_2$ receptor mRNA was not decreased below control levels even following prolonged incubation with 5-HT. The $5\text{-}HT_2$ receptor antagonist ketanserin antagonized the effect of 5-HT while having no effect by itself. (Supported by USPHS Grants MH 48125 and NS 18591.)

198.9

DESENSITIZATION OF THE $5\text{-}HT_3$ RECEPTOR IS ALTERED BY A SINGLE AMINO ACID SUBSTITUTION. J.L. Yakel*, A. Lagrutta, J.P. Adelman, R.A North. Vollum Institute, Oregon Health Sciences University, Portland, OR. 97201

The cloned $5\text{-}HT_3$ receptor (Maricq et al., Science: 254, 432, 1991) was expressed in Xenopus oocytes to study the relation between primary structure and the kinetics of desensitization. 5-HT (300 nM to 100 μM) evoked an inward current at -60 mV, which became larger with hyperpolarization and reversed near 0 mV. The current decayed by 50% during the continual application of 5-HT (30 μM) in 47 ± 3 sec (mean ±s.e.m.; n=13). The site-directed mutant L286F, whereby the leucine at position 286 was replaced by phenylalanine, desensitized much faster (the current decayed by 50% in 1.2 ± 0.1 sec [n=9]), while the L286T mutant (a leucine-to-threonine substitution) desensitized much slower (the current decayed by 19 ± 4% [n=9] during a 1 min application of 5-HT). The concentration of 5-HT that produced a half-maximal current was not greatly altered by these mutations. The direction of these changes in desensitization kinetics is similar to that observed for the neuronal nicotinic receptor expressed in Xenopus oocytes (Revah et al., Nature: 353, 846, 1991), in which smaller, more polar amino acid substitutions at the equivalent position reduced the kinetics of desensitization.

198.10

5-HT1B and 1D RECEPTORS IN MAMMALIAN BRAIN: VISUALIZATION OF THEIR mRNAs AND BINDING SITES BY IN SITU HYBRIDIZATION AND LIGAND BINDING AUTORADIOGRAPHY. R. Cortés[1]*, G.Mengod[1], B. O'Dowd[2], F. Artigas[1], and J.M. Palacios[3]. [1]C.I.D., Centro Investigación y Desarrollo, C.S.I.C., Jordi Girona,18-26, 08034 Barcelona (Spain), [2]Dept. of Pharmacology, University of Toronto, Ont. Canada M5S 1A8. [3]Research Institut Almirall Laboratories, Cardener, 68-74, 08024 Barcelona (Spain).

Cloning of the genes coding for 5-HT1B and 5-HT1D receptors in several mammalian species has shown that these receptors, although presenting marked species differences in their pharmacology, are homologous members of a sub-family of 5-HT receptors. We have used oligonucleotide probes from the sequences of the 5-HT1B and 1D genes to visualize the distribution of these two receptors mRNAs in the rat and other mammalian species including man. In parallel, the distribution of putative 5-HT1B and 1D sites has been studied using receptor autoradiography with ligands such as ^3H 5-HT, ^{125}I -GTI and ^{125}I CYP in the presence of selective displacers in all the species examined. Both receptors were predominant in the basal ganglia but not in the substantia nigra. Furthermore, both receptors mRNAs were also expressed in the nucleus raphé dorsalis.5-HT1B receptor mRNA was much more abundant than 1D in the other brain regions. Mismatches between mRNA and binding sites were observed. These studies indicate areas in the mammalian brain where 5-HT1B receptors can be studied separately from 1D sites.

198.11

EXPRESSION IN BACTERIA OF RECOMBINANT HUMAN SEROTONIN RECEPTORS OF THE 5-HT_{1A} AND 5-HT_{1D} SUBTYPE. W.H.M.L. Luyten*, I. Van de Weyer, W. Gommeren and J.E. Leysen. Department of Biochemical Pharmacology, Janssen Research Foundation, Beerse, B2340 Belgium.

The coding region of a human 5-HT_{1A} receptor clone, purchased from TULCO (Durham, North Carolina, U.S.A.), was inserted into the pMalp bacterial expression vector (New England Biolabs, Massachusetts, U.S.A.). A full-length human 5-HT_{1D} receptor cDNA was amplified by the polymerase chain reaction and also inserted into the pMalp vector. Both constructs generate MalE fusion proteins, whose N-terminus should be translocated to the periplasm. Bacteria harbouring these expression constructs were maintained in rich media supplemented with glucose (1%) and ampicillin (100 µg/ml). Induction of early log-phase cultures with isopropyl-β-D-thiogalactopyranoside led to a rapid flattening of the growth curve and progressive expression of the above-mentioned receptor proteins as judged by radioligand binding. Maximal expression was reached after 3-4 hours for the 5-HT_{1A}- and after ± 2 hours for the 5-HT_{1D}-receptor.

The 5-HT_{1A} receptor, measured with 2-(N,N-di[2,3(n)-^{3}H]propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphtalene (8-OH-DPAT), displayed an affinity of 10-20 nM when expressed in bacteria, compared to 0.5-1 nM when expressed in the HA-7 eukaryotic cell line or measured in rat hippocampal membranes and 1.5-3 nM when the non-hydrolysable GTP-analogue GppNHp was added to the latter two preparations. For a large number of reference compounds the affinity (K_i, calculated from experimentally derived IC_{50}s) was 10-100-fold lower in bacteria, compared to the HA-7 cells or hippocampal membranes. However, for ergotamine, spiperone and metitepine, affinities were comparable in the three systems. The 5-HT_{1D} receptor, measured with 5-hydroxy[G-^{3}H]tryptamine, displayed an affinity of ±5 nM when expressed in bacteria or COS cells, compared to ±0.5 nM when measured in calf substantia nigra membranes. The K_is for sumatriptan and metergoline were comparable in the three systems.

Most likely, the measured affinity differences result from differences in the presence of or interaction with appropriate G-proteins, which allosterically modulate receptor affinity for agonists.

198.12

A NOVEL 5–HT RECEPTOR SUBFAMILY MEMBER HIGHLY ENRICHED IN THE HIPPOCAMPUS. M.G. Erlander*[1], T.W. Lovenberg[1], B.M. Baron[2], M.W. Dudley[2], K. Cannon[1], P.E. Danielson[1], J.E. Burns[1], C.M. Craft[3], J.G. Sutcliffe[1]. [1]Dept. Mol. Biol., The Scripps Res. Inst., La Jolla, CA 92037. [2]Marion Merrell Dow Res. Inst., Cincinnti, OH 45215. [3]Univ. Texas Southwestern Med. Sch., Dallas, TX 75235.

We have used a polymerase chain reaction (PCR)–based strategy to isolate novel cDNA clones of mRNAs encoding 5–HT receptor–like proteins (seven transmembrane family). By using degenerate primers specific to known 5–HT receptors, we isolated two PCR cDNAs from rat brain designated MR22 and MR77 (for description of MR77 see abstract of Lovenberg et al.). A single full–length clone was initially isolated from rat hypothalamus cDNA; MR22 encodes a 370 amino acid protein with 30–34% overall sequence identity with known catechol and 5HT receptors. The tissue distribution of MR22 mRNA is highly enriched in hippocampus (10–20 fold greater) when compared to hypothalamus, thalamus and pons and MR22 mRNA is not detectable in cortex, striatum, cerebellum as well as liver, kidney and heart. Transient expression of MR22 cDNA in CosM6 cells suggests that MR22 encodes a 5–HT receptor in that > 80% of the binding of [^{125}I]–LSD could be inhibited by 100 uM 5–HT (IC_{50} = 1 uM). In addition, binding was sensitive to 5–carboxyamidotryptamine (IC_{50} = 230 nM) and methiothepin (IC_{50} = 82 nM), but insensitive to sumatriptan (IC_{50} > 1 uM). The pharmacological data suggest that MR22 cDNA encodes a novel 5–HT receptor and may represent a unique 5–HT receptor subfamily.

198.13

CLONING AND FUNCTIONAL EXPRESSION OF A NOVEL RAT 5–HT_1 –LIKE RECEPTOR. T.W. Lovenberg*[1], M.G. Erlander[1], B.M. Baron[2], M.W. Dudley[2], P.E. Danielsen[1], J.E. Burns[1], C.M. Craft[3], J.G. Sutcliffe[1]. [1]Dept. Mol. Biol., The Scripps Res. Inst., La Jolla, CA 92037. [2]Marion Merrell Dow Res. Inst., Cincinnati, OH 45215. [3]Univ. Texas Southwestern Med. Sch., Dallas, TX 75235.

In order to identify new members of the 5–HT receptor family, we used a polymerase chain reaction (PCR) with highly degenerate primers corresponding to conserved transmembrane domains three and five of known 5–HT receptors. This approach resulted in two novel sequences, MR77 and MR22 (For description of MR22, see abstract of Erlander et al.). PCR–amplified cDNA was used to isolate a full length genomic clone (MR77) containing an intronless open reading frame encoding a putative 366 amino acid protein related to the rat 5–HT_{1A}, 5–HT_{1B}, rat 5–HT_{1C}, dog 5–HT_{1D}, and human S31 receptors with 34%, 46%, 32%, 50%, and 56% overall identity, respectively. Greater than 90% of the binding of [^{125}I]–LSD to membranes prepared from MR77–transfected CosM6 cells could be inhibited by 100 uM 5–HT (IC_{50} = 59 nM). Binding data both confirms the similarity to the 5–HT_{1D} receptor (sumatriptan IC_{50} = 64 nM) and helps to differentiate the two binding sites [e.g., MR77 appears to be insensitive to 5–Carboxyamidotryptamine (5–CT)]. The amino acid structure of MR77 (long TM 5–6 intracellular loop and a short C–terminal tail) suggested that it may be coupled to the inhibition of adenylate cyclase through a G_i–like G–protein. In HeLa cells cotransfected with MR77 and the hamster Beta–adrenergic receptor, 5–HT (10 uM) inhibited isoproterenol stimulated cAMP accumulation (32%) compared to wild type cells (8%). Together, this data suggests that MR77 is a novel 5–HT receptor in the 5–HT_1 subfamily. The initial pharmacological characterization indicates that MR77 is similar to the 5–HT_{1E} receptor by virtue of its low affinity for 5–CT. Further studies should clarify the correct classification of this unique receptor.

198.14

QUANTITATIVE ESTIMATION OF 5HT_2 RECEPTOR mRNA IN RAT GLIOMA C6BU-1 CELLS BY THE RT-PCR METHOD. M. Ikeda*, M. Mikuni, K. Saitoh, C. Yamazaki and K. Takahashi. Div. Mental Disorder Res., Natl. Inst. Neurosci. NCNP, Kodaira, Tokyo 187, Japan

We have previously shown that 4-hour treatment with 5HT attenuated 5HT-stimulated Calcium mobilization and 24-hour treatment with dexamethasone enhanced it in rat glioma C6BU-1 cells. To evaluate the transcriptional regulation of 5HT_2 receptor in C6BU-1 cells, we applied RT-PCR method to quantify it's mRNA. Total RNA from C6BU-1 cells was extracted by the method of Gough. Priming with random hexamers was used to synthesize cDNA. To normalize sample-to-sample variations, we co-amplified ß-actin cDNA. One primer of each set was radiolabeled by 5' end-labeling. To avoid excess amplification of ß-actin cDNA, primer set for amplification of ß-actin cDNA was added after 15 cycles. The products of PCR were removed after various cycles and fractionated by polyacrylamide gel. Radioactivity was measured by the Bio-imaging analyzer (Fujix BAS2000). Work is in progress to determine the effect of 5HT and dexamethasone on the transcriptional regulation of 5HT_2 receptor gene.

198.15

GENERATION OF A cDNA PROBE SPECIFIC FOR THE 5-HT_{1B} RECEPTOR IN THE OK CELL, AN OPOSSUM KIDNEY CELL LINE. D.R. Cerutis,* N.A. Hass and D.B. Bylund, Dept. of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE 68198-6260.

Serotonin is a neurotransmitter which mediates a wide range of effects by interacting with multiple receptor subtypes. Previous studies from our laboratory have demonstrated the presence of the serotonin 5-HT_{1B} receptor in the OK cell line, an established renal proximal tubule epithelial cell line. In order to generate a probe for this receptor, the published sequences of the canine 5-HT_{1D} and the rat 5-HT_{1B} were used to design degenerate primers directed at the first and sixth transmembranes (TM) domains. OK cell RNA was isolated using standard guanidinium thiocyanate methodology. Approximately 2 µg of this RNA was reverse transcribed using AMV reverse transcriptase, and the resulting cDNA's were subjected to 30 cycles of PCR with the above primers. The resulting product of about 0.8 kb is consistent with the calculated size of this region. This product was cloned into pBluescript KS+ (Stratagene) and sequenced by the dideoxy chain termination method. This cDNA fragment exhibits > 90% similarity (within the TM domains) at the amino acid level with the published mouse and rat 5-HT_{1B} receptors. This fragment was radiolabeled and used as a probe on an OK cell mRNA Northern blot, showing a message size of about 6 kb. This fragment is currently being used to screen an OK cell λgt11 cDNA library. (Supported by NIH grant MH47354).

198.16

CLONING OF A HUMAN PSEUDOGENE RELATED TO THE 5-HYDROXYTRYPTAMINE 1D RECEPTOR GENE. T. Nguyen, P.H. Wu, A. Marchese, W.M. Burnham* and B.F. O'Dowd. Addiction Research Foundation and Department of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

In an effort intially planned to isolate novel adenosine receptors, we prepared degenerate primers based on the third and sixth transmembrane (TM) regions of A1 and A2 receptors. The gene structure of these adenosine receptors had not been reported, however, many G protein-coupled receptors are encoded on single exons. The primers were used to amplify genomic DNA in the polymerase chain reaction, and DNA in the size range 350 to 1000 bp was subcloned. One of these clones, BD-20 with an insert size of 350 bp, contained some sequence homologous to the serotonin receptors. BD-20 was used to screen a human genomic library, and nineteen probe binding phage were plaque purified. Deduced amino acid sequence of one of these clones (TN-1) possessed six TM regions most closely homologous to the 5HT1D receptor, however, several stop codons and deletions were present in the coding region suggesting that TN-1 could not encode a functional receptor. Sequence analysis revealed that the sequence of TN-1 following TM 6 was disrupted by insertion of an Alu element, including a poly A tract of 20 bp. Northern blot analysis using clone TN-1 in rat and human showed the presence of mRNA transcripts in many organs, including brain. However, Northern analysis using clone BD-20 (lacking the Alu repeat) did not show any detectable hybridization. We have previously reported the presence of two dopamine pseudogenes in the G protein coupled receptor gene family, however, this report is the first of a serotonin pseudogene.

198.17

MOLECULAR CLONING AND CHARACTERIZATION OF A cDNA ENCODING A SEROTONIN-RELATED G PROTEIN-LINKED RECEPTOR. Y. Shen*, F.J. Monsma, Jr., C.R. Gerfen, L.C. Mahan P.A. Jose, and D.R. Sibley. Experimental Therapeutics Branch, NINDS and Laboratory of Cell Biology, NIMH, NIH, Bethesda, MD 20892.

We have recently cloned a cDNA encoding a novel G protein-coupled receptor which exhibits high homology to serotonin receptors. This sequence was initially identified as a cDNA fragment which was amplified from rat kidney proximal convoluted tubule mRNA using the polymerase chain reaction (PCR). Northern blot analysis with this fragment reveals an ~3.6 kb transcript with the following rank order of abundance in CNS tissues: hypothalamus > hippocampus = mesencephalon > olfactory bulb = cerebral cortex > olfactory tubercle > striatum. In peripheral tissues, this mRNA is most abundant in the spleen. *In situ* hybridization analysis confirms the Northern blot data and also reveals a high level of transcript in the thalamic reticular nucleus. Isolation and sequencing of a full-length clone obtained from a rat hippocampal library revealed a long ORF encoding a 404 residue protein the sequence of which is most closely related to the serotonin receptor family. Within the 7 putative transmembrane spanning regions, this receptor exhibits 60% - 43% identity with the following receptors: $5HT_{DRO1} > 5HT_{1D} > 5HT_{1A} > D_{1A} > D_2 > 5HT_2 > 5HT_{1C}$. The rat hippocampal cDNA also appeared to contain 72 bp of incompletely spliced intronic sequence located in the 2nd intracellular loop. PCR amplification and sequencing of hippocampal mRNA using primers flanking this region confirmed the presence of two alternatively-spliced forms of mRNA, one of which represents a correctly spliced transcript. This latter PCR product was subsequently used in the construction of a correctly spliced full-length clone for expression analysis. Thus far, transient expression of this clone in Cos-7 cells has not produced specific radioligand binding activity using a variety of ligands. Further identification of this receptor subtype using functional assays is currently being attempted.

198.18

CLONING AND EXPRESSION OF A NOVEL SEROTONIN RECEPTOR SUBTYPE. Frederick J. Monsma, Jr.*Yong Shen, Lawrence C. Mahan, and David R. Sibley. Experimental Therapeutics Branch, NINDS and Laboratory of Cell Biology, NIMH, NIH, Bethesda, MD 20892.

We have utilized the polymerase chain reaction (PCR) technique to selectively amplify G protein-coupled receptor cDNA sequences from rat striatal mRNA. One cDNA fragment was identified which exhibits high homology with previously cloned catecholamine receptors. Sequencing of a full-length clone isolated from a rat striatal cDNA library, using this fragment, revealed an open reading frame of 1308 bp encoding a 436 residue protein with seven hydrophobic regions representing putative transmembrane spanning domains. Within these hydrophobic regions, this receptor was found to exhibit 44% - 36% identity with the following receptors: $D_{1A} = D_{1B} > D_2 = D_3 = D_4 > 5HT_2 > 5HT_{1C} > 5HT_{1D\alpha} > 5HT_{1A} > 5HT_{1D\beta}$. Northern blot analysis revealed an ~3.8 kb transcript with the following rank order of abundance in CNS tissues: striatum >> olfactory tubercle > cortex > hippocampus. Expression of the full-length cDNA in Cos-7 cells resulted in the appearance of high affinity and saturable [^{125}I]-LSD binding ($K_d \approx 3$ nM). Among all endogenous biogenic amines tested, only 5-HT completely inhibited [^{125}I]-LSD binding, exhibiting a K_i of ≈ 300 nM. The rank order of potency for inhibition of [^{125}I]-LSD binding by other serotonergic agonists was: 5-MT > 5-HT > 5-CT > TFMPP > mCPP >>8-OH-DPAT. The rank order of antagonist binding was: mianserin >> (-) propranolol >SCH 23390 > ketanserin = spiroperidol. As a group, ergoline derivatives displayed the highest affinities: lisuride > lergotrile > bromocriptine > dihydroergotamine > 2-Br-LSD > dihydroergocriptine. This pharmacological profile does not appear to correlate with any previously described 5-HT receptor subtype. The functional properties of this novel 5-HT receptor are currently under investigation.

HISTAMINE AND OTHER BIOGENIC AMINES

199.1

EFFECTS OF HISTAMINE ON HYPOTHALAMIC 5-HYDROXYTRYPTAMINE NEURONS IN THE RAT A.E. Fleckenstein*, K.J. Lookingland and K.E. Moore Dept. Pharmacology and Toxicology, Michigan State Univ., East Lansing, MI 48824

Although substantial evidence demonstrates histamine as a neurotransmitter in the mammalian central nervous system, the central functions of the amine remain uncertain. The purpose of the present study was to examine the influence of histamine on 5-hydroxytryptamine (5HT) neurons in the rat hypothalamus by measuring the concentration of neurotransmitter (5HT), its synthesis [accumulation of 5-hydroxytryptophan (5HTP) after administration of a decarboxylase inhibitor] and metabolism [concentration of 5-hydroxyindoleacetic acid (5HIAA)] in brain regions containing terminals of these neurons. The intracerebroventricular administration of histamine increased 5HTP accumulation, dose- and time-dependently decreased 5HT levels, and either increased or had no effect on 5HIAA concentrations in various hypothalamic nuclei. The H_1 antagonist mepyramine maleate blocked, whereas the H_2 antagonist zolantidine dimaleate did not affect the ability of histamine to increase hypothalamic 5HIAA and decrease 5HT concentrations, respectively. These results indicate that exogenously administered histamine has a stimulatory effect on hypothalamic 5HT neurons through an action at the H_1, but not the H_2 receptor. Depletion of neuronal histamine by the histidine decarboxylase inhibitor α-fluoromethylhistidine decreased basal 5HIAA levels. This suggests that histamine may tonically activate hypothalamic 5HT neurons in male rats (supported by NIH grant NS 15911 and a fellowship from the Pharmaceutical Manufacturers Association Foundation).

199.2

HISTAMINE AND METHYLHISTAMINE IN SEIZURE-PRONE RATS. L. Tuomisto*, K. Onodera, M. Ylinen, U. Tacke, M.M. Airaksinen. Dept. of Pharmacology and Toxicology, Univ. of Kuopio, Finland, SF-70211

Brain histaminergic system has been implied as one of the neurotransmitter systems modifying the response of audiogenic seizure (AGS) sensitive rodents to audiogenic stimulation. Lowered histamine (HA) levels have been found in the brains or specified brain areas of genetically epilepsy prone deermice and KM rats. This study was aimed to locate possible differences in histamine turnover between the KM strain and the resistant Wistar-homologue (W). All KM but no W rats studied responded (ARS 5) to 10 KHz, 110 dB sound 1 week earlier. Male rats were decapitated 4 hours after HA synthesis inhibitor alfa-fluoromethylhistidine (FMH, 100 mg/kg i.p.) or saline. Histamine and tele-N-methyl-histamine (MH) were analysed (HPLC and GC-MS,resp.) in 11 brain parts. FMH lowered HA levels both in KM and W rats, but after FMH the overall HA level was lower in KM rats ($p=0.000$, ANOVA). Interestingly, there were regional differences in MH/HA ratio with higher values in KM rats: hippocampus ($p=0.008$, t-test), amygdala ($p=0.005$) and dorsal cortex ($p=0.001$). The dissimilar turnover of HA in brain areas involved in convulsive phenomena suggests a regulatory role for HA in seizures.

199.3

CHARACTERIZATION OF HISTAMINE RELEASE USING SUPERFUSED SLICES OF RAT HYPOTHALAMUS. A. Rodrigues, A. Fink-Jensen*, P.D. Suzdak. Novo Nordisk A/S, CNS Division, DK-2760 Maaloev, Denmark.

Pharmacological studies have demonstrated the presence of presynaptic histamine H_3 receptors (Arrang et al., Nature 302, 1983) in addition to the postsynaptic H_1 and H_2 receptors. The release of histamine (HA) from the hypothalamus of male rats was measured using superfused brain slices preloaded with 150 μCi [^{3}H]-histidine. The amount of de novo synthesis [^{3}H]-HA in the superfusates and tissue was determined by cation-exchange chromatography (H. Timmerman et al., Eur. J. Pharmacol. 138, 1987).

The release process was shown to be Ca^{2+}-dependent and stimulated by high levels of K^+. The H_3 agonist R-α-methylhistamine inhibited, in a concentration-dependent manner, the histamine release evoked by 30 mM K^+ with an approx. IC_{50} of 300 nM and a maximal inhibition of 70%. This effect was fully antagonized by 1 μM of Thioperamide, an H_3 receptor antagonist.

It is concluded that histamine modulates its own release from hypothalamus neurons by interacting with H_3 histamine autoreceptors similarly to other brain regions.

199.4

CHARACTERIZATION OF GLUTAMATERGIC REGULATION OF HISTAMINE RELEASE FROM RAT HYPOTHALAMUS.

K. Okakura-Mochizuki[1], A. Yamatodani*[2], T. Mochizuki[1], A. Horii[3] and H. Wada[1]. Departments of [1]Pharmacol. II, [2]Molec. Physiol. and [3]Otolaryngol., Faculty of Med., Osaka Univ., Suita, Osaka 565, Japan.

A microdialysis method was used to study the effects of glutamate (Glu) on *in vivo* release of histamine from the anterior hypothalamic area of urethane-anesthetized rats. Infusion of 1mM Glu caused about 150% of histamine release over the basal release. N-methyl-D-aspartate (NMDA, 100 μM) caused a similar increase. Glu-evoked histamine release was completely blocked by D(-)-2-amino-5-phosphonopentanoic acid (APV, 100μM), a specific antagonist of NMDA-receptors. APV alone also reduced histamine release. Infusion of TTX (100nM) reduced histamine release, but had no effects on Glu-evoked release. These results clearly indicate that Glu enhances histamine release through NMDA-receptors located on histaminergic nerve terminals, and suggest the presence of tonic glutamatergic regulation of the release. On the other hand, kainic acid (KA, 50μM) caused delayed increase of histamine to about 180% of the basal release. KA-induced histamine release was reduced by APV while the increase was not blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10μM) or 6,7-dinitroquinoxaline-2,3-dione (DNQX, 100μM), antagonists of non-NMDA receptors. In the presence of TTX, KA caused no significant increase in histamine release. These results suggest that the increase of histamine release is induced by Glu which is enhanced by KA.

199.5

CISTERNAL CSF VOLUME AND HISTAMINE CONCENTRATION IN LEWIS RATS WITH EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS.
Edward L. Orr*, Sushanta Mallick, Martin T. Taylor, and Jean H. deSchweinitz. Dept. Anat. & Cell Biol., Texas College of Osteopathic Medicine, Fort Worth, TX 76107

Lewis rats with experimental autoimmune encephalomyelitis (EAE) exhibit significant changes in brainstem and spinal cord histamine and water content (edema) in concert with the clinical course of EAE. To determine if CSF levels of histamine (HA) are also altered in EAE, we measured the concentration of HA present in CSF obtained from the cisterna magna of control and EAE rats. EAE was induced by inoculation of female Lewis rats with an emulsion of guinea pig spinal cord homogenate (gpsch) in complete Freund's adjuvant; control rats received an identical inoculum lacking gpsch. Beginning on day 7 post inoculation (pi), rats were monitored daily for clinical signs of EAE. Whole spinal cords, brainstems, and samples of cisternal CSF were obtained from anesthetized control and EAE rats on days 7, 9, 11, 14, and 16 pi. The tissues and CSF samples were stored frozen until assayed for HA using radioenzymatic methods.

Significant changes in spinal cord and brainstem HA and wet weight occurred in parallel with the clinical course of EAE. Similarly, CSF concentrations of HA also fluctuated in concert with the clinical course of EAE. This increase in CSF HA was accompanied by a significant decrease in the volume of CSF one could obtain from the cisterna magna. These results indicate that cisternal CSF HA concentration parallels the changes in CNS HA levels which occur during EAE in Lewis rats. In addition, the decrease in CSF volume obtained from the cisterna magna of rats with clinically significant EAE is consistent with the significant CNS edema observed in rats with EAE. (Supported by a grant from the National Multiple Sclerosis Society.)

199.6

Histamine metabolites in CSF of patients with schizophrenia: their relationships to other aminergic transmitters and ratings of schizophrenic behavior.
GD.Prell[1], JP.Green[1]*, CA.Kaufmann[2] J.Khandelwal[1], D.Kirch[2], M.Linnoila[3], and RJ.Wyatt[2]; [1]Dept. Pharmacology, Mount Sinai School of Medicine, NY, 10029; [2]NIMH Neuroscience Center, at St. Elizabeth's Hospital, Washington, D.C. 20032; [3]DICBR, NIAAA, Bethesda, MD 20205 USA

Levels of the histamine metabolites, *tele*-methylhistamine (t-MH) and *tele*-methylimidazoleacetic acid (t-MIAA), other aminergic transmitters and their metabolites were measured blindly in cerebrospinal fluid from 36 inpatients with chronic(RDC) schizophrenia(SZ) and 7 neurological inpatients (controls). In both groups, patients' mean ages, durations of illness, and periods of withdrawal from medication were similar. Mean t-MH level of controls was similar to t-MH levels seen previously in healthy volunteers. The mean t-MH level in patients with SZ was 2.5-fold higher ($p<0.001$) than controls; 25 (74%) had levels that exceeded the range of controls. There was no difference ($p>0.05$) in levels of other analytes between the two groups. Those taking (n=10) or withdrawn (n=26) from neuroleptics had nearly the same mean levels of t-MH; each was higher than controls (ANOVA: $p< 0.05$). Among all SZ patients, levels of t-MH, t-MIAA and their sum correlated positively with levels of HVA, DOPAC, NE, MHPG and 5-HIAA; but only t-MH levels correlated with positive scores on the Brief Psychiatric Rating Scale (BPRS) ($r_s= +0.45$, $p<0.02$). Negative scores alone did not correlate with any substance. However, among patients with predominantly deficit schizophrenia (ratio neg/pos BPRS scores >2), the ratio correlated ($r_s=+0.82$,$p<0.02$) only with levels of t-MH. These results suggest increased histaminergic activity in brains of patients with chronic SZ. (Supported by MH-31805 and NS-28012)

199.7

Measurement of histamine in brain by gas chromatography-mass spectrometry.
E Douyon, AM Morrishow and GD Prell*; Department of Pharmacology, Mount Sinai School of Medicine of CUNY; New York, NY 10029 USA

Levels of histamine are often measured in brain tissue, diasylates or cerebrospinal fluid. However, residual presence of drugs used to probe histaminergic function may affect histamine methyltransferase (HMT) used to measure histamine by the radioenzymatic method. Also, recovery of histamine may be problematic. To overcome these difficulties we developed a method to measure histamine by GC-MS. Internal standard, d_4-histamine, is added to aqueous sample homogenates or standards (0.1-100ng). Samples are basified, extracted into butanol-chloroform, then back extracted into 0.1N HCl, and dried. Following derivatization with heptafluorobutyryl anhydride in toluene, the reaction is quenched with TRIS. The organic layer is retained, dried with Na_2SO_4, then derivatized with ethylchloroformate. The monoheptafluorobutyryl-N-ethoxycarbonyl derivative of histamine (MW 379) is separated on a polycyanopropylphenylmethylsiloxane capillary column. Protonated molecular ions m/z 380 and 384 were acquired utilizing methane-positive chemical ionization (CI) GC-MS selected ion monitoring (SIM) for derivatives of histamine and d_4-histamine, respectively. Electron impact ionization yielded ions at m/z 94, 306, 379 and 98, 310 and 383 for derivatives of histamine and d_4-histamine, respectively. Measurements of levels of histamine in whole brains or brain regions of rats using CI-SIM or the radioenzymatic method gave comparable results. This GC-MS method is particularly useful for measuring histamine in samples containing HMT inhibitors such as metoprine or tacrine or other tetrahydroacridines. (NS-28012)

199.8

Characterization of the Putative Promoter for the Histidine Decarboxylase Gene and Regulation by Steroid Hormones in Rat Brain.
C.A. Zahnow* and D.E. Millhorn, University of North Carolina, Chapel Hill, N. C. 27599.

The biogenic amine, histamine, is synthesized from L-histidine by the enzyme Histidine Decarboxylase (HDC). 1.3kb of the putative promoter region for the HDC gene was amplified via the polymerase chain reaction from a 9kb gene fragment and cloned into M13mp19 for sequence analysis. Sites resembling a TATA box, SP1 sites, AP1 and AP2 sites, octamers, CTF/NF-1 and glucocorticoid response elements (GRE) have been identified based upon homology to sequences known to be recognized by sequence-specific transcription factors. Our previous data suggested that HDC mRNA levels are regulated by high (1mg) doses of dexamethasone, and dihydrotestosterone. This regulation may be occurring at the level of the gene through actions on the GRE. It is necessary, however, to determine whether lower, physiological doses of these steroid hormones are also able to regulate HDC mRNA levels. HDC mRNA (2.6kb) was detected via Northern blot analyses in both the hypothalamus and hippocampus. However, the only neuronal source thus far identified for HDC mRNA is found in the hypothalamus (Mol.Cell Neurosci., 1: 1990) In an effort to rule out the possibility that HDC mRNA may be axonally transported (EMBO J.,10: 1991) from the hypothalamic cell bodies to the hippocampus, colchicine, a blocker of axonal transport, was injected into the lateral cerebral ventricle of 44 day old rats and the animals were sacrificed 24 hours later. Northern blot analysis revealed an approximate three fold decrease in hypothalamic HDC mRNA in the colchicine treated rats as compared to PBS treated rats. Treatment did not block the presence of mRNA in the Hippocampus. This suggests that HDC mRNA is transcribed within a cell type in the Hippocampus.

199.9

BRAIN HISTAMINE LEVELS AND HISTIDINE DECARBOXYLASE ACTIVITY IN MAST CELL DEFICIENT RATS(WS/WS).
K. Maeyama , H. Onoue , E. Sakurai , T. Kasugai , Y. Kitamura and T. Watanabe*. Dept. of Pharmacology, Tohoku University School of Medicine, Sendai 980, Dept. of Pathology, Osaka University Faculty of Medicine, Suita 565, Japan.

In the brain, there are at least two types of histamine storing cells, i.e. neurons and mast cells, and histamine derived from mast cells has hampered studies of neuronal histamine. In 1978, Kitamura *et al.* found that *W/W^V* mice are deficient in mast cells, and last year they found that similar rats(*Ws/Ws*) which have mutation of c-kit receptor are completely deficient in mast cells. To clarify the contribution of non-mast cell histamine in the brain, histamine contents and the activities of histidine decarboxylase(HDC), a histamine-synthesizing enzyme, in *Ws/Ws* and congenic (*+/+*) rats were compared. Histamine levels in the forebrain, midbrain, pons-medulla and cerebellum of *Ws/Ws* rats are 0.25, 1.17, 0.31 and 0.12 nmoles/g, respectively and 60.9, 107, 80.7 and 64.4 % of each tissue of congenic *+/+* rats. HDC activities of brains of *Ws/Ws* are not different from those of *+/+* rats.

It is concluded that the brain histamine in *Ws/Ws* rats represents non-mast cell histamine and these mutant rats will be useful for pharmacological research of functions of neuronal histamine.

Aknowledgement: *Ws/Ws* rats were kindly donated by Drs. K. Kondo and A. Niwa(Lab. of Experimental Animal, Yagi Memorial Park, Gifu, Japan).

199.10

DURATION OF STRESS CAN AFFECT HISTAMINE LEVELS IN INTACT AND ADRENALECTOMIZED RATS.
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Central histamine (HA) has been thought to regulate ACTH release, however, no consistant relationship has been found between HA and the exposure to stress. We examined HA levels in several rat brain areas using cold restraint stress for 15 min or for 2 h. In the paraventricular nucleus, median eminence, supraoptic nucleus, suprachiasmatic nucleus, posterior hypothalamus, and the dentate gyrus, HA levels increased after 15 min of stress with a decrease in HA following 2 h of stress. Other areas such as the frontal cortex, septal nucleus, bed nucleus, periventricular nucleus, mammallary bodies, ventromedial nucleus, and the CA1 and CA3 cell fields demonstrated progressive decreases in HA with longer periods of stress. In all areas examined, lower HA levels were seen following 2 h stress when compared to controls or 15 min stress. In sebsequent experiments, 15 min stress with adrenalectomy (7 days prior to testing), attenuated changes in HA levels in the posterior hypothalamus, mammallary bodies, and median eminance. In the suprachiasmatic nucleus and the supraoptic nucleus, adrenalectomy enhanced the decrease in HA followed by 2 h stress. Both effects were reversed with a low basal level of corticosterone replacement. These results show that 1) HA responds to stress in brain regions known to regulate ATCH release, 2) the histaminergic response can depend upon the duration of the stress, and 3) circu-lating corticosterone can affect these levels.

199.11

CLONING OF A HISTAMINE H_1 RECEPTOR. [1]H. Fukui, [1]M. Yamashita, [2]K. Sugama, [1]Y. Horio, [2]S. Ito, [1]K. Fujimoto, [1]Y. Abe, [1]H. Mizuguchi, [1]N. Inagaki* and [1]H. Wada. [1]Dept. Pharmacol. II, Faculty of Med., Osaka Univ. and [2]Osaka Bioscience Institute, Suita 565, Japan.

Histamine acts as a neurotransmitter in the brain. The H_1 subtype of histamine receptors expressed on target cells mediates histaminergic neurotransmission. Although the second messenger of the H_1 receptor was extensively investigated, little was known about the molecular structure the histamine H_1 receptor. We cloned a cDNA encoding the H_1 receptor from a cDNA library of bovine adrenal medulla using in vitro transcription and electrophysiological assay with Xenopus oocytes. The H_1 receptor cDNA clone was 2,960 nucleotide long and encoded a protein of 491 amino acids with seven putative tansmembrane domains and a characteristically large third intracellular loop. Binding affinities to histamine receptor related compounds of the H_1 receptors expressed in COS-7 cells were comparable with those of native H_1 receptors in bovine adrenal medulla. Northern blot analysis showed a band of 3.0-kb nucleotides corresponding to a histamine H_1 receptor mRNA in various bovine tissues including cerebral cortex, lung, small intestine, adrenal medulla, uterus and spleen.

199.12

APPARENTLY COOPERATIVE BINDING TO G PROTEIN-LINKED RECEPTORS LABELED BY [^{3}H]HISTAMINE. W.G. Sinkins* and J.W. Wells. Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada, M5S 2S2.

Tritiated histamine labels two classes of sites in membranes from guinea-pig cerebral cortex. GMP-PNP reduces capacity at both classes with little or no effect on affinity (K_{Pj}) at the sites that remain. The Hill coefficients of 26 histaminergic ligands range from 0.78 to 1.54 among agonists, and from 0.66 to 1.26 among antagonists. The data reveal elements of both H_2 and H_3 specificity, but the abiguity cannot be attributed to a mixture of gene products. Fourteen compounds were studied at 1.4 nM and 11 nM [^{3}H]histamine; in such experiments, the radioligand is both a second independent variable and an internal control. In terms of distinct and independent sites, the relative capacities (F_j) and the inferred affinity of [^{3}H]histamine (K_{Pj}) differ from ligand to ligand; since the discrepancies persist for any number of classes, the model can be rejected unequivocally. All 26 compounds were studied at 1.4 nM [^{3}H]histamine, and two hyperbolic terms [*i.e.*, $F'_jK_j/(K_j + [L])$] are required for 6 agonists and 6 antagonists; F'_j varies widely from ligand to ligand. Agonists reveal a correlation between log (K_2/K_1) and F'_1 $(K_1<K_2)$; antagonists reveal a correlation between log (K_2/K_1) and F'_2. Antagonists thus appear to promote the state to which they bind more weakly. This paradox and Hill coefficients significantly greater than 1 argue against a ligand-regulated equilibrium between two states of mutually independent sites. High values of n_H suggest that binding is cooperative, and ligand-related anomalies in the inferred binding of [^{3}H]histamine are largely avoided by a model in which one equivalent of bound ligand affects the binding of a second. The failure to identify an unambiguous pharmacological specificity may reflect the failure to assess binding in the correct mechanistic context. The approach described here is generally applicable to the evaluation of different models for binding at equilibrium.

199.13

BIOCHEMICAL AND BEHAVIORAL EFFECTS OF CDD-2016, A NOVEL H_3 ANTAGONIST. S. Ghodsi-Hovsepian*, W. Hoss, A. Khan, G.J. Durant, A. El-Assadi, W.S. Messer, Jr., #R.C.A. Frederickson. Center for Drug Design and Development, University of Toledo, Toledo, OH 43606 and #Gliatech, Inc., 23420 Commerce Park Rd., Beachwood, OH 44122

Histamine H_3 receptors are autoreceptors that control the synthesis and release of histamine from nerve endings. A role for brain histamine is indicated in several brain functions, including sleep, arousal and cognition. The ability to regulate pharmacologically the levels of brain histamine raises the possibility of developing therapies based on H_3 receptors as a target and suggests new strategies for the treatment of neurological disorders.

The compound CDD-2016 was synthesized as part of a program to develop novel H_3 antagonists. The biochemical and behavioral properties of CDD-2016 were compared to thioperamide, a potent and selective H_3 antagonist. The inhibition of the binding of 1 nM [^{3}H]-N$^\alpha$-methylhistamine, a selective H_3 agonist, was used to assess binding to H_3 receptors in rat brain membranes. CDD-2016 displayed an IC_{50} value of 23 ± 6 nM compared with a value of 4.0 ± 0.6 nM for thioperamide. (R)-α-methylhistamine, a highly selective H_3 agonist, produced a dose-dependent increase in sleep one hour after i.p. injection in the range of 15 to 35 mg/kg in mice. Both thioperamide (20-70 mg/kg), and CDD-2016 (40-80 mg/kg) were able to inhibit the soporific effects of (R)-α-methylhistamine, respectively.

(R)-α-methylhistamine inhibited the release of histamine from nerve endings in a dose-dependent manner over the range of 10 nM to 1 mM. Both thioperamide and CDD-2016 increased histamine release from nerve endings in the range of 10-100 nM and completely reversed the inhibition of histamine release induced by 1 μM (R)-α-methylhistamine.

In summary, the data indicate that CDD-2016 is a potent and effective antagonist for H_3 receptors. CDD-2016 binds with high affinity to H_3 receptors, blocks the soporific effects of an H_3 agonist *in vivo* and the inhibition of histamine release by an H_3 agonist *in vitro*. Supported by a grant from Gliatech, Inc. to the University of Toledo.

199.14

INTERACTION OF DOPAMINERGIC FIBERS WITH GABAERGIC INTERNEURONS IN RAT MEDIAL PREFRONTAL CORTEX. S.L. Vincent*, R.P. Molloy, F.M. Benes. Department of Psychiatry and Program in Neuroscience, Harvard Medical School and Mailman Research Center, McLean Hospital, Belmont, MA 02178.

The anatomical relationship between dopamine (DA)-containing fibers and GABAergic interneurons was investigated in rat medial prefrontal cortex (mPFC) using a double immunofluorescent method. Rats were perfused intracardially with a solution containing 5% glutaraldehyde and 1% Na-metabisufite in 0.1 M cacodylate-HCl buffer, pH 7.4, at room temperature. Sections (40-μm-thick) were cut on a Vibratome and were incubated in a solution containing 1:100 rabbit anti-dopamine antibody and 1:500 mouse anti-GABA antibody, followed by 1:100 dilutions of FITC-conjugated donkey anti-rabbit and rhodamine-conjugated donkey anti-mouse antibodies. The mPFC was viewed with either epiflucrescence or confocal laser scanning microscopy to asses the degree to which DA-immunoreactive (DA-IR) fibers were in close apposition to GABA-IR neuronal cell bodies. Two types of interaction were identified: (1) fibers with a single terminal varicosity in contact with a soma, (2) fibers en passage with multiple varicosities making contact either around or across a soma. From an examination of all visualized GABA-IR cells in coronal sections of mPFC it was determined that the frequency with which DA-IR fibers formed close appositions was 7%, 39%, and 75% for layers I, II/III, and V/VI, respectively. This gradient of interaction frequency parallels the density of DA afferents to rat mPFC which are most dense in the deeper laminae. Overall, these data suggest that GABAergic interneurons may be targets of mesocortical DA afferents and support the idea that an interaction between DA and GABA may play a role in the intrinsic circuitry of rat mPFC. Supported by MH 00423, a Stanley Award, and the Scottish Rite Foundation.

199.15

EVIDENCE FOR AGONISTIC ACTION OF PICOLINIC ACID ON STRYCHNINE-SENSITIVE GLYCINE RECEPTOR IN THE CENTRAL NERVOUS SYSTEM. T. Kaneko*, T. Tonohiro, M. Tanabe and N. Iwata New Lead Res. Lab., Sankyo Co., 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo, 140 Japan.

Picolinic acid (PA), when administered iontophoretically, suppresses spinal interneurons through activation of strychnine-sensitive glycine receptors (Brain Res., 516, 332-334, 1990). In this study, in order to define further profiles of PA, effects of PA and related compounds on motor functions were compared with those of indole-2-carboxylic acid (ICA) which is reported to be an antagonist for strychnine-insensitive glycine receptors coupled with NMDA receptors. Both compounds were administered systemically in the present experiments.

In mice, PA and ICA suppressed chemically induced convulsions in different manner, at lower doses than those that exerted muscle relaxant effect. PA selectively inhibited strychnine-induced convulsion although ICA reduced all the convulsions following injections of strychnine, bicuculline and pentylenetetrazol. Among PA derivatives, when injected systemically, PA methy ester (mPA) showed the strongest protection against strychnine. In cats anesthetized with α-chloralose, PA and mPA inhibited spinal monosynaptic reflex (MSR) as well as polysynaptic reflex (PSR), whereas ICA depressed PSR sparing MSR. In spinal preparations, suppression of MSR by mPA was unchanged although effect on PSR diminished. Strychnine, when injected after mPA, accelerated recovery of MSR from depression by mPA. On the other hand, ICA failed to show any inhibition in spinal preparations. Furthermore, intracellular recording from feline spinal motor neuron detected hyperpolarization following mPA injection. Conceivably, PA directly inhibits the reflex pathway in the spinal cord, whereas ICA exerts the effect via inhibitory action on supraspinal structures in the brain. PA, especially in its methyl ester form, seems to be useful as a systemically injectable agonist for strychnine-sensitive glycine receptors.

199.16

A SHORT, NOVEL, G-PROTEIN COUPLED RECEPTOR ISOLATED BY PCR USING mRNA EXTRACTED FROM THE OVINE PARS TUBERALIS. P. Barrett*, A. MacDonald, R.J. Helliwell and P.J. Morgan. Rowett Research Institute, Aberdeen, AB2 9SB, U.K.

The ovine pars tuberalis is an important target for the pineal hormone, melatonin. The receptors for melatonin are located on 80-90% of the cells that constitute this tissue. These receptors have been shown to be high affinity receptors coupled to the inhibition of adenylate cyclase through a G_i heterotrimeric protein complex.
Homology between most of the G-protein coupled receptor superfamily is sufficient to design degenerate oligonucleotide primers which will hybridize to DNA sequence homologies in these genes and therefore may be amplified using thermostable DNA polymerases to produce sufficient amount of DNA for sequencing and probes.

Applying the PCR technique to cDNA reverse transcribed on mRNA extracted from the ovine pars tuberalis, a number of receptor sequences were detected. One amplified fragment with G-protein coupled receptor sequence similarity was used to screen a genomic DNA library in λEMBL3. A clone was identified containing a 20kb insert. A 6kb SacI fragment was subcloned into pUC19 and sequenced by primer walking. The identified clone contained an open reading frame of 870bp, encoding a protein of 290 amino acids. The coding sequence is preceded by a sequence which conforms to the consensus sequence proposed by Kozak and ends with a TAA stop codon.

A hydrophobicity plot reveals seven hydrophobic regions which correspond to the transmembrane domains, and these domains contain many of the highly conserved residues found in other receptor sequences. The receptor has the unusual characteristics of a very short loop between transmembrane domains 4 and 5, and a short if non-existent N-terminal cytoplasmic domain. A database search reveals only limited homology with the ligand unidentified receptor edg1 and with the histamine H2 receptor. *In situ* hybridization and Cos cell transfection assays are being used to identify the ligand for this receptor.

199.17

FIRST CHARACTERIZATION OF 6-HYDROXYTRYPTAMINE IN THE RAT MIDBRAIN BY USING SPECIFIC ANTIBODIES. H.Dabadie[1], M.Geffard*[1], C.Berrier[2] and J.C.Jacquesy[2]. [1]Laboratoire d'Immunologie et Pathologie, Université de Bordeaux II, 33076 Bordeaux, France. [2]Laboratoire de Chimie XII C.N.R.S., Université de Poitiers, 86022 Poitiers, France.

The visualization of serotonin (5-HT), 5-methoxytryptamine and tryptamine in the rat midbrain has been made possible by the development of antibodies raised against these conjugated molecules. It has been suggested that 6-hydroxytryptamine (6-HT) might be also a neurotransmitter in this region. In order to test this hypothesis, 6-HT was synthesized and antibodies were raised in the rabbit. The high avidity (IC50 = 5 x 10^{-9} M) and specificity (cross reactivity ratio between 6-HT-glutaraldehyde(G)-bovine serum albumine (BSA) and 5-HT-G-BSA the most immunoreactive compound was 1,500) rendered these antibodies reliable tools for specific molecular detection of 6-HT in the G-fixed tissues. In the dopaminergic region 6-HT-immunoreactivity was noted in the substantia nigra but was particularly intense in the red nuclei where it seems to be localized in the magnocellular division in the form of large 6-HT neurons. In contrast there were few 6-HT neurons in the raphe nuclei. Thus, 6-HT may be a new putative neurotransmitter existing in the ReN, alongside of the other neurotransmitters already described in this region, in the nigrorubral pathway and in the rubral projection from the dorsal raphe nuclei.

TRANSMITTERS IN INVERTEBRATES I

200.1

ACTIONS OF FMRFamide-RELATED PEPTIDES FROM *Fusinus ferrugineus* ON IDENTIFIED CENTRAL NEURONS OF *Helix aspersa*. M.L.Chen and R.J. Walker*. Dept. of Physiology and Pharmacology, Univ. Southampton, Southampton SO9 3TU, UK.

FMRFamide-related peptides have been isolated from ganglia of the mollusc, *F. ferrugineus* which exhibit biological activity. This study investigates the action of *Fusinus* peptides, GSLFRFamide, GSFFRFamide and FMRFamide, and the fragments, LFRFamide and RFamide, and their structure-activity relation. Experiments were performed on neurons, F1 and F2, of suboesophageal ganglia from snail, *H. aspersa*, using intracellular recording and two electrode voltage clamp. On F1, FMRFamide, GSLFRFamide and LFRFamide all exert inhibition due to an increase in K permeability, with thresholds 1 , 1 and 5-10 µM respectively, order of potency: FMRFamide ≥ GSLFRFamide > LFRFamide. On F2, FMRFamide is excitatory increasing Na^+ permeability. In contrast, GSLFRFamide and LFRFamide are inhibitory, thresholds 0.05 and 0.5 µM, respectively. Pressure ejection of GSLFRFamide on F2 induced an outward current, reversal potential -70/75 mV, which was augmented in K^+-free saline and blocked with 1 mM TEA and 100 µM 4-AP. GSFFRFamide and RFamide at 30 µM were inactive on F1 and F2. We conclude GSLFRFamide and FMRFamide act at different receptors and LFRF may be crucial for bioactivity.

200.2

IN VITRO RELEASE OF IR-MET-ENKEPHALIN IN A WHOLE PERIOESOPHAGEAL GANGLION OF Helix aspersa. F. PELLICER; M.ASAI, M.LEON-OLEA, M. SANCHEZ-ALVAREZ. INSTITUTO MEXICANO DE PSIQUIATRIA, MEXICO, 14370 D.F.

The existence of opioid peptides in the nervous system of molluscs has been demonstrated by different techniques: identification of receptors (1), inmunohistochemistry (2), RIA (3), and by behavioral experiments (4). However, the opioid peptide release has not been described. In the present work we study the IR-met-enkephalin (IR-ME) in vitro release from a single whole perioesophageal ganglion. Nine ganglia were dissected and their connective tissue was removed. The ganglia were immersed in a Ringer solution (inmM- NaCl, 75; KCl, 2.5; CaCl2, 10; MgCl2, 5; glucose, 5; Hepes, 5; pH, 7.45). Each ganglion was depolarized with 40 mM of KCl during 10 min, in presence of Phe-Al a (1 mM) as inhibitor. The tissue and perfusates were processed as previously described (3). The results expressed as IR-ME pM/g wet tissue, showed a basal release of 23±4; with high K+ pulse 81±16; post pulse 37±18. The results suggest that in the whole ganglion the in vitro opioid peptide release mechanism is functionally present. (1) Osborne, N. N. et al. J. Phar. Pharmacol. 31:481, 1979; (2) León-Olea, M. et al. Soc. Neurosci. Abs. 17:385.18, 1991; (3) Gutiérrez, R. et al. Comp. Biochem. Physiol. 100:609, 1991; (4) Burrowes, W.R. et al. Life Sci. 33:381, 1983.

200.3

STRUCTURAL FEATURES OF *APLYSIA* BAG CELL PEPTIDES WHICH ARE IMPORTANT FOR REGULATION OF BAG CELL ADENYLATE CYCLASE

R.W. Berry* and R. Sanger Redman. Dept. Cell, Molecular and Structural Biology, Northwestern Univ., Chicago, IL 60611.

The bag cell peptides, α-, β-, and γ-BCP, are a set of structurally related peptides which are produced and secreted along with egg-laying hormone by *Aplysia* bag cell neurons. Among their functions is the modulation of bag cell discharges, as they can influence bag cell excitability by altering cAMP levels. These effects can be excitatory or inhibitory, depending on temperature. We have found that these actions can be reproduced *in vitro* in adenylate cyclase assays, performed in the presence of 200 m*M* Cl^-. In an attempt to define the structural features responsible for enzyme stimulation and inhibition, we have assayed each of the naturally-occurring BCP's, as well as synthetic peptide analogues. At 30° C, each of the BCP's stimulates enzyme activity, just as they elevate cAMP in intact bag cells at elevated temperature. Each stimulates to a maximum of 30%, with potency decreasing in the order α(1-7) > γ ≥ β. Alpha(1-7) and γ inhibit enzyme activity at 15° C, a temperature at which these peptides reduce cAMP levels in intact cells. The β peptide does not share this property, but its stimulation of the enzyme is reduced at the lower temperature. The synthetic peptide RLRF, which corresponds to α(3-6), β(1-4), and γ(1-4), mimics β, but is less potent. The synthetic dipeptides FY [α(6-7)], FH [β(4-5)], and FD [γ(4-5)] are ineffective in this assay at 1 m*M*. These results suggest that binding of the RLRF sequence which is shared by all of the BCP's is sufficient to stimulate adenylate cyclase at 30°, and an electronegative residue immediately C-terminal to RLRF apparently both increases potency and enables the peptide to cause adenylate cyclase inhibition at 15°.

200.4

MODULATION OF NEUROMUSCULAR TRANSMISSION BY EXCITATORY AND INHIBITORY MOTOR NEURONS IN *APLYSIA*. P.J. Church* and P. E. Lloyd. Committee on Neurobiology, The University of Chicago, Chicago, Il 60637.

Buccal muscle I3a is innervated by two excitatory motor neurons, B3 and B38 and the cholinergic inhibitory motor neuron, B47. B38 synthesizes the SCPs and B47 synthesizes myomodulin A (Mma). Application of the SCPs or Mma (10nM) to the muscle enhanced B3-evoked EJPs. We monitored the modulation of B3-evoked EJPs in response to stimulation of B38 and B47 using paradigms designed to release the peptide cotransmitters. When B38 was stimulated in a series of extended bursts during B3 interburst intervals, subsequent B3-evoked EJPs were enhanced, an effect that is due at least in part to release of peptide cotransmitters (SCPs) from B38 during extended bursts. Stimulation of B47 evoked IJPs in I3a. However, extended B47 stimulation during B3 interburst intervals enhanced subsequent B3-evoked EJPs. These results suggest that when B47 is stimulated in short bursts the primary effect is inhibition due to cholinergic IJPs, but when B47 is stimulated in extended bursts, release of Mma contributes to the enhancement of subsequent B3-evoked EJPs. Supported by NRSA 1-F31-MH10240-01 to PJC and NS 23569 to PEL.

200.5

MODULATION OF ACh RELEASE FROM NEUROMUSCULAR SYNAPSES IN *APLYSIA*. G. A. Phares* and P. E. Lloyd. Committee on Neurobiology, University of Chicago, Chicago, Il 60637.

Buccal muscle I5 is innervated by the cholinergic motor neuron, B15. This neuron also synthesizes modulatory neuropeptides: the SCPs and buccalins. The release of peptide cotransmitters from terminals of B15 has been studied in detail.

We have previously demonstrated that terminals of B15 can be loaded with [3]H-acetylcholine ([3]H-ACh) by stimulation of buccal nerve N3 while I5 is bathed in [3]H-choline ([3]H-Ch; Lloyd, 1991). The [3]H-ACh is released upon subsequent stimulation of B15, and most of the released label is recovered as [3]H-ACh since nearly all of the acetylcholine esterase activity is found in hemolymph which is washed from the muscle prior to labeling.

Using this system, we have begun to study the modulation of [3]H-ACh release from B15. After loading B15 terminals with [3]H-Ch, I5 was placed into a microperfusion chamber and superfused with artificial sea water. Superfusate was collected for periods of 3 min. At the beginning of every third collection period B15 was stimulated for one minute with 4 s bursts and 3 s interburst intervals at either 12.5 or 15 Hz. Recovery of [3]H-ACh due to stimulation was determined by subtracting the average radioactivity in the bracketing rest periods from that collected during a stimulation period.

Serotonin (5-HT; 1 μM) caused a transient increase in the [3]H-ACh recovered during 15 Hz stimulation, but only slightly increased recovery at 12.5 Hz. The recovery of [3]H-ACh increased nearly 2-fold for the first stimulation period in 5-HT at 15 Hz, however, this effect was reduced in following stimulation periods although 5-HT was still present. In 1 μM buccalin [3]H-ACh recovery was substantially reduced with stimulation at 12.5 Hz, but only moderate reductions were seen for stimulation at 15 Hz. It appears that frequency of stimulation is an important factor in determining the effects of modulators on ACh release.

200.6

RELEASE OF NEUROPEPTIDES FROM IDENTIFIED *APLYSIA* NEURONS IN CULTURE IS NOT SENSITIVE TO THE PATTERN OF STIMULATION. M.D. Whim* and P.E. Lloyd. Dept. Pharm. Physiol. Sci. & Comm. on Neurobiol. Univ. Chicago, Chicago, IL 60637.

Aplysia buccal neurons B1 and B2 contain the 2 neuropeptides SCPA and SCPB. When placed in primary culture, these cells grow an extensive neuritic field. Newly synthesized peptides can be labeled by the addition of [^{35}S]-met to the culture medium, and using a combination of HPLC and liquid scintillation counting, the SCPs can be shown to be released in a stimulus and Ca-dependent fashion (Lloyd et al, 1986). We used this preparation to investigate how peptide release from individual cells varied with the pattern of stimulation. Initially, total spike number was kept constant while spike pattern was varied. We observed no difference in the amount of labeled SCPs released when the cells were stimulated tonically at 3 Hz compared to 6 Hz for 500 msec every other 500 msec. We then compared 1 Hz tonic stimulation with 6 Hz for 500 msec repeated every 2.5 sec. There was again no difference in the amount of labeled peptide released. Finally we examined the effect of increasing spike number. When cells were stimulated tonically at 1,3 or 5 Hz we observed a linear relationship between spike number and amount of peptide released. Reducing the calcium in the superfusate by 50% resulted in a reduction in peptide release but did not markedly alter this linear relationship. These results suggest that the release of the SCPs from B1 and B2 in culture is insensitive to the stimulation pattern, in contrast to the release of the SCPs from B15 in vivo which have been shown to be very sensitive to the stimulus paradigm (Whim and Lloyd, 1989; Cropper et al, 1990). Supported by NS 23569.

200.7

PRESENCE AND BIOLOGICAL ACTIVITY OF A GnRH-LIKE PEPTIDE IN THE NERVOUS SYSTEM OF *HELISOMA TRIVOLVIS*. J.P. Chang*, S. Patrick, C.J. Price and J.I. Goldberg. Dept. of Zoology, University of Alberta, Edmonton, Alberta, T6G 2E9.

Gonadotropin-releasing hormone (GnRH) constitutes a family of neuropeptides found throughout the vertebrates. While a GnRH-like peptide (GLP) has also been isolated from yeast (α-mating factor), the presence of GnRH has not been clearly demonstrated in several invertebrate phyla. In this study, we tested the hypothesis that GLPs are present and functional in the CNS of the gastropod mollusc, *Helisoma trivolvis*. The presence of GLP was examined by three methods. 1) In immunofluorescence studies using four antibodies generated against different GnRHs, select neurons and putative neurosecretory cells were specifically and consistently labelled throughout the CNS. 2) Reverse-phase HPLC and RIA analysis revealed a GLP which co-migrates with mammalian (m)GnRH. 3) In bioactivity experiments, extracts of *Helisoma trivolvis* CNS stimulated gonadotropin release from dispersed goldfish pituitary cells in culture. Two functional assays were carried out to examine the potential biological roles of GLPs in *Helisoma*. 1) Addition of mGnRH arrested neurite outgrowth in a subpopulation of dissociated *Helisoma* neurons in culture. 2) Intracellular recordings of left-parietal and visceral ganglion neurons revealed diverse electrophysiological responses to mGnRH. These effects were attenuated by a mGnRH antagonist. Taken together, these results strongly suggest that GLPs are important neuropeptides in the molluscan CNS.

(Supported by NSERC grants to JPC and JIG.)

200.8

PHARMACOLOGICAL PROFILE OF GLUTAMATERGIC INFLUENCES IN THE BUCCAL CPG HELISOMA TRIVOLVIS. E.M. Quinlan* and A.D. Murphy Dept. of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois 60608

Feeding behaviors in Helisoma are controlled by a CPG comprised of three subunits (S1-S2-S3). S2 inhibition of S1 and S3 plays a fundamental role in the temporal organization of this CPG. Previous data suggests the S2 neurons are glutamatergic, and that glutamate has direct effects on isolated motoneurons. There are at least two pharmacologically distinct glutamate receptors on identified buccal neurons. The receptor mediating S2 excitation and glutamatergic depolarization resembles a vertebrate kainate receptor. In contrast, S2 inhibition and glutamatergic hyperpolarizations were not blocked by ionotropic glutamate receptor antagonists, nor 24 hour incubation in pertussis toxin (2-6 ug/ml). 1mM external TEA or 1mM 4-AP were also ineffective. However, prolonged exposure to quisqualate ameliorates the S2 ipsps and produces a desensitization to the hyperpolarizing effects of glutamate, ibotenate and quisqualate. Blocking S2 psps changes the timing of action potential bursts in other CPG neurons and motoneurons, allowing us to unravel the temporal organization of this multifunctional CPG.

200.9

PUTATIVE CARDIOVASCULAR ROLE FOR MET-ENKEPHALIN AND α-PEPTIDE IN *LYMNAEA STAGNALIS*. N.M. Ewadinger*, N.I. Syed, R.L. Ridgway, A.G.M Bulloch and K. Lukowiak. Dept. of Med. Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

We have previously shown that two identified, well-characterized cells, Visceral Dorsal 1 (VD1) and Right Parietal Dorsal 2 (RPD2), in the CNS of *Lymnaea stagnalis*, are immunoreactive to a polyclonal met-enkephalin (ME) antibody. Others have shown these cells to also be immunoreactive to an α-peptide antibody. In this study, we found these cells and their fibers to be immunoreactive to a monoclonal ME antibody. Using dot blot analysis, no cross-reactivity between either α-peptide and ME antibody or between ME peptide and α-peptide antibody was observed. Therefore, the two peptides may co-exist within these identified neurons.

Since VD1 and RPD2 innervate the heart, it is possible that both peptides have a functional role in cardiovascular regulation. We used an isolated heart preparation to test the effects of ME and α-peptide on the myogenic heart of *Lymnaea*. During perfusion, effects on heart rate, amplitude, and tonus were examined. Distinct effects of ME (more variable) and α-peptide (consistently excitatory) were recorded. In 27 out of 35 trials, ME was excitatory at concentrations from 10^{-8} to 10^{-5} M. Reliably, α-peptide at concentrations from 10^{-9} to 10^{-6} M caused an increase in both frequency and amplitude of contractions. These results suggest that both ME and α-peptide may function in modulating the heart of *Lymnaea*.

Supported by MRC (Canada). We would like to acknowledge Dr. J. Van Minnen for supplying the α-peptide and the α-peptide antibody. Drs. C. Cuello and A. Côté for providing the monoclonal ME antibody. Drs. B. Brezden and D. Gardner for the use of their heart perfusion system.

200.10

ISOLATION AND CHARACTERIZATION OF MYOMODULIN-CARP-RELATED PEPTIDES (MCRPs) IN MOLLUSCS. W. Lesser*, M.J. Greenberg, K.E. Doble, T.D. Lee, C.G. Morgan, N.A. Pennell and D.A. Price. Whitney Laboratory, University of Florida, St. Augustine, FL 32086-8623.

The MCRPs are a family of neuropeptides that regulate muscle contraction in molluscs, e.g., *Aplysia* (K. R. Weiss *et al.*), *Fusinus*, and *Mytilus* (Y. Muneoka *et al.*). We have used RIA (with antisera kindly provided by Weiss) to monitor HPLC purification of similar peptides from a clam (*Mercenaria*) and a snail (*Helix aspersa*). Of the MCRPs isolated, the sequences of four have been determined by FAB mass spectrometry and microsequencing: i.e., GGFSMLRLamide and GGHAMLRLamide from the clam; and pQLSMLRLamide and GGSGMLRLamide from the snail. We have also determined the relative amounts and distributions of the peptides in individual organs. In the cerebral, visceral, and pedal ganglia of the clam the MCRPs are 5 to 100 times more concentrated than in other tissues. Moreover, immunohistochemistry reveals different staining patterns within each tissue. Supported by NIH HL28440 and NSF grants DIR-8901337 and DIR-9200417.

200.11

CHARACTERIZATION OF AN ALKALINE PHOSPHATASE LOCALIZED IN THE CENTRAL NERVOUS SYSTEM OF *APLYSIA CALIFORNICA*. T. Daza and J.K. Ono*, Dept. of Biological Science, California State University, Fullerton, CA 92634.

Alkaline phosphatase (AP) is commonly used in histochemical studies as a reporter enzyme for depositing a chromogen. However, the *Aplysia* CNS contains a high level of endogenous AP localized in glial cells surrounding neurons and nerve connectives. Several inhibitors of mammalian AP's were tested on the *Aplysia* enzyme. Our results show that the *Aplysia* AP is unlike any one of the several mammalian APs, but in general, is similar to the Liver/Bone/Kidney AP in mammals, which is thought to be the most primitive type. The possible involvement of this AP in transport of neurotransmitters and neurotransmitter precursors into neurons is being investigated.

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200.12

STRUCTURE-ACTIVITY STUDIES OF THE MYOACTIVE HISTIDINE-RICH BASIC PEPTIDE FROM THE R3-R14 NEURONS OF *APLYSIA*. S.L. Knock, G.T. Nagle, A. Kurosky, S.D. Painter.* Marine Biomedical Institute and Department of Human Biological Chemistry and Genetics, Univ. of Texas Medical Branch, Galveston, TX 77555.

The histidine-rich basic peptide (HRBP), a 43-residue peptide product of the R3-R14 neurons of *Aplysia californica*, excites the heart and enhances gut motility *in vitro*. Six peptides were synthesized for structure activity studies; they corresponded to residues 1-43, 6-43, 13-43, 18-43, 23-43 and 32-43 of the native peptide. Each was purified by reversed-phase HPLC. The identity of each peptide was confirmed and the amount of material quantified by amino acid compositional analysis. Biological activity was assessed in an *in vitro* bioassay using *A. californica* crop muscle. The longer peptides, corresponding to residues 6-43, 13-43 and 18-43, were approximately equipotent with HRBP(1-43). In each case, the threshold was 3-10 x 10^{-6} M. The shorter peptides [HRBP(23-43) and HRBP(32-43)] were inactive at 10^{-4} M, suggesting that the NH_2-terminal region of the peptide is not critical for HRBP biological activity. (Supported by NIH NS07185 and NS29261).

TRANSMITTERS IN INVERTEBRATES II

201.1

DIFFUSIONAL PROPERTIES OF EXTRACELLULAR SPACE IN OPTIC LOBES OF THE INVERTEBRATE *SEPIA*. K. T. Potter*, C. Nicholson, R. Williamson and N.J. Abbott. The Marine Biological Association of the United Kingdom, Citadel Hill, Plymouth, PL1 2PB, UK.

Diffusion of molecules within the extracellular space (ECS) of the brain is an important tissue characteristic, likely playing a role in distribution of informational substances, nutrients and metabolic products. The diffusion characteristics of ECS can be defined by tortuosity (λ), which indicates hindrance of diffusion, and volume fraction (α), a measure of the proportion of the tissue that is ECS. Studies of diffusion in vertebrate brain indicate a typical λ of 1.6, and α of 0.2 (20%) (e.g. Rice and Nicholson, J. Neurophysiol., 65: 264, 1991). To determine whether invertebrates have similar ECS diffusion values we studied slices of the optic lobes from *Sepia officinalis* (cuttlefish).

Slices (400 µm thick) of optic lobe were cut with a McIlwain tissue chopper and bathed in cooled, oxygenated artificial seawater. Measurements of diffusion were made by iontophoresing tetramethylammonium ions via a microelectrode and recording the concentration with an ion selective microelectrode located 100 µm or more away. Values of α and λ were estimated by fitting a suitable diffusion model to the data. Analysis of 54 measurements in *Sepia* optic lobe showed $\lambda = 1.86 \pm 0.37$ (mean ± standard error) and $\alpha = 0.29 \pm 0.18$. These data are comparable to those from vertebrate brain slices, suggesting similar ECS architecture in both vertebrates and invertebrates.

KTP received an MBA Bursary and RW was a Wellcome Trust Senior Research Fellow. Supported by Royal Society Marine Science Grant (NJA) and NIH Grant NS-13742 (CN)

201.2

L-GLUTAMATE AND GABA: THEIR EFFECTS ON AFFERENT RESTING ACTIVITY IN THE CEPHALOPOD STATOCYST. Y.J. Tu and B.U. Budelmann*. Marine Biomed. Inst., Univ. Texas Med. Branch, Galveston, TX 77555.

The effects of bath application of excitatory amino acids and GABA on the resting activity (RA) of afferent fibers were studied in the isolated *Sepia officinalis* statocyst. L-Glutamate (threshold 10^{-5}M) and its agonists quisqualate and kainate (thresholds 10^{-6}M) increased the RA in a dose-dependent manner, while D-Glutamate had no effect. Also, no obvious excitatory effects for NMDA and L-aspartate, nor any antagonistic effect of the selective NMDA-receptor antagonist 2-amino-5-phosphonovaleric acid (AP-5) were seen. The spider toxin argiotoxin-636 (threshold 10^{-10}M), 2-amino-4-phosphonobutyric acid (AP-4), glutamic acid diethyl ester (GDEE), and γ-D-glutamylaminomethyl-sulfonic acid (GAMS) had inhibitory effects on the RA and were effective antagonists of L-Glutamate (and its non-NMDA agonists). GABA and the GABA-A agonists muscimol and isoguvacine inhibited the RA. The GABA-A antagonists bicuculline and picrotoxin increased the RA, or reversed the inhibitory effects of GABA and GABA-A agonists. The GABA-B agonist baclofen had no significant effect on the RA.

The data favor the hypothesis that L-Glutamate (via non-NMDA receptors) is a potent excitatory transmitter or modulator of the activity of the afferent fibers in the cephalopod (*Sepia*) statocyst, and that endogenous GABA (via GABA-A receptors) has a tonic inhibitory effect.

Support by NIH grant HAR 5 R01 EY 08312-02.

201.3

LOCALIZATION AND PHYSIOLOGICAL ACTION OF THE NOVEL HOLOTHURIAN PEPTIDE GFSKLYFamide. L. Díaz-Miranda* and J. E. García-Arrarás. Department of Biology, University of Puerto Rico, Río Piedras, P.R. 00931.

The heptapeptide Gly-Phe-Ser-Lys-Leu-Tyr-Phe-NH_2 (GFSKLYFamide: GFS) was recently purified and characterized from gut extracts of the sea cucumber Holothuria glaberrima. This represents one of the first peptides to have been isolated from the phylum Echinodermata and the first from the class Holothuroidea. We have prepared antisera against synthetic GFS and have used them, through indirect immunohistochemistry, to map its distribution in H. glaberrima. Our results revealed that, in the intestine, GFS is expressed in a nerve plexus, where cell bodies are primarily found in the serosa, and a fiber network is associated with the muscular layer. Additional nerve fibers expressing this peptide can be seen in the submucosal layer. Immunoreactive cell bodies showing long processes are also found in the mucosa.

The location of nerve fibers expressing GFS, particularly those closely apposed to the longitudinal muscle cells, suggests that this neuropeptide is involved in regulating the digestive physiology of H. glaberrima. We have used intestinal strips of H. glaberrima to test the potential actions of this peptide. Changes in the tension of the muscle in response to GFS and other peptides were measured under partially isometric conditions. GFS induces a dramatic relaxing action of the muscle tension in a dose-dependent manner. Structure-activity relationships show that the FMRFamide-related peptide, FLRFamide, but not FMRFamide, mimics the action of GFS. In summary, we provide histochemical and pharmacological evidence that indicates that the peptide GFS is present and might be acting as a neurotransmitter in H. glaberrima. [Supported by the Patricia Robert Harris Fellowship to LDM and the NSF (BNS-8801538) and the Department of Biology of the UPR.]

201.4

FMRFamide-RELATED PEPTIDES IN THE FLATWORM *BDELLOURA CANDIDA*. Kevin L. Blair*, David A. Price, and Peter A.V. Anderson. Whitney Laboratory, University of Florida, St. Augustine, FL 32086.

FMRFa-related peptides are defined loosely as any putative neuropeptide that is detectable with an FMRFa-directed assay using techniques such as immunocytochemistry, RIA, or bioassay. *Bdelloura candida*, an ectoparasite on horse shoe crab, is a model for platyhelminth neuro- and muscular-electrophysiology. Immunocytochemistry of whole mounts of brain, whole body, and dispersed brain in culture reveals staining with antisera to FMRFamide, Antho-RFamide and Antho-RWamide. Acetone and acidified acetone (1% TFA) extracts of *Bdelloura* were fractioned by reverse phase HPLC with linear gradients of ACN in aqueous TFA, HFBA and PO_4^{3-}, with the resulting fractions being tested by RIA between each fractionation. Based on differential detection with the antisera: Q_2, S-253, and Yang's, as many as seven different fractions are expected to yield unique peptides. Fractions 57-58 of the acidified acetone extract have been purified to yield a partial sequence of XMFDSPPQVYSPSNFX..? where the identity of X is not clear. Supported by NSF Grant BNS 9109155 to PAVA.

201.5

THE SEQUENCE OF A G PROTEIN-COUPLED RECEPTOR FROM JELLYFISH NEURONAL TISSUE. D.A. Price*, P.A.V. Anderson, M.A. Holman, W. Lesser and M.J. Greenberg. The Whitney Lab of the University of Florida, St. Augustine, FL 32086.

Coelenterates (jellyfish and their kin) are the most primitive animals possessing a nervous system, so we are interested in comparing their neural specific molecules to those of higher animals. Using PCR with degenerate primers based on conserved regions of known G-protein linked receptors, we have isolated a putative receptor clone from a cDNA library prepared from neuronally enriched tissue of a jellyfish (*Cyanea*).

The deduced amino acid sequence of this clone is most similar to those of the tachykinin and NPY receptors. Indeed, the computer program Clustal (PCGene) groups this receptor into a distinct clade which includes the *Drosophila* and mammalian tachykinin and NPY receptors. An array of neuropeptides that have been isolated from coelenterates (primarily by Grimmelikhuijzen and colleagues) have in common the C-terminal sequence Arg-X-amide where X is Ala, Asn, Ile, Phe, Pro or Trp. Since NPY shares this motif, with X = Tyr, we suppose that some of the similarities in sequence among this clade of receptors may reflect similarities in ligands bound. We are currently using two approaches to functionally characterize this *Cyanea* receptor. First, we are attempting to express the receptor in yeast. Second, we are trying to localize it by in-situ hybridization. Support: NSF BNS9109155 (PAVA); NIH HL28440 (DAP & MJG).

201.6

EFFECTS OF FMRFamide RELATED PEPTIDES ON WHOLE CELL CURRENTS IN IDENTIFIED MOTOR NEURONS OF A JELLYFISH. N. Grigoriev and A. N. Spencer*. Department of Zoology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9.

A family of neuropeptides with the carboxy-terminal Gly-Arg-Phe-NH_2 have recently been isolated and sequenced from coelenterates. Two of these peptides are specific to the hydromedusa *Polyorchis penicillatus*: Pol-RFamide I (<Glu-Leu-Leu-Gly-Gly-Arg-Phe-NH_2) and Pol-RFamide II (<Glu-Trp-Leu-Lys-Gly-Arg-Phe-NH_2). Polyclonal antisera to these peptides stain a subset of neurons in both peripheral regions associated with smooth muscle, and in neurons apposed to a network of motor neurons (SMNS) in the nerve-rings (CNS). *In vivo* studies indicate that RFamide peptides are able to cause long duration excitation of these neurons in the absence of any obvious depolarization.

These neurons can be identified in cultures of nerve-rings and whole-cell voltage clamp studies undertaken. Peptides were applied by "spritzing" onto cells through a pipette with an opening of approx. 1μm. Application of 2×10^{-5}M FMRFamide caused a selective inhibition (30 to 40%) of the sustained (I_K-like) potassium current. Based on preliminary experiments using forskolin it appears that this peptide acts through a cAMP-dependent mechanism. This modulation of a voltage activated potassium current may account for the increased excitability seen *in vivo*. Other cultured cells, probably myoepithelial cells, exhibit a variety of ligand-gated responses to pulses of RFamide and Pol 1-RFamide, including fast inward and outward currents.

201.7

IS ACETYLCHOLINE AN INHIBITORY TRANSMITTER IN HEART INTERNEURONS OF THE LEECH?

Joachim Schmidt & Ronald L. Calabrese* Dept. of Biology, Emory University, Atlanta, GA 30233

Inherent tonic activity of heart motor neurons (HE cells) in the leech is organized into rhythmic bursts of action potentials by rhythmic inhibitory input from interneurons of the heartbeat pattern generator, HN cells (Calabrese, 1979). HN cells in the third and fourth ganglion not only form synapses with motor neurons but also form reciprocal inhibitory synapses across the ganglion, thereby constituing half center oscillators. IPSPs in HE and HN cells appear to be Cl^- mediated (Nicholls & Wallace, 1978; Calabrese, 1979).

Synaptic transmission between HN cells and between HN cells and HE cells is blocked by bicuculline methiodide. GABA when applied focally onto the somata of HN cells or when added to the supefusate, has no effect on the membrane potential of HN cells. Acetylcholine (ACh) or the ACh agonist carbachol hyperpolarize HN cells and HE cells when applied focally onto their somata or into the neuropil or when added to the superfusate. IPSP-like responses elicited by focal application of carbachol onto the somata of HN cells and HE cells are blocked by bicuculline methiodide. Focal application of carbachol onto the somata of HN cells and HE cells increases membrane conductance. IPSP-like responses elicited by focal application of carbachol onto the somata of HN cells and HE cells are reversed when Cl^- is injected into the cells. The results indicate, that HN cells use ACh as an inhibitory transmitter, that the postsynaptic receptors for ACh are blocked by bicuculline methiodide and that inhibition of HN cells and HE cells is mediated by an increased Cl^- conductance. (NIH NS24072).

201.8

THE DISTRIBUTION AND MICROSTRUCTURE OF PEPTIDERGIC IMMUNOREACTIVITIES IN THE STOMATOGASTRIC GANGLION OF THE CRAB, *CANCER BOREALIS*. D. Baldwin[1]*, A.E. Christie[2], E. Marder[2] and K. Graubard[1]. [1]Dept. Zoology, Univ. of Washington, Seattle, WA 98195 and [2]Dept. of Biology, Brandeis, Univ., Waltham, MA 02254.

Proctolin, FMRFamide-like, red pigment concentrating hormone (RPCH)-like and cholecystokinin (CCK)-like (using the monoclonal antibodies, C36-9H and C37-4E; Christie *et al., Soc. Neurosci Abst.* 17:278) immunoreactivities within the stomatogastric neuropil were examined using confocal microscopy. Staining consists of individually distinguishable blobs, often identifiable as varicosities running along (and probably enclosed within) fine neurites. A subpopulation of the labeled blobs has a substructure composed of variations in staining density. All immunoreactivities contain blobs ranging in cross-sectional area from <1μm^2 to 20μm^2. FMRFamide-like, RPCH-like and C36-9H staining also show a population of blobs >20μm^2.

Peptidergic immunoreactivity is largely confined to the peripheral and intermediate regions of the neuropil with very limited staining seen in the central core. Peripheral and intermediate labeling varies in three-dimensional distribution in an antibody specific manner. For example, C37-4E immunoreactivity extends past the main neuropil invading the area around and between the neurons immediately posterior to the neuropil. The appearance of the labeled regions also varies. For example, C37-4E staining produces an open, honey-combed appearance, while proctolin staining leaves less open space. Supported by NS 17813(EM), NS 15697(KG), NS 25505(KG) and the Human Frontier Science Program.

201.9

A COMPARISON OF THE MICROSTRUCTURE OF CHOLECYSTOKININ-LIKE IMMUNOREACTIVITY IN THE STOMATOGASTRIC GANGLION OF THREE DECAPOD CRUSTACEA. A.E. Christie[1], D. Baldwin[2], K. Graubard[2]* and E. Marder[1]. [1]Dept. of Biology, Brandeis, Univ., Waltham, MA 02254 and [2]Dept. of Zoology, Univ. of Washington, Seattle, WA 98195.

We used confocal microscopy to compare the microstructure of cholecystokinin (CCK)-like immunoreactivity (using monoclonal antibodies C36-9H and C37-4E; Christie *et al., Soc. Neurosci Abst.* 17:278) in the stomatogastric ganglion of the crab, *Cancer borealis* and the lobsters, *Homarus americanus* and *Panulirus interruptus*. While the superficial appearance of each species' ganglion varies dramatically, the general patterns of peptidergic immunoreactivity remain constant; staining is generally within the peripheral neuropil. C36-9H and C37-4E immunoreactivity consists of individually distinguishable blobs, the majority of which can be resolved as varicosities running along fine neurites. Both antibodies label varicosities ranging in cross-sectional area from <1μm^2 to 20μm^2 in each of the species studied. In *C. borealis*, C36-9H also labels blobs with cross-sectional areas >20μm^2. Some blobs have substructures consisting of regional variations in staining density.

The overall shape of labeled blobs is species dependent. In *C. borealis* and *P. interruptus*, C36-9H blobs appear relatively spherical, while in *H. americanus* the majority of larger varicosities (>10μm^2) are spindle-shaped. The localization of staining within the ganglion also varies. For example in *C. borealis* C36-9H labeled blobs of <20μm^2 cluster in the posterior periphery of the ganglion, rarely invading the central core; while in *P. interruptus* staining is most prevelant in the anterior of the ganglion with occasional ramification in the central core. The results of this study indicate that the microstructure of homologous peptidergic neuropil differs among species of decapod crustacea. Supported by NS 17813(EM), NS 15697(KG), NS 25505(KG) and the Human Frontier Science Program.

201.10

DYNAMICS OF INTRADENDRITIC CALCIUM IN A SEROTONIN-EVOKED PLATEAU-GENERATING CRAB MOTONEURON B. Zhang*[1], K.R. Delaney[2], D.W. Tank[2] and R.M. Harris-Warrick[1] 1).Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853; 2).Biological Computation Research Department, AT&T Bell Laboratories, Murray Hill, NJ 07974.

A set of serotonergic/cholinergic mechanosensory cells, the gastro-pyloric receptor (GPR) cells, exert a variety of neuromodulatory effects in stomatogastric ganglion of the crab, *Cancer borealis*. GPR stimulation evokes a plateau potential of about 15 mV lasting many seconds in the dorsal gastric (DG) motoneuron. Both GPR stimulation and serotonin (5-HT) application produce a slow inward current (SIC) by evoking a conductance decrease (of Ik-Ca) and a conductance increase (of Ih) (Kiehn & Harris-Warrick, 1992) and possibly other as yet unidentified currents.

Several lines of evidence indicate that Ca plays an important role in generation of the DG plateau. The 5-HT-induced SIC persists in TTX-containing saline, but is reduced when the cell is voltage-clamped in the presence of 20 mM TEA (Ik-Ca blocker). Extracellular Cd (200 μM) reduces the amplitude of the plateau leaving a 3-5mV depolarization that lasts several seconds. This suggests that reduction of Ik-Ca, together with increased Ih, may trigger the plateau, but Ca influx is required for full expression of the plateau response. Intracellular injection of the Ca chelator EGTA slows the rising phase of the plateau and prolongs its duration. These results are consistent with a 5-HT-induced Ca-dependent inward current(s) contributing to the plateau.

We are using quantitative fura-2 imaging of dendritic Ca in the presence and absence of excess Ca buffers to determine whether the time course of Ca accumulation and recovery correlates with the amplitude or time course of the 5-HT-induced plateau. Sponsored by NIH NS17323 to RHW.

201.11

Neurones in the head ganglia of the nematode *Ascaris suum*: A possible functional correlate for the nervous system of *C.elegans*. L. Holden-Dye and R.J. Walker. (SPON: Brain Research Association*). Dept. of Physiol. and Pharmacol., Bassett Crescent East, University of Southampton, Southampton, SO9 3TU, U.K.

Stretton *et al.* have pioneered the use of the large parasitic nematode *Ascaris suum* as a functional correlate for the extensively studied nematode *C. elegans*. We have used a whole mount preparation of *Ascaris* head ganglia to intracellularly label neurones and record their electrical properties. These have been combined with immunocytochemical studies to indicate the possible neurotransmitter content of the neurones. Neuropeptide immunoreactivity, particularly FMRF-amide-like, is widespread. The neurones have membrane potentials of about -30 mV, and a relatively high input resistance for their size e.g. 24 megohm for a 100µm cell. Many of the neurones have distinctive morphologies that are similar to neurones in *C. elegans*. All the neurones identified so far in the ventral ganglia are similar to those of *C.elegans* in that they are monopolar and send projections into the nerve ring. Varicosities resembling sites for transmitter release have been observed in this region. These studies will be used to identify simple neuronal circuits in *Ascaris*.

Supported by the Wellcome Trust.

201.12

AMINO ACID SEQUENCE AND MOLECULAR ANALYSIS OF INSECT CARDIOACTIVE PEPTIDES. P.K. Loi*, C.C. Cheung, T.D. Lee, and N.J. Tublitz, Inst. of Neurosci., Univ. of Oregon, Eugene, OR 97403 and Div. of Immunol., Beckman Res. Inst., City of Hope, Duarte CA 91010.

The CNS of the tobacco hawkmoth, *Manduca sexta*, contains several myoactive neuropeptides, the Cardioacceleratory Peptides (CAPs), that are functionally well characterized (Tublitz *et al.*, TINS, 1991). The CAPs have different stage-specific actions, modulating gut contractility in embryonic and larval stages via local release while acting as cardioregulatory neurohormones in the adult moth. Here we report on a series of studies to elucidate the primary structure of the CAPs.

Previous biochemical studies using HPLC isolated two distinct peaks, CAP_1 and CAP_2, which had different retention times but similar CAP-like bioactivities. Refinements to our extraction and chromatography procedures coupled with the use of a syringe pump microbore HPLC system enabled further purification of the CAPs. CAP_2, when subjected to a 5-step chromatographic procedure, fractionated into at least three separate peaks, CAP_{2a}, CAP_{2b}, and CAP_{2c}. Similar analyses on CAP_1 also revealed multiple bioactive peaks. Conventional sequencing and FAB-MS analysis of CAP_{2a} yielded a primary amino acid sequence of PFCNAFTGCamide, which is completely homologous to crustacean cardioactive peptide (CCAP). Biochemical and pharmacological analyses, in which synthetic CCAP and native CAP_{2a} co-chromatographed on 5 different HPLC steps and produced identical dose-dependent responses on the isolated *Manduca* heart bioassay, provided confirmation of the sequence. The primary sequences for CAP_{2b} and CAP_{2c} have also been tentatively established but have not yet been confirmed. Both appear to be short peptides (<10 residues) with a N-terminal pyro-glu and an amide at the C-terminus. These latter two sequences, if confirmed, share these features with the arthropod peptide family that includes the adipokinetic hormones in insects and red pigment concentrating hormone in crustaceans. Supported by grants from NIH and NSF.

201.13

THE REGULATION OF A MUSCARINIC CURRENT IN AN IDENTIFIED INSECT MOTONEURON. B. A. Trimmer*. Department of Biology, Tufts University, Medford, MA 02155.

In larval *Manduca sexta*, brief (500 ms) bursts of activity from mechanoreceptors on the prolegs and ventral body wall evoke slow, long-lasting (5-20 s) depolarizations (sEPSPs) in the identified proleg motoneuron, PPR. The sEPSP has muscarinic pharmacology and can be mimicked by microinjection of the muscarinic agonist, oxotremorine-M (oxo-M), into the neuropil. Both oxo-M and the sEPSP increase PPR's excitability by depolarization and by lowering its spike threshold. Using a single electrode voltage clamp, it has been determined that the muscarinic current (*Iox*) underlying these effects is voltage-sensitive, TTX-insensitive and carried predominantly by sodium. The regulation of *Iox* and the roles of G-proteins and second messengers in insect neurotransmission is now being investigated.

Iox requires the presence of extracellular calcium and is sensitive to agents that affect calcium channels. Hence, nifedipine (40 µM), cadmium (100-400 µM), cobalt (4 mM), magnesium (20 mM) and high concentrations of calcium itself (10 mM), inhibit *Iox*. These results suggest that *Iox* requires, or is regulated by, the entry of calcium.

The injection of PPR's soma with agents that affect G-protein turnover have implicated G-proteins in the control of *Iox*. Using antibodies that recognize subclasses of mammalian G-proteins, Western blotting analysis of *Manduca* nerve cord homogenates reveals a protein, approximately 39 Kd in size, that is immunologically related to Goα. The potential role of this and other G-proteins in the muscarinic modulation of identified neurons is being investigated by immunohistochemistry and ligand binding studies.

Supported by a Sloan Foundation Fellowship and NIH Grant NS30566.

201.14

ECLOSION HORMONE RELEASE AND ITS REGULATION BY ECDYSONE: CHANGES IN THE EXCITABILITY OF THE VM CELLS AT ECDYSIS. R.S. Hewes* and J.W. Truman. Dept. of Zoology, University of Washington, Seattle, WA 98195.

In the tobacco hornworm, Manduca sexta, declining titers of ecdysteroids trigger eclosion hormone (EH) release, and EH in turn stimulates ecdysis, a highly stereotyped behavior used to shed the old cuticle at the culmination of each molt. Intracellular recordings from the EH containing cells (VM cells) before, during, and after hormone release may provide much of the essential groundwork for resolving the mechanisms by which changes in ecdysteroid titers stimulate ecdysis. We have recorded from the VM cells during the molt from the 4th to the 5th larval instar, since at this time they are easily identifiable under a dissecting microscope. It has been possible to confirm their identity with the colocalization of iontophoretically injected biocytin and immunocytochemical staining with an anti-EH antiserum. The most dramatic change seen in the VM cells associated with EH release was a decrease in threshold. In intermolt animals, and up to about 90 min before ecdysis (at which time EH release begins in intact animals), the VM cells required depolariation of 10-30 mV from rest to elicit firing. Threshold voltage then rapidly declined so that by 30 min before ecdysis the threshold had dropped to 0 mV, and the cells fired tonically (2-3 Hz) at rest. Within 2-5 h after the onset of ecdysis behavior, threshold increased again to intermolt values.

201.15

ALLATOSTATIN-IMMUNOREACTIVE NEURONS IN THE COCKROACH *DIPLOPTERA PUNCTATA* AND THE SPHINX MOTH *MANDUCA SEXTA*. N.T. Davis*, H. Agricola, R. Feyereisen, and J.G. Hildebrand. ARL Div. of Neurobiol., Univ. of Arizona, Tucson, AZ 85721.

The allatostatins are a family of neuropeptides that have been shown to inhibit the release of juvenile hormone by the corpus allatum (CA) in cockroaches. Antisera against each of two principal allatostatins, the tridecapeptide (AS-A1) and the octadecapeptide (AS-B2), were used for the immunocytochemical identification of allatostatin-like immunoreactive (ASLI) neurons in the cockroach *Diploptera punctata*. ASLI neurosecretory cells located in the brain and projecting to the corpora cardiaca were stained, and these cells appeared to function in the control of juvenile hormone release. In addition, median neurosecretory cells and neurohemal organs of the ventral ganglia were stained, indicating the possible release of allatostatin-like neurohormones at sites remote from the CA. ASLI interneurons were stained throughout the CNS, suggesting that allatostatin-like peptides may serve as neuroeffectors within the CNS. In addition, projections of ASLI neurons to the midgut, hindgut, and salivary glands and intrinsic neurosecretory cells in the lining of the midgut were stained, indicating that allatostatin-like peptides may be involved in the control of visceral movements and secretion. Comparable neurosecretory cells, interneurons, and efferent visceral neurons were stained in the CNS of larvae and adults of the sphinx moth *Manduca sexta*, indicating that ASLI neuropeptides may occur in insects other than cockroaches.

202.1

ISOLATION AND DISTRIBUTION OF cDNAs WITH HOMOLOGY TO Na^+-DEPENDENT NEUROTRANSMITTER TRANSPORTERS. L.A. Dowd*, H.Q. Nguyen, J.D. Sinor, M. Mercugliano, D.B. Pritchett, and M.B. Robinson. Children's Seashore House; Depts. Pediatrics and Pharmacology, Univ. of Pennsylvania; Phila., PA 19104.

The cDNAs for the GABA, NE, 5-HT and DA transporters have recently been cloned. Since their sequences are highly homologous, these transporters may belong to a gene family. We designed degenerate oligonucleotides directed towards two conserved regions of the rat GABA and human NE transporters and used these as primers for the polymerase chain reaction. Using these PCR products, a rat brain cDNA library was screened for other homologous sequences. We isolated 79 cDNAs which were different from the cloned GABA transporter and characterized 12. Based on restriction digests, most of these 12 cDNAs were unique from the already cloned transporters and were also distinct from each other.

Four of these cDNAs were larger than 3.0 kB and were thought to be full-length clones. Full-length RNA probes from 3 of the 4 cDNAs demonstrated labelling of specific brain regions with *in situ* hybridization histochemistry. cRNA transcripts from clone 29.2 hybridized to mRNA in the substantia nigra pars compacta and ventral tegmental area. RNA transcripts from clone 44.1 hybridized to subregions of the brainstem, while clone 45.2 hybridized to neurons in multiple brain regions, including hippocampus (CA1, CA3, and dentate gyrus) and middle layers of the cortex.

We have also used the original degenerate oligonucleotides in the polymerase chain reaction with the 4 cDNAs as template. Each produced a DNA fragment of approximately 600 bp. This is the distance present between the two regions the oligonucleotides were directed against in the already cloned transporters, suggesting that our 4 cDNAs may be other members of a growing transporter family. (Supported by the Pew Foundation and Grant #GM-34781).

202.2

EXPRESSION OF SODIUM-SENSITIVE ATP-DEPENDENT [^{3}H]DOPAMINE UPTAKE IN A MOUSE CELL LINE TRANSFECTED WITH CLONED HUMAN GENOMIC DNA. A.J. Eshleman, J.C. Fernando, A.T. Eldefrawi*, M.E. Eldefrawi, and R.J. Hickey. Dept. Pharmacol. & Exp. Therap. & the Molecular & Cellular Biology Program, Univ. Md. Sch. Medicine, Baltimore, Md. 21201.

The transfection into mouse fibroblasts of cloned human genomic DNA contained in a cosmid vector has resulted in the identification of mouse cell lines exhibiting sodium-sensitive ATP-dependent uptake of [^{3}H]dopamine (DA). All cells which exhibited [^{3}H]DA uptake had specific [^{3}H](-)-2-ß-carbomethoxy-3-ß-(4-fluorophenyl)tropane 1,5-naphthalene disulfonate ([^{3}H]CFT) binding which was inhibited by micromolar concentrations of desipramine, imipramine, and cocaine. The presence of human DNA in the cell lines was verified by probing Southern blots with the human Alu sequence (pBlur8). A human S. nigra lambda cDNA library was screened with an oligonucleotide probe for the highly conserved region of the first transmembrane domain of the human norepinephrine and rat DA transporters (TM1). The positives were rescreened with a probe made by PCR using the nigral cDNA and two primers, the TM1 and another for the rat DA transporter protein terminus. These clones are being sequenced, and selected clones are being expressed in Cos cells. Positive clones will be used to probe Southern blots of the DNA of the mouse cell lines. (supported by grants to: RJH DRIF 30417; and DA07369; and to M.E.E. DA03680)

202.3

CLONING AND EXPRESSION OF A TAURINE TRANSPORTER FROM RAT BRAIN. K.E. Smith*, L.A. Borden, C.-H.D. Wang, P.R. Hartig, T.A. Branchek, and R.L. Weinshank. Synaptic Pharmaceutical Corporation, 215 College Road, Paramus, NJ 07652.

A complementary DNA clone encoding a taurine transporter was isolated from rat brain by low stringency hybridization with the rat GABA transporter cDNA (GAT-1; Guastella et al., 1990). The nucleotide sequence predicts a protein of 604 amino acids with significant homology to previously cloned neurotransmitter transporters. The functional properties of the cDNA were examined by transient transfection of COS-7 cells. The transporter displays high-affinity for taurine (K_d≈40μM) and is dependent on external sodium and chloride for transport activity, similar to taurine transport *in vivo*. β-alanine, a structural analog of taurine, and GABA significantly inhibit specific taurine transport, while glycine, glutamate, norepinephrine, dopamine, betaine, and L-methionine have no effect. Localization studies demonstrate that the transporter mRNA is present in a variety of peripheral tissues as well as the brain and retina. The cloning of an additional member of the inhibitory amino acid transporter family will facilitate the study of structure\function relationships for this class of molecules as well as the understanding of the physiology of taurine transport in the nervous system.

202.4

EXPRESSION OF GABA TRANSPORTER mRNA IN THE RAT CENTRAL NERVOUS SYSTEM. N. Brecha, C. Weigmann, and E. Messersmith*. Depts. of Anatomy & Cell Biology and Medicine, Brain Research Institute, and CURE, UCLA School of Medicine and VAMC-Wadsworth, Los Angeles, CA 90073.

GABA transporters play an important role in the termination of synaptic activity in the nervous system. The present study evaluates the distribution and developmental regulation of a high affinity GABA transporter in the nervous system using *in situ* hybridization histochemistry. Tissue sections were incubated in antisense or sense RNA, washed at high stringency, and processed for autoradiography. GABA transporter mRNA is abundantly distributed along the entire neuroaxis. Prominently labeled structures include the olfactory bulb, septal nuclei, basal forebrain nuclei, cortex, hippocampus, substantia nigra, superficial layers of the superior colliculus, interpeduncular nucleus, pontine nuclei, cerebellar cortex, spinal trigeminal nuclei and the spinal cord. These studies are in agreement with previous descriptions of the distribution of GABA or L-glutamate decarboxylase immunoreactivity in the central nervous system. Developmental studies show that at birth low levels of GABA transporter mRNA are present in the olfactory bulb, cerebellar cortex, and brainstem nuclei. Other forebrain structures begin expressing GABA transporter mRNA during the first postnatal week. These studies indicate that there is a temporal regulation in the expression of this GABA transporter mRNA.

We thank Dr. J. Guastella for providing the GAT-1 cDNA.
Supported by NEI grant EY 04067 and VA Medical Research Funds.

202.5

ISOLATION AND CHARACTERIZATION OF A cDNA ENCODING A NEW MEMBER OF THE SODIUM-DEPENDENT NEUROTRANSMITTER TRANSPORTER SUPERFAMILY. Eugenia M. C. Jones Committee on Neurobiology and Howard Hughes Medical Institute and Robert A. McCrea* Committee on Neurobiology, Department of Pharmacology and Physiology, University of Chicago, Chicago, Illinois 60637

Sodium-dependent neurotransmitter transporters comprise a superfamily of integral membrane proteins. Degenerate oligonucleotide primers based on regions of amino acid similarity between the GABA and norepinephrine transporters were used to amplify related sequences in bovine retina RNA. Sequence analysis of fifteen PCR products revealed three clones encoding the bovine GABA transporter (there is 94% amino acid identity with the rat brain GABA transporter) as well as a novel member of this superfamily designated as TL-15 having 51%, 58%, 59%, 61% and 62% amino acid identity with the betaine, norepinephrine, serotonin, GABA, and dopamine transporters, respectively. Of the remaining 11 clones, six encoded clusterin and five were not related to any sequences in the GenBank database. RNA blotting indicated that TL-15 hybridized to a single transcript of about 4.4 kb that was present in retina and brain, but not in liver. In situ hybridization showed that TL-15 mRNA was localized to the inner nuclear layer of the bovine neural retina. A similar pattern of hybridization was noted using the bovine GABA transporter cDNA clone. Studies are underway to complete the cloning of this new member of the Na-dependent neurotransmitter transporter superfamily and to identify its ligand. Supported by NRSA 5F31MH09973-02 BPN-02

202.6

DOPAMINE TRANSPORTER PROTEIN: SOLUBLE FRAGMENTS, PHOSPHORYLATION PATTERNS, & MOLECULAR MODELING. C.K. Surratt, S. Yuhasz, M. Amzel, M.T. Brannock, M. Takemura* and G.R. Uhl. *Lab. Mol. Neurobiol., ARC/NIDA & Depts. Neurol, Nsci, Biophysics, JHUSM, Box 5180, Baltimore, MD 21224.*

Efficient expression of dopamine transporter (DAT) proteins can allow production of antibodies, study of phosphorylation and other post-translational modification patterns, and possibly even allow protein crystallization for X-ray structural studies. Bacterial expression of a DAT fusion protein yielded little recoverable product. Expression of rat and human cDNA segments encoding the N-, C-, and second extracellular domains yielded ca. 100 μg peptide/L, with homogeneous material eluted from a single affinity purification step. In preliminary studies, the N- terminal fragment was phosphorylated *in vitro* by protein kinase A. These transporter fragments can elicit polyclonal antibody responses in rabbits. Molecular modelling studies of transporter putative transmembrane helices, in combination with studies of the effects of specific mutations, have yielded insights into possible transporter protein structural motifs and its potential modes of interaction with cocaine, dopamine, and transporter-dependent dopaminergic neurotoxins.

202.7

DOPAMINE TRANSPORTER 1 mRNA DENSITIES DIFFER AMONG NEURONS OF DIFFERENT DOPAMINERGIC NUCLEI. C. Cerruti*, S. Shimada, D. Walther, M. Kuhar & G. Uhl. *Labs. Mol. Neurobiol. & Mol. Pharm. ARC/NIDA & Depts. Neurol. Nsci & Psych., JHUSM, Box 5180, Baltimore, MD. 21224.*

Cloning cDNAs encoding the rat dopamine transporter (DAT1) allows assessment of the regional and cellular bases of its expression in the rat brain. Oligonucleotides and a full-length cDNA complementary to DAT1 mRNA hybridize to a 3.7 kb mRNA prepared from the rat midbrain that is absent in cerebral cortex or cerebellum. *In situ* hybridization using specific oligonucleotides reveals dense hybridization in neurons of the rat substantia nigra compacta, intermediate densities in ventral tegmental area subdivisions and A11, and very low levels in arcuate, olfactory bulb, retina and solitary tract. Different dopaminergic (or putative L-DOPAergic) cell groups thus display different levels of DAT1 dopamine transporter expression. Interestingly, the rank order of expression in several of these groups is parallel to the extent of cell loss in Parkinson's disease (compacta > VTA > arcuate).

202.8

CELLULAR LOCALIZATION OF mRNA FOR A NOREPINEPHRINE UPTAKE TRANSPORTER IN NEURONS AND ENDOCRINE CELLS OF RAT AND MONKEY: AN *IN SITU* HYBRIDIZATION STUDY

R. Elde, B. Meister, M. Eriksson* and T. Hökfelt. *Dept. of Histology and Neurobiology, Karolinska Institute, P.O. Box 60400, 104 01 Stockholm, Sweden.*

Noradrenergic neurotransmission is terminated by reaccumulation of norepinephrine into the presynaptic terminal by an uptake system. A cocaine- and antidepressant-sensitive human norepinephrine transporter (NET) was recently cloned and Northern-blot analysis has shown presence of NET in brain stem and adrenal gland (Pacholczyk *et al.*; Nature 350:350, 1991). In the present study we have synthesized 48-mer oligonucleotide probes from the coding region of the human NET complementary to oligonucleotides 143-190 (probe 1) and 1861-1908 (probe 2). NET mRNA was visualized by *in situ* hybridization in the central and peripheral nervous system and adrenal gland of rat and monkey (*Macaca fascicularis*). Intense hybridization with the NET probes was demonstrated in neurons of the locus coeruleus and in several norepinephrine-containing cell groups of the lower brain stem. Virtually all cells in the superior cervical, inferior and superior mesenteric ganglia contained NET mRNA. Less intense hybridization with the NET probes was demonstrated in chromaffin cells of the adrenal gland, whereas ganglion cells within the adrenal medulla exhibited intense hybridization signal. No NET mRNA was detected in cortex cerebri, hypothalamus, spinal cord, dorsal root ganglia, retina, kidney or pancreas. This study demonstrates presence of NET mRNA in neurons previously known to store and release norepinephrine. Studies on changes in cellular NET mRNA expression may be of importance in order to identify neurons where function is altered in affective disorders and in addiction to cocaine.

(Supported by grant DA 06239 and from the Swedish MRC 04X-2887).

202.9

PRIMARY STRUCTURE AND FUNCTIONAL EXPRESSION OF A CHOLINE TRANSPORTER EXPRESSED IN THE RAT NERVOUS SYSTEM. P. Schloss, W. Mayser and H. Betz*. Dept. of Neurochemistry, Max-Planck-Institute for Brain Research, Deutschordenstr. 46, D-6000 Frankfurt 71, Germany.

Re-uptake of neurotransmitters into the presynaptic terminal is mediated via high-affinity, sodium-dependent transport proteins. These are thought to form a new family of integral membrane polypeptides, which share a common predicted transmembrane topology and significant amino acid identity (30-65%). We have performed polymerase chain reaction (PCR) with degenerate oligonucleotides deduced from the sequence of the GABA transporter (GAT1). This led to the isolation of cDNAs encoding a choline transporter (CHOT1), as shown by functional expression in *Xenopus* oocytes. [^{3}H]Choline uptake in oocytes was of high affinity (Km ≈10 μM) and strictly sodium dependent. Amplification by PCR revealed significant amounts of CHOT1 transcripts in brain, cerebellum, spinal cord and, to a lesser extent, heart, but only very low expression in lung, kidney and muscle.

202.10

DOPAMINE TRANSPORTER mRNA CONTENT IN HUMAN SUBSTANTIA NIGRA: ABRUPT AGE-RELATED LOSS. Michael S. Poosch*, Yue Xia, Dennis J. Goebel, Bader Cassin, Gregory Kapatos and Michael J. Bannon. Departments of Psychiatry (Cellular and Clinical Neurobiology Program) and Pharmacology, Wayne State University School of Medicine, and Wayne County Medical Examiner's Office, Detroit, MI. U.S.A.

Neurotransmitter transporters provide the primary means for terminating synaptic transmission in monoaminergic neuronal systems. Additionally, they constitute a major site of action for psychostimulant drugs and, in the nigrostriatal system, for the uptake of neurotoxins that induce parkinsonism. In this study, a human dopamine transporter (DAT) partial cDNA clone was obtained via reverse transcription of human substantia nigra RNA followed by polymerase chain reaction using primers based on the published sequence for the human norepinephrine transporter. The cloned sequence subsequently was found to exhibit 87% nucleic acid identity (89% amino acid identity) with the sequence for transmembrane domains three through five of the rat homolog. This clone was used to quantitate age-related changes in human DAT mRNA by nuclease protection assay. The postmortem content of DAT mRNA in the nigrae of 18-57 year-old subjects was found to be relatively constant; however, in subjects >57 years of age, an abrupt decline (>95%) in nigral DAT mRNA was observed. In situ hybridization experiments confirmed this profound loss of DAT gene expression in melanin-positive (presumptive dopamine) nigral neurons. In contrast, the tyrosine hydroxylase mRNA content of these samples declined in a more uniform manner with increasing age. These data provide evidence that compensatory changes may be occurring in human dopamine neurons consequent to the normal aging process.

202.11

DOPAMINE TRANSPORTER mRNA CONTENT IN RAT SUBSTANTIA NIGRA: COCAINE-RELATED DECREASE. Yue Xia*, Dennis J. Goebel, Gregory Kapatos and Michael J. Bannon. Departments of Psychiatry (Cellular and Clinical Neurobiology Program) and Pharmacology, Wayne State University School of Medicine, Detroit, MI. U.S.A.

The function of the dopamine transporter is to terminate synaptic transmission in dopaminergic neuronal systems. It is also a primary site of action for such psychostimulant drugs as cocaine and amphetamine. To investigate some of the effects of these drugs on dopamine transporter gene expression, we have taken advantage of the high level of sequence homology between our human dopamine transporter partial cDNA clone and the corresponding sequence in the rat. Using the human clone to make antisense RNA probe, dopamine transporter mRNA in the rat substantia nigra has been successfully visualized by in situ hybridization and quantitated by nuclease protection assay. A preliminary assessment of the effects of cocaine on rat nigral dopamine transporter mRNA content has shown that repeated drug administration (15 mg/kg, twice a day for 6.5 days) produces a >40% decrease in transporter mRNA compared with controls. In contrast, neither acute cocaine administration (4 hr before sacrifice) nor repeated administration followed by 72 hr of abstinence had any effect on nigral dopamine transporter mRNA levels. This suggests that one of the effects of dopamine transporter blockade may be the downregulation of neuronal dopamine transporter gene expression.

202.12

EFFECT OF PROTEIN KINASE C ON GABA TRANSPORTER EXPRESSED IN XENOPUS OOCYTE. N. Saito*, T. Koga, I. Osawa, and C. Tanaka Dept. of Pharmacology, Kobe University School of Medicine, Kobe 650, Japan.

The synaptic neurotransmission is terminated by rapid re-uptake of the neurotransmitter into the presynaptic terminals from the synaptic cleft. GABA, a major inhibitory neurotransmitter, is also transported into glial cell or neuronal cells by variety of the pharmacological kinetics. The cDNA for GABA transporter was isolated and the significant homology between the various neurotransmitter transporters has been identified. However, the modulation of these transporter activities has not yet been elucidated. The presence of multiple putative phosphorylation sites in GABA transporter suggests that the activity of transporter may be regulated by phosphorylation. We have cloned a cDNA for GABA transporter from rat brain library and the effect of various kinases on the GABA transporter was examined in the brain synaptosomal fraction and in the Xenopus Oocytes. The activation of protein kinase C reduced the high affinity uptake of GABA into brain synaptosome but not effected on the low affinity uptake. Similarly, the activation of protein kinase C inhibited the GABA transporter which is expressed in Xenopus Oocyte by injection with the synthesized mRNA for GABA transporter. Using site directed mutagenesis, the regulation site of the transporter activity was also examined.

202.13

IODINATED DERIVATIVES OF TETRABENAZINE FOR MAPPING MONOAMINE STORAGE SITES. M.P. Kung*, D.Canney , Y.Z. Guo , J.Billings and H.F. Kung. Depts. of Psychiatry and Radiology, Univ. of Pennsylvania, PA 19104

Monoaminergic neurotransmission has been implicated in various neurological and psychiatric disorders. Pharmacological characterization of these neuronal systems could be greatly facilitated by the use of specific radioligands.[^{3}H]Reserpine and [^{3}H]dihydrotetrabenazine ([^{3}H]TBZOH) have been evaluated as in vitro probes for the vesicular monoamine transporter. Development of iodinated ligands with higher specific activity and selectivity for monoamine vesicular transport sites would be useful as presynaptic monoamergic markers for radioligand binding and autoradio-graphic studies. Starting with TBZ, an iodinated derivative, iodovinyltetrabenazine (I-TBZ), was synthesized in modest overall yield (28%). Subsequently, the "cold" and [^{125}I]I-TBZ were prepared from the tributyltin derivative by iododestannylation. The racemic mixture was then resolved on a HPLC chiral column into peak A and peak B (absolute configuration not determined). In vivo evaluation of peak A & peak B showed that, despite their similar high liphophilicity, peak A displayed specificity for the rat striatum compared to peak B. This selective in vivo uptake in rat brain after an i.v. injection could also be blocked by TBZ pretreatment. In vitro radioligand binding studies (rat striatal membrane preparations) indicate that peak A (but not peak B) of [^{125}I]I-TBZ binds selectively to a homogenous site corresponding to TBZ site with a Kd value of 0.3nM. The high binding affinity and selectivity of I-TBZ (peak A) suggests that this agent warrants further investigation as a potentially useful tracer for studying CNS vesicular monoamine transport sites. (Supported by Nihon Medi-Physics Inc.)

202.14

STRUCTURE-ACTIVITY RELATIONSHIPS OF SEROTONIN TRANSPORT. Albert S. Chang, Sharon M. Chang, Charles L. Densmore* and Diane M. Starnes. Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381.

A transfectant cell model of high-affinity serotonin transport was used to assess the inhibitory potencies of 5-HT/tryptamine structural analogues in order to assess the significance of 5-HT structural domains in transport interaction. One finding of this study is that methyl substitution at indole-7 position led to enhanced inhibitory potency, suggesting enhanced transport interaction. Further, the discerned SARs are consistent with the hypothesis that the terminal amino group (on the ethylamine side chain) has to be spatially coordinated with the hydroxyl group in order to be recognized by the transport system; the necessary spatial coordination is provided by the aromatic indole nucleus. This hypothesis also seemed to apply to nontricyclic antidepressant (NTCA) structures, including fluoxetine, paroxetine, citalopram and sertraline. Molecular modelling analyses revealed that the same spatial coordination of an electro-negative group (attached to an aromatic system) away from an amino group, as observed in 5-HT, also existed in the NTCA structures. Thus, a common pharmacophore structure seems to exist within structurally diverse inhibitors of 5-HT transport. This pharmacophore presumably accounts for antidepressant interaction with the 5-HT transport system.

202.15

TRANSPORT OF ACETYLCHOLINE DRIVEN BY AN ARTIFICIALLY-INDUCED PROTON GRADIENT. M. L. Nguyen and S. M. Parsons*. Neuroscience Research Institute, University of California, Santa Barbara, California 93106.

Cholinergic synaptic vesicles purified from electric organ that are hypoosmotically lysed and resealed in buffers of pH 5.1-5.8 transiently take up [^{3}H]acetylcholine (ACh) when the external pH is suddenly shifted to 7.8. Less [^{3}H]ACh is taken up when the internal pH is lower or higher than 5.1-5.8. Uptake of the [^{3}H]ACh is due to the artificial proton gradient, whereas the subsequent efflux of the [^{3}H]ACh is due to the decay of the proton gradient, which was monitored by the change in absorbance of acridine orange. Uptake is blocked by vesamicol, which is an allosteric ligand of the ACh transporter. Vesamicol added at the peak of uptake blocks efflux of the [^{3}H]ACh. A 100-fold excess of nonradioactive ACh added at the peak of uptake does not stimulate efflux of [^{3}H]ACh. Vesamicol-sensitive uptake is composed of osmotically sensitive and insensitive components, and the osmotically sensitive component is blocked by low temperature but the osmotically insensitive component is not. [^{3}H]Choline and [^{3}H]-N-methylcarbamoylcholine are not transported, but they are rapidly bound to the ACh transporter in a vesamicol-sensitive manner. The pH jump approach to inducing active transport of ACh will simplify study of the transporter by removing the dependence of transport on the V-type ATPase.

202.16

CHRONIC ESTRADIOL AND PROGESTERONE TREATMENTS AFFECT RAT STRIATAL DOPAMINE UPTAKE SITES. T. Di Paolo* and M. Morissette. Mol. Endocinol., CHUL Res. Centre, G1V 4G2 and Sch. Pharm., Laval Univ., G1K 7P4, Québec, CANADA.

The effects of chronic estradiol (E2) and progesterone (P) treatments to ovariectomized (OVX) rats on striatal DA uptake sites were investigated on tissue homogenates and by quantitative autoradiography with [^{3}H]GBR-12935 binding. Treatments were started the day after ovariectomy for 2 weeks and consisted of E2 (10 µg/0.2 ml), P (0.72 mg/0.2 ml), E2+P or the vehicle. One saturation curve of [^{3}H]GBR-12935 binding was performed using the striatal tissue of each animal. The steroid treatments left affinity (Kd) of [^{3}H]GBR-12935 striatal binding unchanged (OVX: 0.823 ± 0.028 nM). In contrast, the density of these binding sites was significantly increased by these steroids alone or in combination to a similar extent (about 20%): OVX, 4742 ± 287; E2, 5547 ± 287; P, 5822 ± 425; E2+P, 5616 ± 272 fmol/mg of protein. By autoradiography, [^{3}H]GBR-12935 at 2nM was chosen for binding on striatal coronal section (10 µm). No dorsal-ventral or lateral-medial differences were observed for the effects of steroids on [^{3}H]GBR-12935 binding. In anterior and medial striatum, E2 and E2+P treatments increased significantly [^{3}H]GBR-12935 binding (about 25% for E2-treated animals and 45% for E2+P-treated animals) whereas P treatment led to a non-significant increase of binding sites (about 20%). In posterior striatum, all treatments examined produced a significant increase of [^{3}H]GBR-12935 binding (E2, 27%; P, 24%; E2+P, 40%). Chronic exposure to E2 and/or P was shown here to increase striatal DA uptake sites density. The hormonal modulation of DA uptake sites could be involved in the gender related differences in cocaine-induced behavioral sensitization. Supported by the MRC of Canada.

SECOND MESSENGERS III

203.1

EFFECTS OF ANGIOTENSIN II (ANG II) ON INOSITOL PHOSPHATE (IP) HYDROLYSIS IN THE BRAIN OF FEMALE RATS. M K.Steele* and R K.Riemer, UCSF, Depts of Physiology and Obstetrics and Gynecology, San Francisco, Ca. 94143

Ovarian hormones modify the central effects of Ang II on LH and prolactin release. The present experiments tested whether estrogen modifys the intracellular second messenger system of Ang II in the hypothalamus from female rats.

Animals were ovariectomized and treated with either vehicle or ethynylestradiol (25 µg/rat) sc for two days. Whole hypothalamic halves and pituitaries were incubated in Krebs Henseleit buffer with ^{3}H myoinositol. After incorporation of the label, the tissue was stimulated for varying periods of time with 10^{-5}M Ang II.

Ang II stimulated IP production in pituitaries from both untreated and estrogen treated animals. The stimulation was seen by 2 min and lasted for up to 30 min. No stimulation of IP production was seen in hypothalami from untreated animals. In contrast, in hypothalami from estrogen-treated rats, a maximal stimulation was observed at 2 min, which dissipated by 6 min.

These results show that, similar to that seen in the periphery, Ang II stimulates IP production in the brain. Under the conditions of this experiment, this response is short lived and seen only after in vivo estrogen treatment. Modification of Ang II second messenger systems may provide a mechanism by which ovarian hormones modify the efficacy of Ang II on pituitary hormone secretion in female rats (Steele, et al, 1991).

203.2

ENFLURANE INHIBITS MOUSE AND HUMAN BRAIN PHOSPHOTIDYLINOSITOL-LINKED ACETYLCHOLINE AND SEROTONIN RECEPTORS EXPRESSED IN XENOPUS OOCYTES. L.-H. Lin*and R. A. Harris. Dept. of Pharmacology, UCHSC, Denver, CO 80262; Veterans Admin. Med. Res. Serv., Denver, CO 80222.

Modulation of IP_3-mediated signal transduction pathways by the inhalational anesthetic enflurane was studied in *Xenopus* oocytes expressing mouse and human cortical mRNA. We found that enflurane significantly inhibited ion currents activated by m1 muscarinic and 5-HT_{1C} receptors in a concentration dependent manner, with large inhibition (80-89 %) on low and small inhibition (8-44 %) on high concentrations. Similar effects were found with both mouse and human receptors. To investigate the sites of enflurane action, ion currents induced by intracellular injections of GTP-γ-S and IP_3 were examined. Enflurane strongly suppressed the GTP-γ-S-activated current, but not the IP_3-activated current. The results suggest that an inhalational anesthetic can disrupt the function of mouse and human brain PI-linked receptors by selective inhibition on the G protein activity. On the other hand, the results suggest that enflurane exerts little effect on the IP_3-activated Ca^{2+}-dependant Cl^- channels. Together, specific modulation of membrane proteins by enflurane suggests that proteins are the target sites of action for anesthetics.

203.3

INHIBITION BY ETHANOL OF METABOTROPIC RESPONSES TO SEROTONIN AND ACETYLCHOLINE IN XENOPUS OOCYTES. E. Sanna* and R.A. Harris. Dept. of Pharmacology, Univ. of Colorado Hlth. Sci. Cntr. and Denver VAMC, Denver, CO 80262.

In voltage-clamped Xenopus oocytes microinjected with mouse whole brain mRNA, serotonin (5HT) and acetylcholine (ACh) acting through $5HT_{1C}$ and M_1 receptors, respectively, evoke membrane responses through a common biochemical cascade. This involves G-protein-mediated phospholipase C activation, production of IP_3, release of intracellular Ca^{2+} and opening of Ca^{2+}-dependent Cl^- channels. The effect of ethanol (EtOH) on these metabotropic responses was studied. EtOH (25-200 mM) inhibited in a dose-related manner currents evoked by both 5HT (0.1 μM) and ACh (1 μM). The maximal effect (-50% for ACh, and -65% for 5HT) was observed at 150 mM EtOH. Moreover, EtOH (100 mM) produced rightward shifts of both 5HT and ACh dose-dependent curves. To clarify the site of action of EtOH, IP_3 and GTP-γ-S were injected intracellularly. EtOH (100 mM) did not significantly alter the Ca^{2+}-dependent Cl^- currents elicited by either IP_3 (200 fmols) or GTP-γ-S (20 pmols). Treatment of oocytes for 12-18 hrs with 400 nM staurosporine (Stau), a PKC inhibitor, prevented the inhibition by EtOH of 5HT and ACh-induced responses. Moreover, in Stau-treated oocytes EtOH alone was able to induce Cl^- currents which were abolished by intracellular EGTA or at a clamping potential of -20 mV. These data suggest that the inhibition by EtOH of metabotropic responses induced by 5HT and ACh in Xenopus oocytes might be mediated by an alteration of PKC activity, or alternatively, that EtOH effect requires an activated PKC. The role of PKC on EtOH action will be further discussed.

203.4

INHIBITION BY ALUMINUM OF AGONIST-STIMULATED INOSITOL PHOSPHATE RELEASE IN SLICES FROM RAT HIPPOCAMPUS AND CORTEX. T.J. Shafer*, W.R. Mundy, V. Dulchinos and H.A. Tilson, Neurotoxicology Division, HERL, U.S.EPA, Research Triangle Park, NC 27711.

Effects of aluminum chloride (0.1 to 1000 μM) on inositol phosphate (IP) release stimulated by carbachol (CARB), norepinephrine (NE) or quisqualate (QUIS) were examined in rat hippocampal and cortical slices. Release of IPs was measured in the presence of Li^+ by incubation of slices with agonist for 30 min, followed by isolation of ^{3}H-IPs by ion-exchange chromatography. In the absence of agonist, Al^{3+} significantly reduced basal release of IPs only at 1000 μM. In cortical slices, the effect of Al^{3+} on maximal agonist-stimulated IP release was significantly different from the effect of Al^{3+} on agonist-stimulated IP release in hippocampal slices. For CARB-stimulated release, $[Al^{3+}]$ of 50 μM and greater significantly inhibited IP release in cortical slices. 1000 μM Al^{3+} reduced IP release by 55%. In hippocampal slices; 1000 μM Al^{3+} significantly inhibited CARB-stimulated IP release by 40%. Similar effects of Al^{3+} were observed for NE-stimulated IP release. For QUIS-stimulated release, 1000 μM Al^{3+} significantly inhibited IP release in hippocampal slices. However, in cortical slices a biphasic effect of Al^{3+} was observed. 500 and 1000 μM Al^{3+} significantly inhibited IP release, whereas 10 and 50 μM Al^{3+} significantly potentiated QUIS-stimulated IP release. In both hippocampal and cortical slices, 500 μM Al^{3+} significantly inhibited CARB or NE stimulated IP release at all agonist concentrations (1 to 10000 μM) tested. Thus, Al^{3+} inhibits agonist-stimulated IP release in a non-competitive manner. These results suggest that: 1) differences in the sensitivity to Al^{3+} exist in the hippocampus and cortex and, 2) Al^{3+} does not interfere directly with agonist binding to the receptor, but interacts with other components of receptor-stimulated phosphoinositide hydrolysis possibly including G-proteins, phospholipase C and regulatory or modulatory influences such protein kinase C or Ca^{2+}.

203.5

STUDIES OF AGONIST STIMULATION OF EXOGENOUS PHOSPHOINOSITIDE HYDROLYSIS BY PHOSPHOLIPASE C - β IN RAT BRAIN MEMBRANES. P. Kurian and F.T. Crews*, Department of Pharmacology, University of Florida College of Medicine, Gainesville, FL 32610-0267.

A number of neurotransmitters have been shown to activate phospholipase C (PLC) resulting in the hydrolysis of membrane PtdIns(4,5)P_2, leading to the formation of Ins(1,4,5)P_3 and diacylglycerol. To investigate the mechanism of receptor coupling to PLC, membranes were prepared from various regions of rat brain and incubated with a mixture of [3H]PtdIns (87 %), [3H]PtdIns(4)P (10.4 %) and [3H]PtdIns(4,5)P_2 (2.6%) which is comparable to that found in intact tissue. GTPγS stimulated the formation of [3H]Ins(1,4,5)P_3. GTPγS stimulated [3H]Ins(1,4,5)P_3 formation was enhanced by carbachol (220 %) and 5-HT (210 %). Norepinephrine, glutamate and quisqualate did not stimulate [3H]PtdIns(4,5)P_2 hydrolysis in the presence of GTPγS. SAX-HPLC separation of [3H]inositol polyphosphates indicated that there was only one isomer of InsP_3, Ins(1,4,5)P_3, the expected product of PtdIns(4,5)P_2 hydrolysis. No Ins(1,3,4,5)P_4 was formed in this assay. Two isomers of InsP_1 were formed, Ins(1)P_1 and Ins(4)P_1. Both GTPγS - carbachol and 1μM Ca^{2+} stimulated Ins(1)P_1 and Ins(4)P_1 formation. These isomers could be formed by direct hydrolysis of [3H]PtdIns substrate and/or phosphatase activity within our membrane preparation. Both GTPγS-carbachol stimulation and Ca^{2+} stimulated the formation of Ins(1)P_1 (from PtdIns) and Ins(4)P_1 (from PtdIns(4)P and PtdIns(4,5)P_2) suggesting that PLC may act on all the PPI although polyPPI are preferred. Ca^{2+} activated PtdIns(4,5)P_2 hydrolysis at ≈ 3 nM , increasing to a maximum at ≈ 1 μM. The presence of GTPγS and carbachol appeared to left shift the calcium concentration effect curve for Ins(1,4,5)P_3 formation. Membranes from cerebellum showed a large stimulation of PtdIns(4,5)P_2 hydrolysis with GTPγS and Ca^{2+}. In contrast to cortical membranes where carbachol and 5-HT enhanced GTPγS stimulated PtdIns(4,5)P_2 hydrolysis, no increase above GTPγS stimulation by these agonists was observed with cerebellar membranes. Significant increases in quisqualate stimulated Ins(1,4)P_2 formation was found with cerebellar membranes. To investigate the isozymes of PLC involved in neurotransmitter and Ca^{2+} stimulated activity, we used antibodies to PLC β, γ and δ. Antibodies to PLC β, but not PLC γ and δ inhibit GTPγS - carbachol stimulated PtdIns(4,5)P_2 hydrolysis whereas all three antibodies appear to inhibit Ca^{2+} stimulated PPI hydrolysis (Supported by a grant from NIA).

203.6

EFFECTS OF ACUTE AND CHRONIC TREATMENT WITH HALOPERIDOL, LITHIUM AND VALPROATE ON PHOSPHOINOSITIDE TURNOVER IN RAT BRAIN SLICES. R.Li, L.L.Wing, R.J.Wyatt, and D.G.Kirch*. Neuropsychiatry Branch, NIMH, Neuroscience Center at St. Elizabeths, Washington D.C. 20032 and Division of Clinical Research, NIMH, Rockville, MD 20857.

Haloperidol (HAL), lithium (LI) and valproate (VAP) have been shown to be effective in the treatment of bipolar disorder. While these medications treat the same disease, it is unclear whether they work through a common mechanism. The effects of acute (single dose) and chronic (2 weeks and 4 weeks) treatment with HAL, LI and VAP on agonist-induced inositol phosphate (IP) formation were examined. Acute treatment with HAL, LI and VAP had no effects on carbachol- and norepinephrine(NE)-stimulated IP accumulation in both the frontal cortex and striatum, while acute HAL or HAL and LI combined (HAL/LI) reduced cortical and striatal IP basal levels. Four week treatment with HAL, LI and VAP each attenuated carbachol-induced IP formation in the striatum while a significant decrease in the frontal cortex was found only after HAL, LI and HAL/LI treatment. A similar reduction in NE-stimulated IP accumulation was found after HAL and LI treatment for 4 weeks in the frontal cortex, while HAL and VAP each produced a reduction in striatum. Two week treatment with HAL caused a significant reduction in carbachol-stimulated IP accumulation in the frontal cortex and striatum and a decrease in NE-sensitive IP formation in the frontal cortex. While regional and time-dependent differences in IP effects were observed, our data indicate that chronic treatment with HAL, LI or VAP may result in a down-regulation of receptor-mediated IP formation. This suggests that the common action of these drugs on IP systems may be an important factor underlying their mood-stabilizing effect.

203.7

EFFECTS OF U50,488, A KAPPA OPIOID RECEPTOR AGONIST, ON PHOSPHOINOSITIDE HYDROLYSIS IN RAT HIPPOCAMPUS. M. Mosaddeghi, J. M. Moerschbaecher*, D. Paul. Dept. of Pharmacology and Alcohol and Drug Addiction Center, L.S.U. Medical Center., New Orleans, LA. 70112.

Hydrolysis of membrane phosphoinositides (PI) is proposed as a signal transduction mechanism mediating effects of several cell surface receptors. This study examined effects of kappa opioid receptor agonist U50,488 on PI hydrolysis in hippocampus. Tissue slices, labelled with [^{3}H]inositol, were stimulated with KCl and PI hydrolysis were measured in the presence of LiCl. KCl-stimulated PI hydrolysis in a concentration dependent manner (EC_{50}=20 mM) with maximal PI hydrolysis of 0.37 dpm released/dpm incorporated. U50,488 (0.1-1000 mM) did not affect PI hydrolysis. However, U50,488 diminished KCl-stimulated PI hydrolysis in a concentration dependent manner (IC_{50}=100 uM). One mM U50,488 completely abolished 27.4 mM KCl-simulated PI hydrolysis. Time course experiments showed that 100 uM U50,488 did not stimulate PI hydrolysis upto 60 min incubation, whereas, KCl stimulated PI hydrolysis in a time-dependent manner. U50,488 inhibited KCl-stimulated PI hydrolysis after 30 min or longer incubation. The kappa selective antagonist norbinaltorphimine (nor-BNI) (100 uM) blocked the inhibitory effect of 300 uM U50,488 on KCl-stimulated PI hydrolysis. Nor-BNI alone did not alter PI hydrolysis. These data suggest that kappa opioid receptor agonists may exert their central effect, at least in part, by modulating PI hydrolysis. This study supported by NIH-BRSG SO-RR-5376 and DA03573.

203.8

PHOSPHOINOSITIDE HYDROLYSIS IN HIPPOCAMPAL SLICES: MODULATION BY GLUCOCORTICOIDS. K. Kolasa*, L. Song and R. S. Jope. Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama, Birmingham, AL 35294.

Adrenal steroid hormones have many complex effects on the brain, influencing metabolism, signal transduction and behavior. Because glucocorticoids can directly affect the limbic system, especially the hippocampus, we investigated hippocampal phosphoinositide (PI) hydrolysis after adrenalectomy (ADX).

PI hydrolysis induced by norepinephrine(NE), quisqualate, or ACPD, but not by carbachol, was 50% greater in hippocampal slices from ADX (14 days) rats compared with controls. No effects of ADX on PI hydrolysis were detected in cortical or striatal slices. The enhanced response to NE in hippocampal slices after ADX was observed throughout the effective concentration range of NE, was not influenced by in vitro corticosterone or cyclic AMP, and was not due to impaired inhibition of the response to NE which was elicited by activation of protein kinase C or by an inhibitory concentration of quisqualate.

These findings indicate that ADX either removes an inhibitory influence of glucocorticoids on the PI system in the hippocampus or that the neurodegeneration of granule cells in the dentate gyrus following ADX is associated with neurotransmitter-selective increases in PI hydrolysis. These data provide further evidence that glucocorticoids modify signal transduction in the brain and extends their known influence to the PI second messenger system. Investigations of the effects of glucocorticoid administration on hippocampal PI hydrolysis are currently in progress.

203.9

HYDROLYSIS OF [^{3}H]PHOSPHATIDYLCHOLINE IN CYTOSOL AND MEMBRANES FROM RAT BRAIN. M.S. Baird*, L. Song and R.S. Jope. Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama, Birmingham, AL 35294.

Phosphatidylcholine hydrolysis apparently occurs in a manner analogous to phosphoinositide hydrolysis in the transduction of signals to form second messengers. The functions and control mechanisms associated with phosphatidylcholine hydrolysis remain to be clarified.

We adapted methods used by others to measure the hydrolysis of exogenous [^{3}H]phosphatidylcholine incubated with rat cerebral cortex membrane and cytosolic fractions. Both preparations induced hydrolysis of [^{3}H]phosphatidylcholine for at least 60 min, but the rate was higher in the cytosol compared with membranes. GTPγS enhanced hydrolysis in both fractions, supporting the existence of a regulatory G-protein.

Seizures were induced in rats by administration of LiCl (3 mmol/kg) 20 hr prior to pilocarpine (30 mg/kg). After 1 hr cytosolic fractions were prepared and they were found to hydrolyze [^{3}H]phosphatidylcholine at a lower rate than controls, whereas no difference was observed in membranes.

These data show that both cytosolic and membrane fractions contain enzymes that hydrolyze exogenous [^{3}H]phosphatidylcholine, that a G-protein may influence this activity, and that the activity is reduced in the cytosol after seizures induced by lithium and pilocarpine. Studies are underway to identify the enzymes responsible for [^{3}H]phosphatidylcholine hydrolysis and to study the regulation of this process.

203.10

SUSTAINED CALCIUM INFLUX INDUCED BY A NON-METABOLIZABLE INOSITOL TRISPHOSPHATE IN XENOPUS OOCYTES Y. Yao* and I. Parker. Lab. Cellular and Molecular Neurobiology, Dept. Psychobiology, University of California, Irvine, CA 92717.

Ins(1,3,4,5)P_4 (formed by metabolism of Ins(1,4,5)P_3) has been suggested to play a role in phosphoinositide-mediated Ca^{2+} influx (Irvine, R. F. FEBS Lett. 263, 5-9, 1990). To test this hypothesis we injected Xenopus oocytes with a synthetic analogue, 3-Deoxy-3-fluoro-Ins(1,4,5)P_3 (3-F-Ins(1,4,5)P_3), which cannot be metabolized to Ins(1,3,4,5)P_4. Ca^{2+} influx was monitored by applying hyperpolarizing pulses to transiently increase the driving force for Ca^{2+} entry, while recording fluo-3 fluorescent signals and Ca^{2+}-activated chloride currents. Intracellular injection of 100 fmol 3-F-Ins(1,4,5)P_3 (about 100nM final intracellular concentration) induced intracellular Ca^{2+} release followed by a gradually developing Ca^{2+} influx in a manner similar to Ins(1,4,5)P_3. However, in contrast to the transient responses (several minutes) seen with Ins(1,4,5)P_3, 3-F-Ins(1,4,5)P_3 responses lasted several hours, suggesting it is metabolized slowly, if at all. This result indicates a mechanism for Ca^{2+} influx independent of Ins(1,3,4,5)P_4. In some oocytes loaded with 3-F-Ins(1,4,5)P_3, hyperpolarizing or depolarizing steps evoked damped sinusoidal oscillations of Ca^{2+}-dependent chloride current, suggesting that oscillations of intracellular [Ca^{2+}] can be induced by sudden changes in Ca^{2+} flux across the membrane. The ability of 3-F-Ins(1,4,5)P_3 to activate a sustained Ca^{2+} influx should make it a useful tool for studying mechanisms of Ins(1,4,5)P_3-mediated Ca^{2+} influx.

Supported by grant GM39831.

203.11

STUDIES ON ^{3}H-INS[1,2,6]P_3 AND OTHER ^{3}H-INOSITOL PHOSPHATE BINDING SITES IN RAT BRAIN. Heahyun Yoo, Anneli Lindahl, Erol Veznedaroglu* and Claes Wahlestedt. Division of Neurobiology, Department of Neurology and Neuroscience, Cornell University Medical College, New York, NY 10021 and Perstorp Pharma, S-284 80 Perstorp, SWEDEN.

We have studied binding sites of tritiated inositol phosphates (InsPs) in rat brain membranes in an attempt to assess differences and similarities in binding characteristics amongst various tritiated and unlabeled InsP analogs. The present study focused on a novel analog, ^{3}H-Ins[1,2,6]P_3 (PP-56), whose binding characteristics were compared with those of previously studied analogs, *i.e.* ^{3}H-Ins[1,4,5]P_3, ^{3}H-Ins[1,3,4,5]P_4 and ^{3}H-InsP_6. While all four tritiated InsPs displayed specific binding, marked differences in optimum binding conditions were observed. ^{3}H-Ins[1,2,6]P_3 was found to bind to a single population of sites with a K_D of 70 nM. The binding of ^{3}H-Ins[1,2,6]P_3 to rat brain membranes, as well as to cultured vascular smooth muscle and endothelial cell membranes, was greatly enhanced at acidic pH (optimum pH 5) and reduced by alkali ions (while Ca^{2+} had little effect). The conditions favoring ^{3}H-Ins[1,2,6]P_3 binding showed close similarities to those favoring ^{3}H-Ins[1,3,4,5]P_4 binding. In contrast, we found ^{3}H-Ins[1,4,5]P_3 and ^{3}H-InsP_6 to display different pH and ionic requirements for optimum binding, as previously demonstrated also by several other groups. The present data, therefore, suggest the possibility that Ins[1,2,6]P_3 exerts its reported antiinflammatory and antihypertensive actions by interacting with membrane binding sites/receptors, abundantly present not only in smooth muscle and endothelial cells, but also in brain. These sites are probably distinct from ^{3}H-Ins[1,4,5]P_3 as well as ^{3}H-InsP_6 preferring sites. Interestingly, Ins[1,2,6]P_3 showed binding characteristics quite similar to those of Ins[1,3,4,5]P_4, an agent believed to activate Ca^{2+} permeable channels, raising the possibility that Ins[1,2,6]P_3 might affect these same channels.

203.12

PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES FOR αo G-PROTEIN. A.G.Faulk-Greenwood*, X. Li and R.S. Jope. Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama, Birmingham, AL 35294 and S.M. Mumby, Dept. Pharmacology, Univ. Texas Southwestern Medical Center, Dallas, TX 75235.

Guanine nucleotide-binding proteins (G-proteins) mediate many receptor-coupled signal transduction mechanisms. Of the several identified G-proteins, Go is present in brain in the highest concentration and shows some selectivity for nervous tissue. Although several functions for Go have been postulated, definitive evidence of its roles lags behind that obtained for several other G-proteins. In order to facilitate studies of Go, a monoclonal antibody was prepared against the α-subunit.

G-proteins were purified from bovine brain and monoclonal antibodies were prepared by conventional techniques. Western blots with purified G-proteins or brain membranes were used to characterize the monoclonal antibodies. One monoclonal antibody, 1C, reacted specifically with αo. 1C recognized human and bovine αo equally well, and recognized rat, mouse and guinea pig αo to a lesser extent. Native αo reacted better with 1C than did denatured αo. Purified, concentrated 1C reacted maximally with 0.5 μg bovine Go at a dilution of 1:5,000. Several other monoclonal antibodies directed against bovine and rat purified G-proteins are currently being characterized. The development of several specific monoclonal antibodies should facilitate studies of the regulation and function of G-proteins.

203.13

DISTRIBUTION OF CYTOSOLIC G-PROTEIN IN BRAIN AND OTHER RAT TISSUES; EVIDENCE FOR ROLE IN THE ACTIVATION OF CYTOSOLIC PLC BY A MECHANISM OTHER THAN GTP HYDROLYSIS. T Akompong* R L.Spencer and B. S.McEwen. Lab. of Neuroendocrinology, Rockefeller University, New York, New York 10021.

The G-proteins are involved in the transduction of signals initiated by hormones, neurotransmitters and drugs. We examined the distribution of two G-proteins; Giα and Gsα in the membrane and cytosol of rat tissues by western blot analysis. The levels of Giα in the membrane were much higher in brain regions than the peripheral tissues. In contrast the highest levels of Giα in the cytosol were found in spleen and thymus. The two species of Gsα (GsL, and GsH,) were distributed in a tissue dependent fashion in both membrane and cytosol. The effects of calcium, nucleotides, G-protein antibodies and protein phosphatase inhibitors on cytosolic PI-PLC activation were also examined. Calcium in the micromolar range stimulated PLC activity by 10 fold over basal. The calcium stimulated activity was inhibited up to 70 % by antibodies to Gil. GTP and its non-hydrolyzable analogues had no effect on both basal and calcium stimulated PI-PLC activity. NaF which is used to turn on G-protein mediated processes, inhibited the calcium stimulated activity in a dose dependent manner. Our data suggests a mechanism of regulating cytosolic PI-PLC by cytosolic G-proteins that is independent of G-protein hydrolysis required for other G-protein regulated systems.

203.14

SECOND MESSENGER REGULATION OF THE SYNAPTIC VESICLE PROTEIN p65 (SYNAPTOTAGMIN) IN RAT SYMPATHETIC GANGLIA IN VITRO. K.F. Greif*, H. Fahl and E. Lederman. Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

We have demonstrated previously (Linderman and Greif, 1990) that depolarizing agents increase levels of the synaptic vesicle protein, p65 (synaptotagmin), in neonatal rat superior cervical ganglia (SCG) in explant culture. We are investigating the role of second messengers in the signalling pathway. Neonatal SCGs are maintained in explant culture in defined medium. After two days in culture, SCGs are treated with cAMP, Ca^{2+} and protein kinase C (PKC)-associated pharmacological agents for 48 hours. Levels of p65 are determined by radioimmunoassay.

Cholera toxin and forskolin, which elevate cAMP levels, both increase p65 levels. The magnitude of the increase is similar to that produced by depolarization with veratridine, a Na^+-channel ionophore. Surprisingly, 8-Br-cAMP, a nonhydrolysable form of cAMP, blocks the veratridine response, and has no effect on its own. Levels of p65 are slightly increased by the Ca^{2+}-ionophore, A23187. The phorbol ester TPA, which activates PKC, does not appear to increase p65 levels. These results suggest that at least two second messenger pathways contribute to depolarization-associated transsynaptic regulation of p65.

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203.15

STIMULATION OF M_1 MUSCARINIC RECEPTOR-MEDIATED PHOSPHOINOSITIDE TURNOVER BY ALKOXY-1,2,5-THIADIAZOLE DERIVATIVES OF ARECOLINE IN THE RAT BRAIN. S. Periyasamy*, W. S. Messer, Jr., S. Roknich, #P. Sauerberg, W. Hoss, Dept. of Medicinal & Biological Chemistry, University of Toledo, Toledo, OH 43606 and #Pharmaceuticals Research, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark

A series of alkoxy-1,2,5-thiadiazole derivatives of arecoline (alkoxy-TZTPs) was synthesized in an effort to develop M_1 muscarinic agonists. The ligands, including 3-butenyloxy, 2-butynyloxy, cyclopropylmethyloxy, and hexyloxy, stimulated phosphoinositide (PI) turnover in the rat hippocampus. The dose-response curves were biphasic, compared with arecoline, which was monophasic. The maximal effects were approximately 200% stimulation for each derivative with the exception of the 3-butenyloxy compound (85%), compared with arecoline (207%) and carbachol (450%). The cyclopropylmethyloxy and hexyloxy compounds were shown to be partial agonists as expected. In addition, the maximal PI turnover response of arecoline and the hexyloxy compound together was the same as the response of each separately. Pirenzepine was substantially more potent than AF-DX 116 for inhibiting the responses of low concentrations of the thiadiazole derivatives where a high affinity component was activated selectively. The potencies of the two antagonists for inhibiting the response of arecoline were similar. The magnitude of the PI response for the hexyloxy analog was highest in hippocampus, amygdala, neostriatum and cortex and lowest in cerebellum and pons-medulla. Molecular mechanics calculations predicted a conformational preference for the *anti* form with the alkyloxy substituent oriented away from the nitrogen in the tetrahydropyridine ring. The data suggest that the hexyloxy derivative is functionally a selective M_1 agonist. The data are consistent with the hypothesis that the thiadiazoles distinguish between M_1 and M_3 receptors linked to PI turnover. It also is suggested that the *anti* conformer is active at M_1 receptors. The work was supported by a contract from Lilly Research Laboratories and by NS 23929, NS 25765, NS 23598 and NS 01493.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION: GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS

204.1

DIFFERENTIAL MINERALOCORTICOID (MR) AND GLUCOCORTICOID (GR) RECEPTOR EXPRESSION IN LEWIS and FISCHER RATS. C. C. Smith, R. J. Omeljaniuk, H. J. Whitfield, Jr.*, S. Aksentijevich, E. Zelazowska, P. W. Gold and E. M. Sternberg, Clinical Neuroendocrinology Branch, National Institute of Mental Health, Bethesda, MD 20892; Currently: Dept. of Biology, Lakehead University, Ont. Canada P7C 3B2; Dept. of Internal Medicine, Northwestern University, Chicago, IL 60611.

There has been debate in the literature regarding the precise effects of glucocorticoids upon hippocampal and hypothalamic-pituitary-adrenal (HPA) glucocorticoid receptor expression. Results vary depending upon the tissue studied as well as the mode and dose of glucocorticoid replacement. LEW/N and F344/N rats represent two rat strains whose differential inflammatory and behavioral responses to stress are related to their differential HPA axis responsiveness. They lie at two extremes of the spectrum of corticosterone (Cort) responses to stressful stimuli: chronically HPA hypo-responsive LEW/N compared to chronically HPA hyper-responsive F344/N. Thus it might be expected that the amount of MR and GR binding, and the levels of their corresponding mRNA's in various tissues in LEW/N and F344/N rats might reflect the overall integrated levels of Cort to which these receptors have been exposed. We have found that while the binding affinity (Kd) of MR and GR varies between tissues, there is no strain difference in Kd. Receptor number (Bmax), however, varied not only between tissues, but also between strains. MR Bmax in the hippocampus and pituitary was lower in LEW/N compared to F344/N, whereas GR Bmax in LEW/N thymus was greater than that F344/N. MR and GR mRNA levels in the hippocampus parallelled receptor binding data in the two strains. The findings reported here support the notion that these strains represent an excellent physiologic model for assessing the effects of repeated exposure to relatively low versus relatively high levels of Cort during stress. These differences may contribute to the differential inflammatory disease and stress responsiveness of the two strains.

204.2

NORADRENERGIC REGULATION OF CORTICOSTEROID RECEPTORS IN DIFFERENT BRAIN STRUCTURES. S. Maccari, P.V. Piazza, H. Simon, L. Angelucci and M. Le Moal*. INSERM U259 - Rue Camille St Saëns, 33077 Bordeaux France and Farmacologia II, Univ. Roma, P.le A. Moro, 2 00185 Rome, Italy.

The effects of corticosteroids on various brain functions, including the negative feedback control of hypothalamo-pituitary-adrenal (HPA) axis activity, are mediated by two types of receptors (type I or mineralocorticoid and type II or glucocorticoid) in the central nervous system. Although receptor number, especially the number of type II receptors, is thought to be regulated by circulating levels of corticosterone, a direct neural control of these receptors has been hypothesized. In order to demonstrate neuronal control of corticosteroid receptors, we tested the effect of 6-OHDA lesionig of noradrenergic systems at the level of the pedunculus cerebellaris superior produced in adrenalectomized animals in which corticosterone levels were maintained within normal limits by corticosterone replacement implants. Both receptors were studied in hippocampus, hypothalamus and amygdala. The results indicate that: 1) the noradrenergic system regulates corticosteroid receptors; 2) this control depends on the brain region studied. The type I receptor number was enhanced in hippocampus and reduced in hypothalamus and amygdala. The type I receptor affinity was unaltered. The type II receptor number was increased only in the hypothalamus while the affinity of this type of receptor was increased in hippocampus. These different effects of NA lesioning on corticosteroid receptor types may derive from an action on different noradrenergic receptors. Activation of β NA receptors may increase corticosteroid receptors and activation of α_1 NA receptors may decrease these receptors. We have tested this hypothesis by the administration of phenylepryne (α_1 agonist) and salbutamol (β agonist). In the hippocampus we found that salbutamol increase the type I corticosteroid receptor affinity and phenylephrine decreases type I and type II corticosteroid receptor numbers. In conclusion, central NA neurons appear to regulate the plasticity of the two corticosteroid receptors in a different and complex way. This findings may elucidate the mechanisms controlling glucocorticoid secretion.

204.3

METHAMPHETAMINE-INDUCED DECREASE IN NEURAL GLUCOCORTICOID RECEPTORS. M.T. Lowy*. Dept. of Psychiatry, Case Western Reserve University, Cleveland, OH 44106

Methamphetamine (MA) is a potent psychostimulant which is neurotoxic to dopamine (DA) and serotonin (5-HT) neurons. Glucocorticoid receptors (GR) are extensively co-localized in monoamine neurons. The present study was designed to determine if MA could decrease neural GR and to relate such effects to DA and 5-HT levels. MA (6.25, 12.5 and 25 mg/kg) was given every 2 hrs for a total of 4 doses. Rats were adrenalectomized 6 days later and subsequently sacrificed 24 hr later. MA produced dose-related decreases in GR in the striatum, hippocampus, frontal cortex and hypothalamus. Hippocampal mineralocorticoid receptors were not affected by MA. 5-HT was also decreased in all 4 brain regions, whereas DA was decreased only in the striatum. MA did not decrease GR in cerebellum and had no effect on DA and 5-HT in this region. Similarly, MA did not decrease GR or 5-HT levels in the spleen. In contrast, MA decreased pituitary GR, but did not alter DA or 5-HT levels in the pituitary. These results demonstrate that MA produces a decrease in GR in a variety of brain areas, which is related to 5-HT depletions. MA-induced decrease in neural GR may have significant implications for the rewarding and neurotoxic effects of this abused drug. Supported by MH44699.

204.4

CONFOCAL MICROSCOPY OF GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS (GR, MR) IN CULTURED HIPPOCAMPAL NEURONS AND ASTROCYTES. D. L. Barker*, R. Giuliano, Z. Krozowski, M. C. Bohn, Molecular Dynamics, Sunnyvale, CA 94086 and Dept. Neurobiology and Anatomy, Univ. Rochester Med. Ctr., Rochester, NY 14642.

Confocal laser scanning microscopy (CLSM) was used to study the subcellular localization of GR and MR in dissociated neurons and astrocytes prepared from whole E18 rat hippocampus and grown in defined or serum-containing medium. CLSM permits sensitive visualization of fluorescently tagged antibodies against these rare proteins in both thin (0.5 μm) optical sections and in-focus projections of whole cells. Immunofluorescence (IF) was combined with propidium iodide staining to yield dual-labeled images of receptor localization and cell nuclei. Antisera included a monoclonal to rat liver GR, BUGR2, provided by R. Harrison, and an affinity-purified polyclonal, MR4, raised against a fusion protein, expressed in E. coli, from a construct of a 168 amino acid portion of the N-terminus of human MR and glutathione transferase.

In astrocytes fixed in the presence of corticosterone (CORT, 10^{-6}M, 30 min), both GR-IF and MR-IF were concentrated in nuclei, whereas only MR-IF was observed in nuclei in the absence of CORT. In neurons fixed in the absence of CORT, GR-IF was observed in the nuclei of some neurons, but not others. MR-IF was uniformly distributed over nuclei, cytoplasm and processes regardless of the presence or absence of CORT. Serum has no effect on the localization of either receptor. These observations suggest that the subcellular localization of MR and GR is differentially regulated. In addition, the translocation of GR appears to be regulated by a different ensemble of factors in neurons and glia. (Supported by NIH grant NS20832 and Molecular Dynamics.)

204.5

GLUCOCORTICOID RECEPTOR (GR)) AND MINERALOCORTICOID RECEPTOR (MR) mRNA REGULATION IN NEURONS AND ASTROCYTES. M.C.Bohn*, S.Hussain, R.Guiliano, M.K.O'Banion and D.Dean. Dept. Neurobiol. & Anatomy, Univ. of Rochester Med. Ctr., Rochester, NY 14642.

Glucocorticoids produced by the adrenal cortex act through two receptors, GR and MR, to influence gene expression in the brain. Previous studies have shown that both neurons and glia are targets for glucocorticoid action. To establish defined *in vitro* systems to study glucocorticoid effects, we have used the RNAse protection assay to compare levels of mRNAs coding for GR and MR in neurons prepared from embryonic E18 rat hippocampus and type 1 astrocytes prepared from newborn rat cortex .

Protected bands corresponding to GR mRNA and MR mRNA are observed in both neurons and astrocytes. Neurons grown for 5 days express approximately equal amounts of MR mRNA and GR mRNA. *In vivo*, levels of the two messages are also approximately equal in E18 hippocampus, but in postnatal day 10 hippocampus, MR mRNA is 4-5 fold higher than GR mRNA . In contrast, in astrocytes, GR mRNA levels are 5-fold those of MR mRNA. Both messages are easily detected in 2.5µg of total RNA from neurons, whereas 1µg of polyA+RNA is needed to detect MR mRNA in astrocytes. These results suggest that the absolute abundance of the two messages, as well as their relative abundance, is different in neurons and astrocytes.

Study of the effects of culture conditions on mRNA levels showed that the ratio of MR/GR mRNA is significantly higher in neurons grown in the presence of 1% serum or medium conditioned by astrocytes, than in serum-free defined medium. This increase is due to an increase in levels of MR mRNA without a change in GR mRNA levels. Addition of 10^{-6}M corticosterone for 48 hours also increases the MR /GR mRNA ratio in neurons.

These studies suggest that the expression of GR and MR is differentially regulated and is quantitatively influenced by age, cell type and extracellular factors. (Supported by NIH grant NS20832)

204.6

DIFFERENTIAL DISTRIBUTION OF TYPE I CORTICOSTEROID RECEPTOR mRNA VARIANTS IN THE RAT HIPPOCAMPUS. S.P. Kwak*H. Akil, and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Three forms of the mineralocorticoid receptor mRNA, alpha, beta, and gamma, are expressed in the rat hippocampus. These subtypes share a common protein coding domain but differ in their 5'UT sequence. The subtypes of MR mRNA are transcribed from the same gene, although it appears that the variants are transcribed by separate promoters flanking each 5'UT exon and subsequently spliced onto the common exon coding for the MR protein. Interestingly, the 5'UT exon is spliced onto the protein-coding exon 3bp upstream of the putative initiator ATG, but the Kozak sequence is faithfully retained in each case. The physiological relevance of multiple MR mRNA transcripts in the hippocampus is not clear, although it is conceivable that their expression may be differentially regulated since they have separate transcriptional units, and their translational efficiency may be different since they possess varying 5'UTs. To test these possibilities, we determined whether the three variants are loaded on polysomes and therefore translated into proteins; we also examined the mRNA distribution within the hippocampus. We found all three species to be bound by polysomes, indicating that they are all involved in MR protein synthesis. In situ hybridization revealed that the three forms are differentially distributed within the CA fields. The alpha form was enriched in the CA2-3, fasciculum cinereum, and the indusium gresium while other two forms were evenly distributed in CA1-3. These results suggest that the multiple forms of MR mRNA are functionally relevant and are expressed differentially among the subregions of the hippocampus. (work supported by NIMH grant # MH42251).

204.7

POSTNATAL HANDLING IN THE RAT ALTERS HIPPOCAMPAL GLUCOCORTICOID RECEPTOR GENE EXPRESSION. S. LaRocque, D. O'Donnell, C. Gianoulakis*, J.R. Seckl and M.J. Meaney. Douglas Hos. Res. Ctr., Depts of Psychiatry, and Neurology & Neurosurgery, McGill Univ, Montreal, Canada H4H 1R3 and Dept of Medicine, Univ of Edinburgh, Edinburgh, Scotland EH4 2XU

Postnatal handling of rat pups alters the development of hypothalamic-pituitary-adrenal responses to stress. This effect is, in part at least, mediated by the effect of handling on glucocorticoid receptor (GR) density in the hippocampus, a critical target site for the negative-feedback effects of glucocorticoids on HPA activity. In order to study the molecular basis for the difference in GR binding we examined GR mRNA as well as mineralcorticoid receptor (MR) mRNA expression in hippocampal sections from adult animals that were either handled (H) and nonhandled (NH) for the first three weeks of life. mRNA expression was determined using in situ hybridization with cRNA probes and quantified by counting silver grains over invidual hippocampal neurons. There was a highly significant increase in GR mRNA expression in each of the hippocampal cellfields of the H animals (dentate gyrus= + 64%; CA1= + 62%; CA3= + 53%; CA4= + 45%). By contrast, there were no significant changes in MR mRNA expression as a function of postnatal handling. Handling had no effect on hippocampal neuron density or volume. These studies demonstrate that early handling selectively alters GR mRNA expression in the hippocampus.

204.8

REGULATION OF MINERALOCORTICOID RECEPTOR mRNA EXPRESSION IN HAMSTER HIPPOCAMPUS BY PHOTOPERIOD. B.B. Turner*, S.J. Lance, and L.I. Holtsclaw. Dept. of Physiology, College of Medicine, E. Tenn. State Univ., Johnson City, TN 37614.

The secretion ratio of cortisol (F) to corticosterone (B) in hamsters decreases following 8 weeks exposure to short day photoperiods (Krey et al., Neurosci. Abstr. 15:715). An increase in MR binding was also observed at 8 weeks. Since F is the putative principal ligand of MR in brain, it has been proposed that the decrease in F results in enhanced MR binding though up-regulation. In this study, we test this hypothesis by measuring the expression of MR mRNA at a time prior to the reported change in adrenal secretion. Male golden syrian hamsters (n=10) were exposed to either long day length (14:10) or short day length (10:14) for 18 days. A plasmid containing a 1047 bp sequence (p95; from P. Patel) of the DNA binding region of rat MR was amplified. The transcribed cRNA was radiolabelled with ^{35}S-UTP and sections were hybridized overnight with the riboprobe. The density of the film autoradiographs was quantitiated using image analysis. Some sections were also dipped in NTB-2 emulsion for cellular localization of silver grains. Two-way analysis of variance indicated a significant effect of photoperiod ($P<0.0001$). The hamsters exposed to short day length had about 40% more MR mRNA in their hippocampi than did long day animals. Post-hoc Scheffe tests indicated a significant photoperiod effect for 7 of the 8 hippocampal regions. We conclude that changes in brain MR mRNA expression precede, rather than follow, changes in adrenal secretion; hence, changes in receptor number are not the result of decreased F synthesis. Supported by NIH S07 RR05959.

204.9

HIPPOCAMPAL GLUCOCORTICOID AND MINERALOCORTICOID RECEPTOR GENE EXPRESSION IS UNALTERED IN ALZHEIMER'S DISEASE. I.R. Seckl*, D. O'Donnell[1], M.J. Meaney[1], C Yates[2] and G. Fink[2]. Dept. Med.,West. Gen. Hosp., Edinburgh, [1]Douglas Hosp. Res. Ctr., McGill Univ., Montreal, Canada and [2]MRC Brain Metabolism Unit, Edinburgh, UK.

Glucocorticoids acting at the hippocampus via two types of intracellular receptor are thought to mediate shut-off of the hypothalamic-pituitary-adrenal axis. Excess glucocorticoids exert direct and indirect neurotoxic actions on hippocampal neurons. Since glucocorticoid hypersecretion with failure of central feedback occurs in Alzheimer's disease (AD) it has been proposed that a primary reduction in hippocampal glucocorticoid receptor expression leads to failure of feedback, hypercortisolemia and hence further neuronal loss. However, we have recently found that lesions of the cholinergic innervation of the hippocampus - known to be severely affected in AD - increase corticosteroid receptor gene expression in specific subregions of the hippocampus. We have now examined glucocorticoid (GR) and mineralocorticoid receptor (MR) gene expression in individual neurons in human postmortem hippocampus, using *in situ* hybridization in 5 patients with mild-moderate AD (81±3y) and 7 controls (81±7y) without neurological disease. We found similar distributions of MR and GR mRNA expression in control hippocampal neurons, with high expression in dentate gyrus and CA2-4, but significantly lower expression in CA1. There were no differences in GR or MR mRNA expression between AD and control hippocampus in any subregion. These data do not suggest a primary deficiency of biosynthesis of hippocampal corticosteroid receptors in AD. In view of reports of elevated glucocorticoid levels in AD, the maintainance of hippocampal GR and MR gene expression in AD may reflect loss of the cholinergic innervation.

205.1

LOCALIZATION OF ANGIOTENSIN AND NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE DIAPHORASE (NADPH-d) IN RAT HYPOTHALAMUS J. Calka, C.H. Block*, and G.T. Pauer The Research Institute of the Cleveland Clinic Foundation, Cleveland, OH 44195

Immunoreactive (ir) angiotensin (Ang)-(1-7) is found in magnocellular neurons of the paraventricular (PVN) and supraoptic nuclei (SON) with a distribution pattern similar to vasopressin. Recently, nitric oxide synthase, the enzyme which forms nitric oxide from arginine has been demonstrated in these magnocellular hypothalamic nuclei using NADPH-d histochemistry. Further, nitric oxide participates in neuronal plasticity, evidenced by its role in long-term potentiation. We have demonstrated prominent morphologic changes of Ang-(1-7)-ir in the dehydrated rat hypothalamo-neurohypophysial system. Thus, the present study addressed whether there exists an anatomical relationship between the Ang-(1-7) and nitric oxide systems that may subserve plasticity in hypothalamus.

Perfusion-fixed hypothalamic tissue from adult male rats was sectioned at 30 µm. Brain sections were processed for immunocytochemical localization of Ang-(1-7), and subsequently for histochemical localization of NADPH-d.

Microscopic examination of hypothalamus revealed a limited degree of colocalization of NADPH-d and Ang-(1-7)-ir in magnocellular neurons of SON and PVN. Throughout all levels of SON, approximately 5% of the neurons were found to be labeled with both NADPH-d and Ang-(1-7). In PVN at rostral levels, 2 separate cell populations were observed which contained either Ang-(1-7) or NADPH-d. However, at mid-levels through the PVN, a small number of double-labeled cells were viewed in the lateral aspect of PVN. Double-labeled cells were seldom found at caudal levels of the PVN.

This pattern of colocalization of Ang-(1-7)-ir and NADPH-d in magnocellular neurons of the SON suggests that nitric oxide may be involved in the morphologic changes that are observed in hypothalamus during dehydration. Future studies to examine this possibility are proposed.

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205.2

The Effect of Lesions of the Median Preoptic Nucleus on Augmentation of Vasopressin and Oxytocin mRNA Levels in Response to Chronic Osmolality. *M.L. Volkov and J.T. McCabe** Uniformed Services University of the Health Sciences, Bethesda, MD

The median preoptic nucleus (MnPO) is an osmosensitive afferent of the supraoptic nucleus (SON) in rats. With changes in plasma osmolality, it influences the release of both vasopressin (VP) and oxytocin (OT) from the SON and MnPO lesions will attenuate VP release that normally follows acute hypertonic saline administration (Gardiner, et al. '85; Mangiapane et al. '83). Through the use of quantitative *in situ* hybridization, we found that lesions that destroy >70% of the ventral aspect of the MnPO effectively diminished augmentation of VP mRNA levels in SON neurons during chronic hyperosmolality induced by a salt-loading regimen, whereas levels of VP mRNA under basal conditions of water availability were not different in lesioned and controls animals. In comparison to nonlesioned rats, MnPO lesioned rats had higher levels of OT mRNA under basal conditions, and did not show as great an augmentation of OT mRNA levels as a consequence of salt-loading. These results indicate that presynaptic inputs from forebrain regions mediating plasma osmolality affect not only the release of VP from the posterior pituitary, but are also involved in the modulation of intracellular processes determining VP and OT synthesis. Supported by Grants USPHS NS25913 and USUHS RO70AL to JTM.

205.3

PREABSORPTIVE SIGNALS INHIBIT VASOPRESSIN (AVP) SECRETION IN RATS DURING HYPOVOLEMIA. M.G. Scheidler*, J.G. Verbalis and E.M. Stricker. Depts. of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

Plasma volume deficits induced by sc injection of polyethylene glycol (PEG) solution in adult rats stimulates neurohypophyseal secretion of AVP and oxytocin (OT), and also enhances thirst and salt appetite. Eight hours after sc PEG treatment rats implanted with a remote pyloric noose (to prevent gastric emptying of ingested fluid) drank water in proportion to induced volume deficits but they consumed no 0.5 M NaCl. In blood samples taken minutes after drinking, only basal levels of AVP were found despite hypovolemia, whereas plasma OT was elevated appropriately. In other studies PEG-treated rats were additionally fitted with a gastric fistula, to drain the ingested fluids. In this situation, rats drank 20-45 ml of water per hr for several hours but no saline. Again, plasma OT was elevated whereas plasma AVP was not. The results are consistent with previous reports in other species that dehydration-induced AVP secretion is inhibited by drinking. However, pituitary secretion of OT is not similarly inhibited, consistent with its association with inhibition of salt appetite in hypovolemic rats.

205.4

WATER DEPRIVATION INDUCES WIDESPREAD CHANGES IN GLUTAMATE RECEPTOR DENSITIES IN BRAIN: IMPLICATIONS FOR EXCITATORY PATHWAYS WHICH REGULATE WATER BALANCE. R.B. Meeker*, R.S. Greenwood, S.P. McGinnis and J.N. Hayward, Department of Neurology and Neurobiology Curriculum, University of North Carolina, Chapel Hill, NC 27599.

Glutamate synapses are thought to provide excitatory input to vasopressin neuroendocrine cells for the release of vasopressin in response to dehydration. Two days of water deprivation result in an increase in the density of glutamate binding sites in the hypothalamus with no corresponding change in K_D. This increase is thought to reflect compensatory changes within systems involved in the regulation of water balance; principally the supraoptic nucleus (SON). Autoradiographic mapping studies in Long-Evans rats deprived of water for 48 hrs using [^{3}H]glutamate as a ligand verified specific increases in glutamate binding sites in the supraoptic nucleus in water deprived rats relative to non-deprived controls. However, both increases and decreases in glutamate receptor density of equal or greater magnitude were noted in other brain regions. Increases in receptor density were found in the SCN, PVN, amygdala and hippocampus. Decreases in receptor density were found in the tuberculum olfactorium, lateral septum, diagonal band of Broca, arcuate and ventromedial hypothalamus. Thus, glutamate receptors appear to be widely used and highly dynamic within fluid balance regulatory circuits, increasing or decreasing in response to changes in activity. Increases in receptor density were associated with the magnocellular neuroendocrine cells and general limbic circuits whereas, reduced receptor densities were found in areas known to provide synaptic input to the region of the SON.

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205.5

IN SITU HYBRIDIZATION ANALYSIS OF HYPOTHALAMIC MAGNOCELLULAR NEURONS FOLLOWING AXONAL INJURY PRODUCED BY PITUITARY STALK COMPRESSION IN RATS. J. Dohanics*, T. G. Sherman, G. E. Hoffman and J. G. Verbalis, Univ. of Pittsburgh, Pittsburgh, PA, 15261

Pituitary stalk compression (SC) causes almost complete disappearance of vasopressin (AVP) and oxytocin (OT) from the neural lobe and increased water intake characteristic of diabetes insipidus. Similar to neurolobectomy and pituitary stalk transection, the axonal injury produced by SC also leads to death of substantial proportions of AVP (≈60%) and OT (≈30%) neurons in the supraoptic (SON) and paraventricular (PVN) nuclei. In this study we investigated the effect of SC on expression of AVP and OT mRNAs in the PVN and SON via in situ hybridization histochemistry, to determine whether the surviving neurons manifested increases in AVP and OT gene expression to compensate for the loss of neurosecretory units. Adult male rats received SC (n=4) or sham surgery (n=4) 21d prior to euthanasia by decapitation. AVP and OT contents of neurointermediate lobe (NIL) homogenates were determined by RIA. Hypothalamic blocks were serially sectioned to 14 µm using a cryostat. Sections were hybridized with specific AVP and OT exon C [35]S-labeled cRNA probes. Grains were counted in visually identified neurons using Optimas Bioscan software and were corrected for cell area. NIL AVP and OT contents in SC rats were 1.8% and 0.9% of sham operated rats, but plasma [Na+] concentrations were similar in both groups (141.0±0.1 vs. 141.7±0.7, NS). Magnocellular AVP and OT mRNA grain counts/cell area were not significantly different between the sham-operated (n=1006 neurons counted) and the SC rats (n=1178 neurons counted). These data suggest that under conditions where a normal plasma osmolality can be maintained by increased water intakes, there is no significant compensatory increase in AVP and OT mRNA expression in the magnocellular neurons that survive axonal injury. It remains to be determined, however, whether the surviving neurons respond normally to acute and chronic osmotic challenges, as well as whether synthetic activity at the time of SC is related to magnocellular neuronal survival.

205.6

HYPOOSMOLALITY INHIBITS NEUROHYPOPHYSEAL EXPRESSION OF C-FOS AND FOS-RELATED ANTIGENS IN RATS. J.G. Verbalis* A.G. Robinson and G.E. Hoffman. Departments of Medicine and Physiology, University of Pittsburgh, Pittsburgh, PA 15261.

Previous studies from this and other laboratories have shown that neurohypophyseal neurons express c-Fos protein following stimuli that cause secretion of vasopressin (AVP) and/or oxytocin (OT). Hyperosmolality is one such stimulus known to increase secretion and synthesis of AVP and OT, and to stimulate robust expression of c-Fos protein in magnocellular neurons. Because hypoosmolality inhibits secretion and synthesis of AVP and OT in rats, we studied expression of c-Fos and fos-related antigen (FRA) immunoreactivity (IR) in hypoosmolar rats. Hypothalamic sections (25 um) from perfused brains were stained using antisera to c-Fos protein (Oncogene Sciences) and FRA (M. Iadarola, NIH). Compared to controls (pNa=140±1 mmol/L, n=6), rats kept hypoosmolar for 7 d (pNa=109±1 mmol/L, n=5) had complete absence c-fos IR and markedly reduced levels of FRA IR. Additional hypoosmolar rats were infused iv with 1.5 M NaCl @ 1.0 cc/h and blood was collected for plasma sodium (pNa), AVP and OT before perfusion after 0, 2, 4, 6, and 8 h. During correction of hypoosmolality, magnocellular neurons expressed both c-Fos and FRA IR as early as 2 h (pNa=122±1 mmol/L, n=5), and maximally by 4-6 h (pNa=135±4 mmol/L, n=10). Magnocellular c-Fos and FRA expression preceded increased plasma levels of AVP, though not of OT. Therefore: 1) hypoosmolality downregulates neurohypophyseal proto-oncogene expression as well as AVP and OT secretion and synthesis; and 2) subsequent increases in plasma osmolality rapidly induce proto-oncogene expression of both c-Fos and FRA's, for AVP neurons even before measurable pituitary secretion occurs. The latter suggests that during some conditions c-Fos/FRA expression can detect magnocellular neuronal activation before neurosecretion occurs.

205.7

ARGININE VASOPRESSIN (AVP) LEVELS AFTER DAILY INFUSIONS OF ANTISENSE OLIGONUCLEOTIDES INTO THE SUPRAOPTIC NUCLEUS (SON). L.M. Flanagan*, M.M. McCarthy, P.J. Brooks, D.W. Pfaff, and B.S. McEwen. The Rockefeller University, 1230 York Avenue, New York, NY 10021 USA.

Synthetic antisense oligonucleotides have been shown to reduce synthesis of proteins by blocking translation in vitro. In the present experiment we used the hypothalamic-neurohypophyseal system as an in vivo model for antisense pharmacology. Antisense 5' AVP mRNA just downstream from the translation start codon (n=4) or a scrambled sequence control oligonucleotide (n=3) was infused into the bilateral SON (100 ng in 0.5 µl/side/min). After 10 daily infusions, the antisense treatment decreased AVP levels in the pituitary by 60%, and in the SON by 75%, whereas it increased AVP levels in the paraventricular nucleus (PVN) several fold. Compared with the scrambled sequence oligonucleotide control injections, antisense increased plasma osmolality, and decreased urine osmolality at the time of sacrifice. Histological analysis confirmed the location of the cannula tip near the SON. Oxytocin (OT) levels were somewhat reduced in the pituitary, but were elevated in plasma which suggests osmotic stimulation of OT neurons (Brimble, 1978). The increase in the level of AVP in the PVN may result from a compensatory mechanism activated by episodes of diabetes insipidus due to impaired synthesis of AVP in the SON. These preliminary results suggest that antisense oligonucleotides can provide a useful tool for studying control of neuropeptide synthesis in vivo. Supported by NIH grant NS07080 and postdoctoral fellowship NS09026.

205.8

VOLUME CHANGES REGULATE A CATIONIC CURRENT TO TRANSDUCE OSMOSENSITIVITY IN SUPRAOPTIC NEURONS. Stéphane H.R. Oliet* and Charles W. Bourque. *Centre for Research in Neuroscience, Montreal General Hospital and McGill University, Montreal, Canada H3G 1A4.*

The membrane potential and electrical activity of rat supraoptic neurons are known to be modulated by changes in plasma osmolality. Whole-cell patch-clamp experiments on acutely dissociated supraoptic neurons were performed to characterize the mechanism mediating this endogenous osmosensitivity. The amplitude of a non-selective cationic current (NSC; E_{rev}≈-41 mV) was found to be proportional to changes in external osmolality (-30 to +30 mOsm; 1.4 %NSC/mOsm; R=0.99; n=17). Experiments using a confocal laser scanning microscope revealed that changes in cationic conductance were directly correlated (R=0.88) with changes in cell volume. Comparable volume changes directly elicited by pressure (≈1 mmHg) applied through the patch electrode also modulated a cationic current, cell potential and firing rate (n=11). Reversal potential analysis and ionic substitutions revealed that the currents modulated by pressure and external osmolality were, in fact, the same. We conclude that volume-sensitive cationic channels transduce osmosensitivity in rat supraoptic neurons. *Supported by the MRC and Heart and Stroke Foundation of Canada.*

205.9

GLUTAMATERGIC EXCITATION OF RAT SUPRAOPTIC NEURONS FOLLOWING OSMOTIC STIMULATION OF THE ORGANUM VASCULOSUM LAMINA TERMINALIS (OVLT). C.W. Bourque* and D. Richard. *Center for Research in Neuroscience, Montreal General Hospital, Montreal, Canada.*

Osmotically-evoked hormone release by the axon terminals of magnocellular neuroendocrine cells (MNCs) is partly mediated by the OVLT. Intracellular recordings obtained in rat hypothalamic explants examined the nature of OVLT's contribution to the osmoregulation of supraoptic MNC firing. Local hypertonic stimulation (<30 mOsm) excited 14/17 OVLT neurons, a response which was temporally associated with a dramatic increase in the frequency of spontaneous EPSPs and spike discharges recorded in 19 of 23 MNCs. A fast excitatory connection between the OVLT and MNCs has been proposed on the basis that electrical stimulation of the OVLT elicits a CNQX-sensitive compound EPSP in MNCs (Yang & Renaud, S.N. Abs 1991). Confirming the *specificity* of this connection, computer averaging during recordings obtained from 7/18 pairs of neurons revealed a tight correlation between spike discharge in OVLT neurons and the occurence of an EPSP in MNCs. Bath-application of kynurenic acid (200 µM) or CNQX (10-25 µM) reversibly abolished evoked EPSPs (n=6), as well as the OVLT-mediated hypertonic activation of supraoptic MNCs (n=4). We conclude, therefore, that glutamatergic synapses onto MNCs relay osmosensitive information arising within the OVLT. *Supported by MRC, FCAR and the Heart and Stroke Foundation of Canada.*

205.10

ATRIAL NATRIURETIC FACTOR (ANF) MODULATES EXCITATORY TRANSMISSION FROM THE ORGANUM VASCULOSUM OF THE LAMINA TERMINALIS (OVLT) TO THE SUPRAOPTIC NUCLEUS (SON). D. Richard* and C.W. Bourque. *McGill University, Montreal, Canada H3G 1A4.*

Previous studies have implied that ANF modulates the osmotic regulation of neurohypophyseal hormone (NH) release via an action at receptors which are presynaptic to magnocellular neurosecretory cells (MNCs). One possible site for such action may be at the glutamatergic OVLT→SON synapse, which relays osmotic information arising within the OVLT. To study the effects of ANF at this synapse, we obtained intracellular recordings from SON MNCs in rat hypothalamic explants. Electrical stimulation of the OVLT (80 µA; 200 µs) evoked a fast glutamatergic EPSP in each of 20 MNCs tested. In control conditions, voltage-current analysis revealed that the conductance underlying evoked EPSPs (G_{EPSP}) ranged from 0.5 to 1.7 nS. Bath-application of ANF^{1-28} (50-100 nM; n=10) or dibutyryl cGMP (0.5-1 mM; n=3) reversibly decreased the evoked G_{EPSP} by 41.1±3.6%. Bath applied ANF was also found to reversibly abolish the synaptic activation of MNCs following local hypertonic (5-30 mOsm/Kg) stimulation of the OVLT (n=3). These results indicate that ANF may modulate the osmoregulation of NH release via an inhibitory action at the OVLT→SON synapse. *Supported by the MRC, FCAR and the Heart & Stroke Foundation of Canada.*

205.11

INTERACTION OF NEUROTRANSMITTERS AND NEUROMODULATORS ON SUBFORNICAL ORGAN NEURONS H. A. SCHMID*, F. SCHÄFER, E. SIMON. Max Planck Institute f. Physiol. and Clin. Research, 6350 Bad Nauheim, FRG.

The subfornical organ (SFO), a circumventricular organ lacking a blood-brain barrier, is known to participate in osmoregulatory responses in mammals and birds. Blood borne and brain intrinsic Angiotensin II (AII) has been shown to affect the activity of SFO neurons. In this study we investigated the possible interaction of AII with various Atrial Natriuretic Factor (ANF) analogs and cholinergic and noradrenergic agonists on spontaneously active duck SFO neurons in an *in vitro* slice preparation. AII (0.1 µM) excited over 70% of the SFO neurons, while ANF, which antagonizes AII *in vivo*, has no effect, neither alone, nor on the AII induced excitation. Isoproterenol (10 µM) and ACh (30 µM) increased the activity of 85% and 40% of the neurons, whereas clonidine decreased it in 85%. Coapplication of ACh and Isoproterenol together with AII had additive excitatory effects. These results suggest a high degree of signal integration of osmoregulatory important substances within the SFO.
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205.12

Furosemide Alters Development in Neonatal Rats. G. Watkins, J. Diaz*, E. Taylor, and M. Rolfs, Dept. of Psychology, Univ. of Washington , Seattle, WA 98195.

Furosemide (Lasix), a powerful diuretic, is routinely used in human neonates. We examined the acute and chronic effects of Lasix on water compartments in the neonatal rat.

In the acute study, 4 day old rat pups were randomly assigned to one of three groups: 1) Lasix (IP, 15 mg/kg every 2 hours)), 2) saline (IP, every 2 hours), or 3) non-inject controls. After 24 hours all animals were sacrificed. In the chronic study, pups were randomly assigned to one of three groups: 1) gastrostomy-fed, receiving Lasix via a chronic indwelling subcutaneous catheter, 2) gastrostomy-fed, receiving saline via chronic indwelling catheter, and 3) mother-reared, non-injected. Lasix and saline were given at doses modeling a steady-state condition in humans. After seven days of treatment, all the animals were sacrificed. For both studies, wet and dry whole brain, kidney, and spleen weights were recorded for all animals.

Acute Lasix treatment resulted in a significant decrease in body weight and a significantly smaller brain to body weight ratio. In addition to a significant decrease in body weight, chronic Lasix treatment also resulted in significantly smaller wet brain weights, and significantly heavier wet and dry kidney weights. There was no difference in spleen weights.

Lasix, when administered in a dosing regimen which mimics that given neonatal infants, changes the fluid dynamics of the developing brain. Whether this directly affects neuronal development and results in long-term neurological sequelae remains to be determined.

206.1

MODULATION OF CATECHOLAMINE RELEASE BY INTERLEUKIN-2 (IL-2) IN MOUSE HYPOTHALAMUS. F. Villemain*, D. Seto, U.K. Hanisch, R. Quirion and A. Beaudet. Montreal Neurol. Inst. and Douglas Hosp. Res. Center, Montreal, Canada, H3A 2B4.

Immunohistochemical and *in situ* hybridization experiments from our laboratory have previously demonstrated the presence and expression of IL-2 in mouse brain (Neurosci. Abstr., 1991, 475-20). In normal CD-1 mice, high concentrations of IL-2 and Tac antigen (low-affinity subunit of IL-2 receptor)immunoreactivity were detected within the arcuate nucleus/median eminence complex of the hypothalamus. Electron microscopic studies indicated that both antigens were contained in a subpopulation of arcuate neurons. In addition, double labeling studies indicated that some of the Tac immunoreactive cells also stained positively for tyrosine hydroxylase, suggesting that endogenous IL-2 might act upon hypothalamic monoamine neurons. To test this hypothesis, the effect of IL-2 on endogenous catecholamine release was investigated in hypothalamic slices superfused for 30 min in Krebs buffer (KB) and stimulated 40 min with high-K^+ (25 mM) KB in the presence or absence of low (10^{-9}M) or very low (10^{-12}M) concentrations of recombinant IL-2 (Boehringer). The superfusate was collected over the entire duration of the experiment and analyzed for dopamine (DA), noradrenaline (NA) and metabolites using an HPLC system. At low concentrations of IL-2, the evoked NA efflux was significantly reduced at the very onset of stimulation. At very low concentrations, the inhibitory effect only became apparent after 30-min of stimulation. A slight, but non significant inhibition of dopaminergic release was also evident after stimulation at low concentration of the cytokine. These results support the role of neuronal IL-2 in the modulation of monoamine release in the ventrobasal hypothalamus. Supported by HFSP and MRC.

206.2

INTERLEUKIN-2 MODULATES THE EVOKED RELEASE OF [^{3}H]DOPAMINE FROM MESENCEPHALIC CELLS IN PRIMARY CULTURE. R. ALONSO*, J. DIORIO, R. QUIRION and P. BOKSA. Douglas Hospital Research Centre, Dept. of Psychiatry, McGill University, 6875 Lasalle Blvd, Montreal, Quebec, Canada, H4H 1R3.

Beside its immunological functions, Interleukin-2 (IL-2) is known to interact with several neuronal systems. In particular, our laboratory previously showed that IL-2 modulates the release of acetylcholine in the rat hippocampus (Seto et al, Neurosci.Soc.Abstr., 1991). It has also been suggested that IL-2 is involved in the regulation of striatal dopaminergic transmission (Lapchak, Soc.Neurosci. Abstr., 1991). In an attempt to further investigate the interaction of IL-2 with dopaminergic neurons, we evaluated the effects of this cytokine on [^{3}H]dopamine (DA) release from rat cultured mesencephalic cells. Since this cell culture model contains a high proportion of GABAergic neurons, modulation of [^{3}H]GABA release by IL-2 was also investigated. IL-2 (10^{-14}-10^{-8} M) modulated K^+-induced [^{3}H]DA release with a biphasic dose response curve. Very low concentrations (10^{-12} M) of IL-2 increased, while higher (10^{-8} M) concentrations decreased, K^+-induced [^{3}H]DA release. Very low concentrations (10^{-12} M) of IL-2 also increased NMDA ($5x10^{-5}$ M)-induced [^{3}H]DA release while 10^{-8} M IL-2 had no effect on the NMDA response. IL-2 alone did not modify spontaneous [^{3}H]DA release. Under our experimental conditions, the release of [^{3}H]GABA evoked by K^+ or by NMDA is mostly Ca^{2+}-independent. IL-2 did not modulate the [^{3}H]GABA release evoked by either K^+ or NMDA. This study demonstrates that low concentrations of IL-2 selectively interact with dopaminergic neurons in cultured mesencephalic cells to modulate [^{3}H]DA release evoked either by K^+ depolarization or NMDA receptor activation. Mesencephalic cell cultures should provide a useful model to further investigate the mechanisms involved in these IL-2 effects. Supported by MRC of Canada.

206.3

EFFECT OF PROTEIN KINASE C ACTIVATION ON INTERLEUKIN 6 PRODUCTION BY RAT CORTICAL ASTROCYTES. M. Grimaldi, C. Ventra, O. Meucci*, A. Scorziello, A. Avallone and G. Schettini. Dip. Scienza delle Comunicazioni Umane, Sez. Farmacologia, II Facolta' di Medicina e Chirurgia, Via Pansini 5, 80131 Naples, ITALY.

It has been demonstrated that astrocytes release low amounts of IL-6 in resting conditions. The aim of our study has been to evaluate the effect of protein kinase C activation on IL-6 production by primary cultures of rat cortical astrocytes. We found that IL-1 beta dose-dependently stimulates IL-6 production. The activation of protein kinase-C by phorbol 12-myristate 13-acetate (PMA) also increases IL-6 release. The simultaneous stimulation with IL-1 beta and PMA shows a synergistic effect on the cytokine production. Preliminary results seems to confirm the synergistic effect of IL-1 and PMA coincubation also at level of IL-6 gene expression. In conclusion, the activation of protein kinase C per se strongly increases IL-6 production and potentiates the effectiveness of IL-1 beta in stimulating IL-6 production from cultures of rat cortical astrocytes. (CNR grant # 90.01554 to G.S.)

206.4

CORTICOTROPIN RELEASING HORMONE INCREASES PLASMA LEVELS OF INTERLEUKIN - 6. Z.A. Kronfol*, B. Gosnell, M. Kluger. Departments of Psychiatry and Physiology, University of Michigan Medical Center, Ann Arbor, MI 48109

Several reports have now confirmed the existence of complex and bidirectional interactions between the central nervous system and the immune system. We have earlier reported that open field stress increases core body temperature along with a rise in plasma levels of interleukin - 6 (IL-6). Since many of the effects of stress can be mimicked by corticotropin releasing hormone (CRH), we hypothesized that CRH injections would be accompanied by similar increases in the plasma levels of IL-6, and that this effect will be blocked at least in part by the CRH antagonist α-helical CRH. To test this hypothesis, we injected male Sprague-Dawley rats in which we had previously placed intracerebro-ventricular cannulae with either CRH (1.0µg) or saline ICV. Twenty or sixty minutes later, the animals were sacrificed and plasma levels of IL-6 were measured using a B9 bioassay. The same experiments were later repeated with two additional groups: one group receiving α-helical CRH and one group receiving both α-helical CRH and CRH. The results indicated a significant increase in the plasma levels of IL-6 in the CRH-treated group. The group receiving bothα-helical CRH and CRH had somewhat lower levels of IL-6. These results indicate that CRH given ICV, like stress, is accompanied by significant elevations in the plasma levels of IL-6, and that this effect of CRH can be partially reversed by α-helical CRH.

206.5

NEUROLOGIC DISEASE INDUCED IN TRANSGENIC MICE BY THE ASTROCYTE-SPECIFIC EXPRESSION OF INTERLEUKIN-6. J.L. Campbell, M.B.A. Oldstone and L. Mucke*. Dept. Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Cytokines, including interleukin-6 (IL-6), are known to have a spectrum of CNS effects. Although inappropriate expression of cytokines within the CNS has been reported in a variety of CNS diseases including multiple sclerosis, AIDS dementia complex (ADC) and Alzheimer's disease their precise pathogenetic role in these diseases remains unknown. We have investigated this question by using a transgenic approach to target the inappropriate expression of IL-6 to astrocytes in the CNS of mice.

The complete cDNA encoding for IL-6 was inserted in the first exon of the murine glial fibrillary acid protein (GFAP) genomic DNA. Following microinjection of the GFAP-IL6 into the germline of mice, a number of transgenic offspring were found to have a phenotype characterized by growth retardation, tremor, hunched posture, ataxia, hind-limb weakness and seizures. In all such mice high levels of IL-6 transgene expression were found in the brain. No disease phenotype was seen in non-transgenic littermates nor in transgenic mice without detectable IL-6 gene expression. Histopathological examination of the brain in IL-6 expressing mice was on the whole unremarkable except for the presence of occasional scattered perivascular mononuclear cell accumulation in cerebellar fissures and some neovascularization. Neither dysmyelination nor neuronal loss were evident. However, immunocytochemical staining and northern blot analysis revealed the presence of a pronounced astrocytosis present in hippocampal formation,cerebellum and white matter tracts.

Therefore overexpression of IL-6 by astrocytes in the CNS of mice produces a severe neurological disease in association with marked astrocytosis - these features have similarity to some human CNS diseases eg. ADC. This model should prove extremely valuable for elucidating the role of cytokines in neuropathogenesis.

206.6

HISTAMINE INDUCES INTERLEUKIN 6 RELEASE FROM THE HUMAN ASTROCYTOMA CELL LINE, U-373 MG. D.C. Waters, R.E. Bruns*, J.J. Howbert, and B.D. Gitter. Depts. of Central Nervous System Research and Biochemical Pharmacology Research, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285.

Histamine is a mediator of allergic, inflammatory, and immune responses. Although it is produced by neurons and mast cells in the brain, histamine's potential role as a mediator of CNS inflammation has not been described to date. Histamine receptors linked to phosphoinositide hydrolysis have been identified on primary rat astrocytes and human astrocytoma cell lines. Since astrocytes have been shown to secrete inflammatory mediators such as prostaglandins and cytokines, we studied the ability of histamine to induce the secretion of interleukin 6 (IL-6) and prostaglandin E_2 (PGE_2) by human U-373 MG astrocytoma cells. Histamine stimulated IL-6 release in a concentration-dependent manner, reaching 50% of the maximum response at 10µM. However, histamine did not stimulate PGE_2 production at concentrations as high as 50µM. Detectable levels of IL-6 were produced approximately 1 hour after stimulation and peaked after 24 hours. IL-6 release induced by histamine was inhibited by the H1 antagonist, pyrilamine ($IC_{50} \approx 5$nM), but was not affected by the H2 antagonist famotidine or the nonpeptide substance P antagonist (±)CP-96,345 at concentrations ≥ 5µM, indicating that the IL-6 response was specifically mediated through H1 receptors. In addition, histamine-stimulated phosphoinositide (PI) turnover was also inhibited by pyrilamine, suggesting that IL-6 secretion was linked to this second messenger. Histamine in the CNS may, therefore, contribute to neuronal inflammation by inducing astrocytes to secrete IL-6, a key mediator of immune and inflammatory responses.

206.7

SYMPATHECTOMY ENHANCES INTERLEUKIN-2 (IL-2)-INDUCED PROLIFERATION BY SPLENOCYTES FROM SPONTANEOUSLY HYPERTENSIVE RATS (SHR). Edwin S. Purcell* and Vincent H. Gattone, II. Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS 66160.

The SHR is known to possess increased sympathetic innervation of a number of tissues and a T cell immunological deficiency. We have previously described increased catecholaminergic innervation of the thymic parenchyma and splenic white pulp (Purcell and Gattone, Exp Neurol, in press, 1992) and an impaired proliferative response of splenocytes to IL-2 (Purcell and Gattone, Anat Rec 229:70A, 1992) in SHR as compared to Wistar-Kyoto (WKY) and Fischer 344 (F-344) rats. To determine if there is a causal relationship between the increased innervation and the immune dysfunction, we performed neonatal sympathectomy using a combination of guanethidine and NGF-antisera. At 12 weeks of age, splenocytes were obtained and cultured with or without 50 units/ml recombinant human IL-2. Subsequently, cells were pulsed with tritiated-thymidine and thymidine incorporation was determined by scintillation counting. The stimulation index (cpm of stimulated cells/cpm of unstimulated cells) of sympathectomized SHR displayed a significantly higher stimulation index than sham treated control SHR (con 1.28 $\pm$ 0.17/symX 1.97 $\pm$ 0.27 , $p \leq 0.05$). Sympathectomy did not enhance the robust proliferative response of WKY splenocytes to IL-2 (con 3.85 $\pm$ 1.06/symX 3.41 $\pm$ 0.52). However, it did increase the responsiveness of F-344 splenocytes (con 2.00 $\pm$ 0.34/symX 3.54 $\pm$ 0.24, $p \leq 0.05$) to a level similar to the WKY. These results suggest that noradrenergic innervation contributes to the immune deficit of the SHR by decreasing the proliferative response to IL-2. Supported by MH46511.

206.8

INTERLEUKIN-2 ENHANCES SCOPOLAMINE-INDUCED AMNESIA IN THE MOUSE. M. Bianchi and A.E. Panerai* Dept. Pharmacol. University of Milano, School of Medicine, 20129, Milano, Italy.

Evidence has been reported that IL-2 exerts several neuromodulatory effects, including the inhibition of acetylcholine release in specific CNS regions and a dose dependent inhibition of long-term potentiation in the rat hippocampus (1,2). These observations might explain the memory impairment observed in patients during immunotherapy with IL-2 (3).

We evaluated the effects of human rIL-2 on scopolamine-induced amnesia for a passive avoidance response in mice (4). Pre-training i.p. administration of 1250 or 2500 I.U. of the cytokine significantly potentiates the amnesic effect of scopolamine 1mg/kg i.p.. Higher (5000-10000 I.U.) or lower (625 I.U.) doses of IL-2 were completely ineffective. The post-training administration of IL-2, as well as IL-2 alone, does not affect memory retention.

Our results are in agreement and further extent the electrophysiological and clinical observations, and suggest that the pharmacological modulation of the cholinergic system could be useful in order to reduce some neurological side effects of IL-2 on learning and memory.

1. Araujo D.M. et al., Brain Res. 498:257, 1989;
2. Tancredi V. et al., Brain Res. 525:149, 1990;
3. Bocci V. et al., J.Biol.Reg.Homeo.Agents 2:107,1988;
4. Galliani G. et al., Med. Sci. res. 15:313, 1987

206.9

ANOREXIC EFFECTS OF MICROINJECTION OF INTERLEUKIN-1β (IL-1β) INTO THE VENTROMEDIAL HYPOTHALAMUS (VMH) OF THE RAT. S. KENT*, F. RODRIGUEZ, K.W. KELLEY and R. DANTZER. INSERM U.176, rue Camille Saint-Saëns, 33077 Bordeaux Cedex, France (SK, FR and RD); Dept. of Animal Sciences, Univ. of Illinois, Urbana, IL 61801 (KWK).

IL-1 is a cytokine that is released by activated macrophages and monocytes and mediates many of the local and systemic responses to inflammation. Intracerebroventricular injections of IL-1β produce anorexia, however, the specific site(s) of action within the central nervous system have not been elucidated. We report here that bilateral infusion of recombinant human IL-1β (Glaxo, Geneva; 5 and 30 ng) into the VMH of adult male rats dose-dependently induces anorexia in two animal models. (1) Daily consumption of food and water were measured in rats fed *ad libitum*. IL-1β (5ng) reduced food intake to 56 ($p<0.01$) and drinking to 70 % ($p<0.05$) of baseline. Larger decreases were observed after infusion of 30ng. Amounts returned to near control levels after 24 h. Body weight was reduced for 2 d post-IL-1β. (2) Rats were food-restricted and trained on an operant schedule (FR10) for food reinforcement. They were tested for 5 min before injection of IL-1ß and then retested 1, 2, 4, 8, and 24 h later. Both doses produced profound decreases in responding, with maximal effects 2-4 h post-injection. Four h post-infusion responding was reduced to 15$\pm$7 % (5ng) and 3$\pm$3 % (30ng) of baseline ($p<0.01$). These results suggest that the VMH may serve as a central site of action for the depressive effects of IL-1β on food intake.

(Supported by DRET (RD) and ONR (KWK))

206.10

EFFECT OF IL-1ß INJECTIONS IN PGE2-SENSITIVE mPOA HEAT GAIN SITES OF ADX RATS WITH mPOA CORTICOSTERONE REPLACEMENT. G.E. Resch*. Schl. Biol. Sci., Univ. Missouri at Kansas City, Kansas City, MO 64108.

The literature shows the loss of body temperature during cold stress that occurs in adrenalectomized (Adx) rats was restored with whole body corticosterone (Cort) replacement. In our previous reports Cort was replaced in PGE2-sensitive thermogenic sites within the medial preoptic area (mPOA) and tolerance to cold was restored in Adx rats. PGE2 injections into the mPOA sites that normally elicit a rise in colonic temperature (Tc) in intact rats failed to do so in Adx rats. The response was restored with mPOA Cort replacement indicating the PGE2 response was Cort-dependence. Some evidence indicates IL-1ß acts via a PGE2 mechanism, consequently in this report the pyrogenic effects of IL-1ß were tested for a similar cort-dependence. Stainless steel guide tubes (24 ga) were implanted under ketamine + acepromazine anesthesia in the mPOA and PGE2 injected stepwise dorsal to ventral to identify PGE2-sensitive thermogenic sites as previously reported. Cold tolerance tests were used to verify effective Adx. IL-1ß (1 au) was then injected into these sites in 5 intact rats and in 5 Adx rats, before and again after 6 to 8 days of Cort injections into mPOA sites(100ng 2X/day). IL-1ß elicited a 0.57°C +/- 0.69) rise in Tc in Adx rats, (0.92°C +/- 0.28) in the intact controls and 1.42°C +/- 0.41) in 8 day Cort replaced rats ($P<.05$, Adx vs Rpl) These data do not appear consistent with Chowers report of Cort attenuation of a pyrogen fever; however site specificity and differences in methods may account for the data. Compared with previous PGE2 responses, Adx attenuated the IL-1ß response but did not abolish it. These data support the notion of IL-1ß acting through a PGE2 transduction mechanism, however the incomplete blockade of IL-1ß by Adx suggests IL-1ß may operate through more than one mechanism of action. Supported by AFOSR 870297.

206.11

INTERLEUKIN-6 AND TUMOR NECROSIS FACTOR INDUCTION BY CENTRAL ENDOTOXIN M. G. De Simoni*, C. Gemma, A. De Luigi, A. Manfridi and P. Ghezzi. Istituto di Ricerche Farmacologiche "Mario Negri", via Eritrea 62, 20157 Milan, ITALY.

Our previous studies[1] have shown that intracerebroventricular (icv) interleukin 1 (IL-1) induces high levels of circulating interleukin 6 (IL-6) indicating that central IL-1 is able to induce cytokine-mediated actions in the periphery. To better elucidate the communication between brain and periphery, the present study analyses the effects of central endotoxin (LPS) administration on cytokine production. Different doses of LPS have been injected to rat cerebral ventricles and serum IL-6 and tumor necrosis factor (TNF) have been mesured. Central LPS resulted in more marked induction of circulating IL-6 than the same dose of LPS given systemically, the maximal response being obtained in both cases 2 hours later. Differently from IL-1 central injection, LPS-induced IL-6 was not prevented by IL-1 receptor antagonist indicating that LPS central effect is not mediated by IL-1.

Serum TNF levels have also been measured following central and systemic LPS injection: also in this case the icv administration was more efficient than the systemic one. The time-course analysis indicated that the peak of TNF induction is delayed after icv compared to systemic injection. LPS icv (but not intraperitoneus) induced IL-6 and TNF in various brain areas.

The data show that central endotoxin administration induces high circulating levels of IL-6 and TNF indicating the existence of a pathway that could explain how lesions confined to the central nervous system result in systemic alterations.

[1]De Simoni et al.(1990) J. Exp. Med. 171:1773.

206.12

ELEVATED TUMOR NECROSIS FACTOR-ALPHA LIKE ANTIGEN LEVELS (TNFL) IN BRAINS OF DYSTROPHIC HAMSTERS. E. H. Schlenker*& J. A. Burbach. Depts. of Physiology and Pharmacology & Lab. Medicine, USD School of Medicine, Vermillion, SD 57069.

The BIO 14.6 dystrophic hamster (DH) like patients who have euthyroid sick syndrome have low serum triiodothyronine (T3) levels. Mooradian and coworkers (J Clin Endocrin Met 71:1239,1990) reported that such patients also exhibited elevated serum tumor necrosis factor-alpha (TNF) levels. The purpose of this study was to test the hypothesis that DH have elevated TNFL levels. Measurements of TNFL were made using recombinant murine TNF ELISA. Because TNF in hamsters has not been fully characterized, we chose to call the antigen that cross-reacted in the ELISA tests TNFL. We used normal hamsters (NH) as a negative control and normal hamsters injected i.p. with 5ug/kg endotoxin (EH) as a positive control. Values were expressed as pg of TNFL/mg protein for brain, spleen and lung from animals in the 3 groups are presented below.

	NH (n=3)	EH (n=4)	DH (n=4)
Brain	0.25 $\pm$ 0.14	2.41 $\pm$ 0.38	2.02 $\pm$ 0.41
Spleen	1.97 $\pm$ 0.73	16.53 $\pm$ 3.42	19.50 $\pm$ 7.10
Lung	1.26 $\pm$ 0.77	20.28 $\pm$ 1.84	16.41 $\pm$ 2.70

The results indicate that both EH and DH have elevated TNFL levels relative to those of NH in all 3 tissues evaluated ($P<0.03$, Mann-Whitney Test). The physiological significance of the association of low T3 levels and elevated TNFL levels in DH needs to be investigated further.

206.13

PROSTOGLANDINS MEDIATE ACTH SECRETION IN RESPONSE TO TUMOR NECROSIS FACTOR-α (TNF). J.G. McCoy, S.G. Matta, S.E. Nicol*, and B.M. Sharp. Minneapolis Medical Research Foundation and Depts. of Med., Hennepin County Medical Center and Univ. of Minn., Mpls., MN 55404

We have previously shown that the cytokines IL-1ß and TNF-α given i.v. stimulate the release of pituitary ACTH to a comparable magnitude, with the same time course. However, IL-1ß acts within the hypothalamus, whereas TNF-α has no direct central effect when administered i.c.v. or adjacent to the median eminence. To determine if prostaglandins (PGs) might be mediators of the interaction between TNF-α and the hypothalamo-pituitary-adrenal axis, indomethacin (Indo, 1 mg/kg, i.v.) was administered prior to TNF-α (1µg/rat, i.v.) in freely mobile, alert rats. While Indo alone had no significant effect on plasma ACTH levels [at 20 min peak (mean±sem): 26±13 pg/ml compared to buffer 26±10 pg/ml], it completely blocked the response to TNF-α from 299±54 pg/ml to 28±8 pg/ml. This dose of Indo had no effect on the ACTH response to CRF 1 µg/kg i.v.(at 10 min: CRF alone = 461±69 pg/ml; Indo/CRF = 403±76 pg/ml), demonstrating that there was no direct inhibition of pituitary responsiveness. Since it has been reported that Indo elevates plasma corticosterone (B) levels, the blockade of TNF-stimulated ACTH release by Indo could be due to a rapid feedback inhibition by B, rather than a direct involvement of PGs. However, in additional experiments, inhibition of ACTH secretion by Indo was shown to be dose-dependent, whereas plasma B levels were elevated to the same degree, independent of Indo dose. Furthermore, Indo failed to block the ACTH response to an unrelated ACTH stimulus - insulin-induced hypoglycemia (area under response curve: Insulin alone = 31131±2794 pg x min/ml; Indo/Insulin = 32919±3582 pg x min/ml). In adrenalectomized, B-replaced rats, TNF (1 µg/rat, i.v.) elevated ACTH to the same levels seen in sham animals. Indo inhibited these ACTH responses to the same extent in both groups. Thus, Indo inhibited the ACTH response to TNF-α by a mechanism independent of corticosterone feedback. These results demonstrate that circulating TNF-α stimulates ACTH secretion through a PG-dependent mechanism.

206.14

SELECTIVE MODULATION OF EVOKED HIPPOCAMPAL ACETYLCHOLINE RELEASE BY CYTOKINES

U.-K. Hanisch*, D. Seto, and R. Quirion. Douglas Hospital Research Centre and Department of Psychiatry, McGill University, Montreal, Quebec, Canada.

Signalling peptides of the immune system, i.e. cytokines, also exert effects on glial and neuronal cells. Produced in the CNS or having access to brain targets via passage of the blood-brain barrier, cytokines may control neuronal activity, especially when intrinsic or circulating cytokines are elevated (i) upon challenge of an immune or inflammatory response, (ii) during immunotherapy, or (iii) as a consequence of injury or pathologic processes. By means of superfusion and static incubation of rat brain slices interleukin-2 (IL-2) was shown to modulate the evoked release of acetylcholine (ACh) from hippocampus and frontal cortex, but not from striatal tissue *(Soc.Neurosci.Abstr. 1991: 313.13)*. For the hippocampus, the dose-response relation has a biphasic profile, with inhibition at 1 nM and transient augmentation at 0.1 pM. Subsequently, we investigated the specificity of the IL-2 effect in experiments involving other cytokines, including tumor necrosis factor (TNF). Although several cytokines, like IL-1, IL-3, IL-5, and IL-6, were ineffective at nanomolar concentrations in modulating hippocampal ACh release, we obtained some evidence for a TNFß-mediated inhibition. Taking together, the data suggest a preferential sensitivity of hippocampal ACh release to a modulation by IL-2. (Supported by the FRSQ, the MRCC, and the HFSPO.)

REGULATION OF AUTONOMIC FUNCTION: GASTROINTESTINAL AND VISCERAL AFFERENTS

207.1

THE WEANLING RAT LATERAL HYPOTHALAMIC SYNDROME: INTERMEDIARY METABOLISM. L.Bernardis*,J. Natale,C. Fink and A.Awad. DVAMC Buffalo, Dept. Med., SUNY/Buffalo 14215 and Nutrition Program, SUNY/Buffalo, Buffalo NY 14214.

Weanling rats with ventromedial hypothalamic nucleus lesions show dramatic changes in intermediary metabolism of liver, epididymal fat pad (PAD) and diaphragm (DIA) in the presence of normophagia, normal body weight (BW) and obesity even in the absence of the pituitary gland. No data are available on the weanling rat with lateral hypothalamic lesions (LHAL rat), which shows severe reductions in food intake (FI) and BW. LHAL rats were maintained for 1 mo. post-op (POP). One control (CON) group was fed ad libitum (CONAL), another CON group was pair-fed/gained to LHAL (CONPFPG). Incorporation of 14C-U-glucose into CO_2 (OXID) was significantly (SIG) elevated in liver of LHAL vs CONAL but was normal vs CONPFPG. In PAD, OXID was SIG elevated in LHAL rats vs CONAL, but was also higher than in CONPFPG. No SIG differences were found in DIA. BW and FI were SIG reduced in LHAL vs CONAL and most LHAL rats had to be gavaged for 10 POP days when they began to eat chow pellets. The liver data suggest that the metabolic changes might be due to the severe hypophagia that is seen in LHAL rats, but the PAD data indicate an effect independent of FI that results in enhanced catabolism.

Supported by DVAMC funds (LB) and SUNY/Buffalo funds (JN,CF,AA).

207.2

BLOCKADE OF GABA RECEPTORS IN THE HYPOTHALAMIC DORSOMEDIAL NUCLEUS (DMN) CAUSES PARASYMPATHETICALLY-MEDIATED INCREASES IN JEJUNAL AND COLONIC MOTILITY IN ANESTHETIZED RATS. J. A. DiMicco* and B. Greenwood. Dept. of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis IN 46202, and Eli Lilly and Co., Indianapolis, IN 46285

In our previous studies blockade of $GABA_A$ receptors in the DMN of the rat induces cardiovascular and behavioral changes resembling those associated with emotional stress. Another consequence of emotional stress is altered gastrointestinal (GI) motility. The purpose of the present study was to test the hypothesis that microinjection of the GABA antagonist bicuculline methiodide (BMI) into the DMN of anesthetized rats would produce changes in intestinal and colonic motility. Male rats (250-350 gms) were anesthetized with equithesin. A femoral artery was cannulated for recording of arterial pressure, and heart rate was monitored using a cardiotachometer triggered by the systolic pulsation. Motility was monitored manometrically using saline-filled cannulae placed in the jejunum and colon and connected via pressure transducers to a chart recorder. In some animals, loose ligatures were carefully placed around the cervical vagi for later transection. Microinjection of BMI 15-30 pmol/15 nl into the DMN resulted in increases in both jejunal and colonic motility in addition to the marked tachycardia and moderate increases in arterial pressure previously reported. The enhanced motility was characterized by increases in both amplitude and frequency. The time course paralleled that for the associated tachycardia. In a given animal, the BMI-induced stimulation of GI motility appeared to be reproducible and dose-related. Section of the cervical vagi blocked the increases in jejunal but not colonic motility, whereas intravenous injection of atropine blocked both responses. These observations suggest that: (1) disinhibition of neurons in the DMN of the rat increases jejunal and colonic motility, and (2) the jejunal motility response is mediated through vagal pathways whereas sacral parasympathetic pathways are involved in the stimulation of colonic motility. Taken together with our previous findings, this conclusion points to the possibility of a key role for the DMN in the generation of stress-induced GI hypermotility. (Supported by Eli Lilly and Co., and by USPHS grant NS 19883)

207.3

MICROINJECTIONS OF ANTI-TRH ANTIBODY IN THE DORSAL VAGAL COMPLEX INHIBIT STOMACH CONTRACTIONS PRODUCED BY LATERAL HYPOTHALAMIC LESIONS. C.V. Grijalva*, J. Landeira-Fernandez, M. Prince, G. Ohning, T. Garrick. CURE, BRI, & Depts. of Psychology, Psychiatry, and Medicine, UCLA, Los Angeles, CA 90024.

Lateral hypothalamic (LH) lesions induce stomach erosions which are related to vagally-mediated increases in high amplitude gastric contractions (Garrick et al. *Gastroenterology 99*: 1990, 1213). Microinjections of anti-TRH antibody (TRHa) in the dorsal vagal complex (DVC) block stomach contractility induced by glutamate injections in the raphe pallidus nucleus (RPa) (Prince et al. *Gastroenterology*, 1992). The present study examined whether TRHa into DVC would also block the increase in contractility following bilateral electrolytic LH lesions. In three groups of urethane anesthetized rats, gastric contractions were measured with extraluminal force transducers and analyzed by computer. After a 30 min baseline period, LH lesions were performed in two groups. One hour later one, LH group received bilateral injections of TRHa (1.6 µg/ 0.1 µl) into DVC. The second LH group was injected with an equal volume of BSA vehicle. The third group received a bilateral injection of glutamate (100 pmole/ 0.1 µ l) into RPa and one hour later TRHa was infused into the DVC. Both LH lesions and glutamate injections evoked high amplitudes contractions. Vehicle produced a short term suppression of gastric contractions but the magnitude of the suppression was greater in groups treated with TRHa. Gastric contractions in TRHa-treated LH lesioned rats remained suppressed for over 45 min. These results suggest that LH lesions may induce gastric contractions by activating DVC through the release of TRH. (UCLA PZ-06; CNPq)

207.4

PEPTIDE YY MICROINJECTED INTO THE DORSAL VAGAL COMPLEX INHIBITS CENTRALLY STIMULATED PANCREATIC SECRETION. D.C. Whitcomb, J.T. Curtis, E.M. Stricker and A.F. Syed*. Departments of Medicine and Behavioral Neuroscience, Univ. Pittsburgh, Pittsburgh, PA 15261.

Peptide YY (PYY) is a hormone that is released from the distal intestine in the presence of luminal nutrients. PYY inhibits pancreatic secretion, gastric acid secretion, gastric emptying and intestinal motility. Recent studies suggest that PYY inhibits the vagal component of pancreatic secretion at a site proximal to the pancreas (Putnam, AJP 256:G698-G703, 1989). PYY receptors are present in the dorsal vagal complex (DVC) and circulating PYY can bind to at least some of these receptors (Hernandez, Gastroenterol. 100:A827, 1991). The present study sought to determine whether activation of these receptors by microinjection of PYY into the DVC can inhibit centrally stimulated pancreatic secretion. Male rats were anesthetized with urethane (1.5 mg/kg) and the pancreatic duct was cannulated so that pancreatic secretion could be collected without bile. The rat was placed in a stereotaxic frame and the dorsal surface of the medulla surgically exposed. After the basal pancreatic secretory rate was determined, 2-deoxyglucose (2DG 50 mg/kg iv) was injected to centrally stimulate pancreatic secretion. After 30 minutes, PYY (100 nl of a 0.5 µM solution) or artificial CSF was injected into the DVC bilaterally. 2DG caused a significant (>3x), long lasting increase in pancreatic secretion. Injection of PYY but not CSF into the DVC caused an immediate inhibition of centrally stimulated pancreatic secretion to, or below initial baseline levels. Pancreatic flow typically returned to pre-PYY levels within 20 minutes. These data support the hypothesis that PYY inhibits vagal dependent pancreatic secretion, in part, by acting directly at the DVC.

207.5

DISTRIBUTION OF GASTRIN-RELEASING PEPTIDE IMMUNOREACTIVITY IN THE DORSAL VAGAL COMPLEX IN RATS. R.B. Lynn*, R.R. Cooperman and R.R. Miselis, Dept. of Medicine, Thomas Jefferson Univ., Philadelphia, PA 19107 and Dept. of Animal Biology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Gastrin-releasing peptide(14-27)(GRP), also referred to as bombesin, injected into the dorsal vagal complex(DVC) or intracerebroventricularly inhibits the increase in gastric function in response to numerous stimuli. To determine the neuroanatomic circuitry mediating this inhibitory response we used immunohistochemistry to better define the location of GRP-immunoreactive(IR) fibers and cell bodies within the DVC. GRP fiber and terminal labeling was heavy in the dorsal motor nucleus of the vagus(DMV) and medial subnucleus of the nucleus of the solitary tract (NTS), in contrast to very light labeling in the centralis and gelatinosus subnuclei. Moderate labeling was also found in the dorsal, interstitial, lateral, commissural - caudal to the area postrema(AP) and the rostral intermediate subnuclei. GRP immunoreactivity did not appear in the commissural subnucleus ventral to the AP and the dorsomedial, periventricular portion of the NTS. In colchicinized animals, numerous cell bodies were labeled and were located in the medial and dorsal subnuclei of the NTS. GRP-IR fibers and cell bodies were seen in a number of other areas of the brain with the heaviest labeling in the suprachiasmatic and medial parabrachial nuclei. In conclusion, GRP immunoreactivity in the DVC conforms to the particular subnuclear boundaries we have described in reporting the viscerotopic organization of the NTS. Labeling is abundant in areas of the NTS that receive gastric and cardiac afferent input, but spares those areas that receive afferents from the cecum and esophagus.

207.6

FOREBRAIN MODULATION OF GASTROINTESTINAL FUNCTION IS MOST DIRECT THROUGH PARASYMPATHETIC INNERVATION. R. R. Miselis*, L. W. Enquist, X. Zhao and M. Yang, Dept. Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104

The use of pseudorabies virus (PRV) as a transneuronal tracer enables labeling brainstem and spinal cord autonomic neurons in a viscerotopic manner with short survival times following injections of specific viscera. With increasing survival times the virus passes transneuronally into central afferent sources to the autonomic motoneurons (Miselis et al., Soc. Neurosci. Abst., 17:1361, 1991 and Card et al J. Neurosci. 10: 1990). Transneuronally labeled neurons occur specifically throughout the visceral neuraxis including forebrain sites such as the paraventricular nucleus of the hypothalamus (PVN), bed nucleus of the stria terminalis, central nucleus of the amygdala and the insular cortex. To determine the major route by which PRV travels retrogradely to the forebrain sites, the stomachs of rats were injected at multiple sites with Bartha (Ba - an attenuated strain of PRV), total volume - 6 µl of 9 x10^8 pfu/ml with or without a high complete subdiaphragmatic vagotomy preceding the injection. Rats survived 4 to 6 days before being sacrificed. The vagotomy blocked the transport of the virus to the CNS via the parasympathetic route. The cut prevented major labeling of the vagal complex and all forebrain sites except the PVN. Labeling continued to occur in the intermediolateral cell column of the spinal cord and brainstem sites known to project to the sympathetic outflow. This indicates that the great majority of forebrain modulation of gastrointestinal function is most directly applied through parasympathetic innervation of the gut (assumes both systems can be infected by Ba). The data also continue to support the contention that the virus is moving most rapidly by transsynaptic means rather than by nonspecific spread. Supported by NIH Grant GM 27739

207.7

CATECHOLAMINERGIC CONNECTIONS TO VISCERAL MOTONEURONS ESTABLISHED BY TRANSNEURONAL TRANSFER OF PSEUDORABIES VIRUS FROM INDIVIDUAL VISCERA. M. Yang, S.M. Altschuler*, X. Zhao, L.W. Enquist, and R.R Miselis. Dept. of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104.

It has not been possible to map the distribution of catecholaminergic (CA) neurons as a function of their relationship to specific visceral organs. With viral transneuronal tracers and double labeling it is possible to determine which CA neurons of the brainstem connect to autonomic motoneurons innervating specific organs and whether they influence visceral function via parasympathetic or sympathetic routes. The stomach, esophagus or cecum of a rat was injected with Bartha pseudorabies virus. Some stomach injected rats had a high complete subdiaphragmatic vagotomy preceding the injection. After survival times of 4 to 6 days the brains were processed for double immunoflorescence for viral antigen and either DBH or PNMT CA enzymes. No apparent topology emerged within any CA neuron grouping in relation to a specific viscus. In all cases the stomach injections resulted in the most double labeled neurons in any CA grouping compared to the esophagus or cecum. Vagotomy preceding a stomach injection blocked double labeling in A_1, A_2, and caudal C_2. There was less double labeling in C_1, rostral C_2, C_3, and A_6. There was no change in A_5.

207.8

FOS EXPRESSION IN THE FERRET BRAINSTEM AFTER EMETIC STIMULI. F.M. Boissonade *, K.A. Sharkey and J.S. Davison. Dept. Medical Physiology, University of Calgary, Calgary, Alberta T2N 4N1 Canada

The aim of this study was to map central pathways involved in the emetic reflex by examining the expression of Fos within the ferret brainstem after emetic stimuli. All experiments were performed on halothane anaesthetized ferrets. Local anaesthetic was infiltrated S.C. prior to all skin incisions. The trachea and left carotid artery were cannulated, blood pressure (BP) monitored and rectal temperature maintained at 38°C. Animals were paralysed and ventilated. In 7 animals electrodes were positioned on the supradiaphragmatic vagal crossing branch, 4 were stimulated, 3 were unstimulated controls. In 6 animals chronic duodenal cannulae were inserted 14 days prior to experimentation. On the day of the experiment animals were prepared as described above and given intraduodenal injections of either 1.5M saline (n=3) or normal saline (n=3). Animals were deeply anaesthetized 1h after the onset of the stimulus and perfused fixed with 4% paraformaldehyde. Forty µm frozen sections were incubated with a polyclonal Fos antibody for 48h and tissue was reacted using the ABC technique. For both types of stimulation, induction of a fictive emetic episode was verified by a dramatic increase in BP following application of the stimulus. After electrical stimulation, dense Fos labelling was observed in the medial subnucleus (mn) of the nucleus of the solitary tract (NTS) and moderate labelling in the dorsal/dorsolateral (d/dln) and interstitial (ni) subnuclei of NTS. Labelling was also moderate in the dorsal motor nucleus of the vagus (DMNX), area postrema (AP) and the subnucleus gelatinosus (sg) of NTS. Hypertonic saline induced dense Fos expression in a more discrete region, consisting of the medial portion of the sg and the AP. Labelling was moderate in the mn and sparse in DMNX. In controls, virtually no staining was observed in the dorsal vagal complex.
Supported by the Canadian MRC and AHFMR.

207.9

THYROTROPIN RELEASING HORMONE ANALOGUE AND SEROTONIN INTERACT WITHIN THE DORSAL VAGAL COMPLEX TO POTENTIATE GASTRIC ACID SECRETION. **D. M. McTigue, R. C. Rogers* and R. L. Stephens Jr.. Ohio State University, Dept. of Physiology, Columbus OH 43210.**

The brainstem raphe nuclei send projections to the dorsal vagal complex [(DVC); i.e. the nucleus tractus solitarius and the dorsal motor nucleus]. These vagal nuclei play integral roles in relexes controlling visceral function. The raphe projections to the DVC contain the putative transmitters thyrotropin releasing hormone (TRH) and serotonin (5HT). Co-localization of these agents suggests a possible interaction between these compounds involving the DVC neurons. In this study, the effects of 5HT and a TRH analogue, RX77368, on vagal control of gastric acid secretion were studied. Microinjection of RX77368 (0.66 pmol in 10nl) into DVC evoked a significant increase in acid output. 5HT (8pmol in 10nl) microinjected into the DVC produced no change in gastric acid secreted. When the same doses of RX77368 and 5HT were co-injected, the amount of acid secreted was significantly greater than that due to RX77368 alone. Vehicle control injections into the DVC produced no change in the acid output. Injection of atropine methyl nitrate i.p. eliminated the elevation in acid secretion due to co-injection of RX77368 and 5HT suggesting vagal cholinergic influence over the stomach is involved. The results of the study suggest 5HT amplifies the effects of TRH on DVC neurons. Supported by NS24530 (RCR) and DK42880 (RLS).

207.10

SPINAL INPUT TO INTESTINE-SENSITIVE NEURONS IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS (DMNV). X. Zhang*, W.E. Renehan and R. Fogel. Division of Gastroenterology, Henry Ford Hospital, Detroit, MI 48202.

We have recently shown that 92% of the DMNV neurons which could be driven by a subdiaphragmatic vagal stimulating electrode were inhibited by moderate intestinal distention. Our data suggested that vagal primary afferents excited neurons in the nucleus of the solitary tract which then inhibited the neurons in the DMNV. It is possible, however, that spinal afferents also contribute distention-related information to the vagal complex. To explore this important issue, we recorded the responses of DMNV neurons to intestinal distention in animals which had undergone complete transection of the subdiaphragmatic vagus nerve. Influx and efflux catheters were placed in a 60-70 cm loop of small intestine and normal saline was perfused through the lumen at low pressure. The intestine was distended by raising the efflux catheter to 20 cm above the level of the animal. This manipulation increased intraluminal pressure by 9.0 (SE = 0.63) mm Hg and increased the circumference of the intestine from 16.9 (SE = 0.04) to 21.6 (SE = 0.02) mm. Glass micropipettes filled with 6% HRP or 2% Neurobiotin (Vector Labs) in Tris-KCl buffer were used to intracellularly label physiologically-characterized distention-sensitive neurons in the DMNV. In contrast to the results obtained in normal animals, only one of the 20 labeled DMNV neurons in the vagotomized animals was inhibited by distention. Ten of the 20 neurons were excited by the stimulus and 9 showed no response. The results suggest that an ascending pathway(s) from the spinal cord provides distention-related information to the DMNV. This pathway would seem to oppose the circuit which includes vagal primary afferents, but the vagal afferent circuit is dominant in the intact animal.

207.11

Distribution of 5-HT, substance P and CGRP-containing vagal afferent fibres in the rat dorsal medulla.

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Evidence supports the presence of a wide variety of putative neurotransmitters in sensory neurones of the vagus nerve, based on immunocytochemical and *in situ* hybridization studies on the perikarya of the nodose ganglia. Although these studies suggest a chemical heterogeneity they are unable to provide information regarding the central organization of a particular group of chemically identified afferents. This study examines the presence of 5-HT, substance P or CGRP in the central fibres of the vagus and compares their distribution.

Vagal afferents anterogradely labelled following the injection of cholera-toxin into the nodose ganglia and its subsequent immunofluorescent detection together with immunofluorescent detection of 5-HT, substance P or CGRP using different fluorochromes. Areas of the nucleus tractus solitarius (NTS), dorsal vagal motonucleus (DVN) and area postrema (AP) were scanned using a confocal microscope and the resulting images examined for fibres both anterogradely labelled and containing immunoreactivity for one of the putative neurotransmitters studied.

Afferents containing 5-HT were detected mainly in the AP and the adjacent NTS as well as in the ventral NTS. Afferent fibres containing CGRP were found primarily in the more caudal aspects of the NTS and DVN. Substance P-containing afferents were surprisingly few in number and did not appear to be associated to any particular region.

These results, together with previous demonstrations of a viscerotopic organization of vagal afferents suggest that afferents innervating different organs contain different putative neurotransmitters.

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207.12

SOLITARY TRACT NUCLEUS AS A RELAY IN THE VAGAL CORTICAL ASCENDING SYSTEM IN RATS. S. Ito*. Dept. Physiol., Kumamoto Univ. Med. Sch., Kumamoto,860 Japan.

Both the A- and C- vagal afferents project to the granular insular cortex (GI), presumably through polysynaptic processes at the solitary tract nucleus (NTS), to evoke surface positive-negative field potentials (PNs)(Neurosci.Abstr. 243,10,1991). To clarify the input-output organization of the NTS, the present study 1)mapped vagally evoked field potentials (FPs) at the NTS and 2)stimulated various sites in the NTS to evoke PNs in the GI in SD rats anesthetized with chloralose or barbiturate, immobilized with tubocrarine, and artifitially ventilated. A-afferent-evoked FPs were restricted laterally in the NTS while C-afferent-evoked FPs were distributed broadly throughout it. Stimulation less than 1 μA at the lateral part of the NTS evoked a PN at the GI. The threshold increased as the stimulation site moved medially;it was more than 100 μA at the midline. These results suggest that the caudal NTS is differentiated along the mediolateral axis;the lateral part serves as the relay for both the afferents in the cortical ascending system, but the visceral information processed in the medial part does not contribute to the thalamocortical projection.

207.13

GLUTAMATE RELEASE IN THE CAT NUCLEUS TRACTUS SOLITARIUS (NTS): AN IN VIVO MICRODIALYSIS STUDY.

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Biochemical and pharmacological evidence (eg. Perrone, 1981, Brain Res. 230: 283-293) suggests glutamate may be the neurotransmitter utilized by synaptic terminations of vagal afferent fibres in the NTS. By EM immunohistochemistry we have also shown high levels of glutamate in terminals in the NTS (Batten & Saha, 1991, Soc. Neurosci. 17: 243.11). We have now used *in vivo* microdialsis to examine glutamate release into the extracellular fluid of the NTS of cats anaesthetized initially with halothane, then pentobarbitone (5 mg/kg/hr i.v.). A microdialysis probe (CMA/12, 1 mm membrane) was inserted into the medial NTS at area postrema level and was perfused with Krebs Ringer at 25 μl/min during a 2 h stabilization period, then at 2.5 μl/min during the collection periods (25 min per sample). *In vitro* recovery of glutamate for individual probes varied from 3.5% to 7.5% at this perfusion rate. Basal glutamate release was compared with release during electrical stimulation of the vagus (20 Hz). Each brain was fixed for histological verification of the probe position. Samples were analyzed by HPLC with electrochemical detection, using OPA derivatization (Kehr & Ungerstedt, 1988, J. Neurochem. 51: 1308-1310). Experiments so far (n=10) have failed to show any consistent or significant increase in glutamate release with vagal stimulation. This casts doubt on the idea that glutamate is a neurotransmitter in vagal afferents, and raises the possibility that glutamate measured/localized in the NTS may have a metabolic role.

207.14

VAGAL INNERVATION OF RAT LIVER, BILE DUCTS, PORTAL VEIN, AND ASSOCIATED PARAGANGLIA: AN ANTEROGRADE TRACING STUDY.

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In order to investigate the distribution and structure of the vagal liver innervation, abdominal vagal afferents and efferents were selectively labeled by injecting WGA-HRP (Neuhuber, JANS 20:243-255,1987) or DiI (Berthoud et al. , JCN 319:261-276,1992) into the nodose ganglia, and DiA into the dorsal motor nucleus, respectively.

Vagal *afferent* fibers produced numerous terminal-like structures on both intra and extrahepatic portions of the larger bile ducts, on limited regions of the portal vein, and in most of the paraganglia of the hepatic vagal branch and hilus. Varicose vagal *efferent* fibers were present within the fascicles of the vagal hepatic branch and fine terminal-like structures in a small fraction of the paraganglia. No *efferents* were found to terminate on the few neurons embedded in nerves or paraganglia, and neither vagal afferents nor efferents were found in hepatic lobules and parenchyma. In contrast, an abundance of vagal efferent and afferent fibers and terminals with distinctive distribution patterns and structural characteristics were present in esophagus and gastrointestinal tract. While about two thirds of the vagal *afferent* fibers that originate in the left nodose ganglion and are contained in the hepatic branch bypass the liver hilus area on their way to the gastroduodenal artery, a significant number (approx. 10% of the total) of vagal afferents that does innervate the area, originates from the right nodose ganglion, and projects through the dorsal celiac branch and the periarterial plexus of the common hepatic artery.

It is concluded that vagal intralobular hepatic innervation is largely absent in rats, and that the putative hepatic vagal nutrient and osmoreceptors are located either in the portal vein, bile ducts, or the hepatic paraganglia.

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207.15

VAGAL INNERVATION OF THE RAT PANCREAS: AFFERENT AND EFFERENT PATTERNS COMPARED. T.L. Powley*, C.L. Stanwyck, D.L. Kim, and M-C. Holst. Purdue Univ., W. Lafayette, IN 47907.

Although it is well established that the vagus innervates the pancreas, little is known about the intrapancreatic pathways, target tissues, or endings of vagal fibers. In separate animals, the carbocyanine dye DiI was injected into the left or right nodose ganglion or bilaterally into the dorsal motor nucleus of the vagus. Animals were injected intraperitioneally with fluorogold to label the dorsal motor nucleus, nodose ganglia, and pancreatic ganglia and islets. After the rats were sacrificed, each pancreas was blocked into a standard series of whole mount samples from the head and the tail. These samples were surveyed with epifluorescence and confocal microscopy. Afferent fibers were most numerous in the head of the pancreas near the common bile duct and its branches, where they occurred in many (up to 42±8%) of the nerve bundles. Afferents from left and right vagi were equally numerous in the different regions and followed similar courses. Afferents terminated most prominently as fine puncta within the interlobular ganglia of the pancreas. Whole mounts near ducts in the head had the highest percentage (up to 22±6%) of vagally innervated ganglia. In comparison, vagal efferents were found in a similar number of nerve bundles following the same course as the afferents. The percentages of ganglia innervated by efferents in the different regional samples also paralleled those observed for the afferents (see also Br. Res. 553: 336-341, 1991). Overall, the density of vagal afferents and efferents is consistent with the conclusion that the pancreas receives modest numbers of fibers from the vagus. Finally, the innervation pattern suggests that the vagal branches supplying the proximal duodenum are also the source of parasympathetic connections with the pancreas, an organization which may reflect the embryological derivation of the pancreas from duodenum. (DK27627)

207.16

VAGAL AFFERENTS END ON INTERSTITIAL CELLS OF CAJAL AT THE GASTRODUODENAL JUNCTION OF THE RAT. M-C. Holst*, E.A. Baronowsky and T.L. Powley, Purdue University, West Lafayette, IN 47907.

Although Cajal described interstitial cells in all layers of the gastrointestinal tract, only those in muscle have been well characterized, and little is known about the source(s) of axons contacting them. A combination of a double staining paradigm with laser scanning confocal microscopy provides high resolution and specific information on these cells. In this study vagal afferents of the pyloric region were labeled by injections of the fluorescent carbocyanine dye DiI into the nodose ganglia (left, right or both). Additional animals had injections of DiI in the dorsal motor nucleus of the vagus and/or DiA in the nodose ganglion with or without supranodose vagotomy. Intraperitoneal injections of fluorogold labeled neurons throughout the enteric nervous system, the dorsal motor nucleus of the vagus and the nodose ganglion. DiI labeled vagal afferent fibers in transverse or longitudinal sections (140 μm) or whole mounts of the antrum, pylorus or duodenum were located with a fluorescent microscope. Stacked serial confocal images of muscle, submucosa or mucosa reveal fine afferent fibers and clusters of puncta forming a dense covering over the body and processes of interstitial cells of Cajal. In addition to forming such complexes, vagal afferents also supply puncta or endings to a multiplicity of other sites including the bases of epithelial cells, gastric glands, Brunner's glands, duodenal crypts, and ganglion cells in the myenteric and submucosal plexuses. Although interstitial cells are commonly considered part of an intrinsic network with a pacemaker function, the rich and ubiquitious nature of the vagal afferent/interstitial cell complexes suggests these structures may also participate in sensory functions. Supported by NIH DK27627.

207.17

NADPH DIAPHORASE ACTIVITY IN AUTONOMIC NEURONS WHICH INNERVATE THE RAT ANOCOCCYGEUS MUSCLE. B. Galloway, J. Bordegaray, W.G. Dail and S.L. Rogers*. Dept. of Anatomy, Univ. of NM, School of Medicine, Albuquerque, NM 87131.

The anococcygeus muscle of the rat is supplied by parasympathetic nerves which may contain acetylcholine and vasoactive intestinal polypeptide, yet neither of these neurotransmitters is responsible for inhibition of this muscle. Relaxations of the anococcygeus can be blocked by compounds which interfere with nitric oxide (NO) formation. Since it has been suggested that NADPH diaphorase activity may be a marker for neurons which synthesize NO, the present study reports on the localization of this enzyme. Intramural neurons of the anococcygeus were moderately to intensely stained for NADPH diaphorase. Staining was limited to the cell soma with no evidence of a fiber plexus in the anococcygeus or a reaction in proximal processes of the cell body. Neurons in the pelvic plexus showed the same range of staining intensity as those in the anococcygeus, while there was no staining of retrogradely labeled neurons of the sympathetic chains. The results further support the hypothesis that neurons which synthesize NO may be characterized by a high NADPH diaphorase activity.

207.18

AN ULTRASTRUCTURAL STUDY OF SEROTONINERGIC INPUTS TO NEURONS OF THE MYENTERIC PLEXUS OF THE GUINEA-PIG ILEUM. H.M. Young* and J.B. Furness. Dept. of Physiology, University of Melbourne, Parkville, Victoria, 3052, Australia.

The serotonin-immunoreactive (IR) neurons in the myenteric plexus of the guinea-pig ileum are descending interneurons. In this study we determined whether these serotonin-IR interneurons selectively provide input to any sub-population of myenteric nerve cell bodies.

Myenteric ganglia were processed for pre-embedding immunocytochemistry for serotonin. Serial ultrathin sections were taken through one ganglion from each of two animals. In one ganglion, a sample region was chosen that contained the cell bodies of 43 neurons. We counted the number of serotonin-IR terminals that formed close contacts or synapses with every cell body in the sample area. The sample included the cell bodies of 3 Dogiel type II and 2 serotonin-IR neurons. Each of the Dogiel type II cell bodies received only one close contact each from a serotonin-IR terminal. In contrast, one of the serotonin-IR cell bodies received 4 synapses and 10 close contacts, and the other received 5 synapses and 17 close contacts from serotonin-IR terminals. Other nerve cell bodies received varying numbers of synapses and close contacts from serotonin-IR terminals. In another ganglion the Dogiel type II cell bodies also received very few serotonin-IR inputs whereas the serotonin-IR cell body received many synapses and close contacts from serotonin-IR terminals. It is probable that serotonin interneurons form descending chains.

RESPIRATORY REGULATION: TRANSMITTERS/RECEPTORS

208.1

NON-M1 CHOLINERGIC RETICULAR MECHANISMS PRODUCE A STATE-DEPENDENT DECREASE IN PARABRACHIAL NEURON DISCHARGE. K.A. Gilbert* and R. Lydic. Dept. of Anesthesia, Penn. State University, College of Medicine, Hershey, PA, 17033.

Some parabrachial nuclear complex (PBNC) neurons reveal altered discharge rates during sleep, but mechanisms mediating state-dependent changes in PBNC cell firing rate are not understood. Microinjection of the mixed cholinergic agonist carbachol (0.4 μg) into the medial pontine reticular formation (mPRF) of awake cats produces a state (DCarb) that is similar to rapid eye movement sleep. Recent reports suggest that the cholinoceptive mPRF contributes to state-dependent changes in PBNC cell discharge (Gilbert & Lydic, *Neurosci.Letts., 120*: 241-244, 1990). Encouraged by these findings, this study sought to examine which populations of mPRF cholinergic receptors could contribute to state-dependent changes in PBNC cell discharge. Long-term recordings from a single PBNC neuron revealed a decreased firing rate during DCarb (32.7 Hz) compared to waking (63.4 Hz). A similar decrease in discharge rate was observed following mPRF microinjection of the acetylcholinesterase inhibitor neostigmine (6.3 μg). Similar nicotine injections (0.35 and 3.5 μg) did not alter PBNC cell discharge. Pretreatment of the mPRF with the mixed cholinergic antagonist atropine (5.0 μg) blocked the carbachol-evoked decrease in PBNC cell discharge. An equimolar dose of the M_1-specific antagonist pirenzepine failed to block the DCarb-induced decrease in PBNC cell discharge. These results suggest that activation of an mPRF non-M_1 receptor subtype is required to produce both the DCarb state and the concommitant decrease in PBNC cell discharge.

Supported by grants HL-47749 and HL-40881 to RL.

208.2

GABA INHIBITS CAUDAL HYPOTHALAMIC NEURONS THAT ARE STIMULATED BY HYPOXIA IN SPONTANEOUSLY HYPERTENSIVE AND WISTAR-KYOTO RAT BRAIN SLICES. C.A. Shonis' and T.G. Waldrop. Department of Physiology & Biophysics, College of Medicine, University of Illinois, Urbana, IL 61801.

A GABAergic input onto caudal hypothalamic neurons modulates basal respiratory drive and several cardiorespiratory reflexes. Recent studies from this laboratory have shown that neurons in this same hypothalamic region are stimulated by hypoxia *in vivo* and *in vitro*. The purpose of the present study was to determine if caudal hypothalamic neurons that are stimulated by hypoxia are also inhibited by GABA. Extracellular single unit recordings of hypothalamic activity were performed in a rat brain slice preparation; brain slices (400 μm thick) were placed in an interface chamber that was perfused with nutrient media equilibrated with 95% O_2/5% CO_2. Neuronal responses to hypoxia (10% O_2/5% CO_2/85% N_2 or 5% CO_2/95% N_2) and to GABA (10 mM) were recorded from slices taken from both Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Hypoxia increased the discharge frequency by over 200% in >70% of the caudal hypothalamic neurons studied. Most of the neurons that were stimulated were inhibited following removal of the hypoxic stimulus. GABA depressed the discharge frequency of most (>70%) of the hypothalamic neurons that were stimulated by hypoxia. Similar results were obtained for SHR and WKY neurons. These results demonstrate that caudal hypothalamic neurons that respond to hypoxia are also sensitive to GABA. Thus, GABA-sensitive neurons in the caudal hypothalamus may be involved in the cardiorespiratory responses to hypoxia in both spontaneously hypertensive and normotensive rats. (Supported by NIH 38726 and IL Affiliate-AHA).

208.3

EFFECTS OF $GABA_A$ RECEPTOR ANTAGONISTS ON THE RAPHE MAGNUS-INDUCED RESPIRATORY INHIBITION IN CATS. M. Aoki* and Y. Nakazono. Dept. Physiology, Sapporo Medical College, Sapporo 060, Japan

We previously reported that chemical as well as electrical stimulation of the medullary raphe magnus (NRM) induces marked depressant actions on respiratory activities in the medulla and the spinal cord in cats. In the present study, we examined the possible involvement of putative transmitters in the stimulation induced inhibition by observing the actions of receptor antagonists on the responses to stimulation of the NRM. Cats were anesthetized with pentobarbital sodium (initial dose of 30 mg/kg, i.v.) and artificially respirated. Spontaneous respiratory neuron activities and phrenic nerve discharges were maintained. Putative transmitters mediating these effects were assessed by systemic injection and iontophoretic application of various antagonists of serotonin, GABA, opiates etc. The NRM-induced respiratory inhibition was specifically blocked following injection of the $GABA_A$ receptor antagonists (picrotoxin 0.8-1.25, bicuculline 0.13-0.25 mg/kg). Iontophoretically applied picrotoxin (5 mM, 50-200 nA) and bicuculline (5 mM, 100-300 nA) significantly reduced the NRM-induced inhibition. Iontophoretic application of the $GABA_B$ receptor antagonist 2-OH saclofen (10 mM) showed little blocking action on the NRM-induced inhibition. This study provided evidence for the involvement of a $GABA_A$ receptor-mediated system responsible for the NRM-induced respiratory inhibition.

208.4

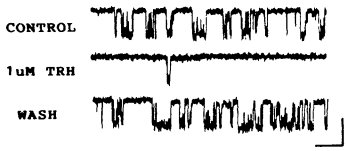
GABA-B ACTIVATED K^+ CHANNELS IN CULTURED BULBOSPINAL NEURONS FROM NEONATAL RATS. P.G. Wagner,* Y. Sun, and M.S. Dekin. Sch. Biological Sciences, Univ. of Kentucky, Lexington, KY 40506.

Activation of GABA-B receptors has been shown to directly inhibit the activity of medullary respiratory neurons *in vivo* (Lalley, *Brain Res.*, 376:392, 1986). To determine the mechanism of action underlying this effect we have studied the properties of GABA-B activated channels in cultured bulbospinal neurons from the dorsal (DRG) and ventral (VRG) respiratory groups of the neonatal rat. Bulbospinal neurons were labeled with fluorescent latex beads injected into the phrenic motor nucleus prior to being placed in culture (Wagner and Dekin, *Neurosci. Abstr.*, 17(1):619, 1991). Using the patch clamp technique in the cell attached configuration, bath application of the GABA-B agonist baclofen (100uM) caused the expression of outward currents in both DRG and VRG neurons. The appearance of these currents was slow in onset (> 3 minutes) and they disappeared when the patch was excised in the inside-out configuration. I-V relationships for the GABA-B activated channel were obtained in bath solutions containing 140 mM KCl to zero the membrane potential. In all cells, the I-V relationship displayed outward rectification at membrane potentials positive to -50 mV. The single channel conductance was 90.4 pS ± 2.6 (n=12). When the concentration of K^+ in the pipette was altered, the reversal potential for these currents followed the predicted equilibrium potential for K^+. These data suggest that GABA-B receptors on DRG and VRG bulbospinal neurons are coupled to a large conductance K^+ channel via a second messenger system. In addition, the outward rectification of the GABA-B activated channels suggests that they modulate the repetitive firing activity of these neurons during depolarization but have little effect on quiescent or actively inhibited neurons. (Supported by NIH grants HL40369, HL02314, and RR07114).

208.5

THYROTROPIN-RELEASING HORMONE INHIBITS GABA-B ACTIVATED K+ CHANNELS IN CULTURED BULBOSPINAL NEURONS OF THE NEONATAL RAT. M.S. Dekin*, P.G. Wagner, and Y. Sun, Sch. of Biological Sciences, Univ. of Kentucky, Lexington, KY 40506

In an accompanying report, the GABA-B agonist baclofen was shown to activate a K+ conductance in cultured dorsal (DRG) and ventral (VRG) respiratory group neurons (Wagner *et al.*, this volume). In whole animal studies it has been suggested that the neuropeptide thyrotropin-releasing hormone (TRH) antagonizes the respiratory depression caused by the central administration of GABA (Hedner *et.al., Acta. Physiol. Scand.*, 117:427, 1983). It was of interest, therefore, to determine the effects of TRH on the activity of the GABA-B activated K+ channel. All recordings were done using the patch clamp technique in the cell attached configuration. An example of GABA-B activated K+ channels recorded in a DRG neuron during bath application of 100 uM baclofen is shown below (top trace). Addition of 1 uM TRH to the bath reduced the channel activity (middle trace). This effect was fully reversible upon washout of TRH (lower trace). Channel open time probabilities were 19% in baclofen, 3% in baclofen and TRH, and 17.8% in the baclofen wash. TRH did not alter either the single channel conductance or the mean channel lifetime. These data demonstrate that TRH inhibits GABA-B activated K^+ channels by modulating either the GABA receptor or its associated second messenger system. (Supported by NIH Grants HL40369, HL02314, and RR07114).



Scale: 3 pA, 24 ms

208.6

NMDA RECEPTOR BLOCKADE AT THE VENTROLATERAL SUBNUCLEUS OF THE NUCLEUS TRACTUS SOLITARIUS (VLNTS) PRODUCES INSPIRATORY APNEA. I.Berger, W.H.Panico, J.McManigle, W. Norman, S.Vitagliano, P.Hamosh, K.L.Dretchen*, R.A.-Gillis and A.M.Taveira da Silva. Georgetown University, Washington, D.C. 20007.

Our purpose was to determine whether blockade of excitatory amino acid (EAA) transmission at the VLNTS affects respiratory activity. Studies were conducted in chloralose anesthetized cats while monitoring respiratory minute volume(VE), tidal volume, respiratory rate (F), inspiratory time(Ti), expiratory time and end-tidal CO2(FECO2). Bilateral microinjections (coordinates:-1.2R;2.6L;1.7D) of the N-methyl-D aspartate (NMDA) receptor antagonist CPP (2.25nM;N=8) produced significant increases in Ti (+4.1±1.6 sec,P<0.05) and FECO2(+1.3± 0.2%,P<0.05) and decreases in VE(-75±14ml/ min,P<0.05) and F(-3.5±0.7 breaths/min,P<0.05). Apneustic breathing and inspiratory apnea ranging from 15 to 80 seconds were seen in all cats. Microinjection of the NMDA receptor antagonist AP-7 (225nM; N=3) into the VLNTS also produced apneustic breathing and inspiratory apnea. Microinjections of the non-NMDA receptor antagonist CNQX (0.05nM;N=3) had no effect on respiratory activity. We conclude that an endogenous EAA at the VLNTS acting at NMDA receptors plays an important role in breathing.

208.7

NON-NMDA NEUROTRANSMISSION IN THE MEDIAL NUCLEUS TRACTUS SOLITARIUS (mNTS) IS NOT INVOLVED IN ALL AFFERENT-EVOKED INSPIRATORY TERMINATION REFLEXES. D.R. Karius*, L. Ling, and D.F. Speck. Dept. of Physiology, Univ. of Kentucky, Lexington, KY 40536.

Reflex termination of inspiration can be elicited by stimulation of the vagus (X), superior laryngeal (SLN), and intercostal (ICN) nerves. Neurotransmission at non-N-methyl-D-aspartate (non-NMDA) receptors in the mNTS is required in the production of the Breuer-Hering reflex in the rat, as well as the inspiratory termination produced by SLN stimulation in the cat. This study tested the hypothesis that non-NMDA neurotransmission in the mNTS of cats is required for inspiratory termination elicited by X or ICN stimulation. Adult cats were decerebrated, vagotomized, paralyzed and ventilated. One or both SLN, the right X, and a left ICN (T6-8) were cut and placed on stimulating electrodes. After recording control thresholds for eliciting inspiratory termination from each nerve, the non-NMDA antagonist DNQX (10 mM, 200-700 nl) was ejected into the mNTS. Each reflex was tested 10, 30 and 60 minutes after ejection. The inspiratory termination elicited by vagal stimulation was abolished by ejections ipsilateral to the stimulated nerve. The X-elicited termination persisted after ejections into the contralateral (left) mNTS, although the termination elicited by the left SLN was abolished. Inspiratory termination elicited by ICN stimulation persisted with no change in threshold following ipsilateral, contralateral and bilateral ejections, even though X- and SLN-evoked terminations were severely attenuated or abolished. This study indicates that non-NMDA transmission in the mNTS, although required for X- and SLN-elicited terminations, is not required for all inspiratory terminating inputs. (Supported by NIH PO1 40369).

208.8

SYNERGISTIC ROLES FOR NMDA AND NON-NMDA RECEPTORS IN BRAINSTEM RESPIRATORY CONTROL IN ADULT RATS. C.A. Connelly* and J.L. Feldman, Systems Neurobiology Laboratory, Dept. of Physiological Science, UCLA, Los Angeles, CA 90024-1527.

Effects of NMDA and non-NMDA excitatory amino acid (EAA) receptor antagonists on the generation and transmission of central respiratory drive were examined in anesthetized adult Wistar rats. Respiratory-modulated neuronal impulses from Bötzinger (Böt), pre-Bötzinger (preBöt) and rostral ventral respiratory group (rVRG) neurons were recorded extracellularly with single or multibarrel electrodes also used to microinject antagonists to non-NMDA (CNQX, 1mM in saline) or NMDA (CPP, 1mM in saline) receptors. Diaphragm electromyogram activity was recorded to monitor respiration. Bilateral microinjection of CNQX (18-54 pmol/side) into brainstem sites containing inspiratory-modulated preBöt and/or rVRG neurons increased the rate of spontaneous breathing in 5/8 rats. Concomitant with these increases, inspiratory timing and burst amplitude became variable in 4 rats. Apnea occurred in 6/7 rats when CNQX microinjection in these regions was combined with systemic MK-801 administration (0.5-1.0 mg/kg) to block NMDA channels. When MK-801 was not administered, bilateral microinjection of both types of EAA receptor antagonists (CPP and CNQX, 18 pmol/ea) into rVRG and/or preBöt markedly perturbed respiratory pattern and timing in 6 rats. Within 1-5 minutes, 5 of these rats developed apnea which lasted 1/2 to 2 hours; arterial pressure was unaffected. Although antagonism of non-NMDA receptors in preBöt and rVRG subregions may substantially perturb the respiratory pattern, breathing is sustained unless NMDA-mediated excitatory transmission through these regions is also impaired. Research supported by NIH grant HL 37941. CAC was supported by the Parker B. Francis Foundation.

208.9

IONIC MECHANISMS INVOLVED IN THE GENERATION AND MODULATION OF RESPIRATORY RHYTHM IN VITRO. S.M. Johnson*, J.C. Smith, G.D. Funk, & J.L. Feldman, Systems Neurobiology Lab, Dept of Physiological Science, UCLA, Los Angeles, CA, 90024-1527

Neurons with voltage-dependent, pacemaker properties in the pre-Bötzinger Complex (preBötC) of ventral medulla, have been proposed to be the kernel of the respiratory oscillator (*Science* 254 '91). To test for involvement of cationic conductances in respiratory rhythm generation, ion channel blockers and neuromodulators were applied to rhythmically active medullary slices of neonatal rats Perturbations of the rhythm in the slice were analyzed from whole-cell recordings of respiratory neurons and recordings of oscillatory motor output.

Increasing concentrations of K^+ channel blockers in the bath solution increased the frequency of rhythmic motor discharge, eventually disrupting the rhythm. In contrast, activation of neurotransmitter receptors ($5HT_{1A}$, $GABA_B$, and α_2 adrenergic) known to be coupled to K^+ conductances (via G-proteins) reduced the discharge frequency; prior application of blockers of inward rectifying K^+ conductances prevented these effects. In the preBötC, respiratory pacemaker neurons received excitatory synaptic drive and exhibited 10-20 mV membrane potential oscillations that increased in frequency with depolarization. These oscillations had a rapid upstroke, a slowly declining plateau-like potential upon which action potentials were either expressed or shunted, and a rapid repolarization to resting potential.

These data suggest that in preBötC cells: (i) An inward-rectifying K^+ conductance potentially coupled to the G-protein second messenger system in part sets the baseline membrane potential and bursting frequency (ii) A strong, slowly inactivating cationic conductance provides the major inward current during the depolarization phase. Support: HL02204 & HL40959 (JCS); HL08524 (SMJ).

208.10

NADPH-DIAPHORASE REACTIVE NEURONS WITHIN PONTO-MEDULLARY CARDIORESPIRATORY NUCLEI OF THE RAT. H.H. Ellenberger*, J. Groomer & J.L. Feldman, Systems Neurobiology Lab, Dept. of Physiol. Science, UCLA, Los Angeles, CA, 90024-1527

We performed a neuronal labeling study to determine whether nitric oxide (NO) is utilized as a neuromessenger within cardiorespiratory regions of rat brainstem. We labeled nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-*d*) containing neuron cell bodies and terminal varicosities to identify presumptive NO producing neurons (Hope et al., *PNAS*, 88:1991). NADPH-*d* labeled cell somata and/or terminals were located in many regions of caudal brainstem, including portions of all reticular nuclei, somatic and visceral sensory nuclei, and cranial nerve motor nuclei. With respect to cardiovascular control, labeled cell bodies and terminal varicosities filled the rostrocaudal extent of many parts of the nucleus of the solitary tract, including commissural, dorsal, dorsomedial, medial, ventral and ventrolateral subnuclei. The dorsal motor nucleus of the vagus contained labeled terminals and lightly labeled cell bodies. Nucleus ambiguus (NA) contained labeled terminals, especially in the rostral compact division. The intermediate reticular nucleus of the lateral tegmental field (intLTF) was prominently labeled, with many darkly stained neurons, axons and terminals in the region extending ventrolaterally from the dorsal vagal complex. The ventrolateral intLTF region (ventrolateral to NA) contained fewer, lightly stained somata and terminals. Many darkly stained neurons were located in the lateral paragigantocellular nucleus extending dorsomedially into the ventral gigantocellular nucleus in rostral medulla. Many darkly stained neurons and terminals were located within the rostral raphe obscurus. Labeled terminals formed a dense horizontal sheet ventral to the facial nucleus in the region of retrotrapezoid nucleus. Labeled cell bodies and terminals were scattered within the pontine LTF and concentrated within discrete portions of lateral and medial parabrachial and Kölliker-Fuse nuclei. These results indicate that NO may be released at pre- or postsynaptic sites to act as a neuromessenger within the brainstem to modulate cardiorespiratory function. Supported by NIH Grant HL 37941.

208.11

NITRIC OXIDE INVOLVED IN THE PONTINE RESPIRATORY FUNCTION OF CATS. L. Ling, D.R. Karius and D.F. Speck*. Dept. of Physiology, Univ. of Kentucky, Lexington, KY 40536.

Previous studies from our laboratory demonstrated that apneusis could be produced by bilateral microinjection of MK-801 (an NMDA receptor-associated channel blocker) into the pontine respiratory group (PRG), indicating that NMDA receptors in the PRG were necessary for the pontine pneumotaxic mechanism. The close relationship between NMDA receptors and nitric oxide in the central nervous system suggests that nitric oxide production by these NMDA-sensitive neurons within the PRG may be involved in respiratory control. Ten adult cats were anesthetized, decerebrated, decerebellated, paralyzed and ventilated. Bilateral microinjection of N_ω-nitro-L-arginine (L-NNA, 1 mM, 200 - 600 nl, inhibitor of nitric oxide synthase) into the PRG significantly prolonged the time of inspiration (Ti) in all animals when lung inflation was withheld. Four cats showed complete apneusis after the injection. N_ω-nitro-D-arginine (1 mM, inactive enantiomer) injections at the same sites did not change the no-inflation Ti. The effect of L-NNA on the no-inflation Ti could be partially reversed by bilateral microinjection of L-arginine (10 mM, nitric oxide synthase substrate) in 3 cats. We conclude that endogenous formation of nitric oxide from L-arginine in the PRG region is involved in the normal function of the pontine pneumotaxic mechanism. (supported by NIH P01 40369)

208.12

EFFECTS OF SUBSTANCE P AND CP-96,345 ON RESPIRATORY PATTERN GENERATION IN THE *IN VITRO* FROG BRAIN. S.F. Perry*, N. Kimura and J.E. Remmers. Dept. of Medical Physiology, Univ. of Calgary, Calgary AB, Canada T2N 4N1.

Substance P (SP) increases the amplitude and/or frequency of neural activity related to breathing in mammalian *in vitro* or decerebrated *in situ* preparations, and the binding of SP to its NK1 receptor is effectively blocked by the nonpeptide antagonist CP-96,345. In order to investigate the role of SP in respiratory pattern generation we superfused the isolated brainstem of the leopard frog, *Rana pipiens*, with HEPES-buffered, 10^{-13} M - 10^{-6} M solutions of SP, NK1 antagonist or a combination of the two, while recording from the trigeminal, facial, vagal and hypoglossal cranial nerve rootlets using suction electrodes. These nerves innervate the respiratory muscles in the frog. SP reduced respiratory frequency in a dose-dependent manner from 10^{-9} M upward, while eliciting high-frequency, low-amplitude bursts reminiscent of buccal pumping activity during the breathing pauses. Amplitude also increased in the trigeminal but not in the vagus nerve. NK1 antagonist (from 10^{-9}M upward) applied alone tended to increase breathing frequency, but when applied together with SP, failed to completely block the frequency-reducing effects and low-amplitude activity caused by the latter. We conclude that the effect of SP on the frequency of respiratory activity in the frog brainstem preparation is opposite to that reported for mammalian preparations. SP thus appears to be an important neuromodulator but is not essential for central respiratory pattern generation in tetrapods. This research was supported by MRC grant MA 9719 to JER.

208.13

EFFECTS OF MORPHINE AND STRYCHNINE ON RECIPROCAL RESPIRATORY MOTOR ACTIVITY IN THE ISOLATED FROG BRAINSTEM. N. Kimura*, S.F. Perry and J.E. Remmers. Dept. of Medical Physiology, Univ. of Calgary, Calgary, AB, Canada T2N 4N1.

The role of opioids and of inhibitory amino acids in respiratory control was studied in the frog, *Rana pipiens*, using two isolated brainstem preparations. Both were superfused with oxygenated HEPES buffer. An *in vitro* preparation, consisting of the brainstem alone, showed spontaneous bilateral bursting activity in the trigeminal, vagal and hypoglossal nerve rootlets. An *in situ* preparation, consisting of the brainstem plus the ventral half of the skull and nerve branches to respiratory muscles, showed a coordinated discharge pattern resembling the respiratory motor output reported *in vivo*. The sternohyoid branch of the hypoglossal nerve (Hsh) showed an augmenting discharge pattern which alternated with a short burst from the main branch (Hm). This reciprocal activity became synchronous when the preparation was exposed to 10 μM strychnine. The laryngeal branch of the vagus nerve (Xℓ) showed a biphasic discharge pattern, an early component commencing near the end of the Hsh burst and extending to the peak of the Hm burst, and a late component following the peak of the Hm burst. Bath application of 5 to 20 μM morphine prolonged the interburst interval or abolished the burst discharge of the nerve rootlets. Morphine prolonged the Hsh burst duration and decreased the amplitude of Hsh and of the late component of Xℓ, but had no obvious effect on the Hm burst. These morphine-induced effects were antagonized by 2.5 to 10 μM naloxone. The results suggest that respiratory motor output in the frog involves strychnine-sensitive synaptic inhibition and is modified by the opioid system as in mammals. (MRC grant MA 9719 to JER)

TEMPERATURE REGULATION AND FEVER

209.1

INHIBITION OF PROTEIN SYNTHESIS ATTENUATES THE FEVER INDUCED BY HYPOTHALAMIC MACROPHAGE INFLAMMATORY PROTEIN-1. W.M. Zawada, R.D. Myers, W.D. Ruwe*. Dept. Physiol. & Biophys., Univ. of Arkansas for Med. Sci., Little Rock, AR 72205 and Dept. Pharmacology., East Carolina Univ. Sch. Med., Greenville, NC 27858.

Macrophage inflammatory protein-1 (MIP-1), a novel cytokine composed of α/ß subunits, is released from macrophages during infection. When injected IV in rabbits and into the anterior hypothalamic preoptic area in rats, MIP-1 causes an intense fever which is not blocked by indomethacin. The purpose of this study was to determine sensitivity of ventral septal area (VSA) to MIP-1 and the role of protein synthesis in the MIP-1 fever. Guide cannulae were implanted bilaterally above the VSA and medially above the 3rd ventricle in male Sprague Dawley rats. Bilateral microinjections into the VSA of MIP-1 in a dose of 14pg/0.5μl per site caused a biphasic elevation in core temperatures (T_c) to 0.9 ± 0.2°C within 1 hr and to 1.5 ± 0.2°C in 3 hrs which persisted for over 6 hrs. The infusion of 80μg/10μl of an inhibitor of protein synthesis, anisomycin (ANI), into the 3rd ventricle either 10 or 30 min before the microinjections of MIP-1 attenuated significantly the rise in T_c for 1 to 3 hrs. These results coincide with the findings that ANI inhibits both endotoxin- and IL-1ß - induced fevers. Further, synthesis of new protein factor(s) may be requisite for the induction and maintenance of a non-cyclooxygenase fever. [Supported in part by NIH NS26045 and NSF IBN-91-21656].

209.2

RELEASE OF AVP IN THE VSA OF 1 KIDNEY - 1 CLIP GOLDBLATT HYPERTENSIVE RATS. M.L. Earle*, T. Horn, R. Landgraf & Q.J. Pittman. Medical Physiology & Neuroscience Research Group, University of Calgary, Calgary, Alberta, CANADA. & Section of Biosciences, University of Leipzig, Leipzig, GERMANY.

Release of the peptide arginine vasopressin (AVP) within the ventral septal area (VSA) is inversely correlated to fever height. 1K-1C Goldblatt hypertensive rats display reduced fevers, and in the present study we have tested the hypothesis that this is due, in part to an overactive AVP system. We have therefore measured AVP release in the VSA during the development and maintenance of hypertension. Using male Sprague-Dawley rats (80-100g), following an initial baseline blood pressure recording (using tail-cuff plethysmography), the 1K-1C surgery or sham surgery was performed. In addition, guide cannulae were implanted for push-pull perfusions in the VSA and for icv injections of PGE1. Either 4 or 18 days following this surgery, the blood pressures were taken after which the animals were anaesthetized with urethane, rectal temperature was measured and push-pull perfusates of the VSA were collected before and after icv injection of PGE1 (50 or 100 ng) or vehicle, at 30 minute intervals. The perfusate was then lyophilized and the AVP content measured using a specific radioimmunoassay. We found the level of fever produced in the 1K-1C hypertensive rats to be significantly ($p<0.05$) lower than that produced in the normotensive rats ($n\geq9$) at both day 4 and 18. However, neither baseline levels nor PGE1 stimulated release of AVP in the VSA differed between normotensive and hypertensive animals. Supported by Alberta Heart & Stroke Foundation & NATO Collaborative Research Grant.

209.3

TEMPERATURE RESPONSES OF MICE TO ENDOTOXIN OR POLY-INOSINIC:POLYCYTIDYLIC ACID. **W. Kozak, C. A. Conn, and M.J. Kluger*, Department of Physiology, Univ. of Michigan Medical School, Ann Arbor, MI 48109.**

There are reports that mice, in contrast to other laboratory species, are not capable of developing fevers after injection of endotoxin (LPS) or polycytidylic:polyinosinic nucleotide (Poly I:C). However, it has been shown that murine cells stimulated with LPS "ex vivo" can produce cytokines known as endogenous pyrogens in other mammals.

When body temperature (Tb) and activity (Act) were measured using biotelemetry, unrestrained male Swiss Webster mice kept at 31 ± 1°C ambient temperature responded with fever, accompanied with decreased activity, to ip injection of 2.5 mg/kg LPS. Injection of LPS in mice was followed by a fall in body temperature (ΔTmax -0.87± 0.14°C) lasting for 3 h, and Tb reached a peak after 24 h of LPS injection (ΔTmax +2.04±0.11°C). The mice recovered to normal circadian rhythm of Act and Tb within 48 h of injection. In contrast, injection of Poly I:C (2.5 mg/kg, ip) led to a fall in Tb of -1.89 ±0.16°C, which was of similar magnitude to that seen during influenza infection, with no ensuing fever.

Indomethacin (5 mg/kg), which had no effect on Tb in control mice, blocked LPS fever, and resulted in a prolonged, magnified post-injection fall in Tb. Antiserum against mouse tumor necrosis factor (TNF) significantly enhanced LPS-induced fever and inhibited the initial fall in Tb. These data are consistent with the hypothesis that rodents can produce cryogens that may limit the magnitude of Tb increase during fever, and TNF may play a role in this negative feedback. **Supported by AI27556 & ONRN00014-90-J-1547.**

209.4

IMPAIRED BARORECEPTOR RESPONSES AND FEVER. Y.Takahashi, Q.J.Pittman, W.L.Veale*, Neuroscience Research Group, University of Calgary, Calgary, Alberta, CANADA.

Recent studies indicate a possible interaction between fever and cardiovascular responses. Electrolytic lesions of the rat NTS (the site of baroreceptor afferents) attenuate PGE_1 fever, and carotid sinus-denervated lambs have a reduced endotoxin fever. In the present study baroreceptor afferents in the Aortic Depressor Nerve (ADN) and Carotid Sinus Nerve (CSN) were severed to determine whether these may play a role in fever regulation in the adult rat. Bilateral ADN, CSN transections and sham surgeries were performed on male Sprague-Dawley rats. Intracerebroventricular and intravenous cannulae and temperature telemetry devices were also implanted. Following a 2 week recovery, groups of rats (n=7) were given icv injections of PGE_1 (75 & 150 ng),icv IL-1ß (24 units) or iv injections of E.coli endotoxin (50 ug/kg) and fever responses were monitored. At the end of the experiment baroreceptor denervation was confirmed by testing rats under urethane anesthesia for their cardiovascular responses to iv phenylephrine and nitroprusside. All denervated rats displayed <20 bpm change in heart rate in responses to >75mmHg alterations in blood pressure.

Statistical analysis (2-way Anova for repeated measures) showed no significant difference between control and denervated rats in febrile responses to 75 ng PGE_1, IL-1ß or iv Endotoxin. Fever responses to 150 ng PGE_1 were lower in denervated rats than in controls. Therefore it appears that functional baroreceptor denervation does not necessarily interfere with the development of fever in rats.

209.5

BIPHASIC THERMAL RESPONSE TO CLONIDINE (CLO) MICROINJECTED INTO THE PREOPTIC AREA (iPOA): DIFFERENT GENESIS OF THE TWO PHASES. A. A. Romanovsky, O. Shido, A. L. Ungar, and C. M. Blatteis*. Dept. of Physiology and Biophysics, Univ. of Tennessee, Memphis, TN 38163.

The iPOA microinjection (μinj) of agents often causes local injury, inducing prostaglandin (PG)-mediated core temperature (T_c) rises. However, the iPOA μinj of the α_2-agonist CLO generally evokes a T_c fall. To study the contribution to this response of an unavoidable traumatic injury, we implanted cannulas iPOA into guinea pigs and, 7 days later, measured their T_c during 7 h without treatment (time-control) or 2 h before and 5 h after the bilateral μinj (in 1 μl of pyrogen-free saline [PFS]) of: (**a**) CLO (0, 0.5, 1.5 or 5 μg); (**b**) CLO (0.5 μg) 20 min prior to the PG synthase blocker indomethacin (INDO, 0 or 10 mg/kg in 0.1 ml of PFS, im); or (**c**) the α_2-antagonist rauwolszine (RAU, 0 or 1 μg) 10 min prior to CLO (0.5 μg). The $T_{ambient}$ was 23°C. Compared with the time-control (no significant change in T_c), the iPOA μinj of CLO caused a biphasic hypo-/hyperthermic response. Both the magnitude and the duration of the initial T_c fall were dose-dependent: -0.4 ± 0.1°C and 60 min for 0.5 μg; -0.9 ± 0.1°C and 70 min for 1.5 μg; and -1.2 ± 0.1°C and 90 min for 5 μg. During the second phase of the response, T_c increased to a dose-independent level (1.0-1.5°C) where it remained until the end of the experiment. PFS produced a gradual T_c rise from the outset. The hypothermic phase was completely blocked by RAU, while the hyperthermic phase was significantly attenuated by INDO, but insensitive to RAU. These results confirm our earlier conclusion that the thermal response to the iPOA μinj of an agent, whether hyper- or hypothermizing, is contaminated by an artifactitious, PG-mediated, T_c rise consequent to nonspecific brain tissue injury. (Supported by NIH NS 22716.)

209.6

HYPOTHALAMIC NEURONAL NETWORKS FOR THERMOREGULATORY VASOMOTOR CONTROL. K.Kanosue*, M.Yanase-Fujiwara and T.Hosono, Dept. of Physiol., Dept. of Obst. & Gynecol., Osaka Univ. Med. Sch., Suita, Osaka 565, Japan.

Warming of one side of a rat's preoptic area and anterior hypothalamus (POAH) causes skin vasodilation on both sides of the body. The present study evaluated the extent to which signals from POAH thermosensitive sites cross the midline within and below the POAH. Hind-paw vasomotor responses to unilateral POAH warming were measured for urethane anesthetized (1.2g/kg, i.p.) rats in which the POAH had been midsagittally transected and for rats in which one side of the hypothalamus had been coronally transected just below the POAH. Unilateral POAH warming produced bilateral paw vasodilation in midsaggittaly transected rats but the ipsilateral dilation occurred at lower threshold. Unilateral POAH warming usually produced bilateral vasodilation in unilaterally transected rats and the threshold stimulus temperature eliciting vasodilation was always lower for the paw of the intact side regardless of which side of the POAH was warmed. These results show that information controlling thermoregulatory vasomotion crosses the midline both inside and outside the POAH. Furthermore, local unilateral transection of the medial forbrain bundle (MFB) had the same effect as the unilateral transection of the whole hypothalamus, while the transection medial to the MFB had no effect. Vasomotor signal from the POAH would descend through the MFB.

209.7

SYNAPTIC INTERACTIONS OF HYPOTHALAMIC TEMPERATURE SENSITIVE AND INSENSITIVE NEURONS John D. Griffin* and Jack A. Boulant. Department of Physiology, Ohio State University, Columbus, OH 43210.

The rostral hypothalamus is important in thermoregulation and contains both temperature sensitive and temperature insensitive neurons. Studies indicate that hypothalamic warm sensitive neurons are intrinsically thermosensitive; but, little is known about synaptic interactions within local neuronal networks. To study these interactions, whole-cell recordings were made of neuronal intracellular activity in rat hypothalamic tissue slices. The frequencies of action potentials and postsynaptic potentials (i.e., EPSPs and IPSPs) were recorded during changes in tissue temperature. While warm sensitive and temperature insensitive neurons display very different dendritic morphologies, both types of neurons receive excitatory and inhibitory synaptic inputs. Moreover, the predominent synaptic inputs come from temperature insensitive neurons, suggesting that warm sensitive neurons primarily affect cells outside of this local neuronal network. (Support by NIH NS14644; NS07291; Am. Heart, Ohio.)

209.8

EXPRESSION OF C-FOS IN THE RAT HYPOTHALAMUS AFTER THERMAL AND FLUID CHALLENGES. P.A. MASON[1], L.K. WEIGEL[2], G.A. MICKLEY[3], B.L. COBB[3] AND N. MIRRO[4]. [1]Operational Technologies, [2]Department. of Biology, Trinity University, [3]Radiofrequency Radiation Branch (OEDR), Armstrong Laboratory, Brooks AFB, TX 78235-5301, [4]Systems Research Laboratories.

Expression of the immediate-early gene C-fos occurs in response to a variety of stressors. Thermal stress was delivered by exposing rats to a 600 MHz electromagnetic field (SAR = 10 W/kg) for 20 min. Since thermal challenges may produce changes in blood flow and blood chemistry, we attempted to mimic such alterations using intra- and extracellular dehydration. Rats were water restricted for 48 hr or intraperitoneally injected with 35% polyethylene glycol (PEG)/0.7% NaCl, 35% PEG/4% NaCl or 4% NaCl. C-fos was expressed throughout the hypothalamus in response to the electromagnetic field. Water restriction produced C-fos expression in the periventricular nucleus. PEG produced C-fos expression in the paraventricular nucleus whereas C-fos was expressed in the supraoptic nucleus in response to 4% NaCl. The combination of PEG and 4% NaCl produced C-fos expression in both the paraventricular nucleus and the supraoptic nucleus. Results indicate that there is selective regional expression of the C-fos protein within the hypothalamus in response to these stressors.

209.9

ELECTRICAL STIMULATION OF THE POSTERIOR HYPOTHALAMUS OF ANESTHETIZED RATS KEPT AT 37°C EVOKES SUSTAINED SHIVERING TO INCREASE CORE TEMPERATURE. I. Halvorson and J.A. Thornhill*, Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask., Canada, S7N 0W0.

Experiments were conducted to determine if sustained shivering could be induced by electrical stimulation of the CNS (i.e. posterior hypothalamus, PH) in anesthetized rats kept at 37°C as normally occurs with cold cutaneous stimulation. Male Long Evans rats, anesthetized with urethane (1.5 g/kg ip) were instrumented with an indwelling femoral arterial catheter (blood pressure, heart rate monitored), bipolar needle recording electrodes into the gastronemius muscle (differential EMG changes), thermistor probes for monitoring colonic (core), surface (tail), subcutaneous (abdominal) and gastronemius muscle temperatures, and an insulated stimulating monopolar pin electrode advanced towards the PH (for electrical stimulating of this locus using 0.5 msec pulses of 50 Hz for 30 sec, using currents of 100-300 μA). For the first time it was demonstrated that electrical stimulation of the PH can evoke sustained, current-related increases in EMG electrical activity (shivering) in the gastronemius muscle, immediate graded increases in gastronemius muscle temperature which caused subsequent increases in core temperatures, responses all similar to cold cutaneous stimulation also applied to these animals.

Supported by Medical Research Council of Canada.

209.10

IN VITRO THERMOSENSITIVITY AND C-*Fos* IMMUNOREACTIVITY OF RAT NUCLEUS REUNIENS NEURONS. K.A. Travis*, A.M. Zardetto-Smith, M.Z. Cicha, H.J. Bockholt and A.K. Johnson. Depts. of Psychology and Pharmacology. The University of Iowa, Iowa City, IA 52242.

Functional studies suggest that the nucleus reuniens of the thalamus (Re) may receive temperature information and serve as a site of integration of peripheral sensory information. In vivo c-*Fos* immunoreactivity (c-*Fos* IR) is increased in Re following noxious peripheral heat stimuli (Bullitt, J. Compar Neurol. 296:517-530, 1990).

In vitro neuronal activity was recorded from Re neurons in horizontal tissue slices (350-400 um) of male Sprague-Dawley rats exposed to a change in perfusate temperature (32-42°C). A separate group of tissue slices (n=24) were evaluated for induction of c-*Fos* IR in Re in response to a heat stimulus (40-41°C; 10-20 min).

Of the Re neurons tested for thermosensitivity (n=52): 21.2% were warm sensitive, 1.9% were cold sensitive and 76.9% were temperature insensitive. Compared to control tissue slices (incubated 120 min, 37°C), moderate to heavy c-*Fos* IR was visualized in the Re, posterior hypothalamus and median preoptic nucleus of the hypothalamus in tissue slices exposed to the heat stimulus.

The single unit responses and the induction of c-*Fos* IR in the Re in the slice preparation following temperature changes indicate that Re is part of a circuit integrating thermal information. (Supported by F32HL08442)

209.11

EFFECTS OF SUSTAINED COLD EXPOSURE ON PLASMA LEVELS OF TSH AND CORTICOSTERONE IN AGED 344/N FISCHER RATS. G. Cizza, K. Fukuhara, R. Kvetnansky, A. Moazzez, M.A. Kling*, G.P. Chrousos, and P.W. Gold, National Institutes of Health, Bethesda, MD, 20892.

The hypothalamic-pituitary-adrenal (HPA) axis is traditionally considered to be the neuroendocrine system most responsive to stressful stimuli, while the hypothalamic-pituitary-thyroid (HPT) axis is thought to be much less responsive to external stimuli. However, cold exposure is well-known to activate the HPT axis, though its effects on the HPA axis are less well-delineated. In the present study, we explored the effects of cold exposure on both HPT and HPA function. Because we have shown that aged rats demonstrate both a central hypothyroidism and hypoadrenalism, we also explored the effects of aging and cold-stress mediated changes in HPA and HPT function. To accomplish these tasks, 3-4 month (mo) and 24 mo old 344/N Fischer rats (344/N) (6-7 animals each group) were exposed at 10:00 hr for 24 hr to 4° C or room temperature. Blood was collected by tail artery cannulae at times -15', 30', 2, 4, 8, 12, and 24 hr, and immediately replaced with saline. Plasma was assayed by RIA for thyroid-stimulating hormone (TSH), and corticosterone (B). Basal TSH and B plasma levels were not different between age groups. Cold exposure significantly increased TSH plasma levels ($p < 0.0001$ by ANOVA followed by Scheffe test) in both age groups but more in younger animals. B levels were only subtly increased by cold stress in both young and old animals (ANOVA $p < 0.1$; t test at 30' time-point corrected by Bonferroni: young $p < 0.007$, old $p < 0.07$), with older animals showing the greater but more variable response. We conclude that cold-stress activates the HPT axis more robustly than the HPA axis in the rat. The lack of a profound cold-stress effect on the HPA axis could reflect the known capacity of cold to inhibit the ACTH secretagogue, vasopressin, a peptide that ordinarily lowers body temperature. The attenuated plasma TSH responses to cold-stress in older rats are compatible with their known centrally-mediated hypothyroidism; similarly, their exaggerated B responses to cold-stress are compatible with our previous data showing that, in the context of central adrenal insufficiency, the resultant hypocorticosteronism promotoes disinhibition of pituitary-adrenal responses to centrally-acting stressors despite a relative CRH deficiency.

209.12

EFFECTS OF SERCTONIN ON SIGNAL TRANSMISSION IN HIBERNATING AND COLD-ACCLIMATED HAMSTER HIPPOCAMPAL SLICES. D. J. Horrigan and J. M. Horowitz*. Dept. of Animal Physiology, Univ. of Calif. Davis, CA 95616.

Previously, the temperature dependence of serotonergic modulation of CA1 pyramidal cells in response to Schaffer collateral stimulation has been described in euthermic animals. Here the response from cold-acclimated and hibernating hamsters is examined. Population spike amplitude was measured before, during and after bath administration of 10 μM 5-HT at a bath temperature of 20°C (*J. Comp. Physiol. A.* 167:79-88, 1990). In slices from cold-acclimated hamsters, 5-HT reduced population spike amplitude by 54% (n=5), and in slices from hibernating hamsters this modulatory inhibition was also present - spike amplitude was depressed 81% (n=9). This initial inhibition, seen immediately following application of 5-HT, is blocked by spiperone, a 5-HT_{1A} receptor antagonist. Serotonin binding to a second receptor type (possibly a 5-HT_4 receptor) enhances the population spike amplitude seen as a rebound in spike amplitude after 5-HT is removed from the bath. In slices from cold-acclimated animals the rebound was a 13% (n=3) enchancement over control amplitudes, and in slices from hibernating animals the overshoot was 19% (n=7). Thus, at 20°C 5-HT remains fully effective as a robust neuromodulator of hippocampal neural activity in slices from cold-acclimated and hibernating animals. [NASA grant NAGW-1458].

209.13

CONDITIONING REVERSES NOREPINEPHRINE -INDUCED HYPOTHERMIA IN THE COLD. C. Chancellor-Freeland*, E. Sohn, and A. Ettenberg. University of California, Santa Barbara, Santa Barbara, CA 93117

Research in our laboratory has demonstrated that while acute norepinephrine produces hypothermia when administered in cold environmental temperatures, repeated injections produce an attenuation of this response. The present study examines the potential role of Pavlovian conditioning in the repeat-exposure effect of norepinephrine on body temperature. Male albino rats were used in a four day conditioning paradigm. For days 1-3, nine rats received i.p. administration of norepinephrine (100 ug/kg) in the test environment (5°C), followed by a saline injection in the home cage (23°C). A second group was injected with saline in the test environment, followed by norepinephrine in the home cage. On day 4, both groups received the drug challenge in the test environment. Colonic temperature measurements revealed a mild hyperthermic response in the norepinephrine-test environment group. In contrast, animals with previous norepinephrine-home cage pairings exhibit hypothermia, despite identical handling, cold exposure, and number of injections. These data suggest that the norepinephrine-induced hyperthermia observed in the cold environments, is at least in part related to Pavlovian conditioning.

209.14

THERMOREGULATORY DYSFUNCTION IN RATS EXPOSED TO ACTIVITY-STRESS. N.S. Morrow*[1], P.J. Geiselman[2], G. Ohning[3], and D. Novin[4]. Depts. of Psychiatry[3], Psychology[4], UCLA, Los Angeles, CA 90024, CURE[3], West Los Angeles Veterans Administration Center, Los Angeles, CA 90073, & Dept. of Psychology[2], Louisiana State Univ., Baton Rouge, LA 70803.

Using telemetric monitoring, the relationship between core body temperature, wheel running and gastric erosion formation was examined in female rats housed in activity cages. Rats were sacrificed when body temperature fell to 35.0°C or 30.0°C. A third group was rewarmed with a heat lamp when body temperature dropped to 35.0°C. Group differences in CNS localization of thyrotropin-releasing hormone were also examined. Results indicated that the extent of thermoregulatory disturbances on Day 1 of deprivation was predictive of length of survival. Rewarming the animals significantly extended survival time and attenuated erosion formation. Thermo-regulatory disturbances appear to underlie the development of excessive wheel running and gastric ulceration (NIMH Fellowship 5T32MH17140, NSF BNS-9196142, and UCLA Univ. Research Grant SF86).

209.15

THERMAL STRESSORS PRODUCE NON-UNIFORM BRAIN TEMPERATURE GRADIENTS AND ALTERATIONS IN LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU). G.A. Mickley*[1], B.L. Cobb[1], C.D. Rose[2] and S.T. Farrell[1]. [1]Armstrong Laboratory, Brooks AFB, TX 78235; [2]Trinity University, San Antonio, TX 78235

Last year (Cobb et al., Soc. Neurosci. Abstr., 17/2, 1991, 1460) we reported that certain brain nuclei (e.g., lateral hypothalamus) exhibit significantly different LCGUs depending on the hyperthermic agent (hot-moist air or 5.6 GHz microwaves) used to produce a cephalic temperature rise. After midbrain temperature was raised 2°C, we used the 2-Deoxyglucose method (Sokoloff et al., J. Neurochem., 28, 1977, 897-916) to determine rat LCGU during a 45-minute period of stable hyperthermia. Here, we extend these studies by determining precise local temperatures present in brain structures with either similar (e.g., caudate putamen), or dissimilar (e.g., hypothalamus) LCGUs during exposure to microwaves or hot air. As before, both hyperthermic treatment groups produced similar midbrain and tympanic temperatures. Further, we determined that both hyperthermic methods produced similar hypothalamic and caudate temperatures during the time period of stable hyperthermia when LCGUs were measured. However, microwaves caused a more-rapid rise in the temperature of both caudate and hypothalamus than did hot air exposure. These data suggest that stable differences in local brain temperature may not fully explain the disparity between microwave and hot-air-induced LCGUs. Instead, this difference in LCGUs may be influenced by local temperatures that increase at dissimilar rates or by a variety of inputs from non-uniformly heated afferents.

SOMATIC AND VISCERAL AFFERENTS II

210.1

CALCITONIN GENE-RELATED PEPTIDE AND SUBSTANCE P IMMUNOREACTIVE NERVE FIBERS IN THE DURA MATER OF CAT AND RAT: AN IMMUNOELECTRON MICROSCOPIC STUDY

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The durae mater encephali of the rat and the cat are richly innervated by calcitonin gene-related peptide (CGRP) and substance P (SP) immunoreactive nerve fibers that originate mainly in the ipsilateral trigeminal ganglion. Part of them may participate in nociception and generation of headaches. To characterize the putative sensory nerve endings morphologically, whole mount preparations of the dura mater were processed immunocytochemically for light microscopic and ultrastructural examinations.

In rat and cat CGRP- and SP-immunopositive nerve fibers formed a dense network around dural blood vessels. However, a considerable number of the peptidergic nerve fibers terminate in the dural connective tissue with complex tree-like endings. In both species CGRP immunoreactive nerve fibers were found much more abundant than SP-positive fibers. In the cat, however, the dura mater generally showed less immunoreactive nerve fibers. Electron microscope immunolabelling revealed that CGRP and SP immunoreactivity is mainly found in thin unmyelinated nerve fibers located at blood vessels and within dense collageneous tissue.

The data show that small diameter unmyelinated nerve fibers of the supratentorial dura mater, a part of which is SP and CGRP immunoreactive, innervate blood vessels but also cross the dense connective tissue terminating in free endings far away from vessels. The peptidergic nerve fibers in both locations may be involved in neurogenic inflammation.

210.2

ORIGIN AND PATHWAY OF PENILE CALCITONIN GENE-RELATED PEPTIDE (CGRP)-IMMUNOREACTIVE (-I) AFFERENT NERVES. C.H. Harris, W.G. Dail, R.E. Papka, J.G. Wood*, N.E. Traugh, and D.L. McNeill. Dept. Anat. Sci., Univ. Okla., Okla. City, OK 73190 & Dept. Anat., Univ. New Mexico Sch. Med., Albuquerque, NM 87131.

The penis receives an abundant innervation by CGRP-I primary afferent nerve fibers. In this study, the location of penile CGRP-I afferent perikarya and the pathway by which CGRP-I fibers reach the penis were examined in male Sprague-Dawley rats. To identify perikarya, the retrograde tracer fluorogold (FG) was injected into the dorsum of the penis. Ten days post-injection, rats were perfused and the L4-S2 dorsal root ganglia (DRG) were removed, sectioned and immunostained for CGRP. To identify pathways of CGRP-I nerve fibers, rats received either a bilateral transection of the hypogastric nerves, pelvic splanchnic nerves or pudendal nerves. Ten days later, penises were removed and immunostained for CGRP. Additional rats received a transection of the left pudendal nerve followed immediately by an injection of FG into the penis. Following a 10 day survival, the L4-S2 DRG were removed, sectioned and immunostained for CGRP. FG-labeled perikarya were confined to the L6 DRG and approximately 40% of FG-labeled perikarya were CGRP-I. No diminution of CGRP immunostaining in the penis was observed following transections of the hypogastric or pelvic splanchnic nerves. However, CGRP immunostaining in the penis was eliminated following bilateral pudendal nerve transections. In rats receiving a transection of the left pudendal nerve and a FG injection, no labeled perikarya were present in the L6 DRG. These data suggest that the pelvic splanchnic or hypogastric nerves do not possess a CGRP-I afferent fiber component. Instead, CGRP-I fibers reach the penis primarily via the pudendal nerves and are presumably somatic in nature. Supp. by the PVA Spinal Cord Res. Fnd., OCAST and the Amer. Fnd. for Urol. Dis.

210.3

PELVIC AND NON-PELVIC INNERVATION OF MALE RAT PUDENDAL NERVE. Pacheco,P.*; R.A.Lucio; J.Manzo; M.Martínez-Gómez; P.Carrillo and M.A.Camacho. Depto.Fis.IIB-UNAM; Inst. Neuro-Etología, Univ. Veracruzana; CIRA, Univ. Autón. Tlaxcala. México.

The L6-S1 trunk forms two major nerves, pelvic and pudendal (PN). The PN gives off branches to: coccygeus muscle (CB); penis (DPN); scrotal and tail base skin (SAB); and the anastomotic branch that constitutes a bridge-like structure which forms part of the sacral plexus. From the bridge emerges branches to: obturator muscle (OB); penile muscles (MB); scrotal basal skin (SBB); and semitendinosus muscle (StB). CB, OB, MB and StB electrical stimulation produces contraction of respective muscles. Scrotal basal skin activates SBB, while scrotal apical and tail base skin activates SAB. Stimulation of penis and prepuce evokes DPN activity. Through the anastomotic branch PN evokes obturator and penile muscles contraction and flexion of the third foot finger. CONCLUSIONS: PN is compound of fibers that travel through sciatic nerve (third foot finger); sacral plexus (basal scrotum, penile and obturator muscles); and its own branches to coccygeus muscle, penis and prepuce (dorsal penile nerve) and apical scrotum.

SEP-92 (P.P.); SEP-92 (J.M.); SEP-92 (M. M-G.)

210.4

ELECTRON MICROSCOPY OF POLYMODAL RECEPTORS OF THE TESTICULAR TUNICA VASCULOSA

D. Anderson, L. Kruger*, T. Kumazawa[1], K. Mizumura[1] and Y. Yeh. Dept. of Anatomy and Cell Biology and the Brain Research Institute, UCLA Medical Center, Los Angeles, CA 90024. [1]The Institute of Environmental Medicine, Nagoya University, Nagoya, Japan.

Receptive fields of polymodal receptor axons were delimited by von Frey hair stimulation during sustained electrophysiological study *in vitro* of single units of the dorsal spermatic nerve of the dog, dominated by unmyelinated axons. The sample included A delta and C fibers, selecting for zones of low innervation when feasible and marking the surround of the receptive field with fine insect pins inserted perpendicularly through the tunica albuginea. The blocks were trimmed and embedded for serial analysis of functionally characterized endings. Most receptive fields revealed one or more Remak bundles containing several unmyelinated axons. Profiles with aggregates of dense vesicles and mitochondria as well as scattered spherical clear vesicles were common and often constituted varicosities of continuing axons. Axon terminals, identified by serial tracing, usually end at the surface of the bundle, not surrounded by Schwann cell processes but covered by basal lamina; these generally contain numerous tightly-packed clear spherical vesicles, less numerous granular vesicles, and a few dense-core vesicles with distinct halos. Free axons are rare compared with terminals at the periphery of a thin axonal bundle extending beyond the receptive field.

Supported by NIH grant NS-5685.

210.5

DISTRIBUTION OF CALBINDIN D-28K AND PARVALBUMIN-IMMUNOREACTIVE SENSORY NEURONS INNERVATING VISCERAL AND SOMATIC STRUCTURES IN THE RAT. C. N. Honda* Dept. of Cell Biology and Neuroanatomy, Univ. of Minnesota, Minneapolis, MN 55455.

Calcium binding proteins have been localized to subpopulations of neurons in both the peripheral and central nervous systems. In this study, immunohistochemical and retrograde tracing techniques were combined to compare the distribution of the calcium-binding proteins parvalbumin (PV) and calbindin-D28k (CaBP) in somatic and visceral sensory neurons.

Dorsal root ganglion (DRG) cells were retrogradely labeled by the application of Fluorogold (FG) to sural, gastrocnemius, and pelvic nerves. Adjacent pairs of DRG sections were then immunostained for PV or CaBP using a secondary antibody conjugated with cyanine 3.18 (CY3). Counts of neuronal profiles labeled singly with FG or CY3 and double-labeled with FG/CY3 were obtained from reconstructions of each DRG section examined. The distribution of calcium binding proteins localized to sensory neurons projecting through each peripheral nerve is summarized below as the percentage (range) of FG-labeled neurons immunoreactive for each calcium binding protein. Colocalization of PV and CaBP was negligible.

Nerve (DRG)	% PV	% CaBP
Sural (L4-6)	17 (7-27)	24 (14-34)
Gastrocnemius (L4-5)	27 (18-37)	22 (17-26)
Pelvic (L6-S1)	0	23 (18-27)

The distribution of PV and CaBP suggests that these two proteins may be differentially involved in calcium-dependent processes particular to somatic and visceral sensory neurons. Supported by NIH grant NS25658.

210.6

RESPONSES OF UTERINE RECEPTORS TO MECHANICAL AND CHEMICAL STIMULI IN THE CAT. H.C.Han[1]*, S.K.Hong[1], Y.W.Yoon[1] and J.M.Chung[2]. [1]Dept. of Physiology, Med. Coll., Korea Univ., Seoul, KOREA and [2]Marine Biomed. Inst., Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX, 77550, U.S.A.

This study was done to examine the sensory function of uterine afferent fibers in the cat.

Single unit recordings were made from fine filaments dissected from the hypogastric nerve in 21 cats anesthetized with α-chloralose. The mechanical stimuli were applied to the receptive field by von-Frey hair and stretching of intrauterine latex balloon. As a chemical stimulus, algesic substances (e.g. bradykinin etc.) were injected into the uterine artery. Uterine contractility was continuously monitored.

The majority of uterine afferent fibers were unmyelinated C fibers and the remaining were thinly myelinated A-δ fibers. The threshold to mechanical stimuli was variable from 0.3 g to 18 g of von Frey hair and some of the low threshold units were synchronously activated with spontaneous uterine contraction. Most of the units we tested were excited by one or more algesic chemicals and the response was accompanied by intense uterine contraction. There were some evidence indicating that at least some uterine receptors can be excited directly by these chemicals.

The results suggest that there are at least two populations of uterine receptors, low threshold mechanoreceptors and mechanical nociceptors. In addition some of them are likely polymodal receptor.

210.7

DISTRIBUTION OF SACRAL AFFERENT FIBERS IN CAT PARASYMPATHETIC GANGLIA, URINARY BLADDER AND URETHRA. J. Krier* and J. McRorie. Dept. Physiol., Mich. State Univ., East Lansing, MI 48824

The peripheral distribution of afferent fibers (AF) from sacral dorsal root ganglia (SDRG) to pelvic nerve (PN), pelvic plexus (PP)-urinary bladder (UB) ganglia, UB and urethra (Ur) was studied in cat with the anterograde transport of wheat germ agglutinin-horseradish peroxidase (WGA-HRP). WGA-HRP was injected into SDRG (S1-S3) bilaterally or ipsilaterally. Glutaraldehyde background fluorescence was used to visualize neuronal somas within PP and UB ganglia. When SDRG were injected ipsilaterally, anterogradely transported WGA-HRP was detected ipsilaterally in the PN and branches of the PN to the PP, but bilaterally in PP-UB ganglia. Nonvaricose AF were detected in 28 of 30 PP ganglia (n=3) and in 37 of 42 UB ganglia (n=4). Some single AF and afferent bundles were in close proximity to neurons. For UB, AF were distributed to neck, trigone, apex, anterior and posterior regions of bladder musculature. A dense innervation occurred in neck serosa, external muscle layers and in mucosa-submucosa. For Ur mucosa, AF were oriented parallel to the long axis of mucosal cells. In Ur submucosa, AF were oriented longitudinally, obliquely, and circumferentially. For Ur circular-longitudinal smooth muscle and striated muscle, AF were oriented parallel to the long axis of muscle cells. Our data show sacral AF in parasympathetic ganglia and in pelvic visceral organs. (DK-29920)

210.8

EFFECT OF DORSAL RHIZOTOMY AND MK-801 ON BLADDER AFFERENT NEURONS AND FIBERS. D.L. McNeill*, C.H. Harris, R.L. Shew and R.E. Papka. Dept. of Anat. Sci., Univ. of Okla. Hlth. Sci. Ctr., P.O. Box 26901, Oklahoma City, OK 73190

Suprasacral lesions of the spinal cord result in marked decreases in the density of calcitonin gene-related peptide (CGRP)-immunoreactive (-I) primary afferent nerve fibers in the rat bladder which is ameliorated by post-surgical treatment with the NMDA receptor antagonist MK-801. In this study, the potential of MK-801 to restore bladder CGRP-I nerve fibers following a decentralizing lesion, i.e., dorsal rhizotomy (DR) was examined. In addition, the effect of DR and MK-801 on the number of CGRP-I primary afferent neurons was determined. Group 1 rats served as intact controls. Group 2 rats were anesthetized and the L6 and S1 dorsal roots were transected bilaterally. Group 3 rats underwent a similar surgery, but received a daily i.p. injection of MK-801 (1.0 mg/kg) for 14 days. On post-surgical day 14, rats were reanesthetized, the bladder and the L6 and S1 dorsal root ganglia (DRG) were removed and processed for CGRP immunoreactivity. The density of CGRP-I nerve fibers in each bladder was quantified by image analysis. In bladders from Group 2 rats, the density of CGRP-I fibers was significantly reduced from that observed in control rats. In Group 3 rats receiving MK-801, no improvement in the density of CGRP-I nerve fibers was observed. Counts of CGRP-I neurons in the DRG were not significantly different between the 3 groups. These data suggest that decentralization by rhizotomy significantly reduces the density of peripheral CGRP immunostaining and interferes with the restorative potential of MK-801. In addition, DR with or without MK-801 failed to alter the number of primary afferent neurons expressing CGRP. Supp. by the PVA Spinal Cord Res. Found., OCAST and the Amer. Found. for Urol. Dis.

210.9

CHARACTERIZATION OF DISTENSION-SENSITIVE SACRAL PRIMARY AFFERENTS INNERVATING THE COLON OF THE RAT. G.F. Gebhart* and J.N. Sengupta. Department of Pharmacology, The University of Iowa, Bowen Science Building, Iowa City, IA 52242.

Single-unit activity was recorded from the left S_1 dorsal rootlet. Eight fibers that responded to colorectal distension (CRD; 80 mmHg, 20 s) and demonstrated an evoked-response to pelvic nerve stimulation were selected for further study. Of 8 fibers, 7 had ongoing resting activity and one was silent. The mean resting activity was 9.7 ± 2.9 impulses/s. Repeated CRD (80 mmHg, 20 s) for 10 trials at 4 min intervals evoked responses that were reproducible. There was no evidence of facilitation or inhibition of subsequent responses. All fibers demonstrated slow adaptation during the 20 s period of distension. The stimulus-response function (SRF) to graded CRD (5 to 100 mmHg) was studied in 7 fibers, all of which demonstrated linear increases in firing. The mean maximum response of the fibers to 100 mmHg CRD was 29.4 ± 10.5 impulses/s. Three fibers were tested for response to i.a. bradykinin (BK) and all 3 responded. BK administration at a short interval (5 min) produced rapid tachyphylaxis, but tachyphylaxis to BK could be avoided if given at 20 min intervals. The concentration-response function to BK (0.05-300 μg/kg, i.a.) was studied in one fiber which showed maximum in response at 50 μg/kg. The conduction velocity was measured in three fibers; two fibers were C-fibers and one was an Aδ-fiber.

210.10

RELEASE OF OXYTOCIN INTO SPINAL CORD SUPERFUSATES IN RESPONSE TO VAGINOCERVICAL STIMULATION IN RATS. J.L. Steinman*, J.T. Winslow[1], G. Sansone, C. Gerdes, B.R. Komisaruk and T.R. Insel[1], Institute of Animal Behavior, Rutgers-The State Univ., Newark, NJ 07102 and [1]NIMH, Laboratory of Neurophysiology, Poolesville, MD 20837.

Oxytocin (OXY)-containing terminals have been identified in laminae I and X of the spinal cord in rats. The present study assessed whether vaginocervical stimulation (VS) evokes the release of OXY at the spinal cord level. OXY concentrations in spinal cord superfusates (5 min samples) were measured before, during and after VS (300 g force, on 30 sec-off 30 sec for 5 min) in 8 intact urethane-anesthetized rats. The concentration of OXY, measured using radioimmunoassay, was significantly elevated 10-15 min after VS (Mann-Whitney U test, p=.037, 1-tailed) compared to samples collected 10-15 min pre-VS. Pre-VS values ranged from 0.4 to 2.1 pg/ml (mean ± sem = 1.1 ± 0.1) whereas post-VS values ranged from 0.5 to 15.7 pg/ml (2.7 ± 1.0). There was a 130% increase in OXY after the first application of VS over pre-VS values; when administered a second time, 45 min later (n=6), VS produced a 53% increase over pre-VS values. The hormonal state of these animals was not determined; we are currently investigating whether estrogen influences the release of oxytocin in response to VS.

210.11

EXPRESSION OF C-FOS PROTEIN IN LUMBOSACRAL SPINAL CORD IN RESPONSE TO VAGINOCERVICAL STIMULATION (VS) IN RATS. S.Chinapen*, J.M. Swann, J. L. Steinman, and B.R. Komisaruk. Inst. Animal Behavior and Dept. Biol. Sci., Rutgers-The State Univ., Newark, NJ 07102.

The pattern of VS-evoked expression of the proto-oncogene c-*fos* in lumbar 5-sacral 1 spinal cord segments of ovariectomized adult Sprague Dawley rats was mapped using immunocyto-chemistry. Mechanostimulation (10 sec on/10 sec off for 5 min) was applied to the vaginal cervix of experimental rats and to the perineum of control rats (400 and 100g force, respectively) during gentle restraint. Two hours after stimulation all rats were euthanized, perfused, and the spinal cord was removed, sectioned and immunolabeled for c-*fos*. Using 1-tailed multiple t-tests, the total number of neurons expressing *c-fos* was significantly greater in the experimental (n=4) than control (n=3) group (t=2.9 p=0.04). The experimental group showed a significantly greater number of *c-fos*-activated cells than the control group in laminae I (t=3.3; p=0.03), IV (t=3.0; p=0.02), V-VI (t=3.3; p=0.03) and X (t=2.6; p=0.04). The distribution of neurons activated by VS corresponds to that of cell bodies: a) of ascending pathways (spino-bulbar, -hypothalamic and -thalamic), b) of para-sympathetic preganglionics, and c) receiving synaptic input from descending spinopetal pathways. Supported by Grant GM 08223-08 and Sigma Xi.

210.12

QUANTITATIVE ANALYSIS OF VIP-IMMUNOREACTIVE TERMINALS IN CAT SACRAL SPINAL CORD. Q. Li, J.C. Bresnahan*, M .G. Leedy, and M.S. Beattie. Dept. Cell Biol., Neurobiol. and Anat., Ohio State Univ., Columbus, OH 43210.

The purpose of the present study was to provide a detailed quantitative analysis of VIP immunoreactive (VIP-IR) synaptic terminals in different regions of the cat sacral spinal cord. VIP-IR elements were labelled using the ABC technique in sections of cat S1-S2 spinal cord. VIP-IR elements were seen in the tract of Lissauer, laminae I, II, V, lateral VII, X and rarely in IX. The ultrastructural features of VIP-IR terminals (n=292) were analyzed and compared in different regions (Lamina I&II, lamina V&VII in the sacral parasympathetic nucleus [SPN], and lamina X. VIP-IR terminals contained clear synaptic vesicles (73% round; 22% pleomorphic; flat 5%). Almost all terminals (97.95%) also contained DCVs (range 1-128 / terminal; $\overline{X}$ = 17.11). 45% of the VIP-IR terminals were observed in glomerular structures in I&II, 26% in the SPN and 8% in X. Overall, 41.78% of the VIP-IR terminals exhibited synaptic contacts, most occurring in laminae I&II (50.35%), with less in the SPN (34.4%) and X (29.17%). The most common post-synaptic targets were small dendritic profiles and other axon terminals. Only a few labeled terminals were found to synapse on proximal dendrites or somata in any area examined. Supported by NIH grant NS-10165.

210.13

LOCALIZATION OF VIP RECEPTOR SITES IN THE CAT SACRAL SPINAL CORD (SSC). T.J. Brown*, M.S. O'Dorisio, M.S. Beattie, J.C. Bresnahan. Dept. of Cell Biol., Neurobiol. & Anatomy; and Dept. of Pediatrics, Ohio State University, Columbus, OH 43210.

VIP has been localized to a prominent visceral afferent pathway in the SSC. We have examined the binding characteristics, localization, and effects on cAMP production of VIP in the cat SSC. Synaptosomal fractions were used in competitive binding experiments to determine the affinity (Kd) and number (Bmax) of 125I-VIP binding sites. Binding of 125I-VIP to SSC membranes was dependent on the amount of membrane protein over the range of 75-400 ug. Competitive binding showed a Kd = 3.19$\pm$2.33 nM, Bmax = 0.11$\pm$0.08 nM, n=3. VIP has been reported to stimulate cAMP production in homogenates derived from rat cerebral cortex; while 1uM forskolin stimulated cAMP in SSC, 1uM VIP did not. Sections (20um) from the S1 region of the cord were incubated with 0.2 nM 125I-VIP. Autoradiograms revealed specific binding in laminae I,II, and X, with distinct binding to motoneurons in IX. The results suggest that there are relatively few VIP receptors in SSC, and that these are concentrated in regions corresponding to VIP-containing afferents, and on motoneurons. Supported by NS-10165, and NCI-CA41997.

210.14

DORSAL ROOTS CARRYING UTERINE AND CERVIX INPUT TO DORSAL HORN NEURONS IN RATS. P.D. Wall, C.H. Hubscher and K.J. Berkley*. Dept. Psychology, Florida St. Univ., Tallahassee, FL 32306.

Cervix-responsive neurons at thoracolumbar and lumbosacral regions of spinal cord receive convergent uterine (76% and 42%, respectively) and cutaneous (100%) input. The present study addressed the issue of source of uterine and cervical input to these two separated spinal regions. Neuronal recordings pre- and post- dorsal root cuts (31 and 71 units, respectively) from 10 spinalized, decerebrate unanesthetized rats in estrus were obtained. The search stimulus was a simultaneous distension of both uterine horns using water-filled latex balloons. Hypogastric nerve input was removed by cutting the T13-L3 dorsal roots bilaterally. Pelvic nerve input was removed by cutting the L6-S2 dorsal roots bilaterally. At **L1**, neurons still responded to gentle cervix stimulation after removal of the pelvic (6 rats) or hypogastric nerve inputs (1 rat). Neurons in this area also continued to respond to uterine stimuli after removal of the pelvic nerve input but were lost after removal of hypogastric input. Obvious changes, however, occured in the detail of responses of individual neurons at L1 as a result of these manipulations. At **L6** (4 rats), neuronal responses to uterine stimulation were lost after removal of hypogastric input. Those to gentle cervix stimulation remained after such removal but disappeared after subsequent removal of pelvic nerve input. These results demonstrate that the main source of uterine influence at both L1 and L6 is derived from the hypogastric nerve whereas the main source of cervix input at L1 and L6 is derived from both the hypogastric and pelvic nerves. Since some neurons still responded to uterine or cervical stimuli when all nearby roots were cut, it was apparent that neurons could be influenced by inputs 3-6 segments away. Supported by NIH grant RO1 NS11892.

210.15

NEURONAL RESPONSES TO STIMULATION OF UTERUS, CERVIX AND VAGINAL CANAL IN THE RAT GRACILE NUCLEUS. C.H. Hubscher and K.J. Berkley. Dept. Psychology, Florida St. Univ., Tallahassee, FL 32306.

The present study examined input from female pelvic reproductive organs to the gracile nucleus using electrophysiological techniques. Seventeen female rats in estrus were anesthetized with either ketamine/xylazine (10) or urethane (7). Recordings were made from a total of 112 single units located either rostral to (n = 44), at the level of (n = 44) or caudal to (n = 24) the obex. Testing was as follows: gentle brushing of the caudal body surface, gentle probing of the cervix and inflation of water-filled latex balloons in the uterus and vaginal canal to levels at the threshold for provoking escape responses in conscious rats. Almost all neurons had excitatory cutaneous receptive fields (95.5%) to the brush stimulus. Of these, 49.5% also responded with excitation or inhibition to one (27.1%) but sometimes more than one (22.4%) of the visceral stimuli; 23.4% to uterus, 29.9% to vagina and 14.0% to cervix. Although viscerally-responsive neurons were found throughout the entire gracile nucleus, they were particularly concentrated dorsomedially at the level of obex. These results taken together with similar data for spinal cord and thalamus (Guilbaud et al., 1991; Hubscher et al., 1991 - Soc. Neurosci. Abs.) indicate that the internal reproductive organs have a surprisingly large potential for influencing the properties of somatosensory neurons. Supported by NIH grant R01 NS11892.

210.16

VENTROBASAL NEURONAL RESPONSES TO UTERINE AND VAGINAL DISTENSION AND TO UTERINE SUPRAFUSION WITH PROSTAGLANDINS (PGF2a) AND BRADYKININ. K.J. Berkley, G. Guilbaud*, J.M. Benoist and M. Gautron. INSERM, U. 161, 2 rue d'Alesia 75014 Paris, France.

Previous studies demonstrated the existence of neurons in and near the ventrobasal complex (VB) of anesthetized rats responsive to uterine and vaginal mechanical stimulation (Guilbaud et al., 1991). Most of these neurons had somatic receptive fields, particularly to noxious pinch stimuli, and were located at the rostral and dorsal edges of VB. This study examined responses of these neurons to graded stimulation of the uterus and vagina as well as to suprafusion of the uterus with the inflammatory mediators PGF2a and bradykinin (BK). Fifteen estrous virgin rats were anesthetized with halothane (0.7%) and nitrous oxide (67%) in oxygen and paralyzed (pancuronium bromide). Latex balloons were inserted in each uterine horn and the vagina, and a catheter was secured over the uterine body. The rostral 1/3 of VB was searched with a glass microelectrode filled with pontamine sky blue in saline. The first neuron encountered responsive to pinching the contralateral hindpaw or tail was further examined, first by graded distension of one uterine horn (50 - 225 ul) and then graded distension of the vagina (0.5 - 1.25 ml) and finally by suprafusion with 1 ug (in 1 ml) of PGF2a and then 1 ug (in 1 ml) BK. To avoid sensitization, only one neuron was studied per rat. The responses of 11 neurons to uterine distension increased dramatically above background levels at 200 - 225 ul distension. In contrast, the stimulus-response function of 9 neurons to vaginal distension was more linear, with firing levels gradually increasing from 0.5 to 1.25 ml. These response patterns were nearly identical to escape response probabilities obtained in previous behavioral studies. Of 10 neurons tested, 6 responded to PGF2a; of 13 neurons tested, 7 responded to BK. Taken together, the results support the hypothesis that neurons in and near VB are involved in visceral nociception arising from female pelvic reproductive organs. Supported by EPHE (France) and NIH grant NS 11892.

210.17

REPRESENTATION OF BLADDER, COLON AND ESOPHAGUS IN THE LATERAL THALAMUS OF THE SQUIRREL MONKEY. J. Brüggemann*, T. Shi, R.A. Stea, R.T. Stevens, and A.V. Apkarian, Dept. of Neurosurgery, SUNY Health Science Center, Syracuse, New York 13210.

The lateral thalamus is emerging as an important area for processing the discriminative aspects of innocuous and noxious somatic stimuli. However, little is known about its role in the coding of visceral information. Here we report on the response properties of visceroceptive neurons in chloralose- and nembutal-anesthetized squirrel monkeys. Extracellular single unit recordings were performed in the lateral thalamus. The skin, muscles, and joints were stimulated with innocuous and noxious stimuli. The urinary bladder, the distal colon and the lower esophagus were distended with pressures up to 100 mmHg.

Of 87 thalamic neurons studied in 4 animals 73 were viscero responsive. Of these 63 were located in VPL and its border, 7 in ZI and the reticular n. and 3 in VPI and VL. For 5 neurons visceral specific responses were demonstrated. With respect to their somatic receptive fields 44 viscero responsive units were non-nociceptive and 22 were nociceptive (2 not tested). Due to the search strategy most somatic receptive fields of these neurons were located on the hindlimb and tail. Lateral thalamic neurons exhibited a high degree of viscero-visceral convergence: 18 (of 56) received inputs from all 3 viscera, 25 from 2 and 13 only from one viscus. Excitation and inhibition were seen from all 3 viscera. The incidence of both response patterns was comparable for all 3 organs and unrelated to the viscero-visceral convergence or the location of the somatic receptive fields. Neurons with colon input tended to be located more dorsally in VPL, but a viscerotopic arrangement was not obvious. Somatic nociceptive inputs were not predictive of visceral nociceptive inputs.

The results indicate that the lateral thalamus is a major viscero-somatic link, albeit the organization of the visceral representation in VPL is extremely different from that of the somatic organization.

PAIN PATHWAYS: DORSAL HORN

211.1

RESPONSES OF UPPER THORACIC SPINAL CORD NEURONS TO ESOPHAGEAL DISTENSION IN THE RAT.
I. Euchner,* J.N. Sengupta, G.F. Gebhart, S.T. Meller. Dept. of Pharmacology, University of Iowa, Iowa City, IA, 52242

Previously it has been shown that graded esophageal distension (ED) with a Swan Ganz catheter inserted orally into the thoracic esophagus of rats elicits pseudoaffective responses (pressor and visceromotor responses). The purpose of the present study was to characterize the responses of neurons in the upper thoracic spinal cord (T_2-T_3) to graded ED.

In 34 pentobarbital anesthetized and pancuronium paralyzed male Sprague-Dawley rats (425-500 g), graded ED (0.5 - 1.5 ml, 30 s) produced excitatory, inhibitory and biphasic excitatory-inhibitory responses of T_2-T_3 neurons. Responses to ED were graded; the extrapolated threshold for responses of all units was 0.4 ml. Cutaneous receptive fields, only responsive to noxious pinch, were located in the thoracic, axcillar region or on the forearm. One neuron responded to non-noxious and noxious movement of the forearm joints; no somatic input was found for 3 neurons. Recording depth ranged from 242 to 1404 μm and histological reconstruction revealed that neurons were distributed throughout lamina I-VII. Antidromic activation from C_2-C_4 showed ascending projections of 7/23 neurons tested. ED typically produced a intensity-dependent decrease in arterial blood pressure, but intraveneous sodium nitroprusside (2-4 μg/kg) did not change the activity of neurons that responded to ED. Reversible spinalization with a C_2-C_4 cold block revealed tonic descending influences on neurons responding to ED.

These data complement and extend earlier work and establish that ED is a reproducible and reliable stimulus for the study of thoracic visceral sensation.

211.2

THE NOCICEPTIVE RESPONSES TO GASTRIC ARTERY ADMINISTRATION OF BRADYKININ IN THE RAT ARE MEDIATED BY SYMPATHETIC AFFERENTS. J.N. Sengupta,* S.T. Meller and G.F. Gebhart, Department of Pharmacology, University of Iowa, Iowa City IA, 52242, USA.

Bradykinin (BK) is a potent algesic substance when administered to cutaneous tissues, muscle or to the viscera. However, there have been no systematic studies of the effects of gastric administration of BK, or its role in gastric pain. Therefore, the aims of this study were to determine whether administration of bradykinin into the gastric artery is a noxious stimulus and to evaluate which afferents mediate these effects.

Administration of BK (0.05-10 μg/kg) into the gastric artery of awake, chronically-catheterized rats produced an almost instantaneous dose-dependent increase in arterial blood pressure and an accompanying tachycardia. Within 1 s after administration, doses of BK greater than 1 μg/kg produced a distinct pseudaffective response that consisted of a hunching behavior accompanied by scratching of the abdomen, writhing and a contraction of the facial musculature that lasted for 5-10 s. In addition, gastric artery administration of 1 or 10 μg/kg BK produced a passive avoidance behavior that was significantly different from saline. The greater dose of BK produced a more rapid acquisition of the avoidance behavior. The passive avoidance behavior and the pseudaffective but not the cardiovascular responses were unaffected by prior (48-72 h) transection of the subdiaphragmatic vagus nerves.

These data suggest that gastric artery administration of BK is a noxious visceral stimulus possibly acting through sympathetic afferents.

211.3

THE NOCICEPTIVE RESPONSES TO GASTRIC ARTERY ADMINISTRATION OF ACETYLCHOLINE IN THE RAT ARE MEDIATED BY VAGAL AFFERENTS. S.T. Meller,* J.N. Sengupta, I. Euchner and G.F. Gebhart, Department of Pharmacology, University of Iowa, Iowa City, IA 52242, USA.

Administration of serotonin (5-HT), histamine (HA) and acetycholine (Ach) to visceral organs such as the heart have been reported to be algesic. However, other than the accompanying report on BK, there have been no systematic studies of the effects of gastric administration of algesic agents. Therefore, the aims of this study were to determine whether administration of 5-HT, HA or Ach into the gastric artery are noxious stimuli, and to evaluate which afferents mediate these effects.

Administration of HA (1-100 μg/kg), 5-HT (1-100 μg/kg) or Ach (1-100 μg/kg) into the gastric artery of awake, chronically-catheterized rats produced a rapid, dose-dependent decrease in arterial blood pressure and an accompanying tachycardia. Within 1 s after administration, Ach but not HA or 5-HT, in doses greater than 50 μg/kg produced a distinct pseudaffective response that consisted of a pronounced hunching behavior accompanied by agitation, scratching of the abdomen and a contraction of the facial musculature that lasted for 5-6 s. In addition, administration of 100 but not 10 μg/kg Ach produced a passive avoidance behavior that was significantly different from saline. The passive avoidance behavior and the pseudaffective, but not the cardiovascular responses to Ach were abolished by chronic bilateral transection of the subdiaphragmatic vagus.

These data suggest that the gastric artery administration of Ach, but not HA or 5-HT, is a noxious visceral stimulus that activates subdiaphragmatic vagal afferents.

211.4

C-FOS EXPRESSION IN SPINAL NEURONS AFTER NON-NOCICEPTIVE AND NOCICEPTIVE STIMULATION OF THE LOWER URINARY TRACT. L. Birder* and W.C. de Groat, Depts. Pharmacology and Behavioral Neuroscience, Univ. Pittsburgh, Pittsburgh, PA 15261

Spinal neurons receiving afferent input from the lower urinary tract (LUT) of urethane-anesthetized rats were identified by increased c-fos gene expression using immunocytochemical techniques to detect c-fos protein Non-nociceptive stimulation (NNST) of the LUT induced by saline distension of the bladder increased c-fos positive neurons primarily in the region of the sacral parasympathetic nucleus (SPN), whereas nociceptive stimulation (NST; intravesical infusion of 1% acetic acid) produced the majority of c-fos positive neurons in the dorsal commissure region. MK801, an NMDA antagonist, in a dose (3.5 mg/kg; i.v.) that completely blocked reflex bladder activity did alter the c-fos response to NNST but reduced by 50% the number of c-fos positive cells induced by NST. Pretreatment with capsaicin (CAPS) in a dose (125 mg/kg, s.c.) that increased bladder capacity by 35% did not alter the number of c-fos positive cells induced by NNST but reduced by 90% the number of cells induced by NST. These data indicate that non-nociceptive as well as nociceptive stimuli to the LUT increase c-fos expression in spinal neurons and that the two stimuli activate different populations of afferents (CAPS - sensitive and CAPS - resistant). Glutamic acid acting via NMDA receptors appears to play a role in the central processing of nociceptive input from the LUT.

211.5

CENTRAL PROJECTION OF ELECTROPHYSIOLOGICALLY IDENTIFIED TESTICULAR THIN-FIBER AFFERENTS. K. Mizumura*, Y. Sugiura and T. Kumazawa. Dept. Neural Regulation, Res. Inst. Environ. Med, Nagoya Univ., Nagoya 464-01, Japan

The spinal projection pattern of the testicular polymodal receptor (PMR) was studied by intracellular injection of PHA-L. Recordings were carried out in L1 DRG in 2-3 months old puppies under pentobarbital anesthesia (35 mg/kg). One A-δ (3.7 m/s) and two C-fiber (0.9 and 1.7 m/s) testicular PMRs were labeled good enough to trace their central terminations. The mean diameters of labeled C-DRG neurons were 40.7 μm and 36.8 μm. After entering into the spinal cord, axons of C-PMRs divided into rostral and caudal daughter axons that extended over 20-24 mm (about 3 spinal segments), and issued 15 and 16 branches. Majority of branches ran in/along the lateral surface, two in the middle, and one along the medial surface of the dorsal horn. Terminal swellings and en passant enlargements were observed mainly in laminae I, V, and VII, and some in lamina II. A-δ PMR had the similar termination pattern to C-PMRs except that interval issuing branches was shorter (13 branches over 10 mm) and its rostral daughter axon ran further rostrally (traced for 3.6 mm) in Lissauer tract after issuing all branches. Only two branches were found running just above the central canal in an another A-δ neuron in which termination could be only partially traced. The density of terminals of testicular thin-afferents was lower and their rostro-caudal spread was wider than those reported in cutaneous C-PMRs. These results suggest that testicular afferents have diffuse and wide spreading influences on the spinal neurons.

211.6

VAGAL AND CARDIOPULMONARY SYMPATHETIC AFFERENTS EXCITE UPPER CERVICAL (C_1-C_3) SPINOTHALAMIC TRACT (STT) CELLS IN MONKEYS. M.J. Chandler*, Q.-G. Fu, R.D. Foreman. Dept. Physiology & Biophysics, Univ. of Okla. HSC, Okla. City, OK 73190.

Myocardial ischemia activates vagal and sympathetic cardiac afferent fibers. Anginal pain classically involves sympathetic afferent fibers that enter T_1-T_4 spinal cord segments to activate STT cells. Vagal afferents generally inhibit STT cells in C_4-S_2 segments. After sympathectomies, angina still may be referred to the neck and inferior jaw. The purpose of this study was to determine a neurophysiological basis for this pain referral. We hypothesized that vagal afferents excite C_1-C_3 STT cells. In 15 anesthetized monkeys, 40 C_1-C_3 STT cells were identified by antidromic activation from lateral or medial thalamus. Left cardiac, recurrent laryngeal, and thoracic vagus branches were stimulated electrically; left stellate ganglion was stimulated electrically to activate sympathetic cardiopulmonary (CP) afferents; somatic fields were mapped by brushing hair and pinching skin and muscle. Cardiac vagus input excited 10/26 cells tested; recurrent laryngeal input excited 9/28 cells; thoracic vagus excited 21/37 cells and excited/inhibited 1 cell depending on stimulus parameters. Sympathetic CP input excited 25/39 cells, inhibited 1 cell and excited/inhibited 1 cell. Somatic receptive fields generally included or were confined to the neck; 22 cells were excited by innocuous and noxious stimuli (WDR), 15 cells required noxious stimuli (HT) and 2 cells were low-threshold (LT). We conclude that both vagal and sympathetic CP afferents can increase activity of C_1-C_3 STT cells with somatic fields located on or near the neck. This viscerosomatic convergence may account for referral of pain to the neck region during myocardial ischemia. (Supported by NIH grant HL22732).

211.7

SPECTRAL ANALYSIS OF BACKGROUND ACTIVITY AND NOXIOUS HEAT-EVOKED RESPONSES OF SPINAL DORSAL HORN NEURONS IN THE RAT. A. Eblen-Zajjur* and J. Sandkühler.
II. Physiologisches Institut, Universität Heidelberg, F.R.G.

Rhythmic discharges are a characteristic feature of many neurons throughout the CNS. Little, if anything is known about the role of rhythmicity for the encoding of nociceptive information. Here, we report that a large proportion of nociceptive and non-nociceptive neurons in the spinal cord display rhythmic background activity. This rhythmicity is largely depressed during noxious skin stimulation.

Multiple neuron recordings were made via single electrode in the lumbar spinal cord of pentobarbital anesthetized rats. Single neuron discharges were identified using a template matching algorithm and were analyzed after stationarity test. To evaluate rhythmicity of background activity (BA) and heat-evoked responses (HER) during stationarity phase (48°C, 100 s), auto-correlation (AC) and auto-spectral (AS) analysis was performed, a specific stochastic method for spectral analysis of point processes. The BA of a total of 135 neurons was analyzed. Twenty seven (20%) of which were non-nociceptive (class 1), 85 (62.9%) were multireceptive (class 2) neurons. Fifty four of all neurons (40%) had rhythmic activity. The distribution of the fundamental frequencies has a bimodal shape. The first peak 0.5-2 Hz consisting of 12 neurons overlaps with the respiratory rhythm (1-2 Hz) the second one at 6-13 Hz (16 neurons) is not related to the cardiac rhythm (5 Hz). Class 1 neurons have a higher incidence of rhythmicity (59.3%) than class 2 neurons (34.1%, $p < 0.01$). Only one (5%) of the 20 neurons with a projection to C2 had rhythmic BA. Activation of heat-sensitive nociceptors within the cutaneous receptive field of class 2 neurons had a strong antirhythmic effect in 12/21 (57.1%) neurons. No changes in spectral pattern were observed in non-rhythmic neurons. It is concluded that rhythmicity exist in sensory neurons of the spinal dorsal horn which is affected by activity in primary afferent nociceptors.

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211.8

THE ROLE OF NITRIC OXIDE IN SPINALLY MEDIATED HYPERALGESIA IN MOUSE AND RAT K. F. Kitto, J. E. Haley, M. Cook, P. Dosha and G. L. Wilcox*. Department of Pharmacology and Graduate Program in Neuroscience, University of Minnesota, Minneapolis MN 55455

N-methyl-D-aspartate (NMDA) receptors may be involved in long term potentiation (LTP), "wind-up" and hyperalgesia. Nitric oxide (NO) also appears to participate in these phenomena. The present study was designed to determine whether NO participates in central hyperalgesia following NMDA receptor activation in spinal cord.

Male ICR mice were injected intrathecally (i.t.) with NMDA (0.3 μg/5 μl) and nociceptive effects were measured with the tail flick test (baseline 3.9 s ± 0.1). In male Sprague-Dawley rats, i.t. injection of L-NAME (N^W-Nitro-L-arginine methyl ester, a NO synthase inhibitor, 1 mg/10μl, i.t.) was immediately followed by a left hindpaw injection of 2.5% formalin (50μl). Over the next 50 min rats were observed for flinching and licking/biting of the formalin-injected paw.

NMDA-induced hyperalgesia (-0.75 s ± 0.17 SEM at 10 min) in mice was completely blocked by 4 h pretreatment with L-NAME but not with its inactive stereoisomer D-NAME. L-NAME was effective both intrathecally and systemically and its effects could be partially reversed by coadministering the NO precursor L-arginine (90-300 μg i.t.). Concurrent administration of hemoglobin (600 μg i.t.), which purportedly inactivates extracellular NO, also blocked NMDA-induced hyperalgesia. Intrathecal sodium nitroprusside (0.1-3 μg i.t.), which spontaneously degrades to NO and ferrocyanide, was hyperalgesic for up to 3 hr (-0.75 s ± 0.1 SEM at 90 min) in the tail flick test. In the rat, L-NAME inhibited the flinching and licking/biting behavior elicited by intraplantar injections of formalin.

These results indicate that spinal NO production is necessary for increased synaptic efficacy following nociceptive input or NMDA receptor activation in rodent spinal cord. (Supported by NIDA grants R01-DA-01933, R01-DA-04274 and K02-DA-00145).

211.9

DIFFERENTIAL EFFECTS OF OPIOID AND ALPHA2 NORADRENERGIC AGONISTS ON METABOTROPIC AND OTHER GLUTAMATE RECEPTORS.
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Excitatory amino acids, which act on glutamate receptors, induce biting and scratching behavior in rodents when injected intrathecally. Whereas most glutamate receptor ligands (e.g., NMDA and AMPA) act on ligand-gated cation channels to produce their effects, the glutamate receptor agonist (+/-)-1-amino-1,3-cyclopentane-*trans*-dicarboxylic acid (ACPD) acts through a G-protein-coupled metabotropic glutamate receptor. When injected intrathecally, biting and scratching behaviors were elicited in a dose dependent manner by NMDA (0.03 - 0.5 nmol), AMPA (1.0 - 10.0 ng) and ACPD (0.03 - 1.0 μg). However, the ability of the μ- and δ-opioid agonists DAMGO and DPDPE, respectively, and the α_2 noradrenergic agonist, UK14304-18, to block the behavior varied for the three glutamate receptor agonists. Behavior induced by NMDA was only reversed by DAMGO whereas biting and scratching induced by AMPA was only reversed by UK14304-18. ACPD-induced biting and scratching was reversed dose-dependently by DAMGO, DPDPE and UK14304-18.

As we had observed earlier (Kitto and Wilcox, Soc. Neurosci Abstr. 17:71, 1991), the behavior produced by ACPD and its reversal by DAMGO, DPDPE and UK14304-18 was almost identical to that seen with the neurokinin peptide Substance P (SP). The relationship between the metabotropic glutamate receptor and the SP neurokinin receptor suggests an intimate association between these two signaling systems. One can speculate that these receptors share a common cellular localization or signaling mechanism. (Supported by K02-DA-00145, RQ1-DA-04274 and -01933)

211.10

FUNCTIONAL PROPERTIES OF SPINOMESENCEPHALIC TRACT (SMT) CELLS AND UNIDENTIFIED NEURONS IN THE LUMBOSACRAL SPINAL CORD OF THE RAT. S.H. Park* and R.P. Yezierski. Department of Neurological Surgery and The Miami Project, University of Miami, Miami, FL 33136.

The present study was carried out in order to characterize the functional properties of two different populations of neurons in the lumbar spinal cord of urethane anesthetized rats. Extracellular single-unit recordings were made from unidentified neurons as well as cells antidromically activated from midbrain stimulation sites, including the deep layers of the superior colliculus, periaqueductal gray, and midbrain reticular formation. Recording sites were located in laminae I-VIII and X of spinal segments L4-L6. For each cell receptive field properties, including responses to calibrated mechanical stimuli, were determined.

Thus far, 20 SMT cells and 31 unidentified neurons have been studied. Single SMT units were classified into four groups: wide dynamic range (58%), deep/tap (21%), high threshold (11%) and non-responsive (10%). Unidentified neurons included wide dynamic range (58%), deep/tap (23%), low threshold (16%), and high threshold (3%). Simple excitatory receptive fields were found for most SMT and unidentified cells recorded in laminae I-IV. Large complex inhibitory and/or excitatory fields were commonly found for cells in laminae V-VIII and X.

In conclusion, the results of the present study have shown rat SMT cells to have functional properties similar to those described in other species. Furthermore, it is suggested that sensory neurons in the rat dorsal horn have a functional role that is different from ventral horn neurons in relation to the integration and processing of sensory input, including that derived from noxious mechanical stimuli. Supported by NS19509, NS28059 and the Miami Project.

211.11

BLOCKADE OF NOCICEPTIVE INPUTS TO DORSAL HORN NEURONS BY 6-CYANO-7-NITROQUINOXALINE-2,3-DIONE (CNQX): AN INTRACELLULAR STUDY USING THE RAT SPINAL CORD-HINDLIMB PREPARATION.

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The role of non-NMDA receptors in synaptic transmission from cutaneous mechanoreceptors to dorsal horn neurones has been assessed using the selective antagonist CNQX.

Intracellular data were obtained from 40 dorsal horn neurons responding to noxious stimulation (pinch) of the hindlimb skin, 14 cells were 'nociceptive specific' and 26 cells were 'multireceptive'. The response to pinch consisted of an initial large amplitude depolarization (8-16 mV) typically followed by long duration (up to 20s) slowly decaying EPSPs. In 27 cells the depolarization elicited cell firing but in 13 neurons the excitatory potentials were entirely subthreshold. Superfusion of CNQX (2-5μM) to 13 dorsal horn neurons (9 'multireceptive' and 4 'nociceptive specific') reduced the amplitude (80-90%) of the depolarization in response to pinch and completely abolished cell firing. This effect was reversible. In the 'multireceptive' neurones the response to light touch was also antagonized by CNQX.

These data support a fundamental role for non-NMDA receptors in the transmission of noxious sensory information to second order dorsal horn neurons in the mammalian spinal cord.

This research is supported by the Wellcome Trust.

211.12

TEMPOROMANDIBULAR JOINT STIMULATION IN THE RAT INDUCES C-FOS IN THE CAUDAL SPINAL TRIGEMINAL NUCLEUS.

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Central neural mechanisms underlying sensory and autonomic responses to nociception in deep craniofacial structures are not well-defined. This study examined the distribution of c-fos protein in brainstem neurons following injection of 10 µl of 20% mustard oil (c-fiber-specific irritant) into the left temporomandibular joint (TMJ) of barbiturate-anesthetized rats. The proto-oncogene c-fos is known to be induced rapidly and transiently in neurons by synaptic activity. Animals were processed for fos-like immunoreactivity (FLI) at 2h and 5h after injection using the avidin-biotin method. To assess the effect of adrenal steroids on FLI induced by TMJ stimulation, adrenalectomized (ADX) animals were included in the 2h group. Nuclei showing FLI were found throughout the trigeminal subnucleus caudalis (Vc) and in the caudal subnucleus interpolaris (Vi). Induction of FLI was greatest ipsilaterally in superficial laminae of the dorsal horn near the Vc/C1 junction (i.e. > 4 mm caudal to obex) and bilaterally in dorsal and ventral pockets in Vi about 1 mm caudal to the obex. About 20% of dorsal horn FLI cells were found in the deeper laminae of Vc. FLI in sections rostral to the obex was rare. At periobex levels, significant FLI was also seen bilaterally in the paratrigeminal nucleus, solitary tract nucleus, inferior olive, and ventrolateral medulla. Treatment effects were mainly changes in quantity rather than location of FLI. At all rostrocaudal levels, FLI was reduced at 5h survival when compared to 2h survival. ADX generally promoted an increase in FLI over that of the intact group particularly in the contralateral Vc and Vi. Ipsi- vs. contralateral differences in FLI varied with rostrocaudal position and survival time suggesting differences between sites in the nature and/or degree of response to TMJ stimulation. Extensive FLI induction from obex to cervical cord may indicate a functional diversity in the trigeminal involvement with the processing of nociceptive information from the TMJ. Supported by NIH grant NS26137.

211.13

DIFFERENTIAL EXPRESSION OF C-FOS IN TRIGEMINAL SUBNUCLEUS INTERPOLARIS AFTER CHEMICAL AND THERMAL CORNEAL STIMULATION IN THE RAT.

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The pathways that underlie autonomic responses to noxious corneal stimuli are uncertain. In the rat, the magnitude of select autonomic responses to corneal stimulation is stimulus-dependent. For example, plasma ACTH increases significantly after the application of the specific c-fiber irritant mustard oil (MO) to the cornea, whereas noxious corneal heat has little effect on ACTH. Both stimuli increase arterial pressure and heart rate in a similar fashion. This study examined if the differential ACTH response to MO and to noxious corneal heat was associated with a difference in the distribution of fos-like immunoreactivity (FLI) within the spinal trigeminal nucleus (Vsp). In addition, projection sites for FLI positive neurons to autonomic areas were studied using the retrograde tracer fluorogold (FG). The distribution of FLI within subnucleus caudalis (Vc) and interpolaris (Vi) was quantified in barbiturate-anesthetized rats after noxious corneal heat (52 °C, 20 s pulses for 15 min) or after 20% MO applied to the cornea. After 2h survival, rats were perfused and FLI was visualized by the avidin-biotin method. Bilateral injections of FG (100-200nl, 2%) into medial hypothalamus or parabrachial regions 7-10 days prior to MO stimulation was used to assess the projections of Vsp cells. Noxious corneal heat caused an increase in FLI mainly in the ipsilateral superficial laminae of caudal Vc. Although MO induced a similar increase in FLI in caudal Vc, a high percentage of FLI positive neurons also were present ipsilaterally within the ventral pole of the periobex Vi. FG injections into medial hypothalamus labeled many cells in ventral Vi, while injections into parabrachial areas labeled many cells in Vc. These results suggest that the difference in ACTH response to noxious stimuli may be related to the activation of second order neurons in periobex Vi. Supported by NIH grant NS26137.

211.14

A NEW RAT TOOTH STIMULATION MODEL: EVOKED REFLEX AND TRIGEMINAL (V) BRAINSTEM NEURON RESPONSES.

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We have developed a new technique to implant bipolar stimulating electrodes into the rat maxillary first molar tooth in order to study dental neural pathways and avoid well-known problems of current spread associated with the use of the rat incisor. The mean (± S.D.) threshold of the tooth-evoked digastric jaw-opening reflex (JOR) in 29 anaesthetized rats was 1.1 ± 0.8 mA and its shortest supra-threshold latency was 4.8 ms. For thresholds < 1.5 mA, a prolonged stable JOR threshold lasting > 4 hours was observed. Brainstem neuronal activity was recorded in the V subnuclei oralis and caudalis in 8 and 10 of these rats, respectively. Neurons (n = 278) were classified according to their cutaneous mechanoreceptive field properties as low-threshold-mechanoreceptive (LTM), wide dynamic range (WDR) and nociceptive-specific (NS) neurons and tested for incidence, threshold and latency of molar-evoked responses. Data were as follows:

	Number	Incidence	Latency	Threshold
Oralis LTM	187	37 %	3.3 ± 1.1 ms	2.7 ± 1.4 mA
Caudalis LTM	67	31 %	4.5 ± 1.9 ms	2.3 ± 1.4 mA
Caudalis WDR+NS	24	63 %	4.6 ± 1.7 ms	2.0 ± 1.7 mA

The findings represent the first documenting reflex and neuronal responses evoked by rat molar stimulation. Supported by NIH Grant DE04786.

211.15

VISUALISATION OF CAPSAICIN SENSITIVE DORSAL ROOT GANGLION CELLS AND THEIR CENTRAL TERMINALS.

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Capsaicin selectively activates a sub-population of unmyelinated and thin myelinated primary afferents (Holzer, Pharmacol. Rev. 43:144, '91). In this study a method used to show capsaicin sensitive cells *in vitro* (Winter, Neurosci. Lett. 80:134, '87) has been modified to demonstrate the distribution of these neurons in the intact DRG and their central terminal fields in the spinal cord.

Lumbar segments of hemisected spinal cords with DRGs attached were dissected from young rats anaesthetised by Enfluorane and transferred into a buffer containing (in mM) sucrose:139; NaCl:57.5; KCl:5; $MgCl_2$: 2; $CaCl_2$:1; glucose:12; HEPES:10; $CoCl_2$:5. Tetrodotoxin (400nM) was added to stop cell firing. Cobalt entry into cells through receptor activated Ca channels was induced by capsaicin (2µM). The Co ions taken up by the capsaicin sensitive cells were reacted with H_2S to form CoS. After fixation and sectioning the CoS precipitate was intensified by a silver developer.

Fifteen percent of the total DRG cell population was stained with cobalt. The diameter of labelled cells was in the range of 10-30µm, while unlabelled neurons measured between 10-50µm. In the spinal cord stained fibres and terminals formed a dense network around lamina I cells. In lamina II and in the medial part of lamina III fine fibres and a number of labelled boutons were found. From the lateral border of the superficial dorsal horn labelled fibres looped around to the lateral part of lamina IV.

The selectivity of this technique provides a powerful new tool to identify the cell bodies and central terminals of a particular class of chemosensitive nociceptors.

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211.16

DISTRIBUTION OF FOS-LIKE IMMUNOREACTIVITY IN THE MEDULLARY AND UPPER CERVICAL DORSAL HORN PRODUCED BY STIMULATION OF DURAL BLOOD VESSELS IN THE RAT.

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Previous studies indicate that sensory signals from cranial blood vessels (superior sagittal sinus and middle meningeal artery) converge on central neurons that also receive nociceptive inputs from facial skin. In order to investigate further the central organization of afferent pathways from vascular and cutaneous tissues, the distribution of fos-like immunoreactivity in the medullary and upper cervical dorsal horn produced by dural vascular stimulation was compared with that found in previous studies following mechanical, thermal, and chemical stimulation of cutaneous facial sites.

Dural stimulation was carried out under Brevital anesthesia in rats that had received a chronic surgical exposure of the transverse and superior sagittal sinuses 2-4 days earlier. The exposed dural surface was stimulated for 30-60 minutes with either mechanical (stroking with a blunt probe), chemical (capsaicin or formalin), or electrical (10mA, 1msec pulses) stimuli. Animals were perfused two hours after the end of stimulation and C4 to the obex was processed for fos-like immunoreactivity.

Dural stimulation produced a pattern of labelling in the dorsal horn that was similar for all of the stimulus modalities and that differed from the pattern produced by cutaneous facial stimulation. The dural-induced labelling in the dorsal horn was evenly distributed across a large rostrocaudal distance that extended from the midlevel of caudalis to the level of caudal C2 or rostral C3. In the transverse plane, the labelling was distributed primarily in the ventral (i.e., ophthalmic) half of the dorsal horn. This is a broader, more diffuse distribution than was found with stimulation of any single facial site, and corresponds to a region that represents approximately the dorsal half of the head and face from the intraural line to the beginning of the snout. The labelling was most heavily concentrated in lamina I, with less labelling in laminae III-V, and very little labelling in lamina II. This laminar pattern differs from that found with noxious facial stimulation, which produces heavy labelling in both lamina I and II. These results suggest that the dural afferent pathway may differ significantly in its central organization from sensory pathways that originate from facial skin.

211.17

POSTSYNAPTIC TARGETS OF CGRP IMMUNOREACTIVE VARICOSITIES IN LAMINA II OF THE RAT SPINAL TRIGEMINAL NUCLEUS: AN IMMUNOCYTOCHEMICAL AND INTRACELLULAR LABELLING STUDY IN FIXED TISSUE SECTIONS. J.V. Priestley* and S. Averill. Departments of Physiology & Biochemistry, U.M.D.S. St. Thomas's Medical School Campus, London, England SE1 7EH

Small diameter trigeminal and dorsal root ganglion cells can be divided into two major populations according to whether they express the neuropeptide calcitonin gene-related peptide (CGRP) or express an oligosaccharide which can be recognized by the monoclonal antibody (LA4) and the lectin *Griffonia simplicifolia* IB4. As part of ongoing research on the central terminations of these two populations, we have investigated the cell types in lamina II (LII) which are contacted by CGRP terminals.

100 µm sections of the spinal trigeminal nucleus were cut on a vibratome from rat medulla perfusion fixed with 4% paraformaldehyde. Under visual guidance, LII neurons were injected intracellularly with lucifer yellow. The dye filled the cell body, proximal and distal dendrites, spines, and occasionally also part of the axon. Injected cells were photo-oxidised, drawn at high magnification, and then cut into 30 µm sections on a freezing microtome. Sections were stained for CGRP using Ni enhanced PAP immunocytochemistry, embedded in Durcupan, and examined directly and after resectioning into 1.5 µm sections.

Stalked cells, islet cells and intermediate cell types were identified. Where dendrites passed through areas of CGRP immunoreactive terminals, contacts between them were observed. For example both stalked and islet cells which were located primarily in inner LII received contacts on their dendrites where they penetrated outer LII. No simple relationship was observed between innervation density and cell type.

211.18

RESPONSE PROPERTIES AND LOCATIONS OF AXONS OF LUMBOSACRAL SPINOTHALAMIC (STT) AND SPINOHYPOTHALAMIC (SHT) TRACT NEURONS IN RATS. J.T. Katter*, R.J. Dado and G.J. Giesler, Jr. Dept. of Cell Biol. & Neuroanat., Grad. Prog. in Neurosci., Univ. of Minn., Minneapolis, MN 55455.

Many neurons in sacral spinal cord are labeled after injections of retrograde tracers into the thalamus or hypothalamus. These neurons are located in regions that receive inputs from primary afferent fibers innervating lumbosacral dermatomes and pelvic visceral structures. We recorded extracellularly from neurons in L6-S2 that initially were activated antidromically with low currents (≤30 uA) from either the contralateral posterior thalamus (n=18) or the contralateral hypothalamus (n=6) in rats of both sexes. Recording points were concentrated in the dorsal part of nucleus proprius, and a few were located in the lateral reticulated area and the area around the central canal. Most receptive fields (RFs) included the perineum. Nine of thirteen neurons tested responded to hair movement within their RFs and did not show increased responses to increasingly intense mechanical stimuli including noxious stimuli. These nine neurons were classified as low threshold (LT) neurons. Two of the four LTs tested also responded to colorectal distention. None of the five LTs tested responded to heat or cooling. These results suggest that some STT neurons in lumbosacral cord of rats transmit information about innocuous cutaneous and visceral stimuli. Using microantidromic mapping techniques, axons of 14 neurons were found in the contralateral lateral funiculus in cervical cord: ten were located in the ventral lateral funiculus (VLF), one in the dorsal lateral funiculus (DLF) and three at the border between the VLF and DLF. Almost all axons were located near the lateral edge of the cord. This supports the idea that STT axons are arranged somatotopically with sacral axons located laterally. Supported by NS29276 and MH10059.

211.19

DIFFERENTIAL EFFECTS ON TRIGEMINAL NOCICEPTIVE SUBNUCLEUS CAUDALIS NEURONES OF INFLAMMATORY IRRITANT APPLIED TO CUTANEOUS VERSUS DEEP TISSUES. X.-M. Yu*, J.W. Hu and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, M5G 1G6 Canada.

We recently showed that injection of the inflammatory irritant mustard oil into the tongue muscle induces a significant and reversible expansion of both cutaneous and deep mechanoreceptive fields (RFs) of nociceptive skin/mucosa + deep convergent (S + D) neurones in subnucleus caudalis, and that the expansion is significantly greater for deep RFs. The present study tested if the relative expression of this cutaneous and deep RF neuroplasticity might depend on the specific source of the inflammatory irritation (i.e. cutaneous versus deep). Mustard oil (5µl) injected into the tongue muscle of anaesthetized rats produced a significant ($p < 0.01$) cutaneous and deep RF expansion, although there was no significant difference in incidence of expansion between S + D neurones with ($n = 8$) or without ($n = 11$) a documented tongue input. As a control, mustard oil injected into the gastrocnemius muscle did not change either cutaneous or deep RF properties of caudalis S + D neurones ($n = 3$). When mustard oil was applied to the facial cutaneous region overlapping the deep RF but outside the cutaneous RF of another 7 caudalis S + D neurones, no deep RF expansion occurred but a significant ($p < 0.05$) expansion of the cutaneous RF was documented for 6 of the 7 neurones. Thus, the mustard oil-induced expression of neuroplasticity in S + D neurones may depend on whether deep or cutaneous regions are the source of the inflammatory irritation. The changes in both cutaneous and deep RF properties by deep injections of mustard oil, as opposed to the more limited changes with its cutaneous application, may explain clinical reports of greater sensory disturbances in pain conditions involving deep tissues than those involving cutaneous tissues. Supported by NIH grant DE09559.

211.20

C-FOS EXPRESSION IN THE LUMBAR SPINAL CORD OF THE DEVELOPING RAT INDUCED BY NOXIOUS STIMULI. D. Yi and G. A. Barr*. Biopsychology Doctoral Program, Dept. of Psychology, Hunter College-CUNY, NY, NY 10021 and Dept. Developmental Psychobiology, , New York State Psychiatric Institute, NY, NY 10032.

There are a number of important unanswered questions regarding how pain is processed during early development. In this study, we used the *c-fos* oncoprotein as a marker to examine the development of pain systems in the spinal cord of infant rats using different types of noxious stimuli. One to two-day-old unanesthetized rat pups were injected with 15 µl of 10 % formalin into the right hindpaw and were sacrificed 2 hours later. The spinal cord was processed for immunocytochemistry using a commercial antibody for the *c-fos* protein. *c-Fos*–like immunoreactivity was found ipsilaterally in the superficial dorsal horn and in lamina V of the lumbar spinal cord. At 14 days of age, under light methoxyflurane anesthesia, the right hindpaw was submerged in the hot water bath, 52 °C, four times for 10 seconds each at 5 minute intervals. Pups were sacrificed 2 hours after the termination of the stimulation. Ipsilateral *c-fos* –like labeling was observed in the medial portion of the superficial layer of the lumbar enlargement of the spinal cord. The results obtained from the younger animal are consistent with previous results that suggest that the processing of noxious stimulation occurs at least at the level of the dorsal horn shortly after birth. The differences observed in the pattern of c-fos labeling for the two age groups could either be due to the age difference or to the difference in the type of noxious stimulus used. Further studies are examining those differences.

PAIN MODULATION: COGNITIVE, AUTONOMIC AND ENDOCRINE

212.1

MORPHINE ATTENUATES THE HABITUATION OF NOVELTY-INDUCED HYPOALGESIA. J. Rochford* & E. O'Brien. Douglas Hospital Research Center, , McGill University, Verdun, Quebec, H4H 1R3, Canada.

Exposure to novel stimuli, such as a nonfunctional hot plate apparatus, has been shown to induce hypoalgesia. With repeated exposure, novelty-induced hypoalgesia (NIH) habituates. The present experiments were conducted to determine the effect of morphine administration on the rate of habituation.

Male, Wistar rats (275-300g) were administered 5 mg/kg morphine (Group MOR) or saline (Group SAL) prior to being exposed to a nonfunctional hot plate apparatus for three days. On the fourth day all rats were administered saline and tested for pain sensitivity on a functional (48.5°, 50.0° or 52.0° C) hot plate. This four day sequence was then repeated twice more. Paw lick latencies (PLLs) declined over repeated tests. However, the rate of decline was retarded in group MOR (as indicated by longer PLLs), suggesting that morphine attenuated the habituation of NIH. Futher experiments have demonstrated that (1) the hypoalgesic efficacy of morphine is lower in group MOR than in group SAL; (2) both the attenuation of NIH and the lowered hypoalgesic response to morphine are not observed if animals are exposed to a stimulus other than the hot plate apparatus. Thus, morphine administration alone is not sufficient to account for these effects, they require both morphine administration *and* exposure to the plate apparatus; (3) the longer PLLs observed in group MOR are not affected by pretreatment with 7 mg/kg naltrexone; (4) morphine attenutes the habituation of NIH within a dose range of 5-20 mg/kg.

These data indicate that morphine administration can influence changes provoked by learning on a stress-induced hypoalgesic response, and that such an influence in turn alters the pain inhibiting properties of the drug itself. Funded by NSERC

212.2

BILATERAL LESIONS OF THE CENTRAL OR BASOLATERAL NUCLEUS OF THE AMYGDALA ATTENUATE THE ANALGESIA THAT RESULTS FROM FEAR-INDUCING STIMULI. B.M. Morrison and C.A. Sorenson*. Neuroscience Program, Amherst College, Amherst, MA 01002.

The roles that the central (CNA) and basolateral (BLA) nuclei of the amygdala play in analgesia induced by environmental challenges were investigated through selective bilateral electrolytic lesions (mean destruction: 93.5 percent of CNA or 68.5 percent of BLA). Either lesion attenuated the analgesia, as measured by the tail-flick apparatus, that resulted from cat exposure or classically conditioning an environment to footshocks. Cat exposure and classical conditioning were chosen as the environmental challenges because they induce fear through unconditioned and conditioned means, respectively. Previous experiments had determined that the BLA is involved in the analgesic response to conditioned fear, but this experiment is the first to show that lesions of the BLA attenuate unconditioned fear-induced analgesia. This result, along with the equal magnitude of attenuation of analgesia that resulted from CNA and BLA lesions, support the hypothesized role of the BLA as providing a sensory input to the CNA. In addition, the manipulations that reduced analgesia in this experiment have been shown in other studies to reduce fear, indicating that cat exposure and classical conditioning may induce analgesia through a fear mechanism.

212.3

ADRENALECTOMY BLOCKADE OF OPIATE ANALGESIA IS NOT DUE TO STRESS-INDUCED RELEASE OF HORMONES. L Sutton, M. Fleshner, S.F. Maier* & L. Watkins. Department of Psychology, University of Colorado, Boulder, CO 80309 USA.

Inescapable tail shock (Sk) produces 3 sequential analgesic states as numbers of Sks (5 s, 1 mA) increase: an early opiate (after 2 Sks), a non-opiate (after 5-40 Sks) & a late opiate (after 80-100 Sks). We tested if adrenalectomy (ADX) would affect any of these analgesias &, if so, whether such blockade(s) was due to shock-induced release of adrenal hormones.

We first tested ADXs vs. shams 2 wks post-surgery. Rats received 7 mg/kg naltrexone or saline 15 min before testing baseline pain sensitivity (tail flick [TF]). Sk began right after baseline testing. TF latencies were recorded after 2, 5, 10, 20, 40, 60, 80 & 100 Sks. Both ADX & naltrexone blocked 2 Sk & 80-100 Sk opiate analgesias. The fact that 2 Sk analgesia was blocked implied that Sk-induced hormone release could not be critical, since release is minimal at this time. Since steroids are tonically required for synthesis of various neurotransmitters, perhaps this was the mechanism involved.

If this idea were correct, then it should be possible to test ADX rats shortly after surgery, before long-term changes in neurotransmitter levels have occurred. In this case, despite no circulating adrenal hormones, no reduction in either 2 or 80-100 Sk analgesia should occur. This is indeed what we observed at 48 hrs after ADX. Thus, stress-induced release of adrenal hormones cannot account for the effects observed at 2 wks after surgery.

To determine whether basal levels of cortical or medullary factors are critical, rats were tested 2 wks after ADX, after receiving chronic basal adrenal replacement in their drinking water. Basal steroid replacement abolished the effect of ADX at 2 wk post-surgery.

In sum, these data call for a re-evaluation of the suggestion that some forms of analgesia can be classified as hormonally mediated. Instead, it may be that such analgesias simply rely on neurotransmitters which require adrenal steroids for their synthesis.

212.4

HYPERALGESIA BY SUCKLING IN LACTATING RATS IS ADRENAL GLAND MEDIATED. Martínez-Gómez,M.*; Y. Cruz; P.Carrillo; A.Loranca; F.Mena and P.Pacheco CIRA,Univ. Autón. Tlaxcala; Instituto de Neuro-Etología, Univ. Veracruzana; IIB-UNAM, MEXICO.

As compared with virgin rats Tail Flick Latency (TFL) shows reduction during the three thirds of lactation. Hormonal condition, suckling, pups presence and mammary gland emptying seem to participate in this phenomenon. Present study analyzes this possibilities. After pups separation (6 hs) mothers showed TFL higher than those during suckling. Pups presence did not reduce TFL in both lactating and virgin rats in which maternal behavior was induced. On the other hand, in these virgin rats pups presence and suckling-like stimulation increased TFL. Milk ejection blockade by ducts glued did not impeded suckling hyperalgesic effect (SHE). An hour after suckling a second period performed by new and hungry pups (when mammary glands still are emptied) produced SHE again. Ovariectomy on day 7 or 14 of lactation did not alter the low levels of TFL. Adrenalectomy on day 14 of lactation increased TFL to virgin or weaning levels. CONCLUSIONS: During lactation suckling determines hyperalgesia through adrenal activation.

SEP 92 (MM-G) SEP 92 (PP)

212.5

HYPOALGESIA IN RESPONSE TO NOISE STRESS IS BLOCKED BY LESIONS OF THE AMYGDALA AND PERIAQUEDUCTAL GRAY P.S. Bellgowan,* & F.J. Helmstetter, Department of Psychology, University of Wisconsin,Milwaukee, WI 53211

Prior studies have demonstrated that a single brief exposure to moderately intense (75-95 dB) white noise is sufficient to activate opioid-mediated antinociceptive systems in the rat. This form of stress-induced hypoalgesia is attenuated by systemically administered benzodiazepines and may represent a response to anxiety, sensitization or fear. Our recent data indicate that a neural circuit which includes the amygdala, periaqueductal gray and rostral medulla is critical for the expression of hypoalgesia in response to auditory stimuli that have been paired with footshock during Pavlovian conditioning. This study was conducted to find out if this same neural circuit is responsible for hypoalgesia following noise stress. Male rats received small bilateral electrolytic lesions of the central or lateral nuclei of the amygdala or the ventrolateral periaqueductal gray. Following several baseline radiant heat tail flick (TF) trials subjects were exposed to a single 60s presentation of 90 dB white noise. TF testing continued at 2 min intervals for 12 min. In all cases, baseline TF latencies for lesioned animals did not differ from corresponding groups of sham-operated controls. Control rats showed a time-dependent hypoalgesia following noise. The elevation of TF latency following noise presentation was abolished or attenuated in all lesion groups. These results support the idea that anxiety-related hypoalgesia and defensive behavior share a common neural substrate.

212.6

INVOVEMENT OF PITUITARY BETA-ENDORPHIN IN ANALGESIA PRODUCED BY STIMULATION OF HYPOTHALAMIC ARCUATE NUCLEUS. Q.-Z. Yin, F.Gu, M.-Y. Zhu, N.Zhang and L. Mei * Lab.of Neurobiology, Su-Zhou 215007, P.R.China.

Previous studies have demonstrated that hypothalamic arcuate nucleus(ARC) stimulation could result in a naloxone-reversible analgesia accompanied by an elevation of brain β-endorphin (β-END) level. As the ARC isone of the nuclei in hypophysiotropic area and the pituitary β-END can flow retrogradely via portal vessel system into the brain, hypophysectomy and dexamethasone block were used in this experiment to investigate the role of pituitary β-END in analgesia produced by ARC stimulation.Sham ARC stimulation and sham hypophysectomy were performed as control. As previously, electrical stimulation of ARC produced a marked analgesia with concomitant increase of brain β-END level. The analgesia could be blocked by dexamethasone injection. After hypophysectomy ARC stimulation could neither produce analgesia nor increase the brain β-END level. The results suggest that the pituitary β-END pool might be activated and released by ARC stimulation and flow retrogradely into the brain to result in the analgesia.

212.7

THE EFFECT OF CONDITIONED STRESS AND MORPHINE ON GLUCOSE UTILIZATION IN THE RAT. Gescuk, B.,* Lang, S. and C. Kornetsky. Boston Univ. Sch. of Med. Boston, MA 02118.

This experiment determined the effects of morphine sulfate (MS) on local cerebral metabolic rates for glucose ($LCMR_{glu}$) in male F-344 rats previously exposed to chronic, escapable footshock (conditioned stress or CS). Four groups of animals were studied: control-saline, control-MS, CS-saline, and CS-MS. The two CS groups were exposed to 11 days of half hour sessions of footshock. $LCMR_{glu}$ experiments were carried out without stimulation in the same shock-chamber. All animals were given MS (4mg/kg sc) or saline 7 days, 3 days, and 10 min prior to the start of the experiment. The only significant effects of CS compared to saline control were decreases in the diagonal band and medial habenula. MS significantly decreased metabolic activity in 22 brain regions. In several of these regions, MS in the presence of CS caused a further decrease in $LCMR_{glu}$ when compared to MS alone. However, there were significant increases in 3 thalamic nuclei: anteroventral, gelatinosus, and parafasicular. Interestingly, in the hippocampus (dentate gyrus, CA1), MS alone caused significant increases in $LCMR_{glu}$; but, in the presence of CS, MS decreased metabolism. These results suggests that the effects of MS on brain activity can vary (especially in thalamic nuclei, hippocampus, and some limbic regions) as a result of exposure to a stressful environment. (Supported by grant DA02326 and DA00099 to CK).

212.8

PHYSIOLOGIC RESPONSE AND PERCEPTION OF PAIN IN THE AGED. F Porter, JP Miller, J Morris, L Berg* Washington University School of Medicine, St Louis, MO 63110.

As a prelude to a study of pain in the aged with dementia, ECG and respiration were continuously recorded during undisturbed BASEline (13.2±3.0min), PREParation (tourniquet/cleansing/needle visualized) (.8±.8min), required VENIpuncture (1.6±.3min), and undisturbed RECovery (10±2.6min) from 48 (33 female; 45 white) visually intact, non-demented, ≥65 yr old (X=76±5 yrs) adults. Physiologic response was assessed by change in heart rate (HR), HR variability (HRV), heart period (HP), and respiratory rate (RR). Anxiety, pain and fearfulness were rated by subjects on 10-point visual analog scales and correlated with physiologic responses. A battery of neurocognitive tests was administered to assess cognitive performance. Significant increases in HR and RR and decreases in HRV and HP occurred in response to PREP. In contrast, HR and RR decreased and HP increased significantly from PREP to VENI yet differed at VENI from BASE. All measures returned towards BASE during REC. Poorer cognitive performance was associated with less physiologic response to PREP and greater physiologic response to VENI. Magnitude of physiologic response was not associated with age. Mean anxiety at PREP was 2.7; mean pain rating at VENI was 1.1. Magnitude of physiologic response to VENI was significantly related to ratings of pain sensitivity and fearfulness at BASE. We conclude that: 1) a cognitively based event (PREP) elicits greater magnitude physiologic response than does a mild physiologic insult (VENI); and 2) cognitive performance is differentially related to the magnitude of physiologic response to cognitive vs physical events. These data suggest that cognitive preparation may reduce response to pain among older adults. (Supported by 5P50AG05681 and AG06815)

212.9

FORMALIN INDUCED PAIN DOES NOT AFFECT ACTH AND CORTICOSTERONE PLASMA LEVELS IN RAT. A.M. Aloisi and G. Carli. Ist. Fisiologia Umana, Università di Siena, via Laterina 8, I-53100 Siena, ITALY.

It was the aim of the present research to evaluate the amount of Corticosterone (C) and ACTH release during a condition of persistent pain, indipendently from others environmental factors. Male Wistar rats were group-housed, familiarized with the open field and tested during the dark phase. Groups varied according to a) treatment: formalin (50 µl at 0.1 and 10%), sham injection, restraint (30 min) and control; b) time of blood collection (30 and 60 min after treatment). Pain levels, assessed according to licking duration, flexion and paw jerk were higher in the 10% than in 0.1% formalin group. C displayed the same levels in formalin treated, sham injected as well as in control group, being elevated in the restraint one. ACTH was found increased 30 min after formalin (10%) and in the restraint group with respect to all the others. In conclusion, it is possible to elicit tonic pain without a clear involvement of the pituitary-adrenal axis.

212.10

CARDIOPULMONARY INPUT TO PUTATIVE PAIN MODULATORY NEURONS IN THE ROSTROVENTRAL MEDULLA. C.L. Thurston*, and A. Randich, Dept. Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Neurons in the rostroventral medulla (RVM) have been classified based on their response to a noxious stimulus and the evoked withdrawal reflex. ON cells are excited and OFF cells are inhibited by noxious stimuli. We recently showed that ON cell activity is negatively correlated and OFF activity is positively correlated with mean arterial blood pressure (MAP). The present study demonstrated that increases in MAP excites OFF cells and inhibits ON cells whereas decreases in MAP inhibits OFF cells and has no effect on ON cells. In rats with bilateral sinoaortic deafferentations, spontaneous correlations between RVM neurons and MAP are still evident. However, experimentally-produced changes in MAP do not affect RVM neural activity. These data suggest that ON and OFF cells not only receive baroreceptor input, but also either produce changes in MAP or receive input from other neurons that produce changes in MAP.

212.11

EFFECTS OF LESIONS OF THE ROSTRAL MEDULLA AND VENTRAL PERIAQUEDUCTAL GRAY ON ANTINOCICEPTIVE AND CARDIOVASCULAR CONDITIONAL RESPONSES

S.A. Tershner*& F.J. Helmstetter, Department of Psychology, University of Wisconsin, Milwaukee, WI 53211

Upon presentation of an auditory stimulus that has been paired with footshock rats will respond with hypoalgesia and a transient increase in mean arterial blood pressure. The present study was designed to determine if the performance of these Pavlovian conditional responses is dependent on the rostral ventral medulla (RVM) and periaqueductal gray (PAG). Independent groups of subjects were first exposed either to paired or unpaired presentation of an auditory stimulus and footshock. After training all rats were prepared with chronic femoral catheters for measurement of arterial blood pressure (AP). Separate groups of rats within the paired and unpaired training conditions were given electrolytic lesions of the RVM or PAG. Control rats had electrodes lowered but no current was passed. Following adaptation to restraint subjects were tested using the radiant heat tail flick (TF) test. In non-lesioned animals that received paired training, presentation of the auditory signal resulted in significant elevation of TF latency and AP. Unpaired controls did not show these responses. Lesions of the ventrolateral PAG, and RVM lesions that primarily involved the n. raphe magnus and n. paragigantocellularis eliminated hypoalgesia but did not affect conditional elevations in AP. These data support the idea that the PAG and RVM are critical structures in the neural circuit subserving hypoalgesia as a conditional response.

212.12

Central cholinergic inhibition of the vesicoanal (VA) reflex model of urinary bladder irritation. K.B. Thor* and M.A. Muhlhauser, CNS Research, Lilly Research Laboratories, A Division of Eli Lilly and Co., Indianapolis, IN 46285

Previous studies showed that endogenous and exogenous cholinergic muscarinic (Ach-M) agonists can suppress somatic nociception. The present studies were conducted to determine if Ach-M systems can suppress visceral nociception. Bladders of urethane-anesthetized rats were cannulated thru the dome for continuous-infusion cystometrogram recordings. EMG electrodes were inserted into the anus and the peri-urethral musculature for recording sphincteric activity. Infusion of 0.5% acetic acid into the bladder produced modest increases in bladder and urethral sphincter activity and pronounced increases in anal sphincter activity (i.e. VA reflex). Oxotremorine (OXO, 1-30 µg/kg iv) and compound 296182 (0.01-1.0 mg/kg iv), centrally-active cholinergic agonist and partial agonist, respectively, produced a dose-dependent inhibition of the anal sphincter activity (to 10% of control) that was reversed by atropine (ATR, 1-3 mg/kg), but not by doses of methylscopolamine (MSC, 1-30 ug/kg) that did block carbachol-(1µg/kg), 296182- and OXO-induced bradycardia. This indicates that OXO- and 296182-induced inhibition of the VA reflex was centrally mediated. Furthermore, ATR (but not MSC) given alone transiently (15 min.) increased anal sphincter activity, indicating that endogenous acetylcholine was partially suppressing the VA reflex via tonic muscarinic mechanisms. Finally, physostigmine, but not neostigmine, also suppressed the VA reflex in a dose-dependent, ATR-sensitive manner, reinforcing the proposal of endogenous Ach-M suppression of the VA reflex.

212.13

Vesicoanal (VA) reflex activity in the rat: a model of urinary bladder (BL) irritation. M.A. Muhlhauser* and K.B. Thor, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285

Irritation of the BL activates "silent nociceptors". While *c-fos* expression is used to monitor BL nociceptor activity, the technical limitations complicate pharmacological experiments. Thus, the following model was developed. BL of urethane-anesthetized rats were cannulated through the dome for cystometrogram recording. Anal (AS) and urethral (US) sphincter EMG activity were recorded. AS potentials were of 2 types, short (1-3 msec) and long (10-50 msec) duration that were succinylcholine- and methylscopolamine-sensitive, respectively, indicating striated and smooth components. US activity was completely succinylcholine-sensitive. During saline infusion, BL contractions always evoked US activity but only occasionally striated AS activity. (AS smooth muscle activity was never correlated with BL activity and is not discussed further.) In every rat, infusion of 0.5% acetic acid (AA) produced robust increases (2-4 fold) in striated AS activity (*i.e.* VA reflex), which was highly-correlated with BL activity. Topical BL application of capsaicin (CAP) desensitized the AA-induced increase in AS activity. In saline-infused BL, topical CAP mimicked the AA-induced BL-related increases in AS activity. CAP pretreatment (50-125 mg/kg s.q., 4-5 days prior) eliminated AA-induced increases in AS activity. Finally, analgesic compounds, *i.e.* cholinergic agonists and substance P antagonists, reduced the AA-induced increase in AS activity. These studies indicate that striated AS activity is a sensitive indicator of BL nociceptor activity and that the VA reflex model is useful for pharmacological studies aimed at suppressing these nociceptors.

212.14

RELEASE OF ß-ENDORPHIN-IR FROM BRAIN IS REGULATED BY A HYPOTHALAMIC NMDA RECEPTOR F.W. Bach, T.L. Yaksh, and M. Lauritzen*, Dept. Anesthesiology, University of California San Diego, La Jolla, CA 92093-0818

ß-endorphin (ß-EP) is located in cell bodies in the arcuate nucleus of the hypothalamus and its major projections to the periaqueductal gray area in the brainstem are believed to play a role in antinociception. Systematic studies of the pharmacology of receptors governing the release of ß-EP are lacking. We examined the effects of the glutamate receptor agonist NMDA on the release of ß-EP-ir from arcuate nucleus neurons and on the motor response to a noxious heat stimulus. Rats were anesthetized with halothane. A microinjection cannula was stereotaxically implanted in n. arcuatus, and perfusion with artificial CSF was established through cannulae in the lateral ventricle and cisterna magna. Rats were kept at 1-1.2% halothane after the operative procedure. After 30 min of perfusion 0.5 µl of drug or saline was injected, followed then by collection of three 10 min post-injection CSF samples. Antinociception was evaluated by the latency to tail flick after dipping the tail in water heated to 52.5°C immediately before and 4 min after injection. Ten µg, but not 1 µg NMDA significantly increased the release of ß-EP-ir (2-way-ANOVA, $p<0.001$), as compared to saline. Pretreatment with 1.5mg/kg MK-801 i.p. blocked the ß-EP-ir release. The tail dip response was blocked by injection of 10µg NMDA in 10/12 rats, by 1µg NMDA in 6/9, by MK-801+10µg NMDA in 1/6, and by saline in 0/9. These results suggest a possible role for glutamatergic input into ß-EP cells in the hypothalamus, as part of a system which regulates the antinociceptive response to a high threshold stimulus.

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212.15
AUTONOMIC SENSITIVITY AND REACTIVITY IN HUMAN PAIN.
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Human pain responsiveness was previously demonstrated in a tonic pain model of cold pressor test (Chen, PAIN 37:143-160, 1989) whereby healthy subjects clearly being categorized into pain sensitive (PS) and pain tolerant (PT) groups. This report extends the observation of differences in EEG activities (Chen, PAIN 37:129-141, 1989) to the autonomic activities between the PS and PT groups under the cold pressor test. The PS group (n=11) endured a mean+/-s.e. of 48.82+/-3.06 sec while the PT group (n=23) could tolerate the entire 3 min of the tonic pain test (t=35.22; p<.0000). The PS group rated a VAS score of 78.69+/-5.99 vs 58.29+/-4.08 for the PT group (t=2.77; p<.007). Autonomic sensitivity and reactivity showed significant differences in these two groups. At the resting baseline state, PS showed marked higher diastolic blood pressure (82.08+/-3.45 mmHg) than that (66.52+/-0.49 mmHg) of the PT group (t=3.65; p<.0009). In response to the cold pressor test, the PS and PT groups exhibited significant opposite patterns of systolic blood pressure change (decrease of 6.18+/-2.91 vs increase of 1.13+/- 2.06 mmHg, respectively; t=2.05, p<.048). Most strikingly, the diastolic blood pressure displayed a drastic contrast between the PS and PT groups (decrease of 9.83+/-3.72 vs increase of 11.04+/-2.74 mmHg, t=4.51, p<.0001). Further, in the group as a whole, the resting systolic blood pressure was found inversely correlated with the subjective pain rating upon the cold pressor test, primarily through the influence of the PT group alone. These results taken together indicates that the autonomic sensitivity and reactivity can differentiate the individual differences in human pain responsiveness. Plausible neurobiologic mechanisms for the autonomic sensitivity and reactivity to pain in man will be addressed.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CORTEX II

213.1
PRIMATE FRONTAL CORTEX: EFFECT OF INSTRUCTIONAL SIGNIFICANCE ON ACTIVITY FOLLOWING IDENTICAL VISUAL CUES.
S.P. Wise* and D. Boussaoud. Lab. Neurophysiology, NIMH, Poolesville, MD 20837
We examined neuronal activity in dorsal (PMd) and ventral (PMv) premotor cortex and in prefrontal (PF) cortex of two rhesus monkeys. Each monkey fixated a 0.2° white square in the center of a video display while depressing a switch located between two touch pads. Red or green squares (2x2°) served as cues. On each trial, a spatial/attentional cue (SAC) was presented for 0.8 s. The SAC consisted of one square, red or green, and its location indicated where a relevant motor instructional cue (MIC) would appear 1.8 to 3.3 s later. The MIC consisted of either one square (red or green) or both a red and green square presented simultaneously. A green square at the previously cued location instructed a forelimb movement to the right touch pad; red instructed movement to the left. At the offset of the MIC (duration 1 to 3 s), the monkey had to execute a forelimb movement within 1 s (and could break fixation).

Because the SAC could occur in different locations, a MIC with two squares could be physically, retinotopically and craniotopically identical, but instruct diametrically opposite limb movements. Thus, we tested the hypothesis that task-related activity reflects the direction of limb action rather than the sensory stimuli that guide movement.

Our results from a sample of 91 frontal neurons show that the majority in PMd (65%), but only a small minority in PF (18%), have phasic and tonic activity that depends on the motor significance of the stimulus. In PMv, 41% of tested neurons show such a movement effect. Thus, for most cells in PMd, the activity during the MIC and before the onset of movement reflects the direction of the upcoming movement and appears to code for aspects of action rather than the sensory signals that guide the act. By contrast, cells in PF appear to have activity more closely linked to the instructing stimuli, whereas PMv cells have intermediate properties.

213.2
FUNCTIONAL CLASSIFICATION OF CELLS IN PRIMATE MOTOR CORTEX BASED ON SPIKE TRAIN ANALYSES.
M. Taira* and A.P. Georgopoulos. Brain Sciences Center, Veterans Affairs Medical Center, Minneapolis, MN 55417.
In extracellular recordings, the cell type (e.g. pyramidal or local interneuron) is unknown. We developed a statistical technique to classify spike trains to a minimum of cell types. We analyzed spike trains from 1,925 cells recorded in our laboratory while monkeys held their arm in a stable posture preceding the onset of a visual target. First, we used a clustering analysis to identify the measures that would separate most effectively the cells into 3 groups. These were the mean firing rate, the trimean of the inter-spike interval (ISI) distribution, the percent of ISI<20 ms, and a composite measure of bursting activity. This classification was then validated using a discriminant analysis which showed that 96.3% of the cells were correctly classified in their respective groups. The 1st group (59.6% of cells) had low discharge rate, high trimean ISI, small percentage of ISI<20 ms, and low bursting. The 2nd group (19.3%) had low discharge rate, high trimean ISI, intermediate percentage of ISI<20 ms, and high bursting. The 3rd group (21.1%) had high discharge rate, low trimean ISI, high percentage of ISI<20 ms, and low bursting. These groups are very similar to those distinguished by other investigators in the neocortex: low frequency or bursting (presumably pyramidal) and high frequency (presumably local interneurons). Finally, the percentage of directionally tuned cells was very similar in all three cell groups. This finding suggests that directional operations are distributed in the motor cortex among presumably pyramidal and local interneurons.

213.3
RELATIONS OF SINGLE CELL ACTIVITY TO MOVEMENT PARAMETERS IN MOTOR CORTEX AND AREA 5.
A.P. Georgopoulos* and J. Ashe. Brain Sciences Center, Veterans Affairs Medical Center, Minneapolis, MN 55417.
The relations of ongoing single cell activity in the arm area of the motor cortex and area 5 to parameters of evolving arm movements in 2D space were determined using a multiple linear regression model in which the trial-by-trial time course of cell activity, at time $t+\tau$, was expressed as a function of the position, velocity and acceleration of the hand at time t, and of a constant corresponding to the visually defined target direction; τ was a time lag (-100 to +200 ms). We analyzed the impulse activity of 290 motor cortical and 207 area 5 cells recorded previously in our laboratory. (a) In all cases, the regression model above was statistically significant (F-test, $P<0.05$) at the lag with the highest R^2. (b) The median R^2 was 0.55 and 0.46 for motor cortex and area 5, respectively. (c) The median lag at which the highest R^2 observed was -110 ms and +20 ms for motor cortex and area 5, respectively. (d) For the motor cortex, velocity was the most important parameter in accounting for variation in cell activity in 41.72% of the cells, followed by the constant target direction (41.38%), position (8.62%), and acceleration (8.28%). For area 5, the most important parameter was velocity (47.34% of cells), followed by the constant target direction (33.82%), position (14.01%) and acceleration (4.83%). (Supported by NIH.)

213.4
MOTOR CORTICAL ACTIVITY PRECEDING A MEMORIZED MOVEMENT TRAJECTORY WITH A 90° DIRECTIONAL BEND.
J. Ashe*, N. Smyrnis, M. Taira, T. Georgakopoulos, J.T. Lurito, and A.P. Georgopoulos. Brain Sciences Center, Veterans Affairs Medical Center, Minneapolis, MN.
Two monkeys were trained to make a previously memorized arm movement with a 90° directional bend, first up and then to the left (⌐), following a waiting period. They held a 2D handle over a spot of light at the center of a planar working surface. When this light went off, the animals were required to hold the handle there for 0.6-1 s and then move it up-and-to-the-left to receive a liquid reward. There were no external *go* signals. Following 20 trials of making the memorized movement trajectory, 5 trials of visually triggered movements in 8 radially arranged directions were performed. The activity of 143 arm-related single cells in the motor cortex was recorded extracellularly during performance of the task. 58% of these cells changed activity during the waiting period. During the waiting period, the neuronal population vector began to grow in length approximately 170 ms following the time at which the center light went off. The population vector pointed first in the direction of the endpoint of the movement (↖) and then changed direction gradually during the remainder of the waiting period towards the direction of the initial (↑) component of the upcoming movement. (Supported by NIH, HFSP.)

213.5

ARE EXTENT AND FORCE INDEPENDENT MOVEMENT PARAMETERS? Alexa Riehle[1], William A. MacKay[2*], [1]Cognitive Neuroscience Lab., CNRS, 31 ch. J. Aiguier, 13402 Marseille 09, France; [2]Dept. of Physiology, University of Toronto, Toronto M5S 1A8, Canada.

Movement extent and force can be independently controlled in motor performance. Therefore, independent representations of extent and force should exist in the CNS. To test this hypothesis, two levels of frictional resistance were applied to a manipulandum by means of which wrist flexion movements of two extents were performed. A first, preparatory signal (PS) provided complete, partial or no information about extent and/or force of the movement, which had to be performed in response to a second response signal (RS). The activity of about 400 neurons of the primary motor cortex (MI), the premotor cortex (PM), the primary somatosensory cortex (SI) and the parietal cortex (PA) was recorded in two monkeys. Preliminary analysis of the data suggests that different neuronal processes underly the programming of force and extent. On the one hand, partial information about either movement parameter shortened reaction time, when compared with the condition of no prior information. On the other hand, there were, among others, two discrete populations of neurons, one related only to extent, the other only to force. Furthermore, we conducted trial-by-trial correlation analyses between reaction time and neuronal activity during the preparatory period for all conditions of prior information. The mean correlation coefficient was significantly higher in the condition of information about movement extent than of information about force, and it was significantly higher in MI/PM than in SI/PA.

Supported by ONR grant number 00014-89-J1557.

213.6

NEURONAL SPECIFICATION OF DIRECTION AND DISTANCE DURING REACHING IN THE PREMOTOR AREA AND PRIMARY MOTOR CORTEX OF MONKEYS. Q.-G. Fu*, J.I. Suarez and T.J. Ebner, Depts. of Neurosurgery & Physiology, Univ. of Minnesota, Mpls., MN 55455.

In two primates we studied the activity of 186 neurons in the premotor area and motor cortex during a reaching movement. The task involved reaching to 48 targets in the horizontal plane (8 different directions at 6 distances). The neuronal activity before the onset of movement (PT) and during the movement (MT) was analyzed. Based on a cosine tuning function the majority of neurons were related to movement direction either in PT (73.7%) or in MT (68.3%). The cell's preferred direction did not change with movement distance. Using a simple linear regression model many cells had a significant relationship with distance in PT (46.8%) and in MT (68.8%). Distance modulation was not correlated with a cell's preferred movement direction. Both the correlation coefficient and the regression slope were distributed uniformly over the movement directions relative to a cell's preferred direction. Using a multivariate regression model based on distance, direction as well as interaction terms, a continuum of response types were described ranging from 1) cells with only direction encoding, 2) cells with only distance encoding and 3) cells requiring both distance and direction to describe the response. The model accurately predicted the response profile in 79.3% of the cells. The results show in the premotor area and primary motor cortex both direction and distance are encoded in parallel. Supported by Grant NSF/BNS-8707572 and the Human Frontier Science Program.

213.7

CORRELATIONS OF PRIMARY MOTOR CORTICAL SINGLE-UNIT ACTIVITY WITH EMG DURING A BEHAVIORAL TASK. J. L. Adams* and A. B. Schwartz. Barrow Neurological Institute, 350 W. Thomas Road, Phoenix, Arizona 85013.

We compared single-unit activity in the primary motor cortex to EMG activity of the proximal arm muscles as a monkey (Macaca mulatta) performed drawing movements. We were particularly interested in the way this correlation evolved throughout the course of the movement. In macaques the corticospinal pathway to proximal arm muscles is multisynaptic. This indirect linkage as well as intratrial nonstationarity make it difficult to apply standard cross-correlation analysis.

One of the problems in detecting correlation between two time-varying neurological signals over a long time period (> 30 msec) is that any rhythmic variation within the individual signals will tend to contribute to the correlation between the signals. This was especially important in our experiments, since the movements the monkeys were performing were periodic, complicating the structure of cross-correlations. This was solved by using the *impulse response function* which removes the periodic changes.

In order to visualize the changes between motor cortical activity and proximal-arm EMG, the impulse response function was calculated in overlapping 350 msec windows throughout the task. Simulated spike trains and EMG patterns were also tested with this analysis. The correlation between spikes and EMG could be examined for particular epochs in the simulation. As well as the impulse response function, standard cross-correlation and spike-triggered averages were calculated. In the collected data we found that most of the cortical cell - muscle pairs showed transient correlation through each movement.

(NIH- NS26375)

213.8

COMPARISON OF RESPONSES FROM CORTICAL AREAS 4 AND 6 DURING DRAWING. A. Kakavand and A.B. Schwartz*. Barrow Neurological Institute, 350 West Thomas Road, Phoenix, AZ 85013.

Single-unit activity was recorded from two cortical areas as a monkey drew spirals on a touch screen with its contralateral arm. The spirals consisted of three cycles in which the radius changed at a fixed rate per degree of rotation; the largest radius was 6 cm and the smallest 1.5 cm. While isolation of each neuron was maintained, the animal drew the spiral from outside inward and in the opposite direction. Each task was repeated 5 times. In addition, each neuron was tested in a different task ('center->out') that determined whether the cell had a direction of movement to which it fired most rapidly, a preferred direction, and whether the activity of the cell could be fit with a cosine tuning function (directionally tuned). Responses from direction specific cells should occur in opposite portions of the cycle when the spirals were drawn from outside inward compared to the trials when they were drawn from the inside outward. On the contrary, location specific cells should tend to fire in the same part of the cycle regardless of the movement direction.

All cells in this experiment were modulated during the drawing tasks and tested with manipulation of the body to insure that they were related to proximal arm movement. In dorsal Area 6 the activity patterns of 78 cells were found to be directionally tuned during the center->out task. Only three of these cells could be clearly classified as directional during the spiral task. Twelve of these units were clearly location specific. In contrast, of the 81 directionally tuned cells in Area 4, 21 showed clear directional sensitivity during the spiral task while only three were location specific. Thus, location specific cells are more likely to be found in Area 6 while those responsive to direction tend to occur in Area 4. (NIH-NS26375)

213.9

DIRECTIONALLY TUNED RESPONSES IN PRIMATE PREFRONTAL CORTEX COMPARED WITH THOSE IN MOTOR AND PREMOTOR CORTEX R.E. Kettner* and J.K. Marcario. Department of Psychology and Program in Neural Science, Indiana University, Bloomington, IN 47405

To study the input, storage, and conversion of directional variables into coordinated movement, rhesus monkeys were trained to view a sequence of two target lights at randomly selected target locations, to delay their response during a delay period when eye and body position was fixed, and to then press the remembered target buttons in the proper order to receive a juice reward. Directional responses were defined using circular regression techniques and were categorized based upon the existence of statistically significant tuning during sensory (s), delay (d), and movement (m) periods. Thus, a neuron with tuned responses during the s and m periods was termed an sm neuron. Based upon 203 prefrontal and 254 motor/premotor cortex neurons that showed significant tuning, the percentages (prefrontal, motor/premotor) of m (43%, 44%), sd (2%, 2%), sm (10%, 13%), s (4%, 6%) and dm (20%, 18%) neurons were approximately equal for the two cortical areas. In contrast, d (12%, 5%) responses were more common in the prefrontal cortex, and sdm (6%, 16%) neurons were more common in the motor/premotor cortex. Our past studies of the motor and premotor cortex suggests that there is a diversity of sensory-motor responses during this task across the extent of motor/premotor cortex. The present results extend this idea to the prefrontal areas studied. While there were more d responses in prefrontal and more sdm responses in motor/premotor cortex, the similarities between these two areas is even more striking. Of particular interest is the large number of m and dm response in both areas that suggest activity correlated with the execution of arm movements to targets in space. (Supported by NSF grant BNS-8919867)

213.10

COMPARISON OF PREFRONTAL NEURONAL RESPONSE PROFILES WITH THOSE OF THE MOTOR AND PREMOTOR CORTICES IN MONKEY. J.K. Marcario* and R.E. Kettner. Department of Psychology and Program in Neural Science, Indiana University, Bloomington IN 47405.

Previously we have described the categorization of response profiles of neurons recorded in the motor and premotor cortices of rhesus monkeys performing a movement-sequence delayed response task (Marcario & Kettner, 1991; Kettner & Marcario, 1991). The task consisted of an initiation period (I), a sensory period (S), an uninstructed delay period (D), and a movement period (M). Each neuron was categorized according to whether it had statistically significant increases in average firing rate during each of the four task periods. In the motor and premotor cortices, the largest number of neurons were primarily movement related (I, 10%; IM, 24%; M, 21%). Other categories were less common. Categories above 1% were: S (10%); D (8%); SD (9%); IS (6%); DM (3%). All categories were distributed throughout motor and premotor cortex.

To date, analyses of 355 neurons from prefrontal areas immediately anterior to premotor cortex suggest a larger number of IS (11%), ISM (3%), and SM (5%) responses and a smaller number of SD (1%) and IM (14%) responses than in the motor and premotor cortices. Other major categories were similar to those in motor and premotor cortices. In particular, there was still a good number of M (18%) and I (8%) responses, which indicates cells active when the monkey is moving to remembered target buttons. It is interesting to note that neurons categorized as SD units were much more prevalent in the motor and premotor cortices than in the prefrontal cortex. These results agree with the companion abstract in suggesting both similarities and differences in responses recorded across motor, premotor, and prefrontal cortices. (Supported by NSF grant BNS-8919867)

213.11

TEMPORAL PRIORITY AND LONGER PROCESSING TIME OF PREMOTOR CORTEX OVER NEARBY AREAS IN RECEIVING VISUAL CUES IN PRIMATES. K. Okano, Y. Inoue* Department of Physiology, Hokkaido University School of Medicine, North 15, West 7, Sapporo, 060, Japan.

Neuronal activities in the premotor cortex (PM, n=41cells), supplementary motor area (SMA, n=43), and precentral motor cortex (MC, n=53) were recorded while monkeys performed a visually triggered task. Kendall's partial correlation coefficient analysis was applied to the data to determine whether changes in neuronal activity were dependent on visual-stimulus or movement-onset. The proportion of cells in which neuronal activity changes were significantly related to visual-stimulus was 22% in PM cells, 7% in SMA cells, and 0% in MC cells.

Cross correlation analysis was performed on 31 pairs of neurons recorded with a single electrode. Neuronal related correlograms which were constructed by subtracting the shuffled correlograms from the gross correlograms had either a positivity or negativity, asymmetric to the zero time, and declined gradually (8/31). Presynaptic cells showed longer periods between onset of activity changes and the onset of the key pressing (mean±sd; 48.8±39.4 ms). Furthermore, PM cells had a longer processing time than nearby areas (PM/non PM; 3/5cells, t-Test, P=0.04). This suggests that the PM possesses the temporal priority over, and has longer processing time than the nearvy areas in receiving visual cues in primates.

213.12

MODULATION OF THE ACTIVITY OF SMA NEURONS DURING PREHENSION. G. Cadoret*, S. Mellah and A.M. Smith, C.R.S.N, Université de Montréal, Québec, Canada H3C 3J7.

A *M.fascicularis* monkey learned to grasp, lift and hold an instrumented device between the thumb and forefinger, and to hold it stationary for 1 second within a position window. The instrumented device allowed the grip and lifting (load) forces as well as the object position to be accurately measured. The texture and weight characteristics of the device could be changed to examine the scaling of grip and load forces during lifting. On selected blocks of trials, a predictable force-pulse perturbation was introduced during the holding phase to produce a slip force on the fingers. This perturbation invariably induced a reflex increase in grip force and, with repetition, a gradual preparatory increase in the static grip force which preceded the perturbation. Single units were recorded in the controlateral SMA in the different conditions of texture, weight and perturbation. Although many SMA neurons had modulated activity during grasping, only 7 cells could be further identified with the control of hand muscles by their response to microstimulation or by the presence of receptive fields on the palm or fingers. However, of these 7 cells, only one demonstrated a reflex response to the perturbation whereas the 6 others had increased activity related to the anticipation of the perturbation.

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213.13

ACTIVITY OF SINGLE NEURONES IN PRIMATE FACE SOMATOSENSORY CORTEX SI DURING TONGUE-PROTRUSION MOVEMENTS IN DIFFERENT DIRECTIONS. L.-D. Lin*, G.M. Murray, J.W. Hu, and B.J.Sessle Fac. of Dentistry, Univ. of Toronto, Toronto, Ont., M5G 1G6, Canada.

We recently reported (J. Neurophysiol. 67, '92) that neuronal population coding of tongue movement direction may be a feature of face motor cortex (MI). The aim of this study was to determine if neuronal activity in face SI also can be related to movement direction. Two monkeys (*M. fascicularis*) were trained to perform a tongue-protrusion task at each of 3 directions, with a force transducer positioned at 0° or 30° to the left or right of the midline. Extracellular recordings were then made from 47 single SI neurones with a mechanoreceptive field involving tongue, lip, or periodontium. The firing rates of 38 neurones (81%) was significantly altered during the tongue-protrusion task performed by the monkey in 1 or more directions (repeated measures ANOVA; $p<0.05$). The firing rate of 27 of these neurones (71%) varied significantly with the direction of tongue protrusion. Of 24 such 'direction' neurones studied during all 3 directions, 19 exhibited a single direction for which the firing rate was significantly greater than for any other direction; the highest firing rate in 18 of the 19 occurred at one of the two asymmetrical positions. The location of the neuronal mechanoreceptive field however could not predict the presence of directional selectivity of neurones or the preferred direction. This suggests that, in accordance with recent findings in face MI, coding of movement direction may also be a feature of face SI. Supported by Canadian MRC.

213.14

THE ACTIVITY OF VIBRATORY-RESPONSIVE MONKEY PRIMARY SOMATOSENSORY CORTICAL NEURONS IS MODULATED PRIOR TO HAND MOVEMENT. M. A. Lebedev and R. J. Nelson*. Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, 875 Monroe Avenue, Memphis, TN 38163.

Primary somatosensory (SI) cortical neurons may be entrained to the frequency of vibratory stimuli presented to the hand as go-cues for wrist movements. While these neurons initially convey high fidelity information about vibratory stimuli, it is unclear if they do so for the entire period before movement. Recent studies suggest that the activity of SI neurons is modulated prior to both movement and EMG onset. Four adult rhesus monkeys (*Macaca mulatta*) were trained to make hand flexion and extension movements in response to vibratory stimuli applied to the palm. They were also trained to maintain wrist position following the same vibratory cues. Accordance with the *NIH Guide for Care and Use of Laboratory Animals, revised 1985* was kept. The discharge patterns of 10, 15, 38 and 8 Area 3a, 3b , 1 and 2 neurons have been analyzed. Most had receptive fields on the hand ,wrist or forearm. The records for each neuron were split into two cases, one for each movement direction. The vast majority showed changes in mean firing rate and stimulus frequency synchronization that occurred before movement. Most changes lead movement onset by 80ms or more and EMG activity by ≈20ms. Cases were split into three groups based on changes in firing rate. Some 60%, 37%, 38% and 50% of the Area 3a, 3b, 1 and 2 cases showed increased discharge before movement. Of the cases for the respective areas, 20%, 33%, 32% and 19% showed mean discharge rate decreases before movement. The rest did not alter firing rate before movement. For each group, most neurons showed decreased synchronicity prior to movement onset. However, spikes related to single stimulus cycles occurred earlier as the onset of movement approached. A model was constructed that could explain the observed changes in mean discharge rate, synchronicity and spike occurrence.

These results suggest that: 1) before movement, SI neurons may exhibit complex firing patterns rather than faithfully conveying the temporal patterns of peripheral input, 2) these firing patterns may reflect modulation by presumably centrally generated inputs during sensorimotor integration. Support by **USAF Grant AFOSR 91-0333.**

213.15

DISCHARGE OF PARIETAL NEURONS DURING STATIC POSTURES, I. FORCE DEPENDENCE. SI Helms Tillery*, JF Soechting, TJ Ebner, Depts of Physiology and Neurosurgery, Univ Minnesota, Minneapolis, MN 55455

Well-controlled movement requires the use of information provided by somatic receptors, inputs from which first arrive in cortex at the primary somatosensory cortex (SI). The signals thought to be most relevant to the encoding of posture arise largely from muscle, joint, and tendon receptors. These receptors have complex discharge properties in relation to movement, with components related to muscle length and force, as well as joint angle and angular velocity. Therefore, it is important to determine the extent to which discharge in SI neurons can be accounted for in terms of posture without taking into account the forces used to maintain the posture. Parietal neurons were recorded while a monkey performed two tasks involving placement of the hand at locations specified by a robot arm. The monkey either 1) grasped a manipulandum which provided support for the arm, or 2) pressed a switch in the face of the manipulandum which did not provide support. We found that the discharge of many neurons is modulated in a consistent manner with changing location of the hand in space, but that the nature of that modulation was often different in the two tasks.

This work supported in part by a grant from the Human Frontiers in Science Program.

213.16

DISCHARGE OF PARIETAL NEURONS DURING STATIC POSTURES, II. RELATION TO POSTURAL PARAMETERS JF Soechting*, SI Helms Tillery, TJ Ebner, Depts of Physiology and Neurosurgery, Univ Minnesota, Minneapolis, MN 55455

One of the possible uses of kinesthetic information in arm movement is to provide feedback about the position of the arm. The posture of the arm, however, can be described in any of a number of possible coordinate systems (eg. Soechting and Ross, Neurosci 13: 595, 1984). To address the question of coordinate representation of posture in the brain, we have recorded from parietal neurons in an awake monkey while the animal placed its hand at various locations in its workspace specified by a robot arm. The posture of the arm during performance of the task was recorded by a video system and the locations of the shoulder, elbow, wrist, and hand in three-dimensional space reconstructed from the images. The discharge observed in single neurons was then related to various possible descriptions of the posture of the arm. The firing frequency of any neuron typically depended in a nonlinear manner on the postural parameters, regardless of which set of parameters was chosen. However, it was possible to identify simplified parameter sets (eg. a plane in space) which could account for the observed discharge. The degrees of freedom required to describe the discharge could thereby be reduced while still accounting for complexities in the cells' spatial properties.

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213.17

SELECTIVITY OF HAND-MOVEMENT-RELATED NEURONS OF THE PARIETAL CORTEX FOR SHAPE, SIZE AND ORIENTATION OF OBJECTS AND HAND GRIPS. H. SAKATA, A. MURATA, G. LUPPINO, M. KASEDA and M. KUSUNOKI*. Department of Physiology, Nihon University School of Medicine, Itabashi-ku, Tokyo 173, Japan.

In the inferior parietal lobule of the monkey, there is a group of neurons that is specifically related to hand movements. We found many neurons preferred a particular type of manipulandum, and most of them received both visual and motor signals (Taira et al, Exp. Brain Res. 83:29, 1990). In order to study the selectivity of these neurons for the spatial characteristics of objects and hand grips, we trained the monkey to grasp and pull a set of objects of different shapes (sphere, cube, cylinder, plate and ring), sizes and orientations, and recorded the activity of parietal neurons during grasping or fixation of objects.

Many of the hand movement-related neurons were preferentially activated during grasping an object of a particular shape. Several neurons showed categorical selectivity in shape, e.g. some cells preferred round objects such as spheres and cylinders to angular objects such as cubes and plates. Some cells were selective for the size of objects and grips, but others were selective only for shape, independent of size. Some cells were selective in orientation of the plate or ring, either vertical, horizontal or diagonal. Only some neurons responded to viewing the object alone but rest of them required the view of grasping hand to be activated.

These results suggest that the parietal neurons integrate visual and motor signals to discriminate the pattern of active hand movements and play an important role in the matching of the hand posture with the object to be grasped.

213.18

SENSORY TO MOTOR TRANSFORMATION WITHIN AREA 5 OF THE POSTERIOR PARIETAL CORTEX (PPC) OF THE MONKEY. M. Akamatsu[1], T. Hasbroucq I. Mouret and J. Seal*, Industrial Products Research Institiute, Tsukuba, Japan[1] and Lab. of Cognitive Neuroscience, C.N.R.S., BP 71, Marseille, France.

The aim of our work was to study the neuronal mechanism of sensory to motor transformation within area 5 of PPC using single neuron recordings in monkeys performing a sound-triggered forelimb movement. We studied the changes in neuronal activity that occurred after the sensory cue and before the onset of movement on a trial by trial basis. Although the onset of change remained relatively stable on a sensory to motor gradient, the peak in neuronal activity became less sensory and more motor as it occurred later in the reaction time. This suggests that peak activity of these neurons is involved in sensory to motor transformation. To investigate the transformation mechanism, time from onset to peak in neuronal activity, amplitude and rate of increase in neuronal activity were estimated using new methods of data analysis. Plotting these parameters against reaction time gave scatter diagrams each with a characteristic form. The relation between these parameters and reaction time could be simulated in a neuron model with variable amplitude of input, rate of increase in activity and threshold with inhibition after a fixed delay. This model gave scatter diagrams similar to the real data.

213.19

TRANSMISSION OF PRELIMINARY PERCEPTUAL INFORMATION TO THE MOTOR CORTEX.

Jeff Miller[1], Alexa Richle[*2], Jean Requin[2], [1] Dept. Psychol., UCSD, La Jolla, CA 92093-0109; [2] Cogn. Neurosci. Lab., CNRS, 13402 Marseille 09, France

Single neurons were recorded in the primary motor cortex of one monkey to discriminate between **discrete models** of sensorimotor information processing, in which one stage begins only when the earlier stage finishes, and **continuous models**, in which the gradual transmission of information implies that one stage can begin before the earlier stage finishes. The monkey had to perform wrist extension/flexion movements. The visual display provided a two-dimension stimulus: side (an easy discrimination between left and right targets), which determined movement direction, and distance (a difficult discrimination between proximal and distal targets), which determined whether or not the movement was to be made. 116 directionally selective neurons were classified into three classes: sensory neurons (activity change time-locked to the stimulus), motor neurons (time-locked to movement onset), and sensorimotor neurons (having two response components, the first time-locked to the stimulus and the second time-locked to movement onset). Activity changes of sensory neurons were similar in GO and NOGO trials, those of motor neurons were weaker in NOGO trials than in GO trials, and sensorimotor neurons behaved in such a way that, in NOGO trials, the first component remained whereas the second component was reduced, compared to GO trials. Thus the results substantiate the conclusion from reaction time and event-related potential studies that preliminary perceptual information may reach the motor system before stimulus analysis is completed. However, it does not end the debate between discrete vs continuous models, since the interpretation of results depends upon the stage mapping conception of cortical organization.

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MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CORTEX III

214.1

WIDELY DISTRIBUTED NEURON ACTIVITY IN PRIMARY MOTOR CORTEX HAND AREA DURING INDIVIDUATED FINGER MOVEMENTS. **M.H.Schieber* Depts. of Neurol. & Neurosurg., Anat. & Neurobiol., Wash U. Sch. Med., St. Louis, MO, 63110.**

Classic surface stimulation studies implied a somatotopic representation of movements of different fingers in the primary motor cortex (MI) hand area. Recent ICMS studies, however, suggest a diffuse representation. But during movements of a given finger performed actively by the subject, are active neurons confined to a particular sub-region of the hand area, or are they present throughout?

Single neuron activity was studied in the MI hand area of 2 rhesus monkeys trained to perform individuated flexion and extension movements of each finger or the wrist. Conventional techniques were used to record all isolable, task-related neurons without differentiating between pyramidal tract (PT) and non-PT neurons. Though some neurons discharged with only 1 of the 12 movements, most neurons discharged in relation to 2 or more movements. Such neurons could be related to various combinations of movements, not necessarily to movements of adjacent digits, or to flexion and extension movements of the same digit(s). Three-dimensional reconstruction revealed that the region containing neurons related to any one individuated finger movement was virtually coextensive with the region containing neurons related to any other.

Neurons participating in any given individuated finger movement thus appear to form a network spread throughout the MI hand area. The network generating any given finger movement overlaps extensively and shares many neurons with the network generating any other.

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214.2

MOVING A SINGLE FINGER OR MOVING ALL FINGERS: AN ACTIVATION PET STUDY OF CORTICAL MOTOR AREAS.

Ph. Remy, M. Zilbovicius, L. Raynaud , A. Syrota, Y. Samson*, CEA Service Hospitalier F. Joliot, 91406 Orsay, France.

We used PET to test the hypothesis that an individuated movement of a single finger involves a greater number of cortical neurons, and perhaps more motor areas, than a less complex movement of all fingers (M.H. Schieber, TINS, 1990, 13: 440). Eight normal volunteers had rCBF measurements with PET ($H_2{}^{15}O$) in three conditions: 1) Rest; 2) All finger movement: repeated flexion-extension of the all five fingers; 3) Medius movement: flexion-extension of the medius only. All movements were self-paced, and subjects were untrained. Four regions of interest were defined on individual MRI in the cerebral cortex contralateral to the fingers movements, and superimposed on PET images: primary sensorimotor cortex (SM), supplementary somatosensory area (SMA), premotor cortex (PM), parietal associative cortex (area 7). All finger condition activates SM (7.9±3.2%, $p<0.001$) and no other region. medius condition activates both SM (+10.6±3.7%, $p<0.001$) and SMA (+5.2±5.1%, $p<0.01$). The rCBF increase was significantly larger in the medius condition than the one found in all finger condition in both SM ($p<0.05$) and SMA ($p<0.001$). Our results support the hypothesis that more neurons of the primary motor cortex may be involved in movements of individual finger than in combined movement of all fingers. They also show that SMA is involved in the realization of an unusual and difficult single digit movement. (Supported by AP-HP, Paris).

214.3

INTRACORTICAL MECHANISMS FOR THE RECRUITMENT OF MOTOR CORTEX NEURONS. P. Istvan, P. Kirchberger & P. Zarzecki*, MRC Group in Sensory-Motor Physiology, Department of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6

During behaviour, motor cortex neurons are recruited in discharge patterns that vary according to the projection targets of their output axons. It is not known how these distinctive discharge patterns are determined, especially for neurons located close together but projecting axons to separate targets. One mechanism is that nearby neurons are affected by distinct patterns of input from pathways projecting into cortex from *extrinsic* sources (Zarzecki, Somatosens Mot Res 8:313,'91). However, because the majority of synapses upon output neurons are of intracortical origin, the *local* circuitry must also be considered.

We investigated the effects of intracortical neurons upon motor cortex output neurons in anesthetized cats. Corticospinal, corticorubral, corticoreticular and corticothalamic neurons were invaded antidromically and spontaneous discharges of three to five additional neurons were recorded simultaneously with the same or a second nearby electrode. Individual neurons were identified and sorted by their characteristic spike waveforms. Discharges of each waveform-identified neuron were cross-correlated with discharges of the invaded neuron or neurons recorded at the same site. Several different shapes of correlograms were found. The most common correlograms had symmetrical peaks centered at zero time, evidence of shared input. Increased probability of discharge of the output neuron that was delayed after the trigger spike was interpreted as serial coupling. Some correlograms were flat. The correlograms generated for each output neuron using the three to five waveform-identified neurons at the same recording site were usually different from one another. This result suggests that intracortical neurons contribute to the selective recruitment of neurons from within clusters of projection neurons.

Supported by the Medical Research Council of Canada.

214.4

PYRAMIDAL TRACT LESIONS AND SKILLED REACHING IN THE RAT. Whishaw*, I.Q., Tetzlaff, W., Pellis, S.M., Gorny, B., Dept. Psychology, Univ. Lethbridge, Lethbridge, AB, Canada, T1K 3M4

A number of contemporary theories associate the pyramidal tract with distal movements, especially independent finger movements. Such movements are not pronounced in rats, yet the size of the rat corticospinal projections as a ratio of cortical volume is among the highest of all mammals. We reexamined the role of the pyramidal tract in a skilled reaching for food task with the purpose of determining whether it is also involved in other aspects of limb movements. High speed videorecording, movement notation, and kinematic methods were used to describe the movements. Rats with selective lesions to the pyramidal tract, just rostral to the decussation, were studied over a prolonged recovery period. Although success in limb use was only decreased if a difficult reaching task was used, the rats did have enduring impairments in limb use. Impaired movements included limb aiming, paw pronation and supination, and food release. Successful reaching was accomplished by compensatory movements of the body and modifications in reaching style. Since aiming and pronation involve upper arm movements and supination involves both arm and wrist movement, the pyramidal tract is involved in both proximal and distal limb movements. Since digit closing to grasp was unaffected but opening to release food was affected, some aspects of distal movements are lost while others are spared. The results suggest a general association between the pyramidal tract and skilled movements irrespective of the location of the controlled body part.

214.5

CONTROL OF THE DISTAL MUSCULATURE IN INDIVIDUALS WITH CALLOSAL AGENESIS. P. Servos*, L.S. Jakobson, M.A. Goodale, & M. Lassonde, Department of Psychology, University of Western Ontario, London, Ontario, and **Département de Psychologie, Université de Montréal, Montreal, Quebec.

Classic work with split-brain monkeys suggests that the reaching limb can be controlled by either cerebral hemisphere but that finger control is largely crossed (Haaxma & Kuypers, 1975). Accordingly, acallosal subjects (AS) should have no difficulty grasping objects in the visual field ipsilateral to the hand used, but should have great difficulty forming their grasp in crossed space. Recently, however, it has been suggested that human callosotomy patients can exercise a substantial degree of ipsilateral control for the thumb and index finger (Trope et al., 1987). This suggests that AS should show good grip formation throughout the visual field.

In the present study, we carried out a kinematic analysis of reaching and grasping movements executed by 4 AS and 4 matched controls. Ss maintained central fixation while reaching with either hand for objects placed in left, central, and right space. Relative to controls, AS took longer to complete reaches directed into contralateral space, and spent proportionately more time decelerating. Moreover, unlike controls, their grip formation appeared to be impaired in all regions of space. Contrary to the predictions mentioned above, these results suggest either that prehension requires cooperation between the hemispheres, or that the corpus callosum in normal Ss exerts a facilitatory influence on intrahemispheric processing (Lassonde, 1986). Supported by grants from MRC (Canada).

214.6

FORCE-RELATED NEURONAL ACTIVITY IN TWO DIFFERENT REGIONS OF THE PREMOTOR CORTEX IN THE ALERT MONKEY.

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In the lateral area 6, neurons related to finger movements have been described by several groups. The question was raised whether the activity of such neurons may encode grip force exerted between thumb and index finger.

We have explored the premotor region in one monkey trained to produce fine-graded forces in a step-tracking task, with two or three consecutive force steps presented in a random manner. Task-related neurons were found in two subregions of area 6, 150 in the most lateral part of the arcuate premotor area (APA) and 162 on the convexity rostral to area 4. The neurons of both regions shared several properties similar to those of area 4, such as firing patterns and receptive field characteristics. A large population of tonic and phasic-tonic neurons increased or decreased their firing rate with force increase. In lateral APA, 63 neurons had clear receptive fields on the fingers, and 26/40 with tonic activity showed highly significant positive or negative correlations between firing rate and isometric force. All these neurons were located in sites where microstimulation elicited discrete movements of the digits for currents of 7 to 30 uA. In the convexity, 92 neurons also had receptive fields on the hand and from a sample of 25 neurons, 14 had positive significant correlations in contrast to a single negative one. These neurons were clustered in a small region where microstimulation occasionally triggered motor reactions of the digits. For some neurons in both regions, the covariation with force was conditional, depending on the expectation of the reward at the end of the trial.

In conclusion, coding of force is present in area 6 neurons located in two circumscribed regions. Thus, the division of area 6 into subregions according to their connectivity is supported by the present functional study.

214.7

A COMPARISON OF HEMISPATIAL NEGLECT FROM FRONTAL EYE FIELD AND POSTERIOR PARIETAL LESIONS IN THE MONKEY. Douglas P. Crowne* and Linda W. Mah, Department of Psychology, University of Waterloo, Waterloo, Ontario, CANADA N2L 3G1

Observation of monkeys with unilateral frontal eye field (FEF) and posterior parietal lesions suggests that the neglect syndromes may be partially distinct. Ipsilaterally-deviated gaze, impaired head and eye movements, and ipsiversive circling are characteristic of FEF but not parietal lesions. There is also a question of their relative severity. This experiment quantitatively compared visual neglect and recovery in monkeys with unilateral FEF, parietal, and principal sulcus (control) lesions. Neocortical lesions of these 3 regions were produced in 9 macaque monkeys, and they were tested for their ability to detect and respond to brief visual stimuli in varying field eccentricities. Both FEF and parietal lesions resulted in neglect. Recovery from the FEF lesion occurred by the 3rd postoperative week, but neglect was strongly evident in the parietal animals in the 8th week. A second homologous lesion produced a more severe and enduring neglect of contralateral stimuli and also reinstated neglect in the hemifield affected by the first lesion. These data confirm symptoms of visual neglect from parietal lesions and are consistent with differentiated functions of the FEF and posterior parietal cortex in directed attention.

214.8

POSITRON EMISSION TOMOGRAPHY STUDIES OF CEREBRAL GLUCOSE UTILIZATION IN MONKEYS PERFORMING VISUO-MOTOR INTEGRATION TASKS. J. Quintana*, A. Alazraki, Z. Allen, R. Woods, S. Cherry, J.C. Mazziotta, L.R. Baxter and M.E. Phelps. Depts. of Neurology, Psychiatry, Div. of Nucl. Med., and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Positron emission tomography (PET) was used to study local cerebral glucose utilization (lCGU) in a rhesus monkey performing a visually guided motor grasping task while seating in a primate chair, its two hands resting on a horizontal plastic plate. The task consists of grasping a small piece of food reward presented at the bottom of one of four narrow grooves carved in different directions on a vertical plastic panel. Performance requires a target-oriented combined arm/hand reaching movement with a precise coupled position of the thumb and the forefinger. Fluorine-18 deoxyglucose (FDG) was administered intravenously (i.v.) or orally immediately before the animal was exposed to either a repeated performance or a control stable situation during the tracer uptake period (30 or 75 min for the intravenous or oral administration routes, respectively). During the control experiments no movement was required: the monkey sat in the chair in front of the panel and the food reward was administered directly into the mouth. The scanning was carried out on a high resolution Siemens-CTI animal PET device. Differences in regional glucose metabolism due to specific performance-related responses were analyzed by subtracting control from stimulation PET emission data. Results showed an increase in lCGU during task performance after i.v. FDG in posterior parietal (15%), premotor and motor areas of the hemisphere contralateral to the used hand. In addition, smaller increases in the ipsilateral preoccipital parieto-temporal area were seen. No differences in lCGU in those areas were observed between the post-oral and post-i.v. FDG control situations. However, a marked increase in lCGU after i.v. FDG as compared with oral FDG was seen bilaterally in the temporal poles. Thus, the study shows that is possible to perform behavioral studies with PET on monkeys; metabolic and single-unit recording studies might be combined in the same animal to obtain data of cortical activity with high spatial and temporal resolution. Our preliminary data confirm results obtained in human blood flow studies with PET during a visuo-motor tracking task. Parallel PET studies in performing humans and monkeys may ultimately lead to the understanding, at the single-unit recording level, of the functional imaging data collected in numerous human studies so far.

214.9

INTERMEDIATE LINKS IN THE CORTICO-CORTICAL CONNECTIONS FOR VISUAL REACHING.

P.B.Johnson*[1], S.Ferraina[2], R.Caminiti[3] [1]Dept. of Cell Biology and Anatomy, Univ. of North Carolina, Chapel Hill; [2]Dept. of Neuroscience, Univ. Paris VI, France; [3]Ist. di Fisiologia umana, Univ. of Rome, Italy.

Most reaching movements are to visual targets yet the pathways through which the arm regions of the frontal lobe receive visual information are unknown. To address this issue, we have injected multiple retrogradely-transported tracers into the shoulder-related region extending from area 4 to dorsolateral area 6, as identified by single-cell recordings in macaque monkeys performing a reaching task. Within the frontal lobe, the arm-related region of dorsolateral area 6 projects to the border region between areas 4 and 6. This intermediate region, in turn, projects to the arm region of area 4. The parietal lobe inputs to these frontal areas originate from the cortex forming the medial crown of the intraparietal sulcus (areas 2 and 5), the medial bank of the intraparietal sulcus (area MIP), and from a region extending from the most posterior part of the medial bank of the intraparietal sulcus onto the mesial aspect of the hemisphere (areas MDP and 7m). Injections restricted to area 4 retrogradely labeled neurons in areas 2 and 5. Injections in the border region between areas 4 and 6 labeled neurons mainly in MIP, but also in areas 5, MDP, and 7m. Injections in area 6 labeled neurons mainly in MDP and 7m, but also in the adjacent MIP. Other studies have previously shown the existence of direct projections from visual area PO to MIP and 7m (Blatt et al., *J.Comp.Neurol.* 299:421-445, 1990; Cavada and Goldman-Rakic, *J.Comp.Neurol.* 287:393-421, 1989). The projections shown in our study from these parietal areas provide an anatomical substrate by which visual information may influence the frontal lobe mechanisms for the control of arm movement.

214.10

THALAMIC INPUT TO AREA F3 (SMA-PROPER) AND TO AREA F6 (PRE-SMA) IN THE MACAQUE MONKEY.

M. Matelli*, G. Luppino*, R. Camarda* and G. Rizzolatti *(SPON: European Brain and Behavior Society) Istituto di Fisiologia Umana Universita' di Parma Via Gramsci 14 I-43100 Parma Italy.

Recently we showed that the mesial sector of area 6, generally considered coextensive with the so called Supplementary Motor Area (SMA), is composed by two different areas : F3 (SMA-proper) and F6 (pre-SMA) (Luppino et al. J. Comp. Neurol. 311:463-482,1991). On the basis of the neurophysiological properties of the two areas, we proposed that, in motor control, F6 plays a hierarchically higher role than F3.

The present experiments were aimed to verify this proposal by studying the thalamic input to the two areas. Neural tracers (WGA-HRP, FB, TB and DY) were injected in F3 and F6, following their physiological identification with intracortical microstimulation, in four monkeys. The results were the following. The main input to F3 comes from basal ganglia recipient nuclei: VLo (major contribution) , VLm, and VApc. A substantial input arrives also from cerebellar recipient nuclei: VLc, and, to a lesser extent, the caudal part of VPLo. Almost no labelling was observed in area X of Olszewski. Finally, an input arrives to F3 also from the caudal part of MD. The main thalamic input to F6 originates from VApc and area X. Other projections come from VLc and the caudal part of MD. Intralaminar nuclei and, at a much lesser extent, the posterior thalamus project to both F3 and F6.

Taken together these data allow us the following main conclusions: a) F3 and F6 are both targets of basal ganglia and cerebellar outflow. b) The thalamic origins of this outflow is markedly different. c) As far as the basal ganglia are concerned, F3 is a part of the so called "basal ganglia motor loop", whereas, F6 belongs to the "basal ganglia complex loop". This differential basal ganglia input provide further evidence in favour of a higher hierarchical role of F6 in respect to F3.

(Supported by a Grant from Human Frontier Science Program)

214.11

CLUSTERED INTRINSIC CONNECTIONS AMONG REPRESENTATION ZONES IN THE CAT MOTOR CORTEX. A. Keller *. Department of Anatomy, USUHS, Bethesda, MD 20814.

Patterns of intrinsic connections in the cat motor cortex were studied using intracortical microstimulation (ICMS) and extracellular dye injections. ICMS was used to identify cortical sites at which low threshold stimulation (< 20 μA) evoked movements or muscle contractions. The forelimb, hindlimb, and facial representations were discreetly segregated within the motor cortex. Within each of these representation zones, identical movements could be elicited from several non contiguous sites. In each cat, a single small injection of either fluorescent latex beads, HRP, or PHA-L was made into a single site from which wrist flexion was evoked. The distribution of retrogradely labeled somata and anterogradely labeled axons and axon terminals were then correlated with the physiologically defined motor maps. Labeled somata and terminals were distributed in dense clusters across the forelimb representation zone. A more diffuse distribution of somata and terminals occurred within the hindlimb representation area, and only scant labeling was found within the representation of facial musculature. Within the forelimb representation area, clusters of somata and terminals occurred within zones representing movements – in different directions – of digits, wrist, elbow, and shoulder. Tracing of individual PHA-L labeled axons revealed that each intracortical axon forms a number of terminal clusters, and that different clusters of the same axon terminate in zones representing different movements. These data indicate that extensive intracortical connections mediate reciprocal synaptic interactions among groups of cells involved in the execution of different movement segments. These clustered, horizontal connections may be used for coordinating the activity of different cortical representations for the execution of complex movements, and may also be involved in the pliability of motor representation maps. Supported by NIH grant # NS-31078.

214.12

TOPOGRAPHY OF MOTOR CORTEX REORGANIZATION

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Previous studies have shown that after limb amputation there is a use dependent enlargement of motor cortex areas projecting to muscles proximal to the amputation. In order to determine whether in humans such changes conform with motor homunculus topography we performed transcranial magnetic stimulation (TMS) in 10 patients (pts) (4 pts aged 34.8 +/- 7.2 ys with forearm and 6 pts aged 33.2 +/- 6.8 ys with finger amputation). TMS (at 1.5 twitch threshold) mapping was carried out by consecutively placing an eight shaped coil on 80-120 frontal-parietal scalp positions, 0.5 cm apart from each other. EMG responses were recorded from the biceps and (in finger amputees) the extensor indicis muscle.

In finger amputees, off-line analyses of scalp position and EMG response did not show enlargement of the scalp "biceps-field", i.e. the area giving biceps motor responses (>100 uV) to TMS. However, pts with forearm amputation showed a significant enlargement of the biceps field in comparison to their unaffected side ($p<0.01$). In our 4 pts this enlargement resulted from a lateral extension of the field. The forearm is also located more laterally in the human motor homunculus. Therefore the topography of the enlargement of TMS "biceps-fields" strongly suggests, that motor cortex, formerly projecting to the forearm, is used for output to proximal stumb muscles after amputation.

214.13

EXPANSION OF DISTAL FORELIMB REPRESENTATIONS IN PRIMARY MOTOR CORTEX OF ADULT SQUIRREL MONKEYS FOLLOWING MOTOR TRAINING.

G.W. Milliken*, R.J. Nudo, R. Grenda, W.M. Jenkins and M.M. Merzenich. Dept. of Neurobiology & Anatomy, Univ. of Texas Med. Sch., Houston, TX 77030 and Keck Center for Integrative Neuroscience, Univ. of Calif. at San Francisco, San Francisco, CA 94143.

We examined consequences of motor training on the functional topography of primary motor cortex. In normal squirrel monkeys, a detailed map was derived in the zone of representation of hand, wrist, and arm contralateral to the preferred hand using intracortical microstimulation techniques and sterile operative procedures. After a post-operative recovery period, monkeys underwent behavioral training. The task required the animals to retrieve small food pellets from four graded wells on a Plexiglas board. The smallest well required the controlled insertion of only one or two digits into the food well to retrieve pellets. The animal's skill at pellet retrieval was significantly improved by only a few days of intensive training. Training continued for 10-11 days. Within two days after the final retrieval session, a second detailed map of motor hand representations was derived.

In each case, post-behavior maps revealed significant changes in the topographies of movement representations marked by expansion of the cortical sectors representing movements involved in the task. By contrast, in control animals, distal forelimb representations were relatively unchanged. These experiments confirm that the functional representations of primary motor cortex are remodeled by use, throughout life. Supported by NIH NS27974 (RJN), NS10414 (MMM) and the Whitehall Foundation (RJN).

214.14

PLASTICITY IN THE CEREBELLO-THALAMO-CORTICAL PATHWAYS.

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The cerebello-thalamo-cortical pathway (C.T.C.) may be temporarily involved in the learning of new movements (ITO, 1984). The exclusion of the ventrolateral nucleus (VL) of the thalamus does not affect the performance of learned movements, but makes it impossible for subjects to acquire new movements (FABRE and BUSER, 1980). Moreover, simultaneous high frequency stimulation of both the sensory cortex and the VL induced long term potentiation of thalamic input to motor cortical neurons (BARANYI and FEHER, 1979; IRIKI et al., 1989). It was therefore proposed to investigate whether any plasticity in the C.T.C. connections could be observed after classical forelimb flexion conditioning in cats.

A classical conditioning of the ∝-type effectively produced changes in the conditioned motor response about ten days after the beginning of the the conditioning procedures. The probability of the occurrence of this motor response as well as its amplitude were increased.

Motor cortical evoked potentials induced by the conditioned stimulus were also modified. Increasing the excitatory cerebellar inputs was found to affect one or two motor cortical sites, whereas the activation of the other cortical sites was decreased.

These motor and evoked cortical potential modifications were seen to be correlated.

214.15

MAGNETIC TRANSCRANIAL CORTEX STIMULATION TO DOCUMENT MOTOR FUNCTION RECOVERY AFTER STROKE. M.A.Lissens* and W.B.McKay. Dept. Physical Medicine and Rehabilitation, University Hospitals of Leuven, Belgium, and Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, TX 77030.

Information about the integrity of the corticospinal tracts can be obtained by transcranial stimulaiton of the motor cortex. We performed transcranial stimulation using a magnetic stimulator in stroke patients with hemiplegia, in the acute phase and at one month intervals during the motor activity recovery. A case report is presented of a 36-year old female who suffered from a hemorhagic stroke with left hemiplegia. During the first three weeks no motor evoked potentials (MEP's) were obtained anywhere. After one month in the left abductor pollicis brevis muscle a very small MEP of only 50 microvolts (uV) with a slightly delayed latency time was obtained, after two months the amplitude was 140 uV, after three months 450 uV and after four months 1650 uV with a nearly normalized latency time. At this last examination a small response was also obtained in the abductor digiti minimi muscle.

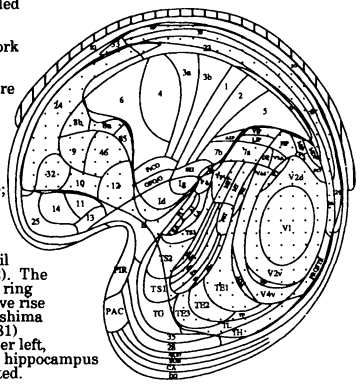
The increase of the initially very small MEP amplitudes and normalization of the originally delayed latency times correlated remarkably with the recovery of motor function.

This clearly demonstrates that MEP's can be used as a quantitative parameter of motor activity recovery, and moreover can motivate patients during rehabilitation.

214.16

AN UPDATED VERSION OF McCULLOCH'S MAP OF THE CEREBRAL CORTICAL AREAS OF THE MACAQUE SUGGESTS A COMPLETE "CIRCLE" OF VISUAL AND OCULOMOTOR AREAS SURROUNDS A CORE OF SENSORIMOTOR CORTEX. E.J.Neafsey*. Dept. of Cell Biology, Neurobiology & Anatomy, Loyola Univ. Medical Center, Maywood, IL 60153.

In 1944 Warren McCulloch published a two dimensional map of the cortical areas in macaque cortex (Physiol Rev 24). His map differed from current "flat" maps (Van Essen, Kaas, etc.) because his map had no artificial breaks or cuts but rather simply "stretched" the limbic cortical areas at the medial edge of the hemisphere. The figure below illustrates an attempt to incorporate some of our present knowledge of macaque cerebral cortical organization into a McCulloch map. The shaded ring of cortical areas can all be classed as visual or oculomotor, based on the work of Van Essen, Kaas, and others. Note in particular that when areas 23 and 24 are included, the ring becomes complete. Several studies have found that area 24 has an oculomotor function and also projects to the superior colliculus (Leichnetz et al, Neuroscience (1981) 6:1023); area 23 has recently been reported to be responsive to the position of the eye in the orbit and to the occurrence of saccades (Musil et al (1991) NS Abstr 17:442). The central core encircled by the ring contains the neurons that give rise to the pyramidal tract (Toyoshima and Sakai, J Hirnforsch (1981) 23:257). Frontal lobe at upper left, corpus callosum around top, hippocampus at bottom. Suggestions invited.



214.17

THREE-DIMENSIONAL RECONSTRUCTION OF SERIAL SECTIONS USING FOURIER DESCRIPTOR ANALYSIS. J.P. Ray*, L.S. Hibbard, and J.L. Price. Anatomy and Neurobiology, and Neurology and Neurological Surgery, Washington University School of Medicine, St. Louis MO 63110.

Our studies of the anatomical organization of the primate prefrontal cortex have used flattened, two-dimensional (2-D) maps of cortex from serial coronal sections using a contour line unfolding technique (Van Essen and Maunsell, *J. Comp. Neurol.*, 191:255, 1980). We are developing computational methods for unfolding the cortex. Preliminary to unfolding is the accurate three-dimensional (3-D) reconstruction of the serial sections. We have adapted Fourier Descriptor (FD) techniques (Zahn and Roskies, *IEEE Trans. Comput.*, C21: 269, 1972) to align sections represented by *xy*-coordinates from tracings of the pial surface and layer IV. After linear interpolation, the N traced coordinates are combined as $u(n) = x(n) + jy(n)$, $n = 0,1,...,N-1$, in a Fourier series equal to the FD $a(k)$

$$a(k) = \sum_{n=0}^{N-1} u(n)\exp(-j2\pi kn/N), \quad 0 \le k \le N-1.$$

The differences between the FDs of adjacent sections dictate the translation and rotation necessary to align the sections. If $a(k)$, $b(k)$ are the complex FDs for coordinate sets $u(n)$, $v(n)$, then the x- and y-translations are given by the differences $[Re(b(0))-Re(a(0))]$, $[Im(b(0)-Im(a(0))]$, respectively. The rotational misalignment and the coordinate starting point offset are extracted from the phase differences of the lower order terms by linear regression. (Support: McDonnell Center for the Studies of Higher Brain Function. Small Grants Program.)

214.18

ASSOCIATION CORTEX AS A DARWINIAN WORKSPACE: Corticocortical Axons Suggest Cloning from Hexagonal Engrams. WILLIAM H. CALVIN*. Univ. of Washington, Psychiatry NJ-15, Seattle WA 98195

The brain might shape up more complex mental images on the milliseconds-to-minutes time scale, using principles similar to how new species are shaped up in millennia and novel antibodies in weeks. *Darwinism* signifies *1)* patterns (genes or memes) that make *copies* of themselves, where *2)* different patterns *compete* with one another for hegemony over a territory, their relative success biased by *3)* a many-faceted *environment*; further, *4) variants* on successful patterns are frequently introduced, occasionally finding a new niche or improving the fit to an existing one. Selective survival may occur without *#1* and *#4*, but *is not appropriately termed darwinism*. Here I show that association cortex has the essential anatomy (standard axon lengths in layer III pyramids) for a true darwinian workspace. 1) **Spatiotemporal patterns** of neuronal activity could clone themselves; because chaotic firing patterns in similar hexagons **synchronize**, **NMDA**-like coincidence facilitation can recruit corresponding points in adjacent unorganized cortex. A **hexagonal mosaic** ensues. Approach/avoidance and remembered environments can 2) promote one pattern over another in boundary disputes. 3) An active pattern may resonate with an underlying synaptic pattern, one **engram** of many in the 0.5mm hexagon. I identify situations where copying errors are corrected, others that 4) encourage variants. **Recombination** occurs; such composite hexapixels might represent **categories**, **ensembles**, and **sequences**. Active patterns ("phenotypes") can be cloned even if novel, successful ones later imprinted in the static connectivity (Lamarckian "genotypes"). Subcortical managers might set good-enough criteria for **memorization** and overt action, serve to speed evolution via "climatic changes." While darwinian procedures seem particularly appropriate for judging novel plans, they may also generate shortcut algorithms that recognize the routine and extrapolate the present situation into one's next act.

VESTIBULAR SYSTEM: NEUROCHEMISTRY

215.1

FOS-DEFINED ACTIVATION OF THE INFERIOR OLIVE DURING CENTRIPETAL ACCELERATION IN UNILATERAL LABYRINTHECTOMIZED RATS. G. D. Kaufman*, J. H. Anderson†, A. J. Beitz. Departments of Veterinary Biology and Otolaryngology†, University of Minnesota, St. Paul, MN 55108 USA.

Polyclonal Fos antibody was used to identify activated neurons in rat brainstem following 90 minutes of centripetal acceleration at 2 G. Long-Evans rats were restrained with the head fixed inside metal cones in darkness and positioned ~50 cm eccentric from the axis of rotation which was earth vertical. Unilateral chemical labyrinthectomies (UL) were performed by middle ear injection of sodium arsanilate, and the rats were sacrificed 24 hours (acute) or 14 to 18 days (chronic) later. When chronic restrained rats with UL were positioned with their long axis tangential (left or right position) to the arc of rotation, the dorsomedial cell column (DMCC), and the beta (IOß) subnuclei, exhibited Fos expression only when the lesioned side was towards the axis of rotation. In acute rats the DMCC was labeled similar to the chronic rats, but the IOß on the side toward the axis of rotation was labeled for both orientations of the rat. Fos activity patterns in other vestibular-related nuclei revealed different patterns in response to the centripetal stimulus, depending on the time since UL, and the orientation of the animal. The vestibulo-ocular reflex, measured with the dual search coil technique, was also quantified. The results suggest that a subset of the otolith receptors was responsible for DMCC and IOß activation. The DMCC might play an important role in adapting to a new gravito-inertial reference, whereas the IOß may be more critical in initial compensation to the UL. Supported by NASA/NGT-50563, NIH DC01086, DC00110, DA06687.

215.2

INFLUENCE OF NMDA ANTAGONIST ON VESTIBULO-OCULAR REFLEX ADAPTIVE GAIN DECREASES. T.L. Carter*[1], R. Baker[2] and J.G. McElligott[1]. [1]Dept. of Pharmacology, Temple University School of Medicine, Phila., PA 19140, and [2]Dept. of Physiology and Biophysics, N.Y.U. Medical Center, New York, NY 10016

Antagonists to the NMDA receptor interfere with neuroplastic electrophysiological and behavioral changes regulated by the central nervous system. More specifically, previous work in our laboratory has shown that the NMDA antagonist MK-801 interferes with adaptive vestibulo-ocular reflex (VOR) gain increases (Soc Neurosci. Abstr. 17 #127.9, 1991). In order to test the generality of this finding, goldfish were trained to decrease VOR gain after a systemic (I.M.) injection of MK-801 (1mg/kg). Goldfish were restrained in the center of a cylindrical test aquarium (dia. = 27.5 cm) which was sinusoidally rotated about the vertical axis at 1/8 Hz ± 20° (vestibular stimulus). Projection of visual stimuli (random dot pattern) onto the wall of the aquarium at the same frequency, amplitude, and in phase with the vestibular stimuli produced VOR gain decreases (towards 0X). The gain of the VOR was assayed by measuring the movements of both eyes using the electro-magnetic search coil technique. Fish were trained to decrease VOR gain over a 3 hour period during which time the VOR in both the light and the dark was measured at 15 minute (1st hour) and 30 minute intervals (2nd and 3rd hour). The systemic injection (I.M.) of fish Ringer's solution (control) or MK-801 (1mg/kg; experimental) did not alter non-adapted VOR gain when measured prior to adaptive training. Furthermore, control and MK-801 injected animals produced identical adapted VOR gain decreases measured during the training period. For both the control and the experimental groups, the onset of VOR gain decrease was immediate and had the same initial rate of gain decrease (0.4/hour). This result is in sharp contrast with that of our previous experiments which demonstrated that systemic injection of MK-801 delays the onset and reduces the rate of adaptive VOR gain increase. These results would indicate that the site of adaptive VOR gain decrease is located at a non-NMDA sensitive area while the site of adaptive VOR gain increase is located at a NMDA sensitive area. (Supported by a grant from NIDCD-NIH # DC 01094).

215.3

LOCALIZED MICROINJECTION AND DIALYSIS OF NMDA ANTAGONIST IN THE VESTIBULO-CEREBELLUM DURING ADAPTIVE VESTIBULO-OCULAR REFLEX GAIN CHANGE. J. G. McElligott* and T. L. Carter, Dept. of Pharmacology, Temple Univ. Sch. of Med., Philadelphia, PA 19140.

The vestibulo-ocular reflex (VOR) has been studied as a model system for investigating neuroplasticity within the central nervous system. Two areas involved in this reflex, the vestibulo-cerebellum and the vestibular nucleus, have been proposed as sites responsible for adaptive VOR gain changes. Our previous studies (Soc Neurosci. Abstr. 17 #127.9, 1991) have found that MK-801, an NMDA antagonist, when administered systemically (I.M.), delays the onset and reduces the initial rate of VOR adaptive gain increases. In order to determine if the vestibulo-cerebellum is an NMDA sensitive site, MK-801 was administered directly into this region. VOR gain changes were measured by means of the electro-magnetic search coil technique. Goldfish were presented with sinusoidal vestibular stimulation by rotating them about the vertical axis (1/8 Hz @ ± 20°) while restrained within a cylindrical test aquarium (dia. = 27.5 cm). Gain increases were produced by projecting visual stimuli (random dot pattern) onto the wall of the test aquarium. These stimuli were presented at the same frequency and magnitude but 180° out of phase with the vestibular stimuli. In all cases, there was no change in the non-adapted VOR gain measured in the light or the dark before and after intra-cerebellar application of MK-801. In addition, the onset and the initial rate of adapted gain change in control and MK-801 microinjected animals (1 to 638ng @ 1.5 ng/nl) was also equal in both groups. A second study using microdialysis probes (dia. = 150 µM, length = 3 mm) was carried out in order to increase the amount of MK-801 available to the vestibulo-cerebellar region. Using this technique, MK-801 was delivered continuously during the entire period of VOR adaptation. Again, there was no difference in the onset time and the initial rate of VOR adaptive gain increase between the experimental and the control infused animals. These results suggest that the vestibulo-cerebellar region is not an area in the goldfish brain where the NMDA antagonist, MK-801, interferes with adaptive VOR gain increases. (Supported by a grant from NIDCD-NIH # DC 01094).

215.4

NEUROGENETIC CLASSIFICATION OF PROJECTION NEURONS IN THE RAT VESTIBULAR NUCLEI. Y.Kitao, S.Okoyama, T.Moriizumi* and M.Kudo. Dept.of Anatomy, Sch.of Med., Kanazawa University, Kanazawa 920, JAPAN.

The vestibular nuclear complex is divided into four subnuclei cytoarchitecturally. Each subnucleus may contain the vestibulo-spinal (Sp), the vestibulo-oculomotor (III) and the vestibulo-thalamic (Th) projection neurons. Present study attempts to make a classification of the projection neurons according to their proliferation periods by combining nuclear bromodeoxyuridine (BrdU) and retrograde cytoplasmic fluorescent dye labeling. 1) The Sp neurons in the medial nucleus were produced through E12-E14 with peak on E13, while most of the SP neurons in the lateral nucleus were generated on day E12. In the inferior nucleus, the Sp neurons arose over days E12 and E13. 2) The III neurons were generated from days E12 to E13 predominantly on E12 taking no different temporal course among subnuclei. 3) The Th neurons were produced on days E13 and E14 without temporal differences among the subnuclei either. The results suggest that the targets of the projections can be destined by the proliferation periods of the neurons.

215.5

IN SITU HYBRIDIZATION OF GLUTAMATERGIC RECEPTORS, GLUTAMATE DECARBOXYLASE AND CHOLINE ACETYLTRANSFERASE ENZYMES IN RAT VESTIBULAR NUCLEI C. de Waele*, M. Abitbol, C. Menini, M. Chat , J. Mallet, P.P. Vidal Lab. de Physiologie Neurosensorielle, CNRS, UPR2, Paris, FRANCE and Lab. de Neurobiologie cellulaire et moléculaire, CNRS, Gif sur Yvette, FRANCE.

Histological distribution of NMDA and metabotropic receptor mRNAs was determined in the vestibular nuclei of the adult rat. Both NMDA and trans-1-amino-cyclopentane-1,3-dicarboxylate (t-ACPD), a specific agonist of glutamate metabotropic receptors, previously had been shown to depolarize strongly medial central vestibular neurons (Vibert et al., Soc. Neurosci. Abst.128.22, 1991). Specific radioactive oligonucleotides were used to probe sections of rat vestibular nuclei according to in situ hybridization methods. Dense labelling of NMDA mRNA appeared in several brainstem regions including the medial, lateral, inferior and superior vestibular nuclei. In addition, these nuclei showed intermediate levels of metabotropic receptor mRNA labellings. In a second step, we studied the localization of mRNAs that encode two forms of glutamic acid decarboxylase (GAD1 and GAD2) and choline acetyl transferase. The findings indicate that both GAD mRNAs were present in all subpopulations of central vestibular neurons. Our data also confirm previous electrophysiological studies which have demonstrated the role of GABA in modulating medial vestibular neuron resting discharges.

215.6

LOCAL INJECTIONS OF ß-NORADRENERGIC SUBSTANCES IN THE CEREBELLAR ANTERIOR VERMIS OF CATS AFFECT ADAPTATION OF THE VESTIBULOSPINAL REFLEX (VSR) GAIN. O.Pompeiano*, P.Andre, P.d'Ascanio and D.Manzoni, Dept. of Physiol. Biochem., Univ. of Pisa, 56127 Pisa, Italy.

In precollicular decerebrate cats sinusoidal roll tilt of the head at 0.15 Hz,±10° was associated with roll tilt of the body in the same direction and at the same frequency, but at the peak amplitude of 12.5°; this stimulus led to 2.5° of neck rotation, which was thus outphase with respect to head rotation. During a 3 hr period of sustained vestibular and neck rotation, the gain of the VSR recorded from the triceps brachii and tested every 15 min progressively increased to reach the mean value of 240.7±41.8,S.E.% of the control (n=11 experiments). This adaptive process was followed up to 1 hr after stimulation. Injection into the zone B of the cerebellar anterior vermis of the ß-adrenergic antagonists propranolol or sotalol (0.25µl at 8µg/µl saline) produced only slight and short-lasting changes in the basic amplitude of the VSR, but decreased or prevented the occurrence of the adaptive changes which affect the VSR gain during sustained head-neck rotation (n=11 experiments). The same agents also suppressed the increase in gain of the VSR which occurred during sustained roll tilt of the whole animal (at 0.15 Hz,±10°) leading to selective stimulation of labyrinth receptors (n=2 experiments); moreover, the ß-adrenergic agonist isoproterenol (0.25µl at 8µg/µl saline) enhanced this adaptive process (n=4 experiments). In conclusion, the results obtained indicate that adaptive changes affect the gain of the VSR in decerebrate cats. Such processes are facilitated by the noradrenergic afferent system acting on Purkinje cells of the cerebellar vermis through ß-adrenoceptors.(NIH grant NS 07685-23 and ASI 1991 RS77).

215.7

CGRP IN THE VESTIBULAR AND COCHLEAR EFFERENT NEURONS IN THE CHINCHILLA. P. Popper*, R.A. Wong Marco, L.F. Hoffman, P.A. Wackym and P.E Micevych. Dept. of Anatomy & Cell Biology, Laboratory of Neuroendocrinology, and Division of Head and Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024.

The distribution of calcitonin gene-related peptide immunoreactivity (CGRPi) among efferent vestibular and cochlear neurons in the chinchilla was investigated. Fluorogold (40 µl, 4% in saline) was injected through the oval window into the inner ear of anesthetized young male chinchillas (6-12 months old). Four days postinjection, the animals were anesthetized and perfused through the heart with 4% paraformaldehyde in phosphate buffer. Alternate 20 µm thick brainstem sections were processed for CGRPi using a fluorescent method. Alternate sections were processed for in situ hybridization using a radiolabeled RNA probe complementary to the 3'end of the αCGRP mRNA. At the levels of the descending facial nerve and the genu of the facial nerve, virtually all of the Fluorogold labeled cells in the perigenual nuclei (group E vestibular efferents) extending to the medial aspect of the medial vestibular nucleus contained CGRPi. All the Fluorogold labeled cells around the dorsal third of the parvocellular reticular nucleus (PCR) and most of the Fluorogold labeled cells in the lateral superior olivary nuclei (cochlear efferents) contained CGRPi. More caudally, 50% of the Fluorogold labelled cells in the PCR had CGRPi. Fluorogold labeled cells in the dorsal lateral aspect of the perifacial zone were not CGRPi. Supported by DC00008 and DC01404.

215.8

NMDA RECEPTOR-MEDIATED CALCIUM INCREASE IN RAT MEDIAL VESTIBULAR NUCLEI. Y.Takahashi, M.P.Takahashi, T.Tsumoto*,K.Doi and T.Matsunaga, Dept.of Neurophysiology, Biomedical Research Center and Dept.of Otorhinolaryngology, Osaka University Medical School, Suita, 565 Japan

An activity-dependent change in synaptic efficacy in the vestibular nuclei may underlie mechanisms of vestibular compensation, and an entry of Ca^{2+} into postsynaptic sites through N-methyl-D-aspartate(NMDA) receptor-linked channels is suggested to play a role in such plasticity of the vestibular system. To test this possibility, we measured changes in $[Ca^{2+}]_i$ with fluorometry using a Ca^{2+} sensitive indicator, rhod-2, in slice preparations of the medial vestibular nuclei (MVN) of young rats (postnatal 4-7 days) following electrical stimulation of afferent pathways and analysed effects of NMDA receptor antagonists on these measures. Single shock stimulation of vestibular afferents or commisural fibers did not induce sizable increase of $[Ca^{2+}]_i$ in the standard medium. In the Mg^{2+}-free medium, however, the fluorescence increased by 3-6 % in response to single shock stimulation, and this increase was blocked by an application of an NMDA receptor antagonist, D,L-2-amino-5-phosphonovaleric acid (APV) (100µM). However, a non-NMDA receptor antagonist, CNQX (10µM) was ineffective. In the standard medium, the fluorescence also increased during tetanic stimulation (30Hz for 3.3 sec) of both pathways by 5-15%. APV reduced such a tetanus-induced increase by 27±11% in case of afferent tetanus and 20±14% in case of commisural tetanus (n=24). A blocker for NMDA receptor-linked channels, MK801 (20µM), also reduced the tetanus-induced increase by 19±10 and 26±9%, respectively (n=31). Thus, tetanic stimulation of vestibular afferents and commisural fibers may induce an influx of Ca^{2+} into MVN neurons partly through NMDA receptor-linked channels in young rats.

215.9

LOSS OF INTEGRATOR FUNCTION AFTER INJECTION OF GABAERGIC SUBSTANCES INTO THE PREPOSITUS HYPOGLOSSI NUCLEI (PPH). J.Yokota, H.Reisine*, and B.Cohen. Depts. Neurol. & Physiol. Mt. Sinai Sch. Med. NY 10029

We have previously shown that velocity storage is most probably generated in the vestibular nuclei (VN) and not in PPH (Yokota et al. 1992). Consistent with this electrical stimulation of PPH of alert cynomolgus monkeys caused ipsilateral eye deviations or nystagmus with ipsilateral horizontal slow phases from 30-45°/s. Peak slow phase velocities were 10-20°/s less than from stimulation of VN or from OKAN. There was no after-nystagmus after PPH stimulation. After microinjections of muscimol (1 $\mu g/\mu l$), the eyes were shifted to the contralateral side, and gaze holding was poor in the ipsilateral field. There was horizontal spontaneous nystagmus with contralateral slow phases. The time constant of the ipsilateral VOR was shortened, although the gain was preserved. Ipsilateral OKAN disappeared; contralateral OKAN was normal. Steady state velocities were lost for ipsilateral but not contralateral OVAR. Vertical eye movements were unaffected. After injection of bicuculline into PPH, changes in eye movements were transient. Animals were unable to hold contralateral gaze positions, and the eyes were shifted to the ipsilateral side. There was horizontal spontaneous nystagmus with ipsilateral slow phases. The time constant of the horizontal VOR was reduced contralaterally with preservation of gain. Contralateral OKAN and OVAR disappeared transiently. We conclude that muscimol sensitive $GABA_A$ receptors are utilized in PPH in the process related to V-T-P integration. They also appear to play a role in producing horizontal slow phases of nystagmus generated by velocity storage. Supported by NS00294 and EY01867.

215.10

EFFECTS OF AMINO ACIDS ON MEDIAL VESTIBULAR NEURONES IN GUINEA PIG BRAINSTEM SLICES. N. Vibert, M. Serafin, A. Khateb, P.P. Vidal° and M. Mühlethaler*. *Department. of Physiology, CMU, 1211 Geneva 4, Switzerland and °Laboratoire de Physiologie Neurosensorielle, CNRS UPR 02, 75270 Paris Cedex 06, France.*

Using intracellular recordings in brainstem slices, we have recently identified in the guinea-pig medial vestibular nucleus (MVN) two main neuronal cell types (A and B MVNn) differing by their intrinsic membrane properties. Both cell types were found to be extremely sensitive to inhibitory (IAA) and excitatory (EAA) amino acids. Almost all MVNn were consistently depolarized and excited by selective EAA agonists, including AMPA (45 out of 46 MVNn), NMDA (49 out of 52 MVNn) and trans-ACPD (73 out of 73 MVNn). In contrast, L-AP4 had no effect in either cell type (8 out of 8 MVNn). The effects of AMPA and trans-ACPD persisted in presence of TTX, and in a low calcium/high magnesium medium known to block synaptic transmission. NMDA responses also persisted in presence of TTX. Almost all MVNn had their firing rate reduced by IAA, including GABA (35 out of 39 MVNn), muscimol (selective GABA A agonist, 83 out of 83 MVNn) and baclofen (selective GABA B agonist, 61 out of 65 MVNn). Whereas for baclofen this inhibition was associated with a membrane hyperpolarization, in the case of muscimol and GABA it could occur concomitantly with either a membrane depolarization (in about half of the cases in A as in B MVNn) or hyperpolarization. The effects of IAA analogs persisted on both cell types in presence of TTX and in low calcium/high magnesium solution, but the depolarizing responses eventually obtained in normal medium were all reversed to hyperpolarizing ones. Since the application of bicuculline always induced a membrane depolarization, we suggest that GABA in the MVN can play a role either directly (postsynaptic hyperpolarizing action) or indirectly (through presumed inhibitory interneurones). These data confirm the major physiological importance of both EAA and IAA as transmitters in the medial vestibular nucleus (supported by the Swiss NSF).

VESTIBULAR SYSTEM: MORPHOLOGY AND PHYSIOLOGY

216.1

CONTRIBUTION OF REGULAR AND IRREGULAR DISCHARGING SEMICIRCULAR CANAL AND OTOLITH AFFERENTS TO LOW FREQUENCY VOR. A.A. Perachio*, D.E. Angelaki and C.L. Strunk. Dept. of Otolaryngology, Univ. of Texas Medical Branch, Galveston, TX 77555.

The functional significance of the diversity in the dynamic characteristics of regular and irregular discharging vestibular afferents and their role in different motor outputs has recently been addressed (Highstein et al., *JNP* 58:719-738, 1987; Minor & Goldberg, *JN* 11:1636-1648, 1991). Minor & Goldberg have shown that the vestibular nerve input to the high frequency VOR comes almost entirely from regular semicircular canal afferents. The present study examines the relative contribution of regular and irregular vestibular afferents to the low frequency VOR by selectively ablating irregular afferents with 100 uA anodal (inhibitory) currents delivered bilaterally to both labyrinths in squirrel monkeys. Eye movements were monitored using EOG electrodes. The contribution of regular and irregular semicircular canal afferents to the low frequency VOR was examined with 3 and 5 sec duration pulses of anodal currents delivered bilaterally at various intervals during a step of angular velocity about an earth-vertical axis. The relative role of a functional ablation of irregular otolith afferents was examined with 3 and 5 sec duration anodal currents delivered bilaterally during the steady-state otolith-induced ocular response evoked with off earth-vertical axis rotation (OVAR). In contrast to the effects seen during high frequency VOR, ablation of irregular afferents significantly decreases eye velocity during on-axis rotations. The effect is transient and only lasts during the period of electrical stimulation. In addition, ablation of vestibular afferents decreases the steady-state eye velocity during OVAR. This supports previously proposed models that have suggested a role for irregular otolith afferents in the generation of that response. (Hess, *Biol. Cyb.* In press; Angelaki, *Biol. Cyb.* In press, 1992).

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216.2

MORPHOLOGICAL FEATURES OF SINGLE INHIBITORY MEDIAL VESTIBULOSPINAL AXONS RECEIVING HORIZONTAL SEMICIRCULAR CANAL INPUT. Y. Shinoda*, S. Kakei, Y. Sugiuchi and T. Kawasaki. Dept. of Physiol., Sch. of Med., Tokyo Med. & Dent. Univ., Tokyo, 113, Japan.

Single horizontal canal-related type-I medial vestibulospinal tract (MVST) axons were visualized by intraaxonal injection of horseradish peroxidase (HRP) and their morphological features were analyzed in terms of axonal branches reconstructed on serial sections, target motor nuclei, and synapses at an electronmicroscopic level. MVST axons were electrophysiologically identified in anesthetized cats as secondary MVST axons descending in the ipsilateral MLF to the upper cervical cord. In the same cats, motoneurons of different neck muscles were retrogradely labelled with HRP. MVST axons running in the ventral funiculus gave off multiple axon collaterals at multiple segments of the upper cervical cord. Terminal boutons were mainly distributed in laminae VII-IX and also in laminae IV-VI. Single MVST axons terminated in the ventromedial, spinal accessory, and dorsomedial nuclei. In these nuclei, terminal boutons of these axons appeared to make contact with cell bodies and proximal dendrites of retrogradely-labelled motoneurons of various sizes. Electronmicroscopic analysis confirmed direct syaptic contacts on the motoneurons and showed the existence of symmetric synapses and pleomorphic vesicles in the synaptic boutons.

216.3

DIFFERENCES IN CILIARY BUNDLE MORPHOLOGY OF TYPE I AND TYPE II VESTIBULAR HAIR CELLS. **L.L. DiCaprio* and E.H. Peterson. Neurobiology Program, Ohio University, Athens, OH 45701.**

Vestibular hair cells are classically divided into two types based on somatic structure and afferent innervation. Hair cell ciliary bundles also exhibit anatomical diversity, which may affect their mechanical properties, and we ask whether the morphological properties of ciliary bundles vary with hair cell type.

We visualized hair cells in transverse sections through the crista of a turtle, *P. scripta*, using DIC optics or confocal microscopy; the confocal material was double labeled, using dextran rhodamine for afferent terminals and Bodipy phallacidin for hair bundles. We identified type I hair cells by the presence of a calyx. Our results indicate that the ciliary bundles of type I cells have a significantly wider base (from kinocilium to shortest stereocilia) and a greater proportion of short stereocilia than adjacent type II bundles.

Such differences in bundle width could be due to variation in numbers, spacing, or width of individual stereocilia. Scanning electron microscopy of the hair cell apical surface indicates that differences in ciliary number account for at least 70% of the observed variation in bundle thickness. Thus, type I hair cells have significantly more stereocilia than type II cells at the same location on the sensory surface.

Ciliary number is thought to be a major determinant of bundle stiffness. Our results suggest that type I bundles will be stiffer, and will require a greater force to deflect them, than adjacent type II bundles. Thus, type I and type II hair cells may be preferentially responsive to different amplitudes of head acceleration. **Supported by NIH-NIDCD DC00618.**

216.4

CONTRIBUTION OF INDIVIDUAL VERTICAL SEMICIRCULAR CANAL PAIRS TO ESTIMATION OF YAW EYE VELOCITY DURING PITCH WHILE ROTATING. M.Dai, T.Raphan , J-I.Suzuki, S.Yakushin, Y.Arai, B.Cohen* Mt. Sinai School of Med, Brooklyn College of CUNY, Teikyo Univ Sch Med, Tokyo Women's Medical College

Pitching the head while rotating about a spatial vertical axis (PWR) generates continuous horizontal nystagmus whose steady state eye velocity is related to rotational head velocity. This has been shown to be dependent on the integrity of the vertical semicircular canals. Here we studied PWR of a canal plugged animal, but with its right anterior and left anterior semicircular canal (RALP) left intact. Pitching the monkey in the plane of the intact canals, induced vertical and roll eye velocity, but no horizontal component. As the pitch plane was positioned away from the intact canal plane, a horizontal component appeared. The pitch plane that induced the maximal steady state horizontal eye velocity was orthogonal to the plane of the intact canals. A significant steady state eye velocity was achieved with pitch periods ranging from approximately 4 to 25 sec. The data suggest that steady state horizontal head velocity is estimated as a mathematical inner product of the vertical semicircular canal signal with the otolith signal. This signal activates velocity storage to induce steady state eye velocity (Raphan et al, 1983). The direct vestibular pathways from the intact semicircular canals contribute to the modulation in eye velocity. Thus, PWR provides the brain with a way to estimate yaw head velocity in the absence of the lateral semicircular canals. **Supported by:** NIH Grants EY 04148, NS 00294, EY 01867, PSC-CUNY Award #662480.

216.5

AFFERENT AND EFFERENT RESPONSES FROM MORPHOLOGICALLY IDENTIFIED FIBERS FROM THE POSTERIOR SEMICIRCULAR CANAL OF THE TURTLE. A.M. Brichta* and J.M. Goldberg. Dept. Pharm. Physiol. Sci., Univ. Chicago, Chicago, IL 60637

Units innervating the posterior semicircular canal in the isolated head of the turtle, *Pseudemys scripta*, were divided into three groups based on their discharge regularity and on their gains and phases re head velocity of their responses to sinusoidal head rotations (0.1-1 Hz), (Soc. Neurosci Abstr 17: 312). Group 1 consist of regular and irregular units encoding head velocity. Phase leads increase from ≈0° in the most regular units to ≈45° in the most irregular units . Gains increase only 1-3 fold between 0.1-1 Hz Group 2 are irregular units whose phases are nearer head acceleration (≈60°) and whose gains grow ≈5 fold over the same frequency range. Group 3 are irregular units responding near head jerk. Phases are ≈ 150° and gains increase 10-50 fold as frequency is raised.

Intra-axonal labeling was used to characterize the peripheral terminations. Groups 1 and 2 are bouton units innervating the peripheral zone of either posterior hemicrista. The first group are located nearer the *planum semilunatum*, while the second group are found near the *torus*. The third group go to the *papilla neglecta*, a small end organ located at the posterior canal-utricular junction . Calyx units, found in the central zone, are a fourth group: they are silent at rest, but otherwise resemble group 1 units. Recordings were also made in intact animals. Discharge properties of all four groups were almost identical in the intact and isolated preparations. Efferent axons were electrically activated in the anterior ramus of the VIIIth nerve. Group 1 afferents were invariably excited by efferent stimulation, while many group 2 afferents were inhibited.

The results suggest that the isolated turtle ear provides an excellent opportunity to study sensory transduction and efferent control in a vestibular end organ. (Supported by ONR Contract N00014-88-k-0381).

216.6

BRAINSTEM ARCHITECTURE OF VESTIBULAR AXONS FROM THE POSTERIOR SEMICIRCULAR CANAL. J.A. Huwe* and E.H. Peterson. Neurobiology Program, Ohio University, Athens, OH 45701.

Previously we described the architecture of peripheral terminals in the posterior crista in a turtle, *P. scripta* (Brichta and Peterson, '92, Ann. N.Y. Acad. Sci.). In the present study we are developing a parallel classification of the central axons of these afferents. We use a palatal approach to the posterior ampullary nerve and label primaries with biotinylated dextran amine. We use long survival times to allow complete filling of the smallest central processes, reconstruct individual axons, and compare them with the total projection from the posterior canal.

All axons bifurcate upon entering the brainstem and emit ascending and descending branches. Some bear varicosities distal to the bifurcation, at their entrance to the brainstem. At the level of the bifurcation, some axons emit clusters of varicosities in the ventrolateral nucleus.

Ascending branches give off varicose collaterals in the dorsolateral and superior vestibular nuclei (Weston, '36) and deep cerebellar nuclei before entering the cerebellum. Most cerebellar fibers run sagittally, bearing mossy rosettes and smooth varicosities; the axons differ markedly in diameter (0.3-1.8 μm).

Descending branches, at the level of the ventromedial nucleus, emit collaterals that pass under the ventricle and reach the MLF or run in fascicles along the ependymal border. Some axons also have branches that run longitudinally through the descending nucleus as far as the obex. Our data suggest that there are morphological differences between the central processes of vestibular afferents as there are between their peripheral terminals. Supported by NIH-NIDCD DC00618.

216.7

VESTIBULO-OCULOMOTOR CONNECTIVITY IN AN ELASMOBRANCH, THE ATLANTIC STINGRAY, D. SABINA. R.L. Puzdrowski* and R.B. Leonard, Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77555.

The organization of the oculomotor complex in elasmobranchs is different from other vertebrates in that the motorneurons for the medial rectus lie contralateral to the innervated muscle. Based on this, Graf and Brunken ('84) postulated that in elasmobranchs all excitatory vestibulo-ocular neurons should project to the contralateral extraocular motor neurons. To examine this possibility, 0.1-0.2μl injections of 2% wheatgerm HRP-20% HRP were made unilaterally into the oculomotor nuclear complex of stingrays. After survival times ranging from 8-23 days, the animals were perfused transcardially with elasmobranch ringers, followed by a 2% paraformaldehyde-3% glutaraldehyde solution. Brains were sectioned at 40μm and reacted with TMB for visualization of transported HRP. Labeled cells were found in the anterior, magnocellular, and descending octaval nuclei. The cells in the anterior nucleus were ipsilateral to the injection site and those in the descending were predominantly contralateral. The labeled cells in the magnocellular nucleus were similar in size to those in the descending, and also were predominantly contralateral to the injection. Based on this distribution of vestibulo-ocular neurons, we hypothesize that the neurons in the anterior nucleus are inhibitory and those in the contralateral descending nucleus are excitatory. (Supported by NIH grant DC 00036-02)

216.8

THE INTERSTITIAL NUCLEUS OF THE VESTIBULAR NERVE IN THE CHINCHILLA. MORPHOLOGICAL ASPECTS AND PRIMARY AFFERENT INNERVATION. K. Beykirch, A. Newman, L.F. Hoffman, W.S. Lee, Y. Naito, J.P. Segundo*, V. Honrubia, Victor Goodhill Ear Research Center, UCLA Div. of Head and Neck Surgery, Los Angeles, CA 90024

The interstitial nucleus of the vestibular nerve (IN) was investigated in the chinchilla using toluidine blue staining to elucidate the soma morphology, while HRP and biocytin tract tracing techniques [Lee et al :*Cell Biol Internat Rep* 13:635-636,1989] were used to study the afferent innervation from the individual vestibular receptors. The IN was dorsolateral to the spinal trigeminal nucleus, and ventromedial to the ventral cochlear nucleus and resides completely within the vestibular nerve root in the brainstem. It extends rostrocaudally for 0.8 to 1.0 mm. The IN was ellipsoid with its long axis in a rostral dorsomedial to caudal ventrolateral direction. Its long axis was 1.1 to 1.4 mm, parallel to the vestibular primary afferent fibers. It was approximately 0.2 to 0.25 mm in width in the mid-portion. One selected specimen contained 424 neurons ranging in size from 4 - 40 microns, with the larger ones (>20 microns) in the dorsomedial aspect of the IN throughout the rostrocaudal extent.

The individual vestibular endorgans innervated specific regions of the IN. Fibers from the endorgans innervated by the superior vestibular nerve (anterior semicircular canal, ASC; horizontal semicircular canal, HSC; utricle, UTR) were located in the rostral portion of the IN. The ASC fibers were most rostral. More caudal, the HSC fibers were dorsolateral and the UTR fibers were ventromedial. The receptors innervated by the inferior vestibular nerve (posterior semicircular canal, PSC; sacculus, SAC) were located in the caudal portion of the IN. Fibers from the PSC were located rostral and medial to the fibers from the SAC which were located most caudal. The SAC innervated a larger area of the IN than any of the other receptors. The innervation patterns were predominantly axodentritic with boutons *en passant*, though some axosomatic contacts were observed via boutons *termineax* . (Work supported by NIDCD grant DC00008 and NIDCD grant DC01404.)

216.9

PROJECTIONS OF THE INDIVIDUAL VESTIBULAR END-ORGANS IN THE BRAINSTEM OF THE SQUIRREL MONKEY. *A. Newman, Y. Naito, W.S. Lee, K. Beykirch, V. Honrubia. UCLA Sch. of Med., Los Angeles, CA 90024.

The CNS projections of primary afferent neurons from individual vestibular receptors were studied using horseradish peroxidase (HRP) or biocytin labelling in 14 ears from seven adult squirrel monkeys. The technique for labeling individual end-organs was similar to that used in the chinchilla [1].

The specificity of labeling was verified by examining the location of the labeled fibers and cell bodies in the vestibular nerve and Scarpa's ganglion. Labelled primary afferents in the vestibular nerve root exhibited a receptor-dependent segregation. Fibers from the horizontal semicircular canal (HSC) and the superior semicircular canal (SSC) were found rostrally and those from the posterior semicircular canal (PSC) and the sacculus (SAC) were found in the caudal area. Utriculus (UTR) fibers were situated between those areas. A bundle of fibers, probably vestibular efferents, were identified immediately rostral to the UTR fibers. The primary afferent fibers bifurcated into an ascending and descending longitudinal fiber tract of secondary branches at the lateral border of the vestibular nuclei. The secondary fibers from individual endorgans occupied specific locations in that: the UTR fibers were dorsal to the SSC and the HSC fibers; the PSC fibers were found most medially and the SAC fibers occupied the lateral-most area. The secondary UTR fibers overlapped considerably with those of the SSC and the HSC. Receptor dependent segregation of fibers was more prominent in the descending tracts than in the ascending tracts.

Projection areas of the tertiary branches of various endorgans exhibited considerable overlap within the major vestibular nuclei. However, differences in the projection patterns could be identified. Within all these nuclei, the SAC projected primarily laterally, the SSC and the UTR ventromedially to the SAC and the HSC projected slightly medially to the SSC area. The PSC projected most medially. The UTR and the SAC sent numerous fibers to the cerebellum. Fibers from the semicircular canals were found projecting through the rostrodorsal region of SN, presumably also projecting to the cerebellum.

1. Lee W, Newman A, Honrubia V: HRP labelling of afferent vestibular nerve fibers in mammals. Cell Biol Internat Report 13: 635-636, 1989.

(Work supported by NIDCD grant DC00029.)

216.10

MONOSYNAPTIC INPUT FROM THE CEREBRAL CORTEX TO THE VESTIBULAR BRAINSTEM NUCLEI IN THE RAT W. Guldin*, S. Mirring, and O.-J. Grüsser
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The cortical input to the brainstem vestibular nuclei in the rat was investigated by means of the retrograde flourescence tracers rhodamine dextran, FITC-dextran and fast blue. For determining the loci of the injections, single unit recordings were carried out under natural vestibular stimulation conditions with a turntable. Recordings were done in anaesthetized animals.

The vestibular brainstem nuclei receive direct input from different cortical areas, such as the border region of the prefrontal and cingular cortex (Cg3 and Cg2), a region of Fr3 adjacent to the Par1 region, and the Par1 region itself. A band of labelled units was found, running from the middle part of area Par2 into the temporal area T1 (nomenclature according to Zilles). Some labelled units were located at the dorso-caudal border of the insular cortex. The distribution pattern of cortical cells connected directly to the vestibular brainstem nuclei of rats is similar to that found in different primates (Macaca fascicularis, Saimiri sciureus, Callithrix jacchus). The question of homology to vestibular cortical regions in primates will be discussed. (Supported in part by the Deutsche Forschungsgemeinschaft Gr 161)

216.11

MORPHOLOGY OF THE EXCITATORY DISYNAPTIC EXTRA-MLF ANTERIOR CANAL PATHWAY IN THE CAT. Y. Uchino, M. Sasaki, N. Isu, N. Hirai, M. Imagawa, K. Endo and W. Graf*. Tokyo Medical College, Shinjuku-ku, Tokyo, Japan and Lab. Physiol. Neurosensorielle, CNRS, Paris, France.

The three-neuron vestibulo-ocular reflex arc provides the fastest link between the vestibular receptor cells and the extraocular muscles for the generation of compensatory movements. We have now determined by intracellular application of horse radish peroxidase (HRP) the morphology of excitatory second-order neurons of the anterior canal system that course outside the medial longitudinal fasciculus (MLF) towards their target sites. Cell somata of these neurons were located in the superior vestibular nucleus. The main axon ascended through the deep reticular formation beneath the brachium conjunctivum to the level of the rostral extent of the nucleus reticularis tegmenti pontis (NRTP) where it also crossed the midline. Small collaterals branched into the superior central nucleus of the raphe and the adjacent reticular formation. The main axon then reached the caudal edge of the red nucleus from where it coursed back towards the oculomotor nucleus. Within the oculomotor nucleus, collaterals reached superior rectus and inferior oblique motoneurons. Some axon branches recrossed the midline within the oculomotor nucleus and targeted superior rectus motoneurons on that side. The signal content and spatial tuning characteristics of this vestibulo-oculomotor neuron remain to be determined.

216.12

CENTRAL PROJECTIONS OF CANALICULAR PRIMARY AFFERENT NEURONS: CORRELATIONS WITH PERIPHERAL INNERVATION LOCUS AND PHYSIOLOGIC CHARACTERISTICS. L. F. Hoffman* and V. Honrubia. Goodhill Ear Ctr., Div. of Head & Neck Surgery, UCLA School of Medicine, L.A., Ca. 90024.

Individual primary afferent neurons innervating the bullfrog's anterior and horizontal semicircular canal cristae were intracellularly labeled with horseradish peroxidase or biocytin following electrophysiologic characterization. Subsequent to histochemical processing of nerve and brainstem, the central projections of each afferent were reconstructed and evaluated with respect to innervation locus within the crista and physiologic characteristics.

The crista-specific central projections of primary afferent neurons sampled in the present study were found to reflect the combined characteristics of crista innervation locus and physiology. Large axon-diameter primary afferents that originated in the central isthmus region of the crista were found to project to some areas of the brainstem vestibular nuclei that were independent of projections from afferents of comparable axon diameter originating in the more peripheral, planar regions. For example, the central region of the ventral vestibular nucleus (i.e. at the level of the vestibular nerve root) received projections from isthmus originating afferents, where projections from planar-originating afferents were not observed. Conversely, the superior vestibular nucleus was found to receive projections from the planar-originating but not the isthmus-originating afferents. Other areas of the vestibular nuclei appeared to receive projections from afferents originating in both crista regions. These included the rostrodorsal and caudodorsal regions of the descending vestibular nucleus. These patterns of central innervation are independent of axon diameter and spontaneous activity characteristics. Though the isthmus-originating afferents exhibited generally higher gains than those innervating the planar regions, the most marked physiologic difference between these afferents was in their response phase to 0.05 Hz acceleration sinusoids. Isthmus-originating afferents exhibited phases closer to stimulus acceleration compared to planar-originating afferents. The central projections of smaller caliber planar-originating afferents exhibited differences from larger afferents innervating comparable crista loci, concommittant with marked differences in their physiologic characteristics. (Supported by NIDCD DC01404.)

216.13

VESTIBULAR INPUTS TO RAPHE-SPINAL NEURONS IN THE CAT: IMPLICATIONS FOR AUTONOMIC AND MOTOR CONTROL. P.S. Bolton*, T. Goto and B.J. Yates. Laboratory of Neurophysiology, The Rockefeller Univ., New York, NY 10021.

Caudal medullary raphespinal neurons (RSN) include cells which mediate motor, antinociceptive and autonomic effects. We previously showed that over 70% of RSN with either slow (5 m/s or less) or more rapid conduction velocities responded to vestibular nerve stimulation. In the present study we determined the nature of vestibular inputs to RSN by analyzing their responses to sinusoidal whole body tilts in vertical planes (delivered at 0.02-1 Hz).

Experiments were conducted on decerebrate cats which were baroreceptor-denervated, vagotomized and had a spinal transection at C_8. Floating electrodes for antidromic stimulation were placed in C_3-C_4. Over 70% of RSN whose firing rate was modulated by whole body tilts had response gains that remained flat across stimulus frequencies, suggesting that their vestibular inputs came principally from otolith organs. For most cells (over two-thirds), the plane of body rotation which produced maximal excitation was nearer pitch than roll.

The present data suggest that changes in head position in the sagittal plane (pitch) may influence autonomic and motor function via the raphe nuclei.

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

216.14

A QUANTITATIVE ULTRASTRUCTURAL STUDY OF THE NEONATAL CHICK TANGENTIAL NUCLEUS AFTER VESTIBULAR NERVE TRANSECTION. E.M. Aldrich and K.D. Peusner*. Department of Anatomy, George Washington University School of Medicine, Washington, DC 20037.

The mechanism of vestibular compensation, the recovery from deficits after unilateral vestibular nerve lesion, has yet to be determined. Past studies have implicated synaptic terminal sprouting in recovery of function. Behavioral studies of hatchling chicks after vestibular neurectomy have shown that compensation occurs over 7-10 days (Aldrich and Peusner, 1989). We have completed a quantitative ultrastructural analysis of the tangential nucleus (TN/lateral vestibular nucleus) in these animals. The TN principal cells receive large calyciform endings from colossal vestibular fibers. Axons project to abducens and spinal motor neurons (Cox and Peusner, 1990). Following vestibular terminal degeneration, no change was detected in percent soma covered by terminals, mean terminal length, number of terminals per 100 μm, or distribution of synaptic terminal lengths up to 8 weeks after lesion. There was no evidence of TN neuron death. The results suggest that physiologic mechanisms should be investigated. Supported in part by NIH grants NS18108 and DC00970.

SPINAL CORD AND BRAINSTEM III

217.1

INTRINSIC DETERMINANTS OF RAT MOTONEURON REPETITIVE FIRING PATTERN. F. Viana*, D.A. Bayliss and A.J. Berger. Dept. of Physiol. & Biophys., Univ. of Washington Sch. of Med., Seattle, WA 98195.

Together with recruitment of silent motoneurons, the *pattern* and frequency of discharge of individual motoneurons determine the smooth gradation of force generated by a muscle. Intrinsic properties of different neuronal types greatly influence their repetitive firing response to excitatory inputs. We studied the repetitive firing behavior of neonatal rat (P0-P14) hypoglossal motoneurons (HMs) (n=50) in brainstem slices. Transduction properties were studied by measuring the repetitive firing response to intracellularly injected depolarizing current pulses.

All HMs fired repetitively in response to 2-s depolarizing current pulses. Repetitive firing threshold current (I_{thr}) averaged 0.6±0.3 nA and minimal steady firing rate (f_{min}) was 13±6Hz. A good correlation was found between $1/f_{min}$ and AHP duration (r=0.83). Also, I_{thr} was correlated with R_N (r= -0.74). The steady-state f-I relationship was linear, with a single slope (30±10 Hz/nA). The f-I slope weakly correlated with R_N (r=0.38) and with AHP duration (r= -0.44).

Neonatal HMs were classified according to 3 repetitive firing *patterns*: 1) *Type D* HMs were characterized by spike-frequency *adaptation* (SFA). The time course of SFA was complex with an early (< 1s) and late phase; the early phase accounted for more than 80% of the total adaptation. During a train of action potentials, the AHP amplitude summated in *Type D* HMs. 2) *Type I* HMs were characterized by spike-frequency *acceleration*. This firing pattern was well fitted with a single exponential function (τ = 90 ms). In *Type I* HMs, AHP amplitude decreased during trains of spikes. Block of the Ca^{2+}-dependent AHP conductance transformed the *I* pattern into a *D* pattern. 3) *Type NM* (non-modulated) HMs had no change in firing rate during long pulses.

In summary, the pattern and frequency of discharge of neonatal HMs to steady synaptic input may not be specified by the characteristics of synaptic drive alone, but may also be a function of motoneuronal intrinsic properties. (NIH NS 14857)

217.2

EARLY POSTNATAL CHANGES IN RAT HYPOGLOSSAL MOTONEURON RESPONSES TO THYROTROPIN-RELEASING HORMONE (TRH) AND SEROTONIN (5-HT) *IN VITRO*. D.A. Bayliss*, F. Viana and A.J. Berger. Dept. Physiol. & Biophys., University of Washington , Seattle, WA 98195.

Normal maturation of a motoneuronal system may involve changes in motoneuron intrinsic properties or in effects of neuromodulators on those properties. The innervation of motoneurons by the coexisting neuromodulators TRH and 5-HT is derived mainly from the caudal raphe nuclei. In raphe neurons, TRH mRNA is developmentally regulated; barely detectable at birth, it rises steadily to near adult levels by P21 (*FASEB J. 4:*A1109, 1990). Therefore, we determined the motoneuronal response to TRH over this same postnatal period. Conventional intracellular recordings of hypoglossal motoneurons (HMs) in brainstem slices of adult rats (>P21) revealed that TRH (1-10 μM) caused a depolarization and decreased input conductance (G_N) in *all* HMs (n=71). The response was more variable in neonatal HMs (P0-P21); in some, the effect of TRH was similar to that in adult HMs (depolarization with decreased G_N), whereas in others, TRH was either without effect or caused a slight depolarization with no change in G_N. We grouped neonatal HMs according to the effect of TRH on G_N. The percentage of HMs exhibiting "adult-like" responses (G_N to <85% of control) increased from 25% (2/8) at P0-P2 to 50% (4/8) at P3-P5, 62.5% (5/8) at P6-P8 and 100% (14/14) by P14-P21. Thus, the response to TRH in HMs changed over the early postnatal period. The response of HMs to 5-HT also appeared to change during development. 5-HT (100 μM) caused a depolarization with no effect on G_N in young HMs (≤P8, n=11). Also, 5-HT decreased the amplitude of the spike after-hyperpolarization (AHP) in most young HMs (9/11) that resulted in an increased slope of the curve relating firing frequency to injected current (f-I curve). By contrast, in older HMs (≥P20) studied to date (n=4), 5-HT caused a depolarization with decreased G_N. It had little effect on the AHP and caused a parallel leftward shift in the f-I curve. Thus, although 5-HT depolarized both young and older HMs, the mechanisms involved were different.
(NIH NS14857 & Francis Families Foundation)

217.3

NOREPINEPHRINE (NE) EXCITES HYPOGLOSSAL MOTONEURONS. M.A. Parkis, D.A. Bayliss, and A.J. Berger*. Department of Physiology & Biophysics, University of Washington School of Medicine, Seattle, WA 98195.

The presence of NE-containing axons and processes in the hypoglossal motor nucleus was first reported by Levitt and Moore (*J. Comp. Neurol. 186:*505-528, 1979). A subsequent study supported this finding by showing that the hypoglossal motor nucleus contains fibers and varicosities strongly immunoreactive to tyrosine hydroxylase and dopamine β-hydroxylase, but not to phenylethanolamine N-methyltransferase (*Brain Res. Bull. 21:*305-312, 1988). Although NE has been reported to excite hypoglossal motoneurons (HMs) (*Soc. Neurosci. Abstr. 8:*958, 1982), little is known about the mechanism of this excitation.

We characterized the electrophysiological response of HMs to NE *in vitro* using conventional intracellular recording from HMs in 400-500 μm brainstem slices taken from juvenile (<30 d.o.) rats.

In all cells recorded to date (n = 11 HMs), NE (50 or 100 μM) applied in the bathing medium or by pressure-injection caused a reversible depolarization, increased input resistance (R_N, mean increase of 41 $\pm$ 13%, n = 8 cells), and a lowering of rheobase. Analysis of action potentials generated by injecting brief (2-msec) current pulses revealed that in three HMs, NE diminished the amplitude of the medium-duration afterhyperpolarization (mAHP, avg. decrease of 34 $\pm$ 15%) The mAHP of two others was unchanged. Those cells that showed a decrease in mAHP amplitude in response to NE also showed a corresponding increase in the slope of the relationship between steady-state firing frequency and injected current.

The increased R_N accompanying the depolarizing response suggests that the effect of NE involves closure of K^+ channels present in the membrane of HMs. (Supported by NIH grant NS 14857).

217.4

EXCITATORY AMINO ACIDS (EAA) INVOLVED IN MIXED CHEMICAL AND ELECTRICAL SYNAPTIC TRANSMISSION IN FROG SPINAL CORD.

M.-S. Rioult-Pedotti* and H.P. Clamann, Dept. of Physiology, University of Bern, 3012 Bern, Switzerland

Synapses between primary afferents and motoneurons (MNs) in amphibians provide joint electrical and chemical transmission. To study properties of the electrical coupling in isolation competitive antagonists of EAAs were used to block chemical transmission. Responses to supramaximal dorsal root stimulation were recorded from ventral roots and intracellularly in lumbar MNs of isolated frog spinal cords.

Evoked excitatory postsynaptic potentials (epsps) were of long duration (up to 1s) which often evoked action potentials. 100 μM D-2-Amino-5-phosphonopentanoic acid (D-APV) abolished spike activity and a late epsp component. At temperatures between 17-20°C MN responses were entirely blocked by 40 μM 6-Cyano-7-nitro-quinoxaline-2,3-dione (CNQX) while at low temperature, 8-12°C, an early (<1ms) component with a steep rise time and two decay rates, fast and slow remained. This component was blocked by arachidonic acid which uncouples gap junctions.

It is concluded that glutamatergig transmission in the amphibian spinal cord is mediated by NMDA and non-NMDA receptors and that the electrical coupling is temperature labile. (Supp.: SNF Grant 31-27973.89)

217.5

REDUCTION OF Na^+-K^+ ATPase ACTIVITY DOES NOT REDUCE THE LATE ADAPTATION OF MOTONEURON DISCHARGE. A. Sawczuk and M.D. Binder*. Dept. of Physiology & Biophysics, Univ. of Washington, Seattle, WA 98195.

In response to a constant-current stimulus, motoneurons display a pronounced reduction in firing rate during the first second of discharge (early adaptation) followed by a more gradual decline in frequency that continues over tens of seconds or even several minutes ("late adaptation"; Granit et al. *J. Physiol.* 169: 1963). The early adaptation is related to an increase in the K^+ conductances associated with the AHP, but late adaptation appears to involve additional mechanisms. One such mechanism might be a gradually increasing hyperpolarizing current generated by the electrogenic sodium pump (Kernell and Monster. *Exp. Brain Res.* 46:1982). To test this hypothesis, we injected long (>60 s), supra-threshold depolarizing current pulses into hypoglossal motoneurons in brainstem slices of young (3-7 weeks) rats before and after adding ouabain (4-20 μM) to the bathing solution. Prior to the addition of ouabain, the membrane potential displayed marked post-discharge hyperpolarization (-11$\pm$2 mV). The addition of ouabain substantially reduced this hyperpolarization (64$\pm$14%), but did not reduce the extent of late adaptation. We are currently investigating other potential contributors to late adaptation such as Na^+ channel inactivation and slowly-activated K^+ currents. (Supported by NIDR grant DE00161 and NIH grant NS26840)

217.6

SHORT-TERM SYNCHRONIZATION OF MOTORNEURONS DEPENDS ON MAP OF EXCITATORY CONNECTIONS. F.A. Dodge*, IBM Research, T.J. Watson Center, Yorktown Heights, NY 10598.

During tonic reflexes, the fluctuations in synaptic drive resulting from the convergence of many excitatory interneurons (EIN) would be expected to show some degree of correlation among motorneurons (MTN) in a given pool: if all the EIN projected globally to all the MTN like the spindle Ia afferents, then the fluctuations would be highly correlated in all MTN; but if the EIN projected locally only to near neighbors as is know for identified inhibitory interneurons, then the fluctuations in widely separated MTN would be completely uncorrelated. A correlated depolarizating fluctuation would tend to synchronize any tonically firing MTN whose membrane potential is approaching threshold at that time; hence, we might expect a map of the strength of short-term synchronization (C-SYNC) to strongly reflect the map of connectivity of the EIN.

Using a mathematical model that realistically simulates a motor pool (Neurosci. Abstr. 17:647, 1991), I have found that C-SYNC (measured as the probability of synchronized firing in excess of chance) is a stochastic variable whose expected values can be predicted from the correlation coefficient and the magnitude of the synaptic noise in the two MTN, weighted by a factor that depends on how closely their mean spike rates match. The model reveals practical limitations to mapping connectivity by C-SYNC.

217.7

CORRELATED AND UNCORRELATED FLUCTUATIONS OF MOTONEURON EXCITABILITY DURING MONOSYNAPTIC REFLEXES. J.-P. Gossard, M. K. Floeter, S. J. Schiff, Y. Kawai and R. E. Burke* Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892 and Children's Hospital National Medical Center, Washington, DC 20010.

Rall and Hunt (*J. Gen Physiol.* 39:397, 1956) showed that fluctuations of monosynaptic reflexes (MSRs) result from correlated and independent variations in motoneuron (MN) discharge probabilities. We have re-examined this phenomenon in unanesthetized decerebrate and decerebrate-low spinal cats with respect to: 1) the probability profiles of individual MNs recorded simultaneously in teased ventral root filaments as functions of population MSRs in the rest of the same ventral root; 2) correlations between group Ia EPSPs and baseline membrane potential recorded intracellularly in a homonymous motoneuron and MSR fluctuations and MN probability profiles; and 3) the effects of conditioning inputs in muscle, skin, and descending afferent systems. Results to date suggest that: 1) individual MNs show different levels of uncorrelated probability fluctuations, with a tendency to greater indepedence among higher threshold cells; 2) increased MSRs after post-tetanic potentiation is sometimes accompanied by changes in mean unit firing probabilities; 3) conditioning extensor MSRs with stimulation of flexor group I afferents that generate presynaptic inhibition produces little change in average MN firing probabilities despite marked inhibition of population response; and 4) conditioning input in skin afferents that generate short latency excitation in extensor motor pools can produce relatively large shifts in probability profiles in some triceps surae MNs in relation to population MSRs. In general, shifts in MN probability profiles were not accompanied by changes in variance. These observations suggest that the relative excitability of individual MNs within a particular motor pool during MSRs not only vary independently of overall pool excitability but can differ between MNs, presumably due to differences in the organization of synaptic inputs among MNs within the pool.

217.8

ACCELERATION OF MOTONEURON DISCHARGE RATE BY HYPERPOLARIZING CURRENT TRANSIENTS. R.K. Powers* and M.D. Binder. Dept. of Physiology & Biophysics, Univ. of Washington, Seattle, WA 98195.

A change in somatic voltage produced by an injected or synaptic current transient alters the discharge probability of a spontaneously-firing neuron by changing the difference between the membrane potential and the spike voltage threshold (e.g. Ashby and Zilm. *Exp Brain Res 47* : 33, 1982). Conventional models of this process predict that a depolarizing current transient will advance the occurrence of the subsequent spike and that a hyperpolarizing current transient will delay it. We have tested the effects of hyperpolarizing current transients on repetitive discharge in cat lumbar motoneurons by superimposing short (5 ms), hyperpolarizing injected current pulses on long (10 s), suprathreshold, depolarizing current steps. In a few cells, the addition of a hyperpolarizing current pulse paradoxically advanced the occurrence of the subsequent spike. In these cases, it appears that the hyperpolarizing transient produces a decrease in spike voltage threshold that exceeds its effect on somatic membrane potential. We are currently investigating the mechanisms underlying this variation in spike threshold using several motoneuron models. These results suggest that at times a hyperpolarizing synaptic input may increase rather than decrease the rate of dischare of the postsynaptic cell. (Supported by NIH grants NS26840 and NS25206)

217.9

MECHANICAL PROPERTIES OF THE TONGUE: EFFECTS OF WHOLE AND PARTIAL HYPOGLOSSAL NERVE STIMULATION IN RAT. E. E. Gilliam and S. J. Goldberg*. Department of Anatomy, Medical College of Virginia-Virginia Commonwealth University, Richmond VA 23298.

The rat's tongue like man's is composed of extrinsic and intrinsic muscles which function to protrude and retract the tongue. The hypoglossal nucleus as well as the nerve are basically "compartmentalized" into protrusive and retrusive subdivisions (nucleus) or branches (nerve) so that the individual muscle groups may be activated in a coordinated manner. This study was designed to begin the examination of the tongue's mechanical properties in detail.

Male Sprague-Dawley rats (200-350 g) were anesthetized with urethane (1.3 g/kg), tracheotomized, and the XIIth nerve was exposed using a ventral approach. The whole nerve or one of its branches was stimulated with bipolar, silver wire electrodes. A silk suture was placed at the apex of the tongue and attached to a sensitive strain gauge after the animal was stereotaxically placed. A preload of ≈4 g was found to be optimal. Stimulation of the whole nerve unilaterally with single stimuli (2X threshold) produced a net retrusive force of 8-10 g, 13-18 ms twitch contraction times, and ≈20 ms half decay times. Tetanic stimulation at 50 to 150 HZ, in 10 HZ increments, for 200 ms yielded 22-27 g tensions and fusion frequencies from 90-110 HZ. Stimulation of the lateral division at 2X threshold produced a retrusive force of 6-10 g, 14-19 ms twitch contraction times, and ≈13 ms half decay times. Tetanic stimulation resulted in 21-25 g tensions and fusion frequencies of ≈100 HZ.

In a separate study, stimulation of the medial branch of the nerve was done and two strain gauges were placed at 30° to each side of the midline of the tongue so that lateral tongue deviations could be recorded. A preload of ≈2.0 g was used on each strain gauge. Low intensity stimulation yielded protrusion with 0.4-0.5 g twitches, ≈14 ms twitch contraction times, and ≈11 ms half decay times. Tetanic fusion occurred at ≈100 HZ with ≈1.5 g tensions. Current studies involve monopolar stimulation of restricted areas of the XIIth nucleus with glass electrodes (≈10-15μ tip diameter). Areas of the nucleus which produce protrusion and retrusion have been identified.

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217.10

SYNAPTIC MORPHOLOGY OF PRIMATE TRICEPS SURAE MOTONEURONS: INITIAL STUDIES. K.A. Starr* and J.R. Wolpaw. Wadsworth Labs, NY State Dept Health & State Univ NY, Albany, NY 12201.

In order to define anatomical changes associated with operant conditioning of the primate triceps surae (TS) H-reflex, the electrical analog of the stretch reflex (TINS 13:137-142, 1990), we are examining in monkeys (Macaca nemestrina) the synaptic contacts on TS motoneurons at the electron microscopic (EM) level. Motoneurons are labeled by retrograde transport of cholera toxin-HRP and visualized with tetramethylbenzidine (Olucha et al, 1985) stabilized with DAB (Rye et al, 1984). Somatic and dendritic synaptic contacts are analyzed by size and shape of synaptic vesicles, subsynaptic specializations, number of active zones, and location.

Initial analysis of data from two naive (i.e., untrained) animals reveals five categories similar to those of Conradi (1969). F-type boutons, with flattened or pleomorphic vesicles, make up 62% of the population and S-type boutons, with spherical vesicles, make up 18%. Both types range in size from 0.8-3.3μm. Larger C-type boutons (1.2-6.4μm), with postsynaptic subsurface cisterns, make up 11% of the population, and M-type boutons (3.4-4.9μm), with multiple active zones and subsynaptic dense bodies, make up 6%. P-type boutons, which are presynaptic on M-type boutons, are 0.8-1.1μm and make up 3% of the population. (Supported by NIH NS22189.)

217.11

QUANTITATIVE ANALYSIS OF RAT MOTONEURON MORPHOLOGY. X.Y. Chen* and J.R. Wolpaw. Wadsworth Labs, New York State Dept of Health and State Univ of New York, Albany, NY 12201.

As part of developing H-reflex conditioning in rat as a model for learning (Wolpaw & Chen, this vol), we are analyzing the elements of the reflex pathway (motoneurons and Ia afferent connections).

Fourteen physiologically identified rat triceps surae (TS) motoneurons (MNs) were intracellularly stained with HRP and reconstructed with a Eutectic system. Somata were in ventral horn of L4-5 and averaged 35μm diameter and 995μm^2 area in sagittal section. Each had 6-12 primary dendrites. Dendritic fields extended widely through and beyond ventral horn and accounted for 97-99% of neuronal surface area. Ventral extent was positively correlated with soma distance from ventral border of ventral horn. Total dendritic lengths, surface areas, and volumes were 33-78mm, .14-.36mm^2, and 63-266x$10^3\mu m^3$, respectively. Primary dendrites averaged 21 branch points, 22 terminations, and 43 branch segments. Their diameters averaged 5.6μm and correlated with total lengths, surface areas, volumes, number of terminations, and maximum segment orders. Tapering averaged 0.21μm/100μm. Rall's ratio, $(\sum(d_{daughter}^{3/2}))/(d_{parent}^{3/2})$, averaged 1.2.

Compared to cat TS MNs, these rat TS MNs had smaller somata, fewer primary dendrites, and smaller total dendritic surface areas and lengths. However, they had more dendritic segments and terminations. Thus, their dendritic fields were more complex. (Supported by NIH NS22189 and Paralyzed Veterans of America Spinal Cord Research Foundation.)

217.12

QUANTITATIVE ANALYSIS OF PRIMATE MOTONEURON DENDRITIC MORPHOLOGY. D.M. Maniccia, C.L. Lee*, and J.R. Wolpaw. Wadsworth Labs, NY St Dept Health & St Univ NY, Albany, NY 12201 & Dept Physiol, UNC, Chapel Hill, NC 27599.

In order to define the spinal cord plasticity associated with operant conditioning of the primate triceps surae (TS) H-reflex (TINS 13:137, 1990), we are analyzing the components of the reflex pathway, the motoneurons and their primary afferent connections, at the light and electron microscopic levels. In the present work, physiologically identified TS motoneurons are intracellularly stained with HRP and reconstructed by camera lucida and with a Eutectic system.

TS motoneurons are clustered in dorsolateral quarter of L6-7 ventral horn. Dendritic fields are elliptical with longest axis rostral-caudal and shortest axis medial-lateral. Dendritic extent is not correlated with soma diameter. However, number of dendritic segments per neuron is correlated with soma diameter, and number per dendrite is correlated with primary dendrite diameter. Thus, larger motoneurons have more complex, but not more widespread, dendritic trees. The most complex neuron analyzed to date has 271 segments and 141 terminations.

Compared to dendritic fields of cat TS motoneurons, those of primate TS motoneurons appear to be more elliptical, and more variable in complexity and extent. (Supported by NIH NS22189 & Paralyzed Veterans of America Spinal Cord Research Foundation.)

217.13

EFFECTS OF H-REFLEX CONDITIONING ON MOTONEURON PROPERTIES. J.S. Carp*, X.Y. Chen and J.R. Wolpaw. Wadsworth Labs, NYSDOH/SUNY, Albany, NY 12201.

Monkeys (Macaca nemestrina) can increase or decrease the triceps surae (TS) H-reflex (HR), the electrical analog of the monosynaptic stretch reflex. Such conditioning changes the spinal cord (TINS 13:137, 1990). In order to define this plasticity and its role in the behavioral change, intracellular recordings were performed in 504 TS motoneurons (MNs) in animals in which one leg's HR had been trained up (HR↑; n=9) or down (HR↓; n=10), and in 14 naive animals. Animals were deeply anesthetized throughout and sacrificed by overdose.

Differences were found among MNs from trained and control sides of conditioned animals and from naive animals. These data, together with HR data from awake behaving animals and nerve volley data from anesthetized animals, give three different views of HR conditioning effects. For example, HRs and nerve volleys are larger/smaller on the trained side of HR↑/HR↓ animals, while the opposite is seen with EPSPs. Control side HRs and MN properties are similar to those in naive animals, while control side nerve volleys are smaller/larger in HR↑/HR↓ animals than in naive animals. The differences in these views presumably reflect differences in animal state and experimental method, and suggest that HR conditioning changes the spinal cord at multiple sites. (Supported by NIH NS22189 and Paralyzed Veterans of America Spinal Cord Research Foundation.)

217.14

H-REFLEX CONDITIONING IN THE RAT: INITIAL STUDIES. J.R. Wolpaw* and X.Y. Chen. Wadsworth Labs, NY State Dept of Health and State Univ of NY, Albany, NY 12201.

Operant conditioning of the H-reflex (HR), which has been demonstrated in primates, is a new experimental model for defining the CNS substrates of learning (TINS 13:137-142, 1990). In order to increase the practical and theoretical potential of this model, we are seeking to establish it in the rat.

Male Sprague-Dawley rats (300-400gm) are chronically implanted with fine-wire EMG electrodes either in right triceps surae (TS) or in right intrinsic foot plantar flexors (IFF), stimulating cuff on right posterior tibial nerve, and bipolar stimulating electrode in left median forebrain bundle. Electrodes connect to a head-mounted tether cable. Monitored by computer, each rat learns to keep background EMG in a specific range. At a random time, nerve cuff stimulation at M response threshold elicits the HR. In control mode, reward (60Hz intracranial stimulation,20-40μA,250-500ms) begins 200ms later. In HRup or HRdown mode, reward occurs only if the HR is above or below criterion. Rats usually perform more than 7,000 trials/24hrs.

Initial results suggest that rats can increase HR amplitude in response to the HRup mode. As in primates, change progresses over days and weeks. The normally small amplitude of the TS HR complicates use of the HRdown mode. Thus, current efforts focus on the typically larger IFF HR. (Supported by NIH NS22189 and Paralyzed Veterans of America Spinal Cord Research Foundation.)

217.15

ALTERED SYNAPTIC TRANSMISSION FROM Ia AFFERENTS AND TO MOTONEURONS, BOTH CROSS-INNERVATING THE SURAL NERVE OF CATS. J.B. Munson*[1], R.D. Johnson[1], J.S. Taylor[1] and L.M. Mendell[2]. [1]Univ of Florida & [2]SUNY at Stony Brook.

Cats whose medial gastrocnemius nerve had been cross-united into the sural nerve 24 months before were anesthetized and prepared for intracellular recording of composite EPSPs from triceps surae motoneurons, which were characterized by rheobase, duration of AHP and input resistance. Peripheral nerves were activated with single shocks (0.5 and 18 Hz) and bursts (32 shocks at 167 Hz, 2 sec interburst interval), and the resultant EPSPs were averaged. As reported (JNphys 65: p590), the EPSPs in low-rheobase motoneurons exhibited more depression during high-frequency stimulation than EPSPs in high-rheobase motoneurons. AHP duration was also a useful predictor of this behavior, but input resistance was less reliable. Motoneurons with axons in the sural nerve developed large EPSPs which were prone to more profound high-frequency depression than those in controls. Similar, perhaps more profound changes were noted when crossed afferents were activated and their synaptic effects evaluated in normal motoneurons. The EPSP amplitude/depression correlation reflects a property of the synapse; thus we surmise that the change occurs in the synapse itself, probably at a presynaptic locus. These changes could result from alterations in activity levels, trophic factors, or both. Supported by NIH: NS-15913, NS-14899, NS-16996, NS-27511.

217.16

SELECTIVE CONNECTIVITY OF LAST-ORDER INTERNEURONS MEDIATING PAD OF GROUP Ib FIBERS ACCORDING TO THE MUSCLE OF ORIGIN. J.R. Eguibar*, J.N. Quevedo, I. Jiménez and P. Rudomin. Dept. of Physiol. CINVESTAV and ICUAP, México.

Although it seems well established that different last-order interneurons mediate PAD of Ia and Ib fibers (J. Neurophysiol, 50:743, 1983), the question remains on whether there is any selectivity in their connections according to the muscle of origin of the target afferent fibers. We have explored the effects of conditioning microstimulation (μS) of the intermediate nucleus simultaneously on the intraspinal threshold of six pairs of single group I afferents. Stimulating pulses were delivered in alternation through two micropipettes placed in the intermediate nucleus to produce antidromic responses in two single group I (Ia-Ib or Ib-Ib) afferents, one from a GS and the other from a PBSt nerve filament. Stimulus current was automatically adjusted to elicit antidromic firing with a constant probability (0.5). Intraspinal μS (<10 μA) delivered at short conditioning testing time intervals (2-5 ms), produced a monosynaptic PAD in the fiber closest to the stimulating micropipette but not in the other fiber, even though PBSt group I conditioning stimulation produced a substantial PAD in both fibers. Our results suggest that a separate presynaptic control can be exerted by last order PAD mediating interneurons on group I fibers arising from different muscles. Partly supported by NIH NS09196 and CONACyT 039-N9107.

217.17

PAD PATTERNS OF REGENERATED GROUP I AFFERENTS AFTER PERIPHERAL NERVE CRUSH IN THE CAT. M. Enríquez, I. Jiménez* and P. Rudomin. CINVESTAV. México.

In normal cats group Ia and Ib muscle afferents have different PAD patterns involving activation of separate sets of last-order interneurons (J. Neurophysiol. 56:987, 1987). Sectioning or crushing cutaneous fibers in the periphery transiently abolishes their PAD (J. Physiol. 313:287, 1981). We have examined in 6 cats the intrafiber PAD elicited in functionally identified group I fibers 6-10 months after crushing the medial gastrocnemius nerve. 16/39 fibers (79.6±20.1 m/s) were classified as "in parallel" based on their responses to vibration, ramp stretch and twitch. Group I PBSt volleys produced PAD in 14/16 fibers (1.1±0.83 mV). This PAD was reduced by conditioning stimulation of the sural (SU) nerve in 6/14 fibers to 52±29% and by the reticular formation (RF) in 5/13 fibers to 77±8%. In 5/13 fibers RF produced PAD (0.39±0.16 mV). 9/39 fibers (84.8+11.3 m/s) were classified as "in series". 8/9 fibers were depolarized by PBSt (1.01±0.87 mV), 3/8 by SU (0.51±0.93 mV) and 6/9 by RF (0.62±0.62 mV). 14/39 fibers (64±13.4 m/s) were not physiologically activated, but in 9/14 fibers PBSt produced PAD (0.36±0.33 mV) and SU in 5/12 (0.34±0.15 mV), which is smaller than the PAD elicited in functionally regenerated fibers. The PAD patterns of regenerated fibers appear not to be different from those of fibers in intact animals, except for a larger number of Ia fibers with PAD induced by RF (10/48 in intact cats). We conclude that the specificity of the connections of PAD mediating interneurons is resumed following the functional regeneration of the afferent fibers. Supported by NIH NS09196 and CONACyT PCEXCCNA 41739.

217.18

DIFFERENTIAL EFFECTS OF RETICULO-SPINAL STIMULATION ON THE INTRASPINAL THRESHOLD OF CUTANEOUS AND MUSCLE AFFERENTS IN THE ISOLATED FROG NEURAXIS. H. González, I. Jiménez and P. Rudomin*. Dept. of Physiol. CINVESTAV, and Fac. de Ciencias, UNAM. México D.F.

In a previous study (Exp. Brain Res. 88:106,1992), we showed in the isolated nervous system of the frog that stimulation of the reticular formation (RF) depressed the DRPs elicited by stimulation of dorsal and ventral roots and suggested that this depression was due to inhibition of PAD of muscle spindle afferents. In isolated preparations with ventral roots sectioned, glass micropipettes (1-3 MΩ) were introduced at L10 segmental level, in the dorsal horn and in the motor nucleus, to produce antidromic responses in cutaneous (gluteus and superficial peroneus) and muscle (tibial) nerve afferents, respectively. RF stimulation (4 pulses 200 Hz, 10-20 μA, 80 ms before the excitability test pulse) increased the antidromic responses elicited in cutaneous nerves (36-104%, n=6). This effect occluded with the PAD elicited in the same fibers by stimulation of dorsal or ventral roots (n=6). In contrast, RF stimulation produced no changes in the antidromic responses of muscle afferents but inhibited the PAD produced in them by stimulation of dorsal (16-31%, n=5) or ventral roots (10-23%, n=5). It is suggested that, like in the cat, descending inputs may inhibit the PAD of muscle spindle afferents and produce PAD in cutaneous afferents. The effect of RF stimulation on Golgi tendon afferents remains to be elucidated. Partly supported by NIH 09196 and CONACyT 0319-N9107.

217.19

PAD OF PERINEAL AFFERENTS EVOKED BY STIMULATION OF SENSORY AFFERENTS AND DURING MICTURITION IN THE CAT. M.J. Angel, D.A. McCrea*, D. Fyda, S.J. Shefchyk Depts. of Physiology and Medicine, Univ. of Manitoba. Winnipeg, CANADA R3E 0W3.

During micturition, activity in the external urethral sphincter (EUS) decreases, and excitatory sphincter reflexes are suppressed. We are exploring the possibility that during voiding, a reduction in transmitter release from tonically active sensory perineal afferents decreases the excitation of EUS motoneurons. The present experiments determine the effectiveness of peripheral nerve stimulation in evoking primary afferent depolarization (PAD) of dorsal penile (sensory pudendal, PUD) and superficial perineal (SFP) afferents. In addition, PAD of these same afferents during micturition was recorded. Experiments were performed on both decerebrate and α-chloralose anaesthetized male cats. Antidromic action potentials of single afferents were evoked by intraspinal micro-stimulation and recorded in the PUD and SFP nerves. Stimulus current was automatically controlled and PAD inferred from a decrease in the current required to maintain a firing probability of 0.5. All hindlimb cutaneous afferents, especially those in posterior tibial and SFP nerves, produced substantial PAD of PUD afferent terminals. Hindlimb muscle afferents were much less effective. In general, low threshold (<2T) afferents evoked the greatest PAD of fast conducting perineal afferents (>35m/s). During micturiton, PAD occurred in 15% of the fibres; primary afferent hyperpolarization (PAH) was observed in 8% of the fibres. The presence of PAH suggests that a tonic level of PAD may be modulated during micturition. These results support the notion that some of the reduction in EUS motoneuron excitation during voiding may be mediated by changes in perineal afferent transmitter release. Supported by the MRC of Canada.

217.20

FAILURES OF TRANSMISSION IN THE MONOSYNAPTIC EPSPS ELICITED BY SINGLE PRESYNAPTIC NEURONS IN SINGLE RAT TRIGEMINAL MOTONEURONS IN-VIVO AND IN-VITRO. Paul D. Grimwood, Kwabena Appenteng and John C. Curtis. SPON: Brain Research Association. Dept. of Physiology, Univ. of Leeds, Leeds, UK.

For each of a sample of 15 EPSPs, elicited by trigeminal spindle afferents and interneurones in trigeminal motoneurones of rats under pentobarbitone anaesthesia, we have determined both the percentage of sweeps which contained EPSPs triggered by the pre-synaptic neurone, and the incidence of randomly triggered EPSPs. We have subtracted the two estimates to produce a corrected incidence of occurrence of EPSPs triggered by the presynaptic neurone. Corrected incidences of failure have ranged from 8 to 96%, and the incidence was linearly related (r^2 = 0.97) to the amplitude of the averaged EPSP. Similar experiments performed in-vitro have revealed, that for individual presynaptic neurones, linear relationships also exist between these two parameters. We conclude that for averaged EPSPs of less than approximately 150 μV, differences in amplitude of the EPSPs can primarily be accounted for by differences in the incidence of failures of transmission.

218.1

THE INFLUENCE OF CONSTRAINTS ON THE KINEMATICS OF A NATURAL PREHENSION TASK. R.G. Marteniuk*, B. Steenbergen, L.E. Kalbfleisch. Human Motor Systems Lab, Simon Fraser University, Burnaby, B.C. V5A 1S6.

Acquiring coordination, according to Bernstein (1967), is 'the mastery of the redundant degrees of freedom'. In solving a novel or complex movement problem (brought about by the relational influence of diverse constraints, e.g. task constraints, organismic constraints), the organism is required to reorganize it's coordination system such that a minimum number of degrees of freedom remain to be actively controlled. In the present study of natural prehension, a multiple degrees of freedom task was used to examine this notion. The kinematics of the movement were examined as a function of task (grasping a full cup versus an empty one), and organismic (hand used) constraints. Seven right handed subjects were required to reach and grasp a cup (the transport phase) and place it on a designated target (the placement phase) using either their preferred or non-preferred hand. Kinematic analysis revealed a precision effect, displayed through increased movement times in the full cup condition, found to be principally due to an increase in the low velocity phase of the movement for the transport phase. Differential lengthening of the pre-peak velocity time was evident for the placement component of the movement. Subsequent analysis of the rate of angular change of the shoulder, elbow and wrist joints demonstrated that, for both transport and placement low velocity phases of movement, a precision effect existed such that for the full cup condition significantly less joint movement occurred. Results however, could not confirm the notion of a fixed relationship between the transport and grasp component in a prehension task (Jeannerod, 1981, 1884; Wing et al., 1986; Wallace and Weeks, 1988). It is argued that the specific coordination relationship is an emergent result of the imposed task constraints in relation to the dynamics of the system. Furthermore as it has been shown that the imposed task constraints had considerable impact on the length of the low velocity phase of the movement, it is argued that joint freezing was implemented in order to solve the movement task at hand. Hence, minimization of the degrees of freedom to be actively controlled is used as a strategy through which more complex movement problems can be solved.

218.2

MAKING CONTACT: TARGET SURFACES AND POINTING IMPLEMENTS FOR 3D KINEMATICS OF HUMANS PERFORMING A FITTS' TASK. C. L. MacKenzie,* School of Kinesiology, Simon Fraser University, Burnaby, BC, V5A 1S6, Canada

Following Fitts' law, kinematics of aiming movements are used for inferences about underlying planning/control. We defined "precision effect" as the relative lengthening of the deceleration phase of movement with decreases in target size (MacKenzie, Marteniuk, Dugas, Liske & Eickmeier, 1987). Having noted changes in impact velocity, the purpose was to examine effects of target surface and pointing implement on 3D kinematics.

Eight adults made aiming movements. Conditions for Index of Difficulty (ID) included: 2 amplitudes (30, 40 cm) and 4 target diameters (1, 2, 4, 8 cm). These were factorially combined with 2 implements (index finger tip or pen tip) and 2 target types (hole or solid target). On each trial, subjects placed the pointer on a constant start position. At the "Ready, GO" signal, subjects moved as quickly and as accurately as possible to the target. The Optotrak system (Northern Digital, Waterloo) collected 3D coordinates at 200 Hz.

MT results replicated Fitts' Law. Kinematic differences among the pointing implement and target surface conditions showed: the pen was faster, with greater, earlier peak kinematic values than the finger tip; a greater proportion of time after peak velocity with the pen than the finger; the hole target had lower peak kinematic values and a greater proportion of time decelerating than the solid target. Results are discussed in terms of task requirements, contact forces in interaction with objects, and subject initiated deceleration control (see also Milner & Ijaz, 1991; Teasdale & Schmidt, 1991).

218.3

RAPID ADAPTATION OF ARM MOVEMENT ENDPOINT AND TRAJECTORY TO CORIOLIS FORCE PERTURBATIONS. J.R. Lackner* and P. DiZio. Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254.

Subjects pointing to a visual target in a rotating room (10rpm,CCW) showed deviations in movement trajectories and endpoints in the direction of the (rightward) Coriolis forces generated by their arm movements. Initial errors were 47±34mm rightward for fast (305±68ms duration) movements and 38±27mm for slow (730±111ms duration). Trajectories and endpoints returned to normal within 40 movements even though subjects were denied visual and tactual error feedback. Adaptation proceeded quasi-exponentially and was about twice as rapid for fast movements as slow. Movements made after the room was stopped showed mirror-image trajectories and endpoint errors to those initially induced by the Coriolis forces during rotation. Initial post-rotation errors were 36±37mm and 27±25mm leftward for fast and slow movements, respectively. Re-adaptation took approximately the same number of movements as adaptation to rotation had. The findings imply that discrepancies between actual and intended arm trajectories - likely determined by comparing correlated patterns of alpha-gamma motoneuronal co-activation and muscle spindle feedback with expected feedback patterns - lead to adaptive compensations to generate straight movements.

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218.4

MODIFICATION OF FAST ARM MOVEMENT EMG PATTERN BY CORTICAL MAGNETIC STIMULATION.
M. Pause , J. Mohm , R. Tauber , K.-H. Mauritz* Dept. of Neurology, Universitaet Wuerzburg, Dept. of Neurorehabilitation Freie Universitaet Berlin, Germany.

It was shown by Day et al. (Brain 112, 1989) that magnetic stimulation of the human motor cortex before a fast target movement delays the entire triphasic EMG pattern, while stimulation after the beginning of the agonist burst delays only the antagonist and later bursts.We reexamined this paradigm with the question as to whether cortical stimulation results only in a delay or in a deletion of a fixed EMG sequence.

Four subjects performed fast elbow extension movements (30 deg) towards a target in a device where the position of the arm and the rectified EMG of the triceps and biceps muscles were synchronously measured. Magnetic stimulation which was focussed on a region evoking responses predominantly in these muscles was triggered by the onset of the first agonist burst with a systematic variation of the stimulus delay from 0 to 280 ms. In a second series the arm movement was initiated by an acoustic signal, and the stimulus was positioned within the reaction time, i.e. in different intervals before the beginning of the voluntary EMG bursts.

With the second paradigm a minimum interval between stimulation and EMG onset was noted with increasing duration at higher stimulation intensities. Apart from a critical period where the stimulation occurs obviously within 15 ms before the beginning of the agonist burst, the general triphasic pattern and the movement were unchanged suggesting a mere temporal shift. With stimulation after the onset of the agonist burst, a delay of the antagonist burst was also observed relating to the poststimulus silent period, but the amplitude and duration of the delayed burst was markedly reduced as if a part of the burst would have been cut off. The movement was modified according to this but was additionally disturbed by the evoked response.

We conclude that an EMG pattern of a simple movement can be delayed without essential changes to the pattern, when magnetic stimulation occurs before the EMG onset while a similar shift of the antagonist burst only is not possible. Stimulation after the beginning of the pattern interrupts the antagonist burst and partially cancels the EMG activity. This result is compatible with the assumption of a fixed motor program.

218.5

SIMILAR TRAJECTORIES FOR SLOW MOVEMENTS TOWARDS A TARGET AND SLOW MOVEMENTS IN A "STRAIGHT" LINE. J.B. de Graaf, A.C. Sittig, and J.J. Denier van der Gon. (SPON: European Neuroscience Association) Delft University of Technology, Jaffalaan 9, 2628 BX Delft, The Netherlands.

Previously we showed that the trajectories of slow arm movements towards visual targets are systematically curved [1]. A possible cause for this curvature is an incorrectly internally represented target position. This would cause a bias in initial movement direction, which is corrected for during the movement. Another possible cause is that the whole movement trajectory that is followed is internally represented as a straight line. In this study we tested these hypotheses. Five normally sighted subjects and four congenitally blind subjects had to move their arm slowly and accurately in a straight line (Exp 1) or towards a target (Exp 2). Stimuli were used to suggest a starting direction (Exp 1) or to indicate a target position (Exp 2). The sighted subjects performed both experiments with visual stimuli and visual feedback (condition A), and with tactile stimuli and excluded vision (condition B). The blind subjects were presented tactile stimuli. The results show that 1) the curvature of the trajectories when moving in a straight line is comparable to the curvature of the trajectories when moving towards a target position, 2) the trajectories in condition A are significantly curved, and 3) the trajectories are significantly curved for the congenitally blind subjects, but not for most normally sighted subjects in condition B.

We conclude that 1) the curved movement trajectories are internally represented as straight lines, and 2) for the normally sighted subjects the curvature of the trajectories is influenced by the absense of visual information, although the curvature is not caused by visual information processing.

[1] J.B. de Graaf et al. (1991) Exp Brain Res 84: 434-438

218.6

DISTURBANCE OF SPATIOTEMPORAL PATTERNS OF EXPLORATORY FINGER MOVEMENTS IN PATIENTS WITH PARIETAL LESIONS F.Binkofski, E. Kunesch, H.-J. Freund*, Dep. of Neurology, University of Düsseldorf, 4000-FRG

In normal subjects exploratory finger movements during object recognition are characterized by a typical spatial pattern of the digital scan path and by regular temporal sequencing of the finger movements that are preformed at a naturally preferred frequency below 2Hz (Kunesch et al., Exp. Brain Res., 78: 539-546, 1989). In order to study the impact of lesions of parietal cortex (PC) on this sensorimotor task, we analysed the temporal and spatial characteristics of exploratory finger movements during recognition of stereometric objects in blindfolded normal subjects (n=17) and patients with lesions of PC (n=8) using a 2-camera Selspot position analysis system. For comparison 6 patients with premotor damage and clinically normal hand function were examined. In order to provide quantitative assessment of the dysfunction of exploratory movements three parameters were defined: 1.preferred frequency and 2.regularity of the digital palpation, 3.movement space of the thumb. In PC lesions with clinical disturbance of stereognostic functions (n=5) the movement frequencies on the affected hand were lower (mean: 0.49Hz, sd: 0.25) than in normal subjects (mean: 1.22Hz, sd: 0.21). The index of regularity calculated as area under peak divided by area under the remainder of the spectrum was lower than in normal subjects (normals: 0.83, sd: 0.05, PC: 0.47, sd: 0.20). Movement trajectories in PC lesions were grossly deranged and thumb movements more expanded in space (PC: mean movement space: 23.0cm^3, sd: 26; normals: mean: 8.3cm^3, sd: 2.6). The results of the unaffected hand in PC were similar to that in normals. In the cases with PMC lesions preferred movement frequencies were slightly lower (mean: 1.04Hz, sd: 0.27), and the regularity index was severely affected (mean: 0.53, sd: 0.30), but movement space and pattern of the thumb were within the normal range. These results indicate that the PC contributes to the motor programs for explorative and manipulative finger movements, whereas the PMC is involved in the temporal organization and the regularity of digital palpation.

218.7

ARM MOVEMENT ENDPOINT ERRORS INDUCED BY CORIOLIS STIMULATION. P. Dizio* and J.R. Lackner. Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254.

We measured pointing movements to a visual target in an otherwise dark slow rotation room with a WATSMART system. Subjects attempted to touch a target about 35cm straight ahead, starting with the fingertip on the axis of rotation. During rotation (10rpm), reaching movements generated lateral Coriolis forces (C_f) proportional to arm velocity which acted without local contact forces. Before and after the movements, virtually no unusual forces were present. Thirteen subjects made fast (305±68ms duration) movements and 11 slow (730±111ms duration). All initial movements exhibited path and endpoint deviations in the direction of C_f. The lateral endpoint error was 47±34mm for fast movements and 38±27mm for slow. Peak lateral error was only 5±13mm greater than the endpoint error for fast movements and 8±19mm greater for slow. Movements were also foreshortened by about 25mm. When again stationary, subjects accurately mimicked their aberrant trajectories and endpoints. In other observations, when subjects pointed in the air above the target surface, endpoint errors were reduced by 65% for slow but not fast movements. The discrepancies between these and other findings on load compensation likely relate to the tactile cues on the arm unavoidable with other methods.

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218.8

Longitudinal Study of EMG Activation Relative to Reaching Kinematics and Kinetics in the first Year

Spencer, J., Kamm, K. & Thelen, E.* Department of Psychology, Indiana University, Bloomington, IN 47405.

In the first year of life, infants learn to reach objects with speed and precision. This study reports on the muscle activation patterns associated with the onset of reaching and the relation between muscle patterns and kinematic and kinetic measures of reaching skill improvement. We observed the reaches of one infant weekly from 3 to 30 wk of age, then bi-weekly to 52 wk. We recorded 3-D kinematic data from the joint centers of both arms and calculated kinetics using inverse dynamics. In addition, we collected EMG (750Hz) with surface electrodes placed over the muscle bellies of: anterior deltoid (AD), upper trapezius (UT), biceps (B), triceps (TI) and erector spinae (ES).

The infant initiated reaches from different starting positions and background movement conditions throughout the first year, generating a wide variety of kinematic, kinetic and EMG patterns. Despite this variability, a co-activation pattern was present at all ages. Reaches were initiated by UT contraction, followed by co-activation of AD, BI, and TRI. The infant rarely used this co-activation pattern in his spontaneous movements prior to the onset of stable reaching at 12 wk. He used the pattern inconsistently during a period of exploration and rapid improvement in reaching skill from 12 to 21 wk. By 22 wk, co-activation was the predominant pattern. In this later stage of reaching skill, the co-activation pattern was coupled with a stable sub-set of kinematic and kinetic patterns.

We suggest that the infant successively discovered this stable strategy of reaching with reaching practice and that it represented an efficient way of controlling the underlying force dynamics of the reach movement.

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218.9

MECHANISM UNDERLYING SHIFTS IN INTERLIMB MOVEMENT PATTERNS IN INFANCY: A PERTURBATION STUDY. D. Corbetta* and E. Thelen. Department of Psychology, Indiana University, Bloomington, IN 47405.

The development of reaching in infancy is marked by successive shifts between bimanual and unimanual limb coordination. Little is known about the mechanisms of early interlimb coupling and the dramatic shifts in coordination patterns. We report on two experiments designed to (1) identify developmental shifts in the manual strategies used for reaching and (2) test bilateral symmetry coupling by changing the task and the limb weighting. In the first experiment, small and large balls requiring uni- or bilateral reaches were presented at midline. In the second experiment, infants' preferred arms were weighted.

Five infants were tested longitudinally from 5 to 8 months, using WATSMART to record trajectories of hands and shoulder and ball position. In the second experiment, we also recorded EMG activity of biceps, triceps and deltoid.

Shifts in the manual strategy used for reaching occurred around 7 months old. At this age, infants shifted from unilateral to bimanual strategies with the large ball or from bimanual to unimanual with the small ball. Weight perturbations provoked compensatory contralateral activity that shifted the coordination into symmetry if originally asymmetric or broke the symmetry of an originally symmetrical pattern.

These results suggest that the development of manual strategies in reaching depends on the underlying strength of the bilateral activity that links both sides of the body as a single system.

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218.10

LONGITUDINAL ANALYSIS OF REACHING KINETICS IN THE FIRST YEAR OF LIFE.

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During the first year of life, human infants undergo dramatic changes in motor control—as well as limb mass and geometry—and must learn to coordinate an array of muscular and passive forces to reach for an object. To better understand these changes in motor control, we quantified the upper-extremity kinematics, inverse dynamics, and intralimb coordination during development as an infant reached (or attempted to reach) for a toy. The infant's movements were recorded weekly from 3-30 wk of age and bimonthly thereafter until 1 yr of age. In early reaches (5-22 wk), the infant principally used flexor muscle torques at both the shoulder and elbow to counteract the gravitational extensor torque. Muscular torques at the shoulder and elbow became more tightly coupled during weeks 48-51. Muscle-power profiles at the elbow and shoulder joints were quite variable in the early weeks (5-22), but a pattern emerged at later ages with first power generation at both elbow and shoulder joints followed by a combined shoulder joint power generation and elbow joint power absorption. By the last month of the first year, there was a triphasic relationship between elbow- and shoulder-joint muscle power: generation at both elbow and shoulder, then elbow absorption and shoulder generation, followed by power absorption at both the elbow and shoulder joints. Of the torque components due to mechanical interactions among limb segments, inertial torques related to upper-arm and forearm angular accelerations and the linear acceleration of the shoulder joint were most prominent throughout the first year. Elbow and shoulder muscle torques were modulated with respect to the interactive torques, particularly as the hand approached the toy. Although hand trajectories stabilized sooner, refinements in intersegmental dynamics continued throughout the first year. Supported by a grant from NIH (HD 22830).

218.11

HOW DOES THE CEREBELLUM CONTROL COMPOUND MOVEMENTS?

H.P. Goodkin* and W.T. Thach. Depts of Anatomy and Neurobiology and the IWJ Rehab Res Institute. Wash Univ Sch of Med, St. Louis, Mo 63110.

A rhesus monkey was trained to perform 2 single digit tasks - flexion of the thumb or index finger cued by separate LED commands - and a two digit task - simultaneous flexion of the thumb and index finger cued by the two LEDs simultaneously. The intent was to combine two simple movements into a third compound synergic movement. This would appear to be the critical test of the hypothesis that the cerebellum may control compound and not simple movements (Thach, et al, Ann Rev Neurosci, 15, 1992).

Injections of 3 ul (5ug/ul) muscimol were placed into the cerebellar dentate nucleus. Muscimol did not significantly impair the monkey's ability to perform any of the instrumented finger tasks. The only consistent measurable effect was a delay in reaction time of 20 to 50 milliseconds affecting the thumb and index finger during both the single and two digit tasks. A reaction time delay was never seen purely in the two digit task. Nor was the relative timing between the thumb and index finger during the two digit task significantly changed. However, the performance of natural compound movements was affected. The monkey's reaches overshot the target (usually unaccompanied by tremor), and he could not use a precision pinch and would substitute single digit strategies.

In this experiment, the dentate nucleus was not necessary for the control of the instrumented single or two digit finger tasks but was necessary for the control of the natural compound tasks. This finding further supports our hypothesis that the cerebellum preferentially controls compound movements. The finding that the instrumented 2 digit "compound" movement was unaffected raises the possibilities that the monkey performed this task as 2 simple movements simultaneously and independently or that the compound movements need to be made to "natural" visual targets.

218.12

PRISM ADAPTATION IN THROWING IS SPECIFIC FOR ARM AND TYPE OF THROW.

W.T.Thach*, H.P.Goodkin, J.G.Keating, and T.A. Martin, Depts of Anatomy, Neurology, and The IWJ Institute for Rehabilita. Research, Washington University Medical School, St. Louis, Mo. 63110.

In throwing, eyes (and head) fixate the target. Donning wedge prisms (bases right) bends the optic path right, and gaze shifts left to fixate the target. The arm throws in the direction of gaze--left of the target. With practice, the throw shifts right, away from gaze direction, closer to and finally on-target. When the prisms are removed, gaze is now on-target, the arm throws right of target an amount almost equal to the original leftward error, and each throw shifts left closer to and finally on-target (Thach et al, 1991). How specific is this adaptation to the task and to the body parts?

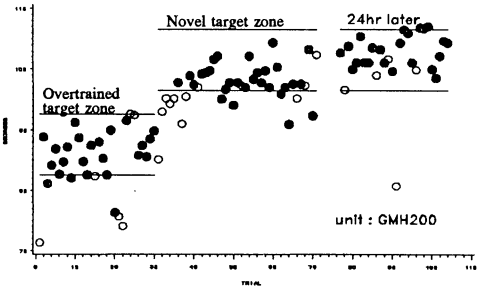
In one experiment, subjects threw with 1) one arm, 2) the other arm, 3) the first arm with prisms, 4) the other arm, and 5) the first arm. Prism adaptation occurred in all subjects in the throwing arm (3), did not affect or abate with throws by the other arm (4), and readapted during throws by the first arm (5). In a second experiment, subjects threw 1) underhand, 2) overhand (same arm), 3) over hand with prisms, 4) underhand, and 5) overhand. On wearing prisms all subjects adapted the overhand throw (3). In 6 subjects, subsequent underhand throw showed no effect of prior overhand adaptation (4). In these subjects, prior overhand adaptation persisted in subsequent overhand throws despite intervening underhand throws, and readapted with repeated overhand throws (5). Two subjects showed carryover from prior overhand adaptation (3) to underhand throw which disappeared with underhand throwing (4), and the amount of residual overhand adaptation (5) was less.

Thus, in most subjects adaptation involved one task and not another task, although many or the same body parts participated in both tasks. This implies separate central channels for adaptation of muscle actions for each task.

218.13

ADAPTATION OF A BALLISTIC MOVEMENT TO A NOVEL ENDPOINT IS ENDURING. J.G. Keating* and W.T. Thach. Dept. of Anatomy and the IWJ Rehab. Res. Ins., Washington Univ. Sch. Med. St. Louis, MO 63110.

We have described an inactivation map of cerebellar cortex produced by systematically injecting muscimol into cerebellar cortex of a Rhesus Monkey. Inactivation of a small area of cortex resulted in prevention (2*u*l of 5*u*g/*u*l) or a marked slowing (2*u*l of 1*u*g/*u*l) of adaptation with no significant change in task performance (Soc. Neurosci. Abs. 551.3, 1991). We now show that normal adaptation is enduring: the adapted state is maintained for at least 24 hours. We conclude that the cerebellar can cortex play an essential role in producing changes of at least this duration in this type of movement adaptation.



End points of ballistic movements are plotted vs. trial number. The first trials 24 hours later end in the adapted target zone, not the overtrained control zone.

218.14

KINEMATICS OF HAND AND ARM ORIENTATIONS IN REACHING AND GRASPING. M.Flanders* and J.F.Soechting. Dept. Physiology, Univ. Minnesota, Minneapolis, MN 55455.

Grasping an object involves a coordinated posture of the arm, the wrist and the hand which is related to the object's location and orientation in space. We have begun to study the kinematic transformations between the parameters defining object location and orientation and those defining arm and hand posture. We presented subjects with a target (a rod 20 cm in length). In some experiments we asked them to remember the object's location and orientation and to move the arm to the remembered target location so as to grasp the virtual target with a power grip or with a precision grip. There were no appreciable consistent errors in subjects' performance on this task. Their performance was comparable when we asked them to reproduce only the hand configuration adequate for grasping, i.e to maintain their arm at the side rather than to move to the target's location. In another series of experiments we left the target in view and also asked the subjects to reproduce only the hand configuration. In this experimental situation, there were appreciable and consistent errors in hand orientation. Subjects appeared to orient the hand relative to the arm in a manner that would have been appropriate had the arm reached the target location rather than remaining at the subject's side. These errors suggest that hand orientation is defined in a reference frame fixed to the arm and not in a reference frame fixed in space.

218.15

EFFECTS OF PRACTICE ON REPRODUCING MOVEMENT DISTANCE AND LOCATION. S. Jaric, D. B. Ilic, D. M. Corcos, G. L. Gottlieb, M. L. Latash*, Institute for Medical Research, Belgrade, Yugoslavia, University of Illinois at Chicago, IL 60608 and Rush Medical Center, Chicago, IL, 60612.

Movements can be controlled by a shift in a command related to joint equilibrium position r from an initial value r_{in} to a final value r_f at a rate ω within a time τ. If a subject learns a movement to a final position, he needs to remember only one value at the control level, r_f and shift r at a rate ω (an r/ω-strategy). If the subject is asked to learn a movement over a certain distance, he needs to remember two values, τ and ω, the product of which gives movement distance (a τ/ω-strategy), since distance itself does not have a single-valued representation at the control level. This control scheme predicts that movements performed by the r/ω-strategy will be more accurate and consistent than those performed by the τ/ω-strategy.

Two groups of subjects (n=6) practiced rapid, self terminating movements to a target. One group of subjects performed 500 movements over a fixed distance from seven different initial starting positions over 36° (a τ/ω-strategy). The other group of subjects performed 500 trials to the same final location from the same seven different initial positions with actual movement distance ranging from 24° to 48° (an r/ω-strategy). Then , the subjects were tested under conditions of both distance and location reproduction. The indices of variability are:

	Practiced					
	CE		VE		$\sqrt{CE^2+VE^2}$	
	Dis	Loc	Dis	Loc	Dis	Loc
Performed Dis	6.7	4.9	5	4.9	8.36	6.29
Performed Loc	1.8	3.8	3	2.3	3.49	4.44

All three indices of variability show that location is better reproduced than distance and are consistent with predictions of the equilibrium point hypothesis.

This work was supported by NIH grants NS 23593, AR 33189, NS 28176 as well as by HHS grant JF-012 through the US-Yugoslav board of scientific cooperation and a grant from the Serbian Research Foundation.

218.16

LOCATION AND DISTANCE CODING IN OPEN LOOP POINTING IN A DEAFFERENTED PATIENT. Bard, C.[1], Fleury, M.*[1], Teasdale, N.[1], Paillard, J.[2], Forget, R.[3], & Lamarre, Y.[3]. Université Laval, Québec.[1] CNRS, Marseille.[2] Université de Montréal.[3]

We tested the ability of a deafferented patient and two neurologically normal subjects to produce alternate wrist pronations and supinations without vision of their moving limb. For 30 seconds, subjects, holding a handle centered on the axis of their right forearm, performed pointings between two visible targets to the left or right of the vertical. From a 0° initial position, subjects pointed at targets, and verbally indicated the end of their movement. The verbal signal triggerred the lighting of the next target immediately or 8 seconds later. Both normal subjects and the patient showed an ability to time the end of their movements with accuracy. The patient produced as stable amplitudes as normals for both delays, confirming recent findings (Larue et al., 1992). Yet, unlike normals, the patient was unable to move her arm to the spatial location of the target. She exhibited an important shift in all series of movements. Rather than an agonist/antagonist length/tension ratio, the deafferented patient seems to modulate her "effort" proportionnaly to the required distance between two targets.

218.17

• THE STUDY OF CONSTRAINED AND PARTIALLY CONSTRAINED REACHING MOVEMENTS • J. Won and N. Hogan.* Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139.

• In early forms of the equilibrium point hypothesis, it was proposed that point to point arm movements were accomplished through an abrupt shift in desired equilibrium position [Feldman, 1966]. Subsequent studies found that a virtual trajectory or a gradual shift of an attractor point from the starting position to the target position was required to provide a simple and complete explanation for experimental observations [Bizzi et al., 1984]. In later work these virtual trajectories were shown through simulations to be remarkably competent in describing unconstrained two joint arm movements. [Flash and Hogan, 1985].

To experimentally test this notion of virtual trajectories in two-joint movements, a new experimental paradigm was designed. Human subjects grasped the instrumented handle of a two-link robot manipulandum and performed specified point-to-point planar arm trajectories. Computer-controlled clutches were used to subtly change the movements by constraining the trajectory to be along an arc of radius equal to the length of one link of the manipulandum. Targets points were arranged to lie along the arc so that the subject could complete the movement even when constrained. Three situations were tested: (1) unconstrained throughout movement, (2) constrained through the entire movement, and (3) initially constrained and then released during movement.

Experimental results showed significant forces during the constraint strongly oriented so as to restore the hand to the unconstrained hand path. In addition when released from the constraint, these forces caused a strong tendency to return the hand to the unconstrained path before the end of the movement was reached. The presence of these restoring forces (even when considering the anisotropy of the end-point arm stiffness) implies that an attractor point exists between the start and target points for a significant portion of the movement. These results extend earlier single-joint results [Bizzi et al., 1984] to the multi-joint case and provides further support for the theory that a single process underlies posture and movement.

218.18

SENSORIMOTOR COORDINATION DURING WRIST MOVEMENT IN NORMAL AND DEAFFERENTED SUBJECTS. M.F. Levin*, Y.Lamarre and A.G. Feldman. Centre de Recherche, Institut de réadaptation de Montréal, CRSN, Université de Montréal, Montreal, Quebec. Canada H3S 2J4.

We examined the relative roles of central and peripheral mechanisms in movement production. Predictions of equilibrium-point model of motor control were compared to EMG, net joint torque and kinematic data from normal (n=8) and deafferented (n=1) subjects who made fast 30° wrist flexion movements. The forearm was immobilized and the hand was clamped at the metacarpals to minimize grasping. After training with no load, the load was randomly presented during the first 700 or 300 ms after which it was suddenly removed. Loading resulted in an undershoot of the target which was regained after the load was removed. In the second experiment, subjects trained to reach the target with the load. In subsequent trials, when the load was randomly not presented, subjects overshot the target. Subjects trained with vision and in experimental trials, they performed movements with and without visual feedback. They were instructed not to correct errors arising from loading or unloading. In spite of the presence of muscle coactivation, wrist stiffness associated with the final position was significantly less for the deafferented subject (0.033-0.084 Nm/deg with vision, 0.022-0.063 Nm/deg without vision) than for normal subjects (0.115-0.269 Nm/deg). This subject was also unable to consistently reproduce the final joint position and had a longer time to peak velocity. Both normal and deafferented subjects produced movements by shifting the torque/angle characteristic to the final position by means of reciprocal (R) commands and by increasing its slope by means of coactivation (C) commands. The low movement precision in the deafferented subject can be explained by a loss of the positional frame of reference for R commands normally provided by muscle afferents and diminished effectiveness of C command due to the absence of peripheral feedback.

218.19

COORDINATION BETWEEN ARM AND TRUNK MOTION DURING RAPID POINTING MOVEMENTS. TR Kaminski*, C Bock & AM Gentile. Dept. of Movement Science, Teachers College, Columbia Univ., New York, NY, 10027

Previous multijoint pointing studies have limited the distance travelled by the hand to within arm's length and have focused on the linkage between the shoulder and elbow joints (Kaminski and Gentile, 1986; 1989). In the present study, the amplitude of hand displacement varied so that successful completion of the movement required the use of 2, 3 or 4 joints (elbow, shoulder, scapulo-thoracic, hip).

Four seated subjects performed pointing movements to a target at five distances; two within and three greater than arm's length. 2-D kinematic analysis indicated that regardless of the number of segments involved, the hand moved along the same path and its velocity profile was smooth and bell-shaped. However, the joint configuration at any given point along that path varied depending on the target location. There was a distal to proximal organization to joint movement cessation: elbow joint stopped first, followed by shoulder, then hip. Thus, for movements beyond arm's length, the pattern of hand motion remains the same while the coordination between the joints is altered.

218.20

SPATIO-MOTOR MAPPING DURING DRAWINGS IN 3D ISOMETRIC CONDITIONS. J.T. Massey*, G. Pellizzer, and A.P. Georgopoulos. Brain Sciences Center, VAMC, Minneapolis, MN 55417; Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55455; and Dept. of Neuroscience, The Johns Hopkins Univ., Baltimore, MD 21205.

Soechting et al. (*Neuroscience*, 17: 295, 1986) described that free hand drawings of circles were characteristically distorted when traced in the sagittal plane. They suggested that the sensorimotor mapping between the motion of arm orientation angles and the intended wrist trajectory is not as well valid for all planes of space. We investigated whether distortions occur when circles are drawn in isometric conditions, that is when the trajectory is defined in the force space. Normal human subjects were asked to exert forces continuously on a 3D isometric manipulandum in order to draw circles in specified planes in the force space. The drawings were performed at a self-chosen pace in the presence or absence of a visual feedback cursor and a visual template. We found that: (a) the orthogonal components of force were modulated in a close to sinusoidal fashion, (b) the phase relation was clustered around 90°, (c) the force amplitude varied relatively more than the timing, (d) these characteristics were not affected by the plane of drawing or by the presence or absence of visual feedback. The results indicate that, in isometric conditions, there were no more distortions for circles drawn in the sagittal plane than for those drawn in other planes. These results suggest that, unlike the movement case, the force modulation is adequately mapped to the intended spatial outcome independently of the plane of drawing. Therefore the distortions observed with free hand drawings in the sagittal planes are likely related to constraints of movement execution. (Supported by NSF and ONR).

LIMBIC SYSTEM III

219.1

AMYGDALAR NEURONAL RESPONSES DURING CROSS-MODAL ASSOCIATION TASK IN AWAKE RATS.
T. Uwano, H. Nishijo, T. Ono, K. Nakamura*, and K. Torii[1]
Dept. Physiol., Fac. Med., Toyama Med. & Pharmaceu. Univ., Toyama 930-01, and [1]ERATO, R&D Corp. of Japan, Yokohama 221, Japan.

The amygdala (AM) receives information from various sensory modalities via the neocortex, and directly from the thalamus. Lesion of the AM decreases emotional responses to various sensory stimuli. In this study, single unit activity was recorded in the AM and ventral parts of the striatum in awake rats. Rats were trained to acquire glucose or intracranial self-stimulation reward, or to avoid electric shock or tail pinch, by identical behavior, licking. Each unconditioned stimulus was associated with a cue tone stimulus (CTS), a light, or an air-puff. The neurons were also tested with various sounds, somatosensory stimuli, and taste solutions through intraoral canulae. Of 645 neurons was recorded, 245 responded to one or more sensory stimuli, and 65 of these responded exclusively to an auditory, a visual, a somatosensory, or an oral sensory stimulus. Most receptive fields of somatosensory neurons included some parts of the head and/or neck. Some auditory specific neurons were tested for extinction, and responses to CTS were diminished when reward was eliminated. Eight multimodal neurons responded to specific combinations of various sensory stimuli. Of these, two responded only to ingestion of glucose and the corresponding CTS. When CTS for electric shock was associated with glucose, these neurons became responsive to the CTS. The results suggest that the AM is one of the foci of convergence of various sensory information, and suggest its involvement in cross-modal and stimulus-affect association.

219.2

CGRP-IMMUNOREACTIVE TERMINALS IN THE LATERAL NUCLEUS OF THE AMYGDALA FORM ASYMMETRIC AND SYMMETRIC SYNAPTIC JUNCTIONS. C. Farb*, C. Aoki, and J.LeDoux. Center for Neural Science, NYU, NY, NY 10003

Projections from medial geniculate body (MGB) to lateral amygdala (AL) have been implicated in the classical conditioning of emotional responses to acoustic stimuli (LeDoux,1990). Recent anatomical studies (Yasui et al., 1991) have suggested that calcitonin gene-related peptide (CGRP) plays a role in neurotransmission in this pathway. In order to elucidate role of this peptide in synaptic transmission, CGRP immunoreactivity in the AL was examined at the electron microscopic level. CGRP-like immunoreactivity was seen in axon terminals, soma, glia and dendrites. Immunoreactive terminals contained many small clear vesicles and 1-5 dense-core vesicles. Labeled terminals formed both asymmetric (excitatory?) and symmetric (inhibitory?) synaptic junctions. Asymmetric synapses were almost always observed on dendritic spines while symmetric synaptic junctions were localized almost exclusively on soma or proximal portions of dendrites. CGRP immunoreactivity in soma and initial-segment axons was seen in vesicles of membrane-bound organelles. These observations are consistent with the possibility that both local inhibitory interneurons and excitatory projection neurons use CGRP in neurotransmission. Since neuroactive peptides are often co-localized with other transmitters, it is possible that CGRP plays a modulatory role neurotransmission in the AL. (Supported by MH 38774 & MH46516).

219.3

OVERLAPPING AND DIVERGENT PROJECTIONS OF CA1 AND THE VENTRAL SUBICULUM TO THE AMYGDALA. R.G. Phillips* and J.E. LeDoux. Dept. of Psychology & Center for Neural Science, New York University, NY, NY 10003.

In recent studies we implicated the hippocampus and amygdala in contextual fear conditioning and suggested that projections from hippocampus to the amygdala are critical. Studies using horseradish peroxidase as a retrograde marker indicate that cells in the ventral CA1/subiculum region of the hippocampal formation project to the amygdala. In the present study we determined whether ventral CA1 and subiculum have differential projections to the amygdala using anterograde and retrograde tracing. The anterograde tracer phaseolus-vulgaris lecouaglutinin (Pha-L) was iontophoretically injected into either ventral subiculum or ventral CA1. Injections in subiculum resulted in a dense projection to the ventromedial part of the lateral nucleus (ALvm), basomedial nucleus (BM), medial nucleus, and intra-amygdala part of the bed nucleus of the stria terminalis, whereas injections in CA1 resulted in transport to the ALvm and BM, but also to the basolateral nucleus (BL). Following iontophoretic injections of FluoroGold in AL or BM, retrogradely labeled cells were found both in the ventral subiculum and CA1, whereas injections of BL resulted in retrograde transport only to CA1. Thus, ventral subiculum and CA1 have overlapping and divergent projections to the amygdala. Processing of contextual information in fear conditioning may be mediated by all or a subset of these projections. Supported by MH38774 and MH46516.

219.4

AMYGDALA EFFERENTS MEDIATING FEAR-POTENTIATED STARTLE: REFRACTORY PERIODS, CONDUCTION VELOCITIES, AND SYNAPSES. **B.A. Pollard* and J.S. Yeomans. Dept. of Psychology, University of Toronto, Toronto, Canada, M5S-1A1.**

Pathways from the amygdala via the caudal ventral amygdalofugal pathway (VAF) have been shown to mediate fear-potentiated startle (Davis, 1991). In the present study, we examined the subsequent connections from VAF to medulla using electrical stimulation and electrolytic lesions. Stimulation of VAF, midbrain, or medulla (two 0.1 ms pulses, 100 to 820 uA) produced an unconditioned startle in behaving rats. Refractory periods ranged from 0.4 to 0.8 ms in VAF sites, and from 0.2 to 0.5 ms in midbrain and medulla sites. To determine connections between sites, 'collision tests' were carried out by delivering a single pulse to one site, and a second pulse to another site. Results suggested ipsilateral midbrain and medulla were connected by axons with a mean conduction velocity of 66 m/s. Ipsilateral VAF and midbrain sites appear to be connected by strong synapses transmitting from VAF to midbrain, with a synaptic delay of less than 0.8 ms. No collision was observed between contralateral sites.

These rats were then conditioned by pairing light with footshock. After fear-conditioning, bilateral lesions via the midbrain electrodes (0.5 mA for 30 s) abolished fear-potentiated acoustic startle, but had no effect on acoustic startle alone. In addition, electrical stimulation of VAF sites could no longer elicit startle behavior, while electrically-evoked startle from medulla sites was decreased but not eliminated.

These results suggest that fear-potentiated startle is synaptically mediated between VAF and midbrain, with fast axons from midbrain to medulla.
(Supported by NSERCC grant A7077 to J.S.Y.)

219.5

GABA-Immunoreactive Terminals Make Synaptic Contact With Identified Projection Neurons in the Rat Extended Amygdala. *N.Sun*, H.Yi and M.D. Cassell* Department of Anatomy(N.S. and M.D.C.) and Central EM Research Facility(H.Y.), University of Iowa, Iowa City, IA 52242.

Recent neuroanatomical studies have suggested that the central nucleus of the amygdala(Ce), lateral bed nucleus of the stria terminalis(BNST) and the dorsolateral sublenticular substantia innominata(SI) comprise the central division of the extended amygdala(CEA). One of the common features of these regions is that they all project to brainstem autonomic nuclei. Previous studies in this laboratory have suggested that intrinsic GABAergic connections seem to link the subdivisions of the CEA which have similar brainstem projection targets and that GABAergic terminals form "basket-configurations" surrounding presumed projection neurons. The present study was designed to determine whether GABAergic terminals make synaptic contact with identified projection neurons in the rat CEA. We combined retrograde transport of HRP-WGA with postembedding immunogold staining for GABA. After injections of HRP-WGA into either dorsal medulla or parabrachial complex of Sprague Dawley rats, neuronal cell bodies in the different subdivisions of the Ce, BNST and SI were peroxidase-labeled. Ultrathin sections from Ce, BNST and SI were immunostained with GABA antisera, using a secondary antibody conjugated to 10nm gold particles. Peroxidase-labeled neurons and their dendrites received synaptic contact from colloidal gold-labeled terminals immunoreactive for GABA. The GABAergic axons contained pleomorphic vesicles and formed symmetric synapses(Gray type II) on the target neurons. The present study demonstrates a widespread GABAergic innervation, largely of intrinsic origin, on CEA neurons projecting to brainstem autonomic nuclei, suggesting a strong GABAergic inhibitory influence on CEA outputs. Supported by NS25139.

219.6

THE PROJECTIONS FROM THE ANTERIOR CINGULATE AND PRECENTRAL AGRANULAR CORTICES TO THE AMYGDALA IN THE RAT. G.D. Fisk*+, T. van Groen and J.M. Wyss. Dept. of Cell Biology and +Psychology, Univ. of Alabama, Birmingham, AL 35294-0019

Several studies in the rat demonstrate that the anterior cingulate (area infraradiata; anterior, IRα; posterior, IRβ) and precentral agranular (Preag) cortices project to the amygdala. The present study characterizes the termination pattern of these projections using the anterograde tracers *Phaseolus vulgaris* leucoagglutinin and Fluororuby. The ventral areas of IRα (i.e., IRaα and IRbα) project predominantly to medial and ventral parts of the amygdaloid complex, specifically medial, basomedial, cortical and central nuclei, the caudal half of the basolateral nucleus and the periamygdaloid cortex. IRaα projects most densely to the medial and the cortical nuclei, and IRbα projects most densely to the medial nucleus and the caudal half of the basolateral nucleus. In contrast to IRaα and IRbα, dorsal IRα (i.e., IRcα) and rostral Preag project primarily to the lateral and basolateral nuclei and avoid the more medial and ventral areas of the amygdala. IRcα projects to the entire basolateral nucleus, but Preag projects only to the rostral half of the basolateral nucleus. Injections in IRβ or caudal precentral agranular cortex do not label axon terminals in the amygdala. Thus, nearly all projections to the amygdala from the IR and Preag cortices arise from the rostral portions of these cortical areas, with ventral IRα projecting primarily to ventral and medial areas of the amygdala, and dorsal IRα and Preag projecting to lateral areas of the amygdala. The preponderance of ventral IRα projections to the medial and central amygdaloid nuclei suggests that ventral IRα influences cardiovascular and affective functions, at least in part, via this pathway.

219.7

DISTRIBUTION OF GABA AND PARVALBUMIN IMMUNOREACTIVE CELLS AND FIBERS IN THE MONKEY AMYGDALOID COMPLEX. A. Pitkänen* and D. G. Amaral, Dept. of Neurology, Univ. of Kuopio, Kuopio, Finland and The Salk Institute, La Jolla, CA 92037.

The aim of this study was to examine the organization of inhibitory interneurons in the primate amygdaloid complex. Coronal sections from the brains of three *M. fascicularis* monkeys were immunohistochemically processed with monoclonal antibodies directed either against GABA or parvalbumin. GABA and parvalbumin immunoreactive (GABA-ir and PARV-ir, respectively) neurons consisted of at least three distinct cell types (small stellate cells; medium to large multipolar cells; and fusiform cells). All labeled neurons appeared to have aspiny or sparsely spiny dendrites. In general, there was a greater number of GABA-ir cells than PARV-ir cells in the amygdala. A higher density of GABA-ir and PARV-ir neurons was found in the deep amygdaloid nuclei (lateral, basal and accessory basal nuclei) than in the more superficial regions such as the central, medial and anterior cortical nuclei. GABA-ir cells were more homogeneously distributed within the deep nuclei than PARV-ir neurons; the latter showed marked variations in intranuclear distribution. The distribution of fibers and terminals demonstrating PARV-ir was similar to the distribution of labeled cells. The highest density of GABA-ir fibers and terminals, however, occurred in the central and medial nuclei rather than in the deep nuclei. Since there was substantial overlap of the distributions of GABA-ir and PARV-ir cells and since they demonstrated similar morphologies, it is likely that the PARV-ir cells form a subset of the GABA-ir cell population.

219.8

DISTRIBUTION OF DIFFERENT TYPES OF AXON TERMINALS TO NEURONS IN THE MEDIODORSAL THALAMIC NUCLEUS (MD) OF THE RAT. M.Kuroda[1]* and J.L.Price[2], [1]Dept.Anat., Toho Univ.Sch.Med., Tokyo 143, Japan, [2]Dept.Anat.& Neurobiol., Washington Univ.Sch.Med., St. Louis, MO 63110

The Golgi electron microscopic study was undertaken to determine the distribution of axon terminals synapsing onto each order dendrite of MD neurons identified by the gold-toning procedure.

In our previous study, four types of axon terminals have been demonstrated in MD ; 1) small axon terminals with round synaptic vesicles (SR type), 2) large axon terminals with round vesicles (LR type), 3) small to medium axon terminals with pleomorphic vesicles (SMP type), 4) large axon terminals with pleomorphic vesicles (LP type). Both SR and LR types of boutons establish asymmetric synaptic contacts, whereas SMP and LP terminals are observed to make synaptic junctions of symmetric specialization. The present study provided evidence that there was no significant difference on the distribution of synaptic organization formed by each type of boutons synapsing along each order of dendrites in different parts of MD, although it was noticeable that each part differed in the number and percentage of each type of boutons. Primary dendrites carried LR, LP and SMP terminals with high proportional density of large-sized boutons regardless of synaptic morphology. No SR terminals were found to make synapses with primary dendrites in each part. Secondary and tertiary dendrites were covered with all types of axon terminals. Higher order dendrites more than tertiary ones were crowded predominantly with SR boutons, but they also carried LR as well as SMP terminals. In addition, SMP boutons were often found to form symmetric contacts with cell bodies. Thus, MD neurons were covered at least with either SMP or LP terminals, which have been shown to be extrinsic inhibitory inputs, at any orders of their dendrites.

The results of the present study suggest that neurons in each part of MD receive the same sets of axon terminals at each order dendrite, though sources of afferent inputs vary with different parts of MD.

Supported by NIH research grant DC 00093.

219.9

IBOTENATE LESIONS OF THE ROSTRAL CENTRAL AMYGDALOID NUCLEUS PRODUCE A MARKED DEFICIT IN DRINKING PASSIVE AVOIDANCE BEHAVIOR IN RATS MORE EASILY UNDER PENTOBARBITAL THAN KETAMINE ANESTHESIA. G.D. Coover* and R.C. Meyer. Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

The rostral third of the central amygdaloid nucleus (rACE) appears to be the most effective target for electrolytic lesions that are "anxiolytic" as assessed by marked deficiency in passive avoidance of drinking (Coover, Soc. Neurosci. Abstr., 16:473). However, ibotenate (IBO) lesions of apparently comparable size did not produce such a marked deficit (Coover, Murison, and Jellestad, Physiol. Behav., in press). The present study examined three doses of IBO under pentobarbital or ketamine-xylazine anesthesia.

A marked deficit in drinking passive avoidance was obtained with a 4 µG dose of IBO (in .4 µL pH 7.4 phosphate buffer), but not with doses of 1 or 2 µG, and only in rats anesthetized with pentobarbital. Vehicle-injected controls required 24 ± 2 (M ± SE) footshocks of incrementing intensity to avoid a water spout for 5 min. In contrast, the group injected with 4 µG IBO under Nembutal required 42 ± 3 footshocks, which is comparable to rats lesioned electrolytically, 45 ± 2 footshocks. The 4 µG/ketamine group required 33 ± 3. Lesser rACE lesion-induced behavioral deficits with IBO under ketamine ($p < .05$ collapsed across dose) may reflect ketamine's antagonism of IBO at the NMDA receptor.

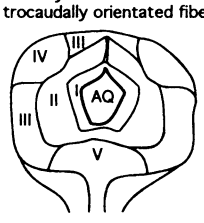
219.10

A NEW SUBDIVISION OF THE PERIAQUEDUCTAL GRAY (PAG) BASED ON DIFFERENT MYELIN CONTENT; A HIGH RESOLUTION LM STUDY G.Holstege*, D.H.Croon, and P.O.Gerrits. Dept. Anatomy, University of Groningen, The Netherlands.

Reports on the myelin content in the periaqueductal gray (PAG) are scarce and confusing. In the nineteenth century Schütz described a rostrocaudally orientated tract in the ventral part of the PAG (Bundle of Schütz or dorsal longitudinal fasciculus). In the nineteen thirties in transverse sections radiating fibers were described running to the periphery of the PAG and adjoining tegmentum.

Using a new glycol methacrylate-resin (GMA) embedding technique for high resolution light microscopy, a cytoarchitectonic map of the normal rat PAG was made, which precisely defines myelin division patterns. Large semi-thin sections stained with Sudan Black B showed excellent morphologic detail with finest myelin sheaths preserved.

The results show that five main areas with myelinated fibers can be distinguished: I. centrally a thin subependymal layer, containing few, thin myelinated axons. This layer is surrounded by area II with rostrocaudally orientated fibers. The number and the thickness of these fibers increase towards the periphery of this zone; area III contains tangentially oriented fibers running to the periphery of the PAG and adjacent tegmentum; area IV is located in the dorsolateral part of the PAG with very few, mostly tangentially orientated fibers; area V is located ventrally and contains a mixture of rostrocaudally oriented fibers and fibers belonging to the visuomotor system.



219.11

THE ORGANIZATION OF PROJECTION FROM THE CINGULATE CORTEX TO THE THALAMIC RETICULAR NUCLEUS. A MULTIPLE TRACER - EM STUDY.

D.A. Lozsadi*, R.W. Guillery; Dept. Human Anatomy, University of Oxford, OX1 3QX, UK.

Although it has previously been reported that cingulate cortex (Cg) sends afferents to the thalamic reticular nucleus (TRN) (Cornwall, 1990. Exp.Br.Res. 80. 57), the apparent lack of input from the TRN to the anterior thalamic nuclei (Steriade, 1984. J.Comp.Neur. 229. 531) suggests that cingulate connections with thalamus differ from those of primary sensory cortical areas. As a first step in an analysis of the pathways linking Cg and thalamus single pressure injections of WGA-HRP, biocytin, neurobiotin or double injections of fluorescent dextrans were made into the rat Cg cortex. All tracers except WGA-HRP present a complete image of the anterogradely labelled structures. The cortico-thalamic fibres enter the thalamus rostrally and give off collaterals throughout the medio-lateral extent of the rostral sector of TRN. The secondary axons are either a few microns long or form more complex branches and terminate in a bouton-like swelling. Correlated electron microscopy demonstrates that these structures are synaptic terminals filled with reaction product, and contact dendritic shafts with asymmetrical synapses. Double tracing experiments show, that there is significant overlap in TRN, of terminals from distinct cortical areas.

Thus we conclude that there is a widespread projection from Cg to TRN, the terminals contact dendritic shafts showing the morphology of excitatory synapses.

219.12

DENERVATION OF FRONTAL CORTEX IN RATS BY RF LESIONS OF THE LATERAL INTERNAL MEDULLARY LAMINA (L-IML).

Yueping Zhang & R.G. Mair*. Dept. Psychol., Univ. New Hampshire, Durham, NH 03824.

In rats, RF lesions of the L-IML produce DNMTS deficits comparable to pyrithiamine-induced thiamine deficiency (PTD). Lesions of either the anterior or posterior portions of the L-IML have no such effect. Previously, we have shown that PTD treatment produces thalamic lesions centered on the IML and is associated with widespread Layer IV cortical denervation. To determine the possible cortical substrates of the L-IML induced deficit, we mapped Layer IV denervation in rats following PTD treatment (N=9) or RF lesions producing complete or partial L-IML destruction (N= 21). Whereas PTD denervated widespread areas of cortex, L-IML lesions denervated layer IV selectively in frontal cortical areas that are innervated by the mediodorsal nucleus of thalamus. Anterior and posterior lesions of the L-IML produced distinctive patterns of Layer IV denervation.

LEARNING AND MEMORY: PHYSIOLOGY II

220.1

RELATIONS BETWEEN ACIDIC FIBROBLAST GROWTH FACTOR (aFGF), LONG-TERM POTENTIATION (LTP), AND ZIF 268 GENE EXPRESSION.

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aFGF, which is released from ependymal cells in the brain by glucose, has memory facilitating effects in rodents, when tested by two tasks, the passive avoidance and the Morris water maze task. We examined relations between aFGF, LTP in rat hippocampal slices, and transcription factor, zif 268 gene expression. Population spikes evoked by stimulation of the stratum radiatum were recorded from the CA1 pyramidal neuron layer. When continuous perfusion of the slices with aFGF of 0.5, 1.0 and/or 2.5 ng/ml concentrations was started 30 min before brief tetanic stimulation, dose-dependent enhancement of LTP occurred. The perfusion of aFGF immediately after or 10 min after tetanic stimulation did not enhance the LTP. No increase of zif 268 related protein was observed in the rat hippocampus with i.p. glucose 2hr before acquisition trial in the passive avoidance task. No increase of zif 268 mRNA was observed when examined 30 min after application of i.p. glucose. The present results suggest that aFGF can influence synaptic efficacy, and that zif 268 gene expression is not related to memory facilitation by aFGF.

220.2

NEURONAL ACTIVATION OF BDNF AND trkB mRNA EXPRESSION IN RAT HIPPOCAMPUS AND ASSOCIATION TO SPATIAL LEARNING.

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The involvement of the hippocampus in cognitive functions such as learning and memory is well established and members of the nerve growth factor family of neurotrophic factors, has been implied of importance in memory related neuronal survival and plasticity. Using in situ hybridization increased expression of brain-derived neurotrophic factor (BDNF) mRNA in the CA1 subfield in the rat hippocampus is shown to be associated with improved spatial memory in the Morris water maze. Furthermore, chemical stimulation of cholinergic and glutamatergic afferents, respectively, is shown to induce BDNF mRNA expression in several regions of the hippocampus. The trkB gene, a member of the trk gene family of tyrosine kinase transmembrane receptors known to mediate cell growth and differentiation, encodes a potential component of a cell surface receptor for BDNF. In order to further elucidate the hippocampal increase in BDNF mRNA expression, following cognitive challenge and afferent transsynaptic stimulation, trkB mRNA expression was subsequently investigated. An injection of quisqualate into the medial septal nucleus caused an increase both in the dentate gyrus and in the CA1 and CA3 regions, and was completely prevented by scopolamine or diazepam pretreatment. In contrast, activation of afferents in the entorhinal cortex resulted in a significant increase only in the dentate gyrus, prevented by diazepam but not scopolamine. Our results show a differential afferent influence on trkB mRNA expression and suggests a relative importance of a cholinergic component in regulating the expression which is not evident in regulation of BDNF mRNA expression where a glutamatergic component seem more manifest. This could mean that septal and cortical afferents to the hippocampus cooperatively regulate the amount of functional trkB-BDNF receptor complexes able to mediate neurotrophic actions. Experiments are currently in progress to determine if cognitive challenge influences hippocampal trkB mRNA expression.

220.3

***C-FOS* AND *C-JUN* PEPTIDES COEXPRESS IN THE RAT BRAIN IN A DIFFUSE, TIME AND NMDA-DEPENDENT MANNER FOLLOWING EXPOSURE TO NOVELTY.**

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The neural consequences on gene expression of exposure to a spatial novelty were mapped in the rat brain by antibodies against the immediate early gene (IEG) products *c-fos* and *c-jun*. Adult male Sprague-Dawley rats were tested for 10 min in a Làt-maze, and corner-crossings, rearings, and fecal boli were counted. Rats were sacrificed at different time intervals (0.5, 2, 6 or 24 h) thereafter. Unexposed handled rats or rats exposed for three days to the maze were used as controls. The latter showed a low and scattered basal positivity. In the *exposed* rats, extensive *c-fos* and *c-jun* positive cells were the granular and pyramidal neurons of hippocampus, and later neurons in all layers of sensorimotor cortex and the granule cells of the cerebellar cortex. The positivity was stronger in rats exposed for the first time to the maze. The *fos* and *jun*-like immuno-reactivity was ***time-dependent***, since it was present between 2 and 6 h after the first exposure, and it was ***NMDA-dependent***, being prevented by pretreatment with the competitive NMDA receptor antagonist CPP at a high (5mg/Kg) but not at a low dose (0.1mg/Kg). The results indicate that exposure to spatial novelty involves activation of the hippocampal formation, followed by that of the cerebral and cerebellar cortex. In conclusion, although its physiological resolution does not allow to dissect specific and non specific effects, IEG brain mapping reveals diffuse, distributed changes in gene expression associated with a non-associative task, supporting the *neural network hypothesis* of information processing at different organizational levels of the CNS.

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220.4

AN INTRAPERITONEAL INJECTION OF ISOTONIC SALINE INDUCES NMDA-DEPENDENT DIFFUSE CHANGES IN *C-FOS* AND *C-JUN* PEPTIDES IN THE RAT BRAIN.

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The aim of this study was to map the neural consequences of an acute blockade of N-methyl-D-aspartate (NMDA) receptors on gene expression in the rat brain by antibodies against the immediate early gene (IEG) products *c-fos* and *c-jun*. Adult male Sprague-Dawley handled rats were given an i.p. injection of the competitive NMDA receptor antagonist CPP at a high (5mg/Kg) or at a low dose (0.1mg/Kg). The controls were given an equivalent volume of 0.9% saline solution or no injection. They were all sacrificed 2 h later. Non perfused brains were processed for immunocytochemistry by the double-antibody technique with avitin-biotin and diaminobenzidine as revealing method. Rats receiving saline or 0.1mg/Kg of CPP showed a diffuse positivity for *c-fos* and *c-jun* in the reticular formation, thalamus, amygdala, the granular and pyramidal neurons of the hippocampus, in all layers of sensorimotor cortex and in the granule cells of the cerebellar cortex. Rats receiving 5.0mg/Kg of CPP showed few, sparse positive cells comparable to non injected controls. *C-fos* and *c-jun* appeared *co-localized* in the same neurons. Thus, the injection-related stress, associated with pain, short lasting restraint and contextual cues, induced IEG expression that was prevented by the isosteric blockade of NMDA receptors at a high but not at a low dose of antagonist. The existence of a pre-wired *neural network* is inferred, which involves different organizational levels of the CNS, is activated by different arousal eliciting mechanisms, e.g. by novelty or painful stimuli, like i.p. injection in this case, and it is likely to be necessary for information processing and storage. Nonetheless, the informational content intrinsic to this activated network is beyond the physiological resolution of brain mapping by IEG.

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220.5

PARALLEL PROCESSING BY cAMP AND CALCIUM DURING HABITUATION IN PC12 CELLS. P. T. Martin and D. E. Koshland, Jr.* Department of Molecular and Cell Biology, University of California, Berkeley CA 94720.

Habituation of norepinephrine secretion in PC12 cells serves as a model for understanding the molecular mechanisms of simple memory processes in neurons. We have investigated habituation in response to repetitive stimulation with high potassium (high K+) and acetylcholine (Ach). For both stimuli, habituation occurred as a result of repetitive stimulation and was identified with decreased calcium levels as habituation progressed. Habituation to both high K+ and Ach was decreased by cAMP through a mechanism independent of calcium elevation. The extent to which habituation was decreased by cAMP depended on the timing of cAMP elevation, and was different for high K+ and Ach. cAMP levels did not change during repetitive stimulation with high K+ or Ach, but both high K+ and Ach reduced cAMP levels when they were elevated by N-ethylcarboxamidoadenosine. These results demonstrate that calcium and cAMP increase norepinephrine secretion through parallel mechanisms, and that calcium serves as a negative feedback on cAMP elevation, under some selected circumstances. This is the second example of parallel processing in the PC12 cell line (McFadden, P. N. and Koshland, D. E., Jr. (1990) *Neuron* **4**, 615-621.), and suggests that parallel processing by second messengers may be a general strategy used by single neurons.

220.6

LATERALIZATION OF MEMBRANE-ASSOCIATED PKC DISTRIBUTION IN RAT OLFACTORY CORTEX SPECIFIC TO OLFACTORY LEARNING BUT NOT AUDITORY LEARNING. J.L. Olds,* U. Bhalla, D.L. McPhie, J. Bower, D.L. Alkon. Computational Neuroscience, California Institute of Technology, Pasadena CA. 91125, Neural Systems Section, NINDS, NIH, Bethesda MD 20892.

The critical role of protein kinase C in the sequence of molecular events subserving associative memory formation has been delineated by several independent laboratories including our own in recent years. Most of these studies have concentrated on changes in the activity or distribution of the enzyme within the mammalian hippocampus. Here we present evidence for learning-specific changes in the distribution of the enzyme within the rat olfactory cortex.

Briefly, operant conditioning was used to train thirsty rats to push paddles for a water reward. For the experimental group (GROUP OLF), training involved two odors (A and B) and an auditory stimulus (C). C was randomly presented independent of whether A or B was presented in a trial. Responding to A and B by pressing the appropriate paddle was rewarded. Control animals (GROUP AUD) received the same three stimuli. In this case A and B were presented to the animal during each trial however their presentation did not have any contingent relationship to reward. For the control animals, reward was given for pressing the appropriate paddle for either C or no C during the trial. Saggital brain sections were analyzed in a manner such that the analyst did not know the experimental groups using quantitative computerized autoradiography for 3H-phorbol12,13-dibutyrate as previously described (Olds et al. SCIENCE 1989). GROUP OLF animals showed a statistically significant increase in the left to right ratio of specific binding within the olfactory cortex when compared to GROUP AUD controls (1.257±.118, vs. 0.965±.084; N=5,6, p<0.04 Two Tailed Student T test). In contrast, the hippocampus did not show significant changes in the lateralization of PKC, though the specific binding for both groups was higher than naive controls.

While lateralization of cortical function has been previously reported for both cortical and subcortical structures within the rat brain, this is the first instance to our knowledge of such a lateralization in olfactory cortex. In addition, this study supports the evolving hypothesis that PKC is at a nexus of molecular events in associative learning

220.7

PKC IN THE HIPPOCAMPUS IS ALTERED BY SPATIAL BUT NOT CUED DISCRIMINATIONS. S.Golski*, J.L.Olds, M.Mishkin, D.S.Olton, D.L.Alkon. Dept. of Psychology, Johns Hopkins Univ., Baltimore, MD 21218.

Protein kinase C (PKC) may play a role in neuronal plasticity, including learning and memory. Changes in membrane-associated PKC were determined by quantitative autoradiography of tritiated phorbol ester ([3H]PDBu) in rats given four different behavioral tests. Cage (CA) rats remained in their cages. The three other groups swam in a water tank surrounded by explicit controlled stimuli. The swim-stimuli group (SS) did not have a discrimination to learn; each SS rat swam for periods of time yoked to a rat in the discrimination groups. The remaining rats learned a discrimination with either space (SD) or a cue (CD) as the relevant stimulus to locate the submerged platform. The SD group had lower PKC levels in the hippocampus than the other groups ($p < .03$, SD vs. CA,SS,CD). [3H]PDBu binding in nCi/g (and standard error) for the hippocampal region for each group was: CA=21.3(1.9); SS=21.4(2.2); SD=16.2(.6); CD=21.3 (1.6). The SD group also had lower PKC levels in the CA1, CA3, and dentate gyrus subfields of the hippocampus. These data suggest that hippocampal PKC is involved in spatial memory and support the conclusions from lesion data, which indicate the hippocampus is required for performance in the SD but not the CD.

220.8

ASSOCIATIVE LEARNING POTENTIATES PROTEIN KINASE C ACTIVATION IN SYNAPTOSOMES OF RABBIT HIPPOCAMPUS. K.Sunayashiki-Kusuzaki, D.S.Lester, B.G.Schreurs and D.L.Alkon. Neural Systems Section, NINDS, National Institutes of Health, Bethesda, MD, 20892.

We have previously documented findings correlating changes in protein kinase C (PKC) localization in the hippocampus and classical conditioning of the rabbit nicitating membrane response (NMR). We have further investigated the nature of these PKC changes and report here that the activation of PKC was significantly potentiated in purified synaptosomes (SPMs) obtained from microdissected regions (CA1, CA2-3) of the rabbit hippocampus that had undergone classical conditioning. The nonstimulated levels of membrane and cytosol PKC levels in SPMs were similar in naive or unpaired control and conditioned animals. Stimulation of SPMs by the combined action of depolarization (40 mM KCl) and phorbol ester (100 nM, phorbol myristate acetate) potentiated the level of membrane-associated (activated) PKC 2- to 3-fold in SPMs of conditioned animals only ($p < 0.01$; Dunnett's test). This response was not found in isolated postsynaptic density fractions prepared from the SPMs, suggesting that this change in the PKC activation properties was specific to the presynaptic plasma membrane. Additionally, there was no difference in the total PKC activities of the SPMs from the different behavioral conditions supporting the notion that the change is in the PKC activation mechanism. It is proposed that there are long term changes in the synaptic membrane in the CA1 and CA2-3 regions of one of the intrinsic lipid PKC activators; diacylglycerol or arachidonic acid. The difference in this biochemical entity may play an important role in memory recall by maintaining a different membrane state that will facilitate subsequent conditioned stimulus-induced PKC activation.

220.9

CHOLINERGIC MODULATION OF THE INPUT/OUTPUT FUNCTION OF RAT PIRIFORM CORTEX PYRAMIDAL CELLS.

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Cholinergic modulation of the excitability of cortical pyramidal cells may enhance the function of afferent input (Hasselmo et al., *J. Neurophysiol.* 67:1230-1246). However, the effect of cholinergic modulation on the full f/I curve (spiking frequency / injected current) has not been described experimentally. This has led to inaccurate models of cholinergic modulation as shifting the slope of an input/output function which is stable over time. Here we show that a cholinergic agonist increases the slope of the f/I curve more for later intervals (during adaptation), while leaving the initial spiking response unchanged.

In brain slice preparations of piriform cortex, we tested the f/I curve of pyramidal cells (n = 32, mean Vm = -74.3 mV, mean spike height = 102 mV) using a 1 sec. intracellular current injection at a range of amplitudes. Adaptation increased the interspike interval and decreased the f/I slope at later stages of current injection. 14 of 32 neurons showed strong adaptation (no spikes after 250 msec. at 2 x threshold current). When possible, the slope of the f/I curve at different intervals was computed with least-squared error. The mean slope was 138.5 ±25.8 Hz/nA (n=9) for the 1st spike interval, 34.2 ±4.4 Hz/nA (n=15) for the 3rd spike interval, and 13.6 ±1.5 Hz/nA (n=15) after 250 msec. of current injection.

Perfusion with the cholinergic agonist carbachol decreased adaptation, increasing the slope of the 3rd spike interval from 30.6 ±4.4 Hz/nA to 39.0 ±10.7 Hz/nA, and the slope after 250 msec. from 9.97 ±2.4 Hz/nA to 18.24 ±5.9 Hz/nA (n = 6). The mean number of spikes at 1.5 x threshold current went from 6.9 ±1 to 10.9 ±2 (n=8). Biophysical simulations allow this modulation to be linked to effects on potassium currents described in voltage clamp preparations. Carbachol (100μM) also suppressed the IPSPs elicited by afferent fiber stimulation, thereby increasing the spiking response to previously subthreshold afferent fiber stimulation. In models of cortical associative memory function, adaptation allows a strong initial response to afferent input during recall, with weakening response during feedback excitation. During learning, cholinergic modulation of adaptation could allow afferent input to drive neuronal responses in a strong and sustained manner.

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220.10

TIME DEPENDENT MODULATION OF HIPPOCAMPAL SYNAPTIC STRENGTH BY SEPTAL CHOLINERGIC INPUT.

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The septohippocampal fibers (SH) provide the major cholinergic input to the hippocampus. Previous research indicates that exogenous acetylcholine (ACh) decreases the amplitude excitatory postsynaptic potentials (EPSPs) in the hippocampus. The present study demonstrates that activation of SH can also influence synaptic strength. Anesthetized (Nembutal) rats were implanted with stimulating electrodes in the medial septum and CA3 hippocampal commissures. A recording electrode was positioned to transverse the CA1 region. SH activation (20 ms, 400 Hz) resulted in a short latency (50 ms) increase and longer latency (peak 200 ms) decrease in EPSP slope and an increase in the population spike area. Slope fluctuations were associated with an immediate decrease and late increase in the paired-pulse (ISI 50 ms) facilitation ratio. SH modulation of synaptic strength was reduced by scopolamine (1mg/kg IP). Population spike facilitation was not altered by scopolamine. Results indicate that septal cholinergic input can alter hippocampal synapses. FRS 441335

220.11

LEARNING-INDUCED CHANGES IN DENTATE NEURONAL ACTIVITY AND IN GLUTAMATE RELEASE ARE BLOCKED BY THE NMDA RECEPTOR ANTAGONIST AP5. C. Rédini-Del Négro and S. Laroche*. Laboratoire de Neurobiologie de l'Apprentissage et de la Mémoire, C.N.R.S., URA-1491, 91198 Gif-sur-Yvette, France.

Learning a tone (CS)-shock (US) association in rats is accompanied by rapid and persistent changes in dentate cell activity in response to the behaviorally significant CS (Behav. Brain Res. 1983, 9:381-387), and is followed by a long-lasting increase in glutamate release in the dentate gyrus (Eur. J. Neurosci. 1990, 2:534-543). Here we report that both cellular and synaptic changes associated with learning require the integrity of the NMDA receptor. Rats were implanted with a cannula and recording electrodes in the dentate gyrus and with a stimulating electrode in the perforant path. The cannula was connected to an osmotic mini-pump delivering AP5 (4mM) or saline (.5µl/hr) for 8 days, during which animals were submitted to 4 daily sessions of 8 paired or unpaired tone-shock trials. In experiment 1, neuronal activity to the CS was recorded in 25 rats. AP5 perfusion, which was shown to block tetanus-induced LTP, reduced significantly the early component of the cellular response, and completely abolished the sustained CS-evoked cell discharge that was normally seen in conditioned controls or in rats receiving saline infusion. Neither spontaneous activity, nor the reactivity of these cells to a loud and novel tone were affected by AP5. In experiment 2, we examined whether the NMDA receptor is involved in the induction of learning-associated changes in glutamate release, as has been shown in LTP. Slices of the dentate gyrus were prepared from 25 rats submitted to 4 conditioning or pseudoconditioning sessions to examine K+-stimulated release of radiolabelled glutamate in the presence of absence of Ca^{2+}. The increase in K+-stimulated, Ca^{2+}-dependent release of glutamate observed in conditioned rats was not affected by saline, but was suppressed by the infusion of AP5 during conditioning. These results support the hypothesis that an NMDA-dependent synaptic plasticity similar to LTP occurs during learning and is necessary for the full development of the hippocampal component of the CS-US associative representation. Supported by grants from HFSP and the EEC (SC1-CT910685).

220.12

THE DOPAMINE D1 AGONIST SKF 82958 REINFORCES OPERANT CONDITIONING OF HIPPOCAMPAL CA1 CELLULAR BURSTING. B. G. XUE AND L. STEIN*. Department of Pharmacology, UCI School of Medicine, CA 92717.

In our cellular analog of operant conditioning, hippocampal CA1 bursting activity is reinforced by local micropressure applications of dopamine, cocaine or the dopamine D2 agonist N0437, but not by the partial D1 agonist SKF38393. Furthermore, dopamine's reinforcing action is blocked by co-administration of sulpiride (D2 antagonist) but not by SCH23390 (D1 antagonist); accordingly, it was concluded that dopaminergic reinforcement of CA1 bursting is exerted mainly at D2 receptors (Stein, L and Belluzzi, J D. Neurosci & Biobehav Rev,13:69-89,1989). Here we report excellent CA1 operant conditioning when the full dopamine D1 agonist SKF 82958 was used as reinforcement. A single-barrelled glass micropipette for simultaneous recording and pressure injection was filled with SKF 82958 (5, 10 and 20 µM in 165mM saline) and aimed at spontaneously active pyramidal cells in the CA1 layer of hippocampal slices. During reinforcement periods, the pressure injector was activated for 10 ms at 15 p.s.i immediately after each burst to deliver an approximately 10 µ-diameter droplet of drug to the vicinity of the cell body. The frequency of bursts was rapidly increased by the 5 and 10 µM doses of SKF 82958, but not by the 20-µM dose. When administered independently of bursting, the effective doses of SKF 82958 did not increase and often suppresed the bursting rates, thus providing a control for nonspecific pharmacological stimulation or facilitation. In other experiments, co-administration of SCH 23390(+) (10µM) eliminated or largely suppressed the reinforcing action of SKF 82958 (10 µM). These in vitro results indicate that activation of dopamine D1 receptors can reinforce hippocampal CA1 bursting activity, and are consistent with recent behavioral data demonstrating that intravenous SKF82958 is avidly self-administered by the rat (Self and Stein, Brain Res. in press).

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220.13

CIRCULATING CATECHOLAMINES AND MEMORY RETRIEVAL: PHYSIOLOGICAL SIGNIFICANCE AND ADRENERGIC RECEPTOR STATE-DEPENDENCY. B. Bohus, S.M. Korte, B. Roozendaal and J. Borrell■. Dept. of Animal Physiology, Univ. of Groningen, Haren, The Netherlands and ■ Cajal Institute of Neurobiology, CSIC, Madrid, Spain (SPON: European Brain and Behaviour Society).

Abundant evidence suggests that epinephrine of adrenal origin assures an optimal condition to consolidation of memory in the rat. The physiological significance of this hormonal action is supported by behavioral findings in adrenalectomized (ADX) or adrenomedullectomized (ADXM) rats with or without epinephrine (E) or norepinephrine (NE) replacement therapy. The present experiments have been designed to investigate the role of circulating E and NE in retrieval of memory. Therefore, plasma levels of E and NE were determined during learning and retrieval of various conditioned responses. In addition, the effects of ADX and ADXM and the effects of E and NE administration on the memory retrieval were studied in a one-trial learning inhibitory avoidance paradigm. The results show (a) the conditioned catecholamine response in various situations appears to be an increment of both E and NE levels; (b) the proactive impairment of memory retrieval by ADX or ADXM is normalized by the administration of E but not of NE before the retrieval; (c) postlearning administration of subthreshold E potentiates the effect of preretrieval E in AD rats; (d) postlearning NE induces NE to affect behaviour during retrieval, but (e) postlearning E fails to prime for retrieval NE actions and postlearning NE does not sensitize for retrieval E. The conclusion of the latter experiment is that the effect on the same population of adrenergic receptors during both consolidation and retrieval of memory is an essential requisite for affecting the expression of conditioned behaviour.

220.14

CENTRAL AMYGDALOID NOREPINEPHRINE INFUSION ENHANCES LATER RETENTION IN ROMAN HIGH-AVOIDANCE AND LOW-AVOIDANCE RATS. B.Roozendaal, J.M.Koolhaas and B.Bohus* (SPON: European Brain and Behaviour Society), Univ. of Groningen, Dept. of Animal Physiol., P.O.Box 14, 9750 AA Haren, The Netherlands.

Memory-enhancement of posttraining norepinephrine given into the CEA was investigated on active and passive behavioral components in a shock-probe/defensive burying paradigm. Genetically selected Roman high-avoidance (RHA/Verh) and low-avoidance (RLA/Verh) rats, displaying active or passive coping strategies in response to emotional stressors, were used. Upon the presentation of the electrified probe during acquisition, both lines spent comparable time in burying behavior. The RLA/Verh rats, but not the RHA/Verh rats, also displayed substantial immobility. During the retention test, the CSF-treated RLA/Verh rats predominantly displayed immobile behavior during the presentation of the nonelectrified probe, whereas the RHA/Verh rats showed neither burying nor immobility. In the RLA/Verh rats norepinephrine (NE) infusion in the high dose (200 ng), and not in the low dose (20 ng), enhanced immobility without affecting the active response. On the other hand, NE given into the CEA of RHA/Verh rats caused a dose-dependent appearance of the conditioned active behavioral component without affecting immobility. The present results suggest a phenotype-dependent effect of intra-amygdaloid NE on memory processes. Differential release of NE in the CEA in the two strains may determine the expression of retention behavior.

220.15

ESTRADIOL ACTS AS AN INTER-AGENT TO ACCELERATE EXTINCTION OF CONDITIONED TASTE AVERSIONS. Y.Wang, D.L.Yuan and K.C. Chambers* Department of Psychology, USC, Los Angeles, CA.

Exposure to a substance that has US properties before acquisition or extinction of a conditioned taste aversion (CTA) can attenuate acquisition and accelerate extinction. This effect is called inter-agent disruption. We have suggested that estradiol (E) accelerates extinction by acting as an inter-agent. If this is the case, then the dose of E that accelerates extinction should induce a CTA when used as a US and exposure to E before extinction should accelerate extinction. In study 1, gonadectomized (GX) females were implanted with 10 mm empty or E-filled silastic capsules after consumption of a 10% sucrose solution. The capsules were removed 18 hours later. Two days after the first sucrose exposure, they again were given access to the sucrose solution. The sucrose consumption of the E-treated females decreased whereas that of the untreated females did not change. In study 2, a CTA was induced in GX females with 0.15 M LiCl (10 ml/kg) after access to a 10% sucrose solution. Two days after acquisition, the females were implanted with 10 mm empty or E-filled capsules. The capsules were removed 8 days later. Daily extinction trials were initiated the next day. The extinction rates of the females given E before extinction were significantly faster than those of the females given no hormone. These data are consistent with the hypothesis that E acts as an inter-agent. BRSG

220.16

REVERSAL OF AGE-RELATED COGNITION DEFICITS IN AUTOIMMUNE MICE BY 647U, A POTENTIAL NEW NOOTROPIC. H. Lal, P.L. Prather, R.R. Luedtke*, and M. J. Forster. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

Autoimmune NZB/BINJ mice exhibit accelerated age-related declines in performance of habituation retention and active and passive avoidance paradigms [*Drug Dev. Res*, **24**:1, 1991]. We evaluated the potential for 647U, a novel drug with antidepressant and cognition-enhancing activity, to improve these spontaneously occurring, age-related deficits. One-way active avoidance training consisted of daily sessions until a performance criterion had been attained. Pre-session administration of 647U at all doses tested (10, 20 and 40 mg/kg) significantly improved both acquisition and retention of the avoidance response. Habituation training consisted of 9 daily 20-min locomotor activity tests where 647U was given immediately after training on days 1 through 4. A dose of 10 but not 20 or 40 mg/kg of 647U produced significant session-related decreases in time spent in the center zone, whereas vehicle treated animals showed no habituation. In contrast, all doses of 647U administered prior to single step-through passive avoidance trial were ineffective in increasing the mean latency to enter the dark chamber at a 24-h retest. The current findings indicate that 647U is efficacious in reversing some of the autoimmune-related learning and memory deficits exhibited spontaneously by NZB/BINJ mice.

220.17

DECREASE IN ASSOCIATIVE LEARNING IN OLDER SPONTANEOUS HYPERTENSIVE RATS. E. Hong* and A. Meneses. Dept. of Pharmacology and Toxicology, CINVESTAV-IPN, Mexico city.
Spontaneous hypertensive rats SHR and their respective controls, the Wistar Kyoto (WKY) rats are frequently used for the study of arterial hypertension. It has been reported that SHR score poorly in learning tasks, therefore, it seems of interest to make a systematic study correlating hypertension and age with learning in SHR and WKY rats of different ages, ranging from 3 to 24 months-old. For this purpose, food-deprived rats, to 85% of their weight were trained on autoshaping during six days. Animals were retrained one month later. Animals were individually trained to find 15 pellet in the food magazine, once that the animal ate the food, a session began. The learning task consisted in illumination of a retractable lever for 8 sec (conditioned stimulus) followed by delivery of a food pellet (unconditioned stimulus) each 60 sec. If the animal pressed the lever (conditioned respones) the trial was shortened and the lever was retracted, the light was turned off and the unconditioned stimulus was delivered. The younger animals learned faster and achieved a greater score than older ones. SHR took a longer time to learn and showed lower scores than WKY rats. The present results suggest that both factors, ageing and hypertension decrease the associative learning.

LEARNING AND MEMORY: PHARMACOLOGY—OPIOIDS

221.1

EXPLICIT AND IMPLICIT MEMORY DISSOCIATED BY ANESTHETIC TECHNIQUE. R. C. Cork, J. F. Kihlstrom and S. R. Hameroff*. Univ. of Arizona Health Sciences Center, Anesthesiology, Tucson, AZ 85724.

Explicit memory entails conscious recollection of some event; implicit memory refers to a change in a subject's behavior attributable to such an event, without conscious recollection of the event. These two types of memory are dissociable with amnesic syndrome and posthypnotic amnesia. This study tested for implicit and explicit memory in surgical patients with two commonly used, qualitatively different, anesthetic techniques.

Human Subjects Committee approval and informed consent were obtained. After barbiturate induction of anesthesia, patients were maintained during surgery with either isoflurane (a volatile fluorocarbon) or sufentanil (a synthetic opiate) with N_2O while one of two lists of commonly associated word pairs was continuously played to the patient via headphones. Patients were tested for free recall, cued recall, recognition, and free association (the one test for implicit memory) after surgery in the recovery room and again two weeks later. Repeated-measures ANOVA was used, $p<0.05$.

Testing was completed for 25 of 30 patients in the Isoflurane Group and 25 of 36 patients in the Sufentanil/N_2O Group. Patients receiving isoflurane were more likely to produce target items from the critical list, presented during surgery, than they were from the control list, a significant priming effect ($p<0.05$). Sufentanil/N_2O resulted in no observable priming effect.

Results showed that isoflurane spares implicit memory, while sufentanil/N_2O does not. Opiates inhibit release of neurotransmitters much better than do volatile anesthetics at clinical levels. Could implicit memory be pre-synaptic and explicit memory post-synaptic? Nitric oxide (NO) has been implicated as a retrograde signal in learning (post-synaptic → pre-synaptic). Could N_2O block pre-synaptic NO actions?

221.2

DYNORPHIN$_{(1-13)}$ IMPAIRS MEMORY FORMATION FOR BOTH AVERSIVELY AND APPETITIVELY MOTIVATED LEARNING IN CHICKS. P.J. Colombo*, K.R. Thompson, J.L. Martinez Jr., E.L. Bennett, M.R. Rosenzweig. Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

Administration of the endogenous opioid peptide dynorphin$_{(1-13)}$ impairs acquisition of memory for aversive training in the two-day-old Golden sex-linked cockerel (Colombo et al., 1990). We examined whether this effect on memory formation is general to both aversively and appetitively motivated learning. Two- or four-day-old White Leghorn cockerels were injected bilaterally with dynorphin$_{(1-13)}$ into the intermediate medial hyperstriatum ventrale - a region important for memory formation in the chick - and trained on either a one-trial peck-avoidance (PA) task, or an appetitive visual discrimination (AVD) task; retention was tested at 24 h. In two-day-old chicks, dynorphin was amnestic for PA training at 0.01 mM. In four-day-old chicks, administration of 0.03 mM and 0.1 mM dynorphin$_{(1-13)}$ impaired memory formation for AVD and PA training, respectively. Thus, the effects of dynorphin on memory formation in the chick are similar for both aversive and appetitive conditioning.

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221.3

THE SEPTAL, BUT NOT THE AMYGDALOID, OPIATE SYSTEM IS INVOLVED IN SPATIAL WORKING MEMORY IN AN APPETITIVELY MOTIVATED TASK. R.Q.Wan*, L. Gorman, K. Pang, and D.Olton. Dept. of Psychology, The Johns Hopkins University, Baltimore, MD. 21218.

The opiate system in both the septum and amygdala can influence memory. Previous studies have typically examined the role of these opiate systems in mnemonic function by using different tasks. Thus, dissociations of the effects of these opiate systems on memory processes are difficult to interpret because many variables differ among behavioral tasks. The present experiment examined the hypothesis that the septal and amygdaloid opiate systems may be involved in different mnemonic processes by keeping all aspects of the experimental procedure the same except for the neuroanatomical location of the opiate manipulation, the medial septal area (MSA) or the central amygdaloid nuclei (CAN). The role of both opiate systems in spatial working memory was tested by using an appetitively reinforced T-maze alternation task. A within-subjects experimental design was used. β-endorphin (β-END, 250 and 1000 ng) or muscimol (MUS, 20 ng) was infused into MSA, and β-END (1000 ng) or MUS (20 ng) was infused into CAN immediately prior to testing. Infusions of β-END and MUS into the MSA, but not into the CAN, impaired choice accuracy. Infusion of either compound did not impair general activity as indicated by the amount of time to complete one session. These results suggest that the opiate system mediates different mnemonic processes in different neuroanatomical areas.

221.4

INTERACTION BETWEEN β-ENDORPHIN AND THE NORADRENERGIC SYSTEM IN THE MODULATION OF MEMORY STORAGE J.L.McGaugh*, I.B.Introini-Collison and L.A.Ford, Center for Neurobio. of Learning & Memory and Dept. of Psychobio., U. of Calif., Irvine, CA 92717.

Intra-amygdala injections of β-noradrenergic (β-NE) receptor agonists facilitate memory storage in a dose-dependent manner. Opioid receptor antagonists facilitate memory, while opiates and opioid peptides impair memory. Propranolol, a β-NE receptor antagonist, blocks the memory enhancing effects of naloxone, suggesting that such effects are mediated by an increased release of norepinephrine (NE). These experiments examined further the interaction between the noradrenergic and the opioid peptidergic systems in the amygdala in modulating memory storage. Male Sprague-Dawley rats (180g, 50-days old on arrival) were trained in inhibitory avoidance (IA) and Morris water maze (MWM) tasks. Injections were given immediately post-training for IA and 5 minutes pre-training for MWM. In the first experiment, rats were given clenbuterol (10.0 or 30.0 ng) concurrently with β-endorphin (0.1 ng) through cannulae previously implanted bilaterally in the amygdala. In both the IA and the MWM, clenbuterol prevented the memory impairing effects of β-endorphin. In the second experiment, rats were given concurrent subeffective doses of propranolol (0.3 μg) and β-endorphin (0.03 ng) intra-amygdally. Neither propranolol nor β-endorphin affected memory when given alone. However, when given together, these doses of propranolol and β-endorphin significantly impaired memory. Considered together the findings of these experiments are consistent with the view that opioid peptides impair memory by decreasing the release of NE in the amygdala.

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221.5

INTRA-AMYGDALA INJECTIONS OF MORPHINE IMPAIR AVOIDANCE LEARNING: ATTENUATION WITH CONCURRENT AMYGDALA INJECTIONS OF GLUCOSE. M.E. Ragozzino* and P.E. Gold. Dept. Psychology, University of Virginia, Charlottesville, VA 22903.

Recent findings from several laboratories demonstrate that modest increases in circulating glucose levels modulate memory processes in rodents and humans. One brain area sensitive to direct glucose administration is the medial septum. For example, intraseptal glucose injections attenuate memory impairments after intraseptal morphine injections, contributing to the view that glucose may act functionally to antagonize opioid actions. The present experiment examined whether, as seen with medial septum, memory processing was susceptible to morphine and glucose injections into the amygdala. Rats were tested for inhibitory avoidance and spontaneous alternation performance. Bilateral morphine (4.0 nmol) injections into the amygdala prior to training impaired later retention of inhibitory avoidance training. Concomitant administration of intra-amygdala glucose (16.6 nmol) attenuated the morphine-induced inhibitory avoidance deficit. Intra-amygdala injections of morphine did not significantly affect spontaneous alternation performance. Thus, both the amygdala and medial septum are sensitive to direct injections of glucose. In both brain regions, glucose attenuates memory deficits after morphine administration. The nature of the deficits, however, varied by task. Injections of morphine into medial septum substantially impaired both spontaneous alternation and inhibitory avoidance performance. Morphine injections into the amygdala impaired inhibitory avoidance learning but had no effect on spontaneous alternation performance. (Supported by NSF BNS-9012239, ONR N0001489-J-1216, NIA AG 07648. M.E.R. is the recipient of a predoctoral fellowship on Behavioral Neuroscience Training Grant NIMH MH 18411).

221.6

RELATIVE SALIENCE OF ENVIRONMENTAL AND DRUG CUES AS CONDITIONED STIMULI. N. M. Bormann and D. A. Overton*. Department of Psychology, Temple University, Philadelphia, PA 19122.

An exp. was conducted to compare the relative salience of environmental (metal box) cues and morphine (6 mg/kg, i.p.) induced cues as conditioned stimuli (CS) when each was paired with a footshock unconditioned stimulus (US), to produce a conditioned suppression of drinking (CR) in water deprived rats. Before training, baseline measures of water consumption were taken 10 min after morphine and saline injections. Then 6 groups (6 rats each) were exposed to the following conditioning procedures: Groups 1 & 2 were placed into a metal box 90 min before saline->US and morphine->US pairings, respectively; Groups 3 & 4 were respectively placed into the box 5 min after saline and morphine injections and received shock 5 min later; Groups 5 & 6 were placed into the box 19 hours before morphine->US and saline->US pairings. For 20 days after conditioning, Ss received drinking test sessions after saline injections in a novel plastic box in order to extinguish any fear in response to the technician and/or injection procedure. Then during subsequent tests Ss were exposed to morphine in the plastic box, to the metal box alone, and to morphine in the metal box before drinking tests. When exposed to morphine in the plastic box, only Group 5 suppressed drinking. When exposed to the metal box alone, Groups 3 and 4 suppressed significantly more than all other groups. When exposed to morphine in the metal box, Groups 1-4 exhibited a profound suppression of drinking. This exp. demonstrates that morphine cues can act as a CS for fear, but that environmental cues presented up to 90 min before US presentation are more salient as CS than morphine drug cues. Supported by NIDA grant DA02405.

LEARNING AND MEMORY: PHARMACOLOGY—MONOAMINES

222.1

ONDANSETRON IMPROVES SHORT-TERM MEMORY AND BLOCKS CHOLINOMIMETIC-INDUCED EMESIS IN RHESUS MONKEYS. H.J. Normile,*[1] H.J. Altman,[1] M.J. Callahan,[2] R.E. Davis[2]. [1]Wayne State University School of Medicine, Detroit, MI 48207 and [2]Parke-Davis Pharmaceutical Research, Warner-Lambert Co., Ann Arbor, MI 48106

The serotonin antagonist Zacopride reverses cholinomimetic-induced emesis in the ferret. We have extended these findings demonstrating that another 5-HT_3 antagonist Ondansetron blocks cholinomimetic-induced emesis in young rhesus monkeys. In addition, Ondansetron improves cognitive performance of aged but not young monkeys at doses similar to those blocking emesis in this species. Pretreatment of young rhesus monkeys with Ondansetron dose dependently decreased the severity of emesis associated with administration of the partial muscarinic agonist CI-979. At one of these doses Ondansetron reliably improved delayed matching-to-sample performance of aged but not young rhesus monkeys. Ondansetron, however, did not enhance attention/vigilance or reverse scopolamine-induced impairments in attention. These results are consistent with previous reports demonstrating cognitive enhancement following administration of 5-HT_3 receptor antagonists and confirm the ability of this class of agents to block cholinomimetic-induced emesis in a second species. Thus, Ondansetron may be useful in ameliorating the GI disturbances commonly associated with cholinomimetic therapy in man. At the same time, combined use of cholinomimetics and 5-HT_3 antagonists may exert synergistic effects on cognitive performance. The combined use of these agents should be considered in treating age-related memory disorders including Alzheimer's disease.

222.2

SEROTONIN DEPLETION IMPAIRS THE PERFORMANCE OF RATS IN SPATIAL WORKING MEMORY TASK. J.Sirviö*, P.Jäkälä, P. Riekkinen Jr. and P.J. Riekkinen. Dept. of Neurology, Univ. of Kuopio, Kuopio, Finland.

The present study investigated the role of the serotonergic system in working memory. The effects of serotonin depletion in brain induced by p-chlorophenylalanine (PCPA, 3x500 mg/kg, ip) on the performance of rats in a continuous operant delayed non-matching to position (DNMTP) task were studied. PCPA treatment impaired behavioural activity (lengthened response latencies, decreased number of trials completed) of rats which had been trained for DNMTP task before the treatment. The percent correct responses were not significantly decreased at any delays (0-16 seconds) tested. Neurochemical analysis showed that frontal and hippocampal serotonin was markedly decreased (>97%) in PCPA treated rats as compared to controls. The present results support the hypothesis that brain (cortical and hippocampal) serotonin is involved in the general organization of behavior, but they do not suggest any important role for serotonin in spatial working memory.

This study was supported by The Finnish Academy of Sciences.

222.3

T-MAZE ALTERNATION DEFICITS IN METHAMPHETAMINE-TREATED RATS. Cooper, B.G., A.E. Butt, & G.K. Hodge.* Department of Psychology, University of New Mexico, Albuquerque, NM 87131-1161.

High doses of methamphetamine (MA), which are neurotoxic to dopaminergic and serotonergic neurons, cause lasting changes in behavior. We examined performance in a T-maze alternation task in MA-treated rats previously tested in an operant conditioning paradigm. Operant conditioning results suggested that these animals were unable to modify learned behaviors when task parameters changed (Cooper, Butt, Hardy, & Hodge, *Soc. Neuroscience Abstr.*, 1991). Since alternation tasks require modification of previously reinforced behaviors and MA treatment disrupts performance when response requirements change, it was expected that MA-treated animals would show impaired learning.

Animals received four subcutaneous injections, two hours apart, of either saline (n=7) or MA (10 mg/kg, n=9; 12 mg/kg, n=8). Eight months post-injection, animals were trained in a T-maze alternation task. Each of eight daily trial consisted of a forced run and a choice run. In the forced run, animals were allowed access to one arm, while access to the other arm was blocked. Animals were reinforced upon entering the unblocked arm where they were held for 20 seconds. Then, in the choice run, animals were allowed to choose between the two arms but were rewarded only for entering the previously blocked arm. Animals were trained to an a priori criterion of seven correct choices per block, for three consecutive blocks, with a perfect score in the third block. Mean number of days required to reach criterion were recorded for each group.

MA-treated animals in the 12 mg/kg group took significantly longer to reach criterion compared to controls ($p<.05$). Results suggest that MA-treated animals have difficulty adjusting their behavior when reinforcement contingencies change.

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222.4

DOPAMINERGIC MODULATION OF LATENT INHIBITION IN PREWEANLING RAT PUPS. C.A. Moody* and L.P. Spear, Dept. Psychology and Center for Developmental Psychobiology, SUNY, Binghamton, NY 13902-6000

The role that dopaminergic neurotransmission plays in the regulation of latent inhibition (LI) was examined in postnatal day 18-19 Sprague-Dawley rat pups. Animals received a single subcutaneous (s.c.) injection of 0, 0.05, 0.1 mg/kg haloperidol or 0, 0.25, 0.5 mg/kg d-amphetamine, 45 or 15 min respectively, prior to the preexposure period which consisted of 0 (0P), 3 (3P), or 9 (9P) min of exposure to banana odor. Twenty min following preexposure or an equivalent amount of time in a holding cage for animals not receiving preexposure, pups received 30 sec of exposure to banana odor during which three 3 sec 1.5 mA footshocks were delivered. Unpaired (UP) animals received footshocks 20 min prior to banana odor exposure. Five min after conditioning (or odor exposure for the UP group), animals were given a 1 min odor preference test between banana and a novel odor (lemon). Time spent on the banana side of the test apparatus was recorded. Non-preexposed animals (0P group) spent significantly less time on banana side relative to their UP counterparts. LI was observed following 9, but not 3, min of preexposure. Similar to observations in adult animals, the high dose of amphetamine disrupted the LI observed in the 9P group. Conversely, haloperidol, typically shown to enhance LI in adult animals, disrupted LI in the 9P pups at the high dose of haloperidol. Studies are currently underway to determine whether this haloperidol-induced disruption in LI is a dopamine autoreceptor-mediated effect. [Supported by NIDA Grants R01 DA04478, and K02 DA00140].

222.5

D1 AND D2 ANTAGONISTS PRODUCE DIFFERENTIAL EFFECTS ON AMPHETAMINE-PRODUCED ENHANCEMENT OF RESPONDING FOR CONDITIONED REWARD IN RATS. **R. Ranaldi* and R.J. Beninger. Dept Psych, Queen's Univ, Kingston, Canada, K7L 3N6.**

Dopamine (DA) D1 receptors may play a role in reward. The effects of D1 and D2 antagonists on amphetamine (AMPH)-produced enhancement of responding for conditioned reward (CR) were evaluated. Rats were exposed to two levers, one producing a 3-s lights-off (LO) stimulus and the other a tone (T), for 5 40-min sessions (S). The levers were then removed and, during 4 Ss, rats were exposed to 80 presentations of LO (on a random time 45-sec schedule) paired with food. Testing consisted of two Ss with the levers again present. Results showed that the saline group increased responding for LO more than for T from pre- to post-pairing, confirming that LO became a CR. AMPH (0.1 to 5.0 mg/kg, ip, 5 min before test Ss) dose-dependently enhanced responding specifically for the CR. SCH 23390, a D1 antagonist (5.0 and 10.0 ug/kg, sc, 2 h before test Ss), shifted the locus of rise in the AMPH dose-response function toward higher AMPH doses. Metoclopramide, a D2 antagonist (1.0 to 7.5 mg/kg, ip, 1 h before test Ss), did not affect the locus of rise but reduced the asymptote of the AMPH dose-response function. These results provide strong support for the hypothesis that reward-related learning may involve a DA signal at D1 receptors. (Funded by N.S.E.R.C.).

222.6

EVALUATION OF CHANGES IN DOPAMINERGIC SYSTEMS AS A BASIS OF LEAD-INDUCED LEARNING IMPAIRMENTS. J. Cohn* and D.A. Cory-Slechta. Environ. Health Sci. Ctr., Univ. Rochester Med. School, Rochester, NY 14642

Even very low-level lead (Pb) exposures are known to impair learning and other behavioral functions. The neurobiological bases of these effects remains obscure, although alterations in neurotransmitter systems have been proposed as one possibility. Using drug discrimination procedures, this laboratory previously reported both a D1 and D2 dopaminergic (DA) supersensitivity in rats exposed to Pb postweaning. To determine whether this DA supersensitivity might underlie Pb-induced learning impairments, the effects of acute administration of the tyrosine hydroxylase inhibitor alpha-methyl paratyrosine (AMPT), the D1 agonist SKF38393 and the D2 agonist quinpirole were compared in control and Pb-exposed rats (0, 50 or 250 ppm in drinking water from weaning) working under a multiple schedule of repeated acquisition (RA) and performance (P). The RA component of the schedule required rats to learn a new 3-member sequence of responses during each experimental session (Center Right Left, RLC, CLR, RCL, or LRC), while the correct sequence of responses for the P component was constant across sessions (LCR). Each of these components alternated twice during the daily experimental session, yielding two RA components (RA1, RA2) and two P components (P1, P2). In control rats, AMPT had little effect on accuracy, which declined by about 25% only in the RA2 component. Quinpirole produced dose-related decreases in accuracy of 20-30% in RA2, P1 and P2, indicating non-specific performance impairments rather than selective learning deficits. SKF38393 also resulted in dose-related decreases in accuracy of 20-30% which occurred in all four components (RA1, RA2, P1, P2), but which was statistically significant only in RA1. While all three DA compounds generally altered response rates in all components of the schedule, only AMPT produced dose-related effects, and only in RA1 and RA2 where rates declined with dose. Pb exposure did not systematically alter any of the observed effects of the DA compounds upon accuracy. It did, however, modify the alterations in response rates produced by AMPT, potentiating the drug-related changes. Taken together, these findings suggest a more pronounced involvement of DA systems with rates of responding rather than with learning processes. Further, they offer little evidence to support the contention that Pb's effects on learning are mediated through DA supersensitivity. NIH ES07026 (JC), ES05017 and ES05903 (DCS).

222.7

INFLUENCE OF CHRONIC BETA-BLOCKER ANTIHYPERTENSIVES ON AROUSAL-MODULATED WORKING MEMORY IN ELDERLY ADULTS. K.A. Franklin and R.A. Jensen*. Biopsychology Laboratory, Department of Psychology, Southern Illinois University, Carbondale, IL 62901-6502.

Level of arousal influences learning and memory. Under conditions of arousal (i.e., sympathetic activation) there is increased release of adrenal catecholamines (CAs) which have primarily peripheral actions since they do not freely cross the blood-brain-barrier. McGaugh (McGaugh, J.L., 1990, *Psychological Science, 1*(1), 15-25) proposed that these substances, by affecting peripheral receptors, may modulate memory through alterations in arousal level which, in turn, affect the memory consolidation process. We have demonstrated that moderate increases in arousal, induced by having human subjects squeeze a hand dynamometer, enhance long-term memory (LTM) in young adults in a variety of tasks. Elderly adults are commonly treated for hypertension. Those who chronically take Beta-blockers may have impaired capacity to modulate memory processes through the release of adrenal CAs. We tested normotensive elderly subjects, elderly hypertensive subjects who chronically take Beta-blockers, and those who take Ca^{2+} blockers or angiotensin-converting enzyme inhibitors (ACEIs). Male and female subjects (mean = 70.7 years) were given a series of reading comprehension tests of working memory and were asked to squeeze the hand dynamometer during the consolidation/retrieval interval. The arousal manipulation produced enhancement of LTM in normotensives, a smaller enhancement in those taking Ca^{2+} blockers or ACEIs, and no enhancement in those taking Beta-blockers. These findings suggest that elderly individuals chronically taking Beta-blockers may be unable to benefit from the memory-modulating effects of arousal, and that medications taken in an attempt to attenuate some of the physiological symptoms of aging may actually contribute to cognitive decline in aging.

222.8

POST-TRAINING D-AMPHETAMINE ENHANCES WIN-SHIFT RADIAL MAZE RETENTION: EVIDENCE FOR A CENTRAL ACTION ON MEMORY. M. G. Packard*, C. L. Williams, J. L. McGaugh. Center for Neurobio. of Learning & Memory and Dept. of Psychobio., U. of Calif., Irvine, CA 92717.

Peripheral post-training injection of the indirect catecholamine agonist D-amphetamine (D-AMP), enhances memory in both aversive and appetitive learning tasks. Previous evidence suggests that the retention enhancing effects of D-AMP seen in tasks using aversively motivated training is mediated by an action of the drug in the periphery. For example, post-training injection of 4-OH amphetamine, an analog with limited capacity to cross the blood-brain barrier, enhances retention in an inhibitory avoidance task. The present experiments examined the contribution of the peripheral effects of D-AMP memory enhancement in an appetitive win-shift radial maze task. On each daily trial in an 8-arm radial maze, male Sprague-Dawley rats (275-300 g) were allowed to obtain food from 4 randomly selected maze arms before being removed from the maze. After a delay, the rats were returned to the maze for a retention test in which only those 4 arms not visited prior to the delay contained food. On the experimental day, the rats were injected (s.c.) with drug or vehicle immediately after completion of the first 4 choices, and a retention test was given after a delay of 18 hours. Injection of 1.0 mg/kg D-AMP enhanced retention relative to saline injected controls, while doses of 0.5 and 2.0 mg/kg were ineffective. Furthermore, injections of 1.0 mg/kg D-AMP administered 2 hours post-training did not affect retention. In contrast to the memory enhancement observed following administration of D-AMP, post-training injection of 4-OH amphetamine (0.5, 1.0, 2.0, and 4.0 mg/kg), did not enhance retention. The findings indicate that peripherally administered D-AMP enhances win-shift retention in the radial maze by acting, at least in part, directly on brain processes.

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222.9

PRAZOSIN ENHANCES RECOVERY OF SPATIAL LEARNING IN ANIMALS WITH HIPPOCAMPAL SYMPATHETIC INGROWTH. L.E. Harrell*, A. Peagler and D. Parsons. Depts. of Neurology and Psychology. VA and University of Ala., Birmingham, AL 35294

We have previously reported that the detrimental effects of hippocampal sympathetic ingrowth (HSI), a neuronal rearrangement induced by hippocampal cholinergic denervation via medial septal lesions (MSL), on spatial learning were ameliorated by phentolamine (α-antagonist) treatment. In this study, we sought to determine the role of α_1-receptors. Adult male rats underwent training on a radial-8-arm maze, with all arms baited, until learning criterion was achieved. Animals were then randomized to 1 of 3 groups: Controls--sham MSL + sham Gx (ganglionectomy); MS--MSL + sham Gx (HSI group); MSGx--MSL + Gx (no HSI). Two days following surgery, testing on the maze was initiated. Prior to the beginning of each daily trial, $^1/_2$ of the animals in each of the groups received prazosin (1 mg/kg IP), while $^1/_2$ received saline. Testing continued until criterion was re-achieved. Prior to surgery, the number of trials to achieve learning criterion was similar among all groups. Following surgery, in the saline treated groups, MS animals required significantly more trials (41.2 ± 10.5 M ± SE) to achieve criterion than MSGx animals (23.5 ± 4.3), who were more impaired than CON (9.7 ± 2.7) ($p < .01$). Prazosin normalized performance of the MS group (10.7 ± 2.5) but was without effect on the MSGx (24.0 ± 4.0) or CON (20.8 ± 8.9) groups. These results 1) confirm that HSI has a detrimental effect on recovery of spatial learning and 2) suggest that the detrimental effect of HSI is mediated through α_1 receptors.

222.10

ALPHA-2-ADRENERGIC MODULATION OF SPATIAL MEMORY PERFORMANCE IN AGED BUT NOT IN ADULT RATS BY MEDETOMIDINE. S.Carlson*, H.Tanila, P.Rämä, E.Mecke and A.Pertovaara. Dept. of Physiol., Univ.Helsinki, Helsinki, Finland

The role of alpha-2-adrenergic mechanisms in spatial memory performance was studied in rats using a highly selective alpha-2-adrenoceptor agonist medetomidine and antagonist atipamezole. The effect on working memory was studied in a T-maze using a delayed alternation task. Testing was performed in two stages, at the age of 8.3 months (mean) and again at the age of 17.6 months (mean). The drugs/saline control were administered i.m. in a double blind fashion.

A low (3 ug/kg) and a high (30 ug/kg) dose of medetomidine improved the performance of the aged rats in the memory task, but a medium dose (10 ug/kg) had no effect. Thus, the non-monotonic dose-response curve of medetomidine resembled that of guanfacine. There was no effect on the younger rats. At the low dose of medetomidine the rats showed no signs of sedation, whereas the high dose was sedative. Atipamezole (0.3-3.0 mg/kg) had no significant effect in the memory task.

Since medetomidine, even at a low dose, has a beneficial effect on the memory performance of aged rats, it could be a good candidate for the treatment of age-associated memory dysfunction.

222.11

EVIDENCE THAT NORADRENERGIC (NE) ACTIVITY PLAYS A ROLE IN DISTRACTIBILITY. B.J.Strupp* & M.D.Bunsey. Division of Nutritional Sciences and Department of Psychology, Cornell University, Ithaca, NY 14853.

The putative modulation of attentional processes by endogenous NE was examined by pharmacologically manipulating this system with idazoxan (IDZ), a drug that blocks α_2 receptors. The drug (0, 0.5 & 1.0 mg/kg, s.c.) was administered to rats prior to testing in two automated discrimination tasks designed to assess different aspects of attention. While IDZ did not affect vigilance or impulsivity, a significant negative correlation was observed between distractibility under the high dose and distractibility in the control condition ($p=.001$). The animals that were the most distractible in the nondrug state were the least distractible when treated with IDZ and vice versa. Further analyses indicated that IDZ primarily affected the animals' tendency to respond prematurely when presented with distractors. The fact that a single dose produced qualitatively different effects on distractibility as a function of baseline distractibility indicates that IDZ's neurochemical effects interacted with the activity of a neurochemical system(s) that plays an endogenous role in modulating this cognitive process.

222.12

EXAMINATION OF ALPHA$_2$-ADRENERGIC AGENTS ON COLD-INDUCED IMPAIRMENT OF DELAYED MATCHING-TO-SAMPLE PERFORMANCE IN RATS. O. A. Morgan, S.T. Ahlers, D. Shurtleff, M.O. Thornton*. Naval Medical Res. Inst., Bethesda, MD 20889-5055.

Modulation of alpha$_2$-adrenergic receptors has been reported to effect working memory performance. The present study examined the effects of clonidine and idazoxan administration in rats, both alone, and under conditions in which performance was impaired by exposure to cold air stress. A delayed matching-to-sample (DMTS) task was used to measure working memory. Under the DMTS task rats were required to respond on one of two levers cued by an illuminated light above the lever on the front wall of an operant chamber. Following a variable delay ranging from 1-16 sec, both lights were illuminated and rats were required to correctly respond on the lever previously cued for a food reward. Rats responded on the back wall lever during the delay interval to prevent position bias. Clonidine (0.005-0.05 mg/kg), idazoxan (2.0 or 5.0 mg/kg), or saline, administered (ip) in a mixed sequence, were given 30 min before a 75 minute session in which rats performed the DMTS task (180 trials). During test sessions the ambient air temperature was either 24°C or 2°C. Administration of clonidine produced dose-dependent impairment of matching accuracy across all of the delays when rats were exposed to 24°C. Idazoxan did not impair matching accuracy at any dose under normothermic conditions. Exposure to 2°C impaired matching accuracy at all delays. Low doses of clonidine (0.005 and 0.01 mg/kg) reduced cold-induced impairment of matching accuracy whereas high doses were ineffective. Idazoxan, at doses up to 5.0 mg/kg, did not alter the effect of cold stress on matching accuracy.

222.13

THE ROLE OF NOREPINEPHRINE IN CONSOLIDATION OF EARLY OLFACTORY MEMORIES. R.M. Sullivan* and D.A. Wilson, Devel. Psychobiology Lab., Dept. Psychology, University of Oklahoma, Norman, OK 73019

Norepinephrine has been shown to be critically involved in olfactory associative learning in newborns. NE blockade during training prevents acquisition of learned behavioral responses to the conditioning odor and their neural correlates. NE is not necessary, however, for expression of the learned responses once they are acquired. In mature animals, levels of NE during the immediate post-training period can influence the consolidation of previously learned associations. The present experiment examined whether NE consolidation processes also occur early in development.

Wistar rat pups were trained on PN5 in a classical conditioning paradigm with citral odor (CS) and intra-oral infusions of milk (UCS). Pups were randomly assigned to either the PAIRED, UCS only or CS only conditions. Immediately following training, pups in each group were injected with either saline, or the ß-receptor antagonists propranolol (10 or 20 mg/kg) or timolol (3 mg/kg). Pups were tested for conditioned behavioral odor preferences on PN6.

The results suggest that post-training blockade of NE impairs early olfactory learning.

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222.14

SOCIAL EXPERIENCE ALTERS RATS' RESPONSES TO ODORS FROM DSP-4-TREATED RATS. C. Cornwell-Jones*, T. Palfai, A. Krause, A. Friedberg, A. McCausland, C. Harfenist, C. Andrew, and T. Kuntz. Psychology Department, Syracuse University, Syracuse, NY. 13244.

Injecting rats with the NE neurotoxin DSP-4, alters the odors the rats produce, as measured by responses of control rats to those odors. The present experiments investigated whether such responses were influenced by the age of the treated animals, or by prior exposure to their odors. Within the period ending on postnatal day 2, male Sprague-Dawley rats were injected subcutaneously with either 50 ug/g of DSP-4, or water. They were housed with the dam in mixed water/DSP-4 litters until weaning. After weaning, some of these litters were divided into separate water-only and DSP-4-only living groups. The remaining litters were divided into water-only and mixed water/DSP-4-treated groups.

Odor preference tests using home-cage bedding as stimuli indicated that juveniles, but not infants, neonatally treated with DSP-4, produce odors that are distinguishable from controls. Mixed groups of water/DSP-4-treated juveniles produce odors which are more aversive to rats housed in the mixed condition than to other rats. These findings suggest that the aversion develops through association with some other negative stimulus within the home-cage, and not because the mixed odors act as an aversive pheromone.

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222.15

IMPROVED ATTENTION FOLLOWING METHYLPHENIDATE HYDROCHLORIDE (RITALIN) IN AN ANIMAL MODEL OF ATTENTION-DEFICIT HYPERACTIVITY DISORDER

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SPON: European Brain and Behaviour Society

Attention-Deficit Hyperactivity Disorder (ADHD) is a disease characterized by attention problems and over-activity. ADHD is frequently treated with psychomotor stimulating drugs. Spontaneously hypertensive rats (SHR) are hyperactive and may be used as an animal model of (ADHD). The present study investigated attention processes of SHR by using a time-discrimination task. In this task, the subject had to space its responses by at least 16 s in order to be eligible for a reinforcer. However, not all responses with sufficient inter-response time qualified, as the reinforcer was set up on the average only every 120 s.

SHR timed their responses less effectively than WKY thereby showing an attention-like deficit. The lowest dose of methylphenidate improved the time discrimination of controls, but only to a very limited degree compared to the effects of low doses of this drug on the behavior of SHR which virtually normalized their time discrimination. Thus, methylphenidate did not have a "paradoxical" effect since both groups are effected similarly by the drug, but SHR are less sensitive compared to WKY.

223.1

SONG-SELECTIVE AUDITORY NEURONS EMERGE DURING VOCAL LEARNING IN THE ZEBRA FINCH. A.J.Doupe* and M. Konishi. Division of Biology, Caltech, Pasadena, CA 91125.

Auditory experience and feedback are essential for normal birdsong learning. Young male birds first hear and memorize a parent tutor song during a stage called sensory learning. Later, during motor learning, they gradually match their own vocalizations to the song memory, using auditory feedback. Therefore, there must exist brain mechanisms for auditory recognition of song and song-like vocalizations, and for providing feedback to the vocal motor system. The neural circuit containing the song nuclei X, DLM, and L-MAN is a possible site for such mechanisms. It plays a crucial but unknown role during song development, and in adult finches all three of these nuclei contain song-selective auditory neurons: these cells respond more robustly to the bird's own song than to songs of conspecific individuals, and they are sensitive to the temporal order of song. We therefore investigated this circuit in juvenile birds, at a stage when it is essential to learning.

We used single unit recordings to characterize the auditory response properties of song nuclei of young male finches of 30-35 days of age, a time when sensory learning is underway and motor learning is just beginning. Each of these birds had been exposed to only one tutor, the male parent, whose song was included in the acoustic stimuli presented during the experiment. We found that X and L-MAN do contain complex auditory neurons at this early stage. Their properties are strikingly different from those in adults, however. These neurons respond to a variety of conspecific songs as robustly as to the tutor song, and they respond well to the tutor song even when the temporal order is reversed.

The presence of auditory neurons in this circuit in young birds suggests that one of the essential functions of this pathway may be the auditory feedback crucial to normal song learning. Moreover, the highly song-selective properties of the adult forebrain song nuclei are not present in young birds, and must emerge during development, in parallel with song acquisition. Further studies of these neurons will reveal which facets of learning their selectivity reflects and may shed light on their critical role in normal song development.

223.2

NEURONS IN FIELD L OF BUDGERIGARS PREFER SPECIES SPECIFIC CALLS TO WHITE NOISE K. Mori* and G. Striedter. Division of Biology, 216-76, California Institute of Technology, Pasadena, CA 91125.

Budgerigars (*Melopsittacus undulatus*) are capable of discriminating between their own calls and those of cagemates. In an attempt to investigate the neuronal correlates of this behavior, we studied the characteristics of neuronal responses in field L to species specific calls as well as to synthetic noise and tones. Adult budgerigars were kept together for several weeks before their calls were recorded. A stereotaxic head pin was affixed to the skull under Xylazine/Ketamine anesthesia several days prior to the recording session. Extracellular activity of single neurons was isolated in field L under Urethane anesthesia (1.4 g/kg). Stimuli consisting of white noise and various calls were delivered to the bird during search, and more than 160 auditory single units were isolated. Units preferring calls to noise were further tested with tone bursts, frequency sweeps and modified calls. Recording sites were later confirmed histologically. We found that more than half of the single units responded more vigorously to its own calls or cagemates' calls than to white noise. Of these units, approximately 90% responded to pure tone bursts of various frequencies. However, half of them did not respond to white noise, which suggests that there is inhibitory interaction among different frequencies. Ninety-five percent of the neurons preferring calls responded to at least one upward or downward frequency sweep ranging from 5 kHz/s to 320 kHz/s. Twenty percent responded predominantly to only one of the calls in our stimulus set, although the frequency-time spectrograms of some of the calls were similar. Forty-one percent of the units tested with forward and reverse calls showed differential responses. Although some of these responses could be explained by the unit's selectivity for direction and speed of frequency sweeps, there were cases where further analysis with modified and synthetic calls revealed more complex signal processing such as interfrequency inhibition in the time domain, and interval tuning for consecutive frequency sweeps. These properties were specific enough to allow neurons to respond to only a single call in our stimulus set.

223.3

ACTIVITY OF SYRINGEAL MUSCLES DURING SONG IN MIMIC THRUSHES. F. Goller and R. A. Suthers*. Medical Sciences, Indiana Univ., Bloomington, IN 47405.

Simultaneous recordings of sound, air sac pressure, bronchial flow and electromyograms of ventral (*m. syringealis ventralis*) and dorsal (*m. syr. dorsalis*) syringeal muscles were made during song in brown thrashers (Toxostoma rufum). Most vocalizations are preceded by strong electrical activity in dorsal, and usually also in ventral, muscles as subsyringeal pressure rises. In neither of these muscles is the EMG prominently correlated with the termination of sound. Electrical activity varies with the frequency characteristics of the vocalizations. Ventral muscles are essentially inactive during simple notes which have little frequency modulation. High pitched FM notes are typically accompanied by high amplitude, long duration EMG's in both ventral and dorsal muscles and by a reduction in the rate of syringeal airflow. Sounds with rapid sinusoidal frequency modulation are accompanied by alternating EMG bursts in the dorsal and ventral muscles, as well as by oscillations in the rate of syringeal airflow and the amplitude of the vocalization. Unilaterally produced vocalizations are often accompanied by similar EMG patterns in the left and right ventralis muscle. The phonating side of the syrinx therefore could not be reliably deduced from bilateral recordings of the EMG. (PHS R01 NS29467-01)

223.4

RIGHT SIDE DOMINANCE FOR SONG CONTROL IN THE ZEBRA FINCH. H. Williams*, L.A. Crane, T.K. Hale, M.A. Esposito, and F. Nottebohm. Biology Dept., Williams College, Williamstown, MA 01267, and The Rockefeller University Field Research Center, Millbrook, NY 12545.

Left-side hemispheric dominance for song control, similar to that described for human speech, has been found in several songbird species. We unilaterally denervated the syrinx or unilaterally lesioned the forebrain nucleus HVC (known to be important for song control) of adult male zebra finches. Disruptive effects on song were greater after right side than after left side operations. After denervation of the right half of the syrinx, the fundamental frequencies of all syllables within a song converged on a value near 500 Hz, and classification by type of nearly all syllables was altered. In contrast, the syllables produced after denervation of the left side of the syrinx largely maintained their pre-operative frequencies, and fewer syllables changed in type. Syllable frequency and syllable structure were less affected by HVC lesions than by syringeal denervation. However, the stereotyped structure of normal zebra finch song was impaired after HVC lesions, and was more strongly affected by right side than left side operations. Although it is apparent both centrally and peripherally, right side dominance for song production in zebra finches may be tied to different aspects of vocal control at different levels of the song system.

Other songbird species with lateral asymmetry for vocal communication have left side dominance for song control, the reverse of zebra finches. The need for a dominant side may be more important than the side of dominance.

223.5

CONNECTIVITIES OF MIDBRAIN VOCAL CONTROL NUCLEUS AND HYPOTHALAMUS: CONTRIBUTION OF ENKEPHALINERGIC PATHWAYS. M.-F. Cheng* and M. Zuo. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102

Recent studies in the ring dove (Streptopelia risoria) have shown that the female's nest-coo calls stimulate her follicular growth. This causal relationship implicates the hypothalamus (Hy), a projection target of the nest-coo control neucleus, the medial n. intercollicularis (mICo). We have used PHAL as an anterograde marker to identify terminal fields in the hypothalamus. We found: one direct projection to the Hy and the other via auditory pathways. One pathway leaving ICo travels medially to the central gray, descends along 3rd ventricle and terminate in the lateral hypothalamus (LHy) and anterior medial hypothalamus (AM). Distribution patterns of immunoreactive enkephalin cell bodies and fibers suggest that LHy/AM projection may be an enkephalinergic pathway. Fluorogold injection was restricted to LHy/AM region and the sections were processed for retrogradelly labelled cell bodies and met-enkephalin immunoreactivity. The 2nd pathway travels laterally and enters the lateral ICo then descends to the lateral lemniscus and follows the auditory pathway to the area adjacent to the OV (thalamic auditory relay), an area previously shown to send projection to the posterior hypothalamus. Parallel enkaphalinergic pathways suggest this too an enkephalinergic pathway.

223.6

NUCLEUS Uva MIGHT BE PART OF A FEEDBACK CIRCUIT FOR SONG PROCESSING

S. Okuhata and F. Nottebohm*, The Rockefeller University, New York, N.Y. 10021

A motor theory of song perception has been proposed to explain the presence of vigorous auditory responses in HVC and other nuclei of the motor pathway for song production. This proposal would require the existence of a pathway to carry feedback from vocal motoneurons, e.g. those in RA and nXIIts, to higher brain centers involved in song discrimination. The thalamic nucleus Uva projects to the neostriatal nuclei NIf and HVC of the song system, and stimulation of Uva elicits strong responses in the descending motor pathway for vocalization from HVC to nXIIts (Williams, 1989). Because of its anatomical position and physiological properties, Uva might be a component of such a feedback circuit.

In order to examine the function of Uva, single unit recordings were performed in awake zebra finches. Antidromic responses to HVC stimulation were used to identify Uva neurons. Most identified Uva neurons respond to sound with a latency of 18-50 ms and some also respond orthodromically to RA stimulation with a latency of 15-25 ms. In addition, respiratory rhythm is observed in Uva. In order to determine the possible sources of these responses, fluorescent beads were injected into Uva unilaterally and backfilled neurons were observed in both the ipsilateral and contralateral medulla. The area of backfilled neurons overlapped with an area (thought to be the nucleus ambiguous) of neurons backfilled following injection of fluorogold into the laryngeal muscles. The response of Uva neurons to RA stimulation might be mediated by this nucleus. The function of Uva might be to initiate song and integrate motor feedback from song production with respiratory and auditory information, and transfer this result to the telencephalon. Thus, the motor pathway for song production forms a loop that sends information back to HVC. This loop and Uva could play an important role in song acquisition, song production and song perception.

223.7

NUCLEUS UVAEFORMIS CONTRIBUTES TO THE TEMPORAL PATTERN OF VOCAL PRODUCTION IN SONGBIRDS.

D.S. Vicario* and H. Williams. The Rockefeller University, New York, NY 10021 and Williams College, Williamstown, MA 01267.

Birdsong is a learned vocal behavior used in intraspecific communication. The motor pathway serving learned vocalizations includes forebrain nuclei NIf, HVC and RA; RA projects to midbrain and brain stem areas that control the temporal and acoustic features of song. Nucleus Uvaeformis of the thalamus (Uva) sends input to two forebrain nuclei, NIf and HVC, but heretofore has been thought not to be important for song production.

We have used three experimental approaches to re-examine Uva's function in adult male zebra finches. 1) Electrical stimulation applied to Uva activates HVC and the vocal motor pathway, including tracheosyringeal motor neurons that innervate the bird's vocal organ. 2) Bilateral lesions of Uva affect the normal temporal organization of song; specifically, damage to the dorso-medial "horn" of Uva seems to account for the effects. 3) Chronic multi-unit recordings from Uva during normal song and calls show bursts of pre-motor activity that lead the onset of individual syllables, and also larger bursts that appear to signal the occurrence of repeated song phrases.

Together, these results clearly implicate Uva in the production of learned vocalizations. The lesion and recording data further suggest that Uva may contribute more to the temporal structure than to the acoustic characteristics of song.

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223.8

GABA-LIKE IMMUNOREACTIVITY IN THE SONG SYSTEM OF THE ZEBRA FINCH. W. Grisham* and A.P. Arnold. Dept. of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

To increase our knowledge of the neurotransmitters that might be involved in the learning or the production of birdsong, we studied the distribution of GABA-like immunoreactivity (GABA-LIR) in the song control regions of the zebra finch brain. Eight male and 4 female brains were fixed with 1% paraformaldehyde/1.25% glutaraldehyde, sectioned at 40μ, and incubated with a GABA-gluteraldehyde antibody (Chemicon). Binding was visualized using an avidin-biotin system with diaminobenzidine as a chromagen.

Area X of males and the LPO of females had many densely packed, small light to moderate GABA-LIR somata. Males had intense neuropil GABA-LIR in Area X, but females did not. Both males and females had scattered neurons in MAN with GABA-LIR. HVC in males usually had intense neuropil GABA-LIR and large, densely-packed somata with moderate GABA-LIR. HVC had no detectable GABA-LIR in females. RA in males had intense neuropil GABA-LIR and large, widely-scattered neurons with intense GABA-LIR. The RA of females had no detectable neuropil GABA-LIR and consisted of small, densely-packed somata with light GABA-LIR. RA neurons with GABA-LIR were significantly larger in males than in females. ICo had small, densely packed neurons with light GABA-LIR in both sexes. These data suggest that GABAergic neurons may be involved in the learning and/or production of song. Supported by NIH NS09040 to W.G. & DC00217 to A.P.A.

223.9

A VOLUMETRIC STUDY OF THE SONG CONTROL NUCLEI OF MALE AND FEMALE STARLINGS. S.L. RICH*[1], F. GOLLER[2] and D.R. SENGELAUB[1] Program in Neural Science[1] and Medical Sciences Program[2], Indiana University, Bloomington, IN 47405.

While song is considered to be largely a male-specific behavior, both male and female starlings (Sturnus vulgaris) have the capability to produce an elaborate and varied song repertoire (Adret-Hausberger and Jenkins, Behav. 107:156 '88; Hausberger and Black, Ethol. Ecol. Evol. 3:337 '91). The neuroanatomical basis for this complex song in starlings has not been examined in detail, but investigation of song production in other birds has revealed a set of discrete brain nuclei involved in vocal behavior (Nottebohm et al., J. Comp. Neurol. 165:457 '76). These nuclei are generally sexually dimorphic, and both the relative size and degree of dimorphism correlate with song behavior (Nottebohm et al., Brain Res. 213:99 '81; Brenowitz et al., Brain Res. 343:104 '85). In this study the size of telencephalic nuclei involved in song were compared in male and female starlings.

Brains from 2 male and 5 female adult starlings were frozen sectioned (60 µm), stained with cresylecht violet, and examined with light microscopy. Three principal song nuclei (HVc, Area X, and RA) were identified, their perimeters traced, and volumes determined. In all cases the volumes displayed a marked sexual dimorphism, with male:female ratios of 5.11 in HVc, 3.28 in RA, and 2.89 in Area X. Ratios of Area X and HVc to RA are consistent with volumetric measurements taken in birds with similar song dimorphisms. The similarity of the relative sizes and dimorphisms of the song control nuclei of starlings with other species is interesting given the complex vocal ability of starlings and their dynamic vocal repertoire.

223.10

MK801 BINDING DECLINES STEADILY WITH AGE IN A NUCLEUS INVOLVED IN AVIAN SONG LEARNING. S.M Aamodt*, E.J. Nordeen, and K.W. Nordeen. Dept. Psych., U. Rochester, Rochester, NY 14627.

N-methyl-D-aspartate (NMDA) receptors are linked to various forms of experience-dependent plasticity and early learning. Recently, physiology and autoradiography have revealed this receptor type in brain regions controlling song learning and production in zebra finches (*Poephila guttata*). We have shown that in one nucleus involved in song learning, the lateral magnocellular nucleus of the anterior neostriatum (lMAN), binding of the NMDA receptor antagonist ^{3}H-MK801 is much greater in males at 30 days, at the beginning of song learning, than in adulthood, after song learning is complete. To understand better how this regional decline in NMDA receptor binding relates to vocal learning, we have elaborated its timecourse and begun to examine how it is affected by auditory manipulations that could extend the period during which song learning can occur.

Using autoradiography, ^{3}H-MK801 binding was measured in lMAN of 30d, 55d, 80d, and adult (4-6 mos and 7-20 mos) males. Binding declined steadily between 30d and adulthood, but did not differ between the two adult groups. Values were 0.176 ± 0.012 pmol/mg tissue at 30 days, 0.125 ± 0.003 at 55 days, 0.104 ± 0.002 at 80 days, and 0.080 ± 0.005 in adults. Since song learning can be extended by restricting early auditory experience, we also examined MK801 binding in the lMAN of males raised either deaf or isolated from conspecific song. In 80d deaf males, binding in the lMAN (0.107 ± 0.007 pmol/mg) was no different from binding in age-matched controls. However, in two 80d males isolated from hatching, MK801 binding was higher (0.126 ± 0.013), and more comparable to levels in 55d control birds. Additional birds are currently being examined to confirm this finding.

223.11

SEXUAL DIMORPHISM IN THE VOLUME OF SONG CONTROL NUCLEI OF EUROPEAN STARLINGS: ASSESSMENT BY A NISSL STAIN AND AUTORADIOGRAPHY FOR MUSCARINIC CHOLINERGIC RECEPTORS D.J. Bernard*, J.M. Casto, & G.F. Ball Dept. of Psychology, Johns Hopkins Univ. Baltimore, MD 21218

In European starlings (*Sturnus vulgaris*), male song is more complex than female song and is produced at a higher rate. In order to investigate the degree to which this behavioral dimorphism is reflected in the brain, we compared the volume of song control nuclei (SCN) of male and female photosensitive starlings in brain sections stained with cresyl violet. Area X is approximately 2.2 times larger in males than in females. Other SCN, such as the robust nucleus of the archistriatum (RA) and the caudal part of the ventral hyperstriatum (HVc) are approximately twice as large in males as in females. This sex difference agrees well with studies on other songbird species where the degree of behavioral dimorphism parallels the volumetric dimorphism in the SCN. Previous work has suggested that there are several neurochemical markers of the SCN that also demonstrate this volumetric dimorphism. We therefore assessed the volume of the SCN using autoradiographic methods. Area X is well defined by a high density of muscarinic cholinergic receptors in comparison to the surrounding caudate. We localized muscarinic cholinergic receptors by film autoradiography in alternate sections with the use of [^{3}H] N-methyl scopolamine (NMS) as the ligand. The density of cholinergic receptors defined the boundaries of area X in good agreement with the Nissl stain: the volume of area X as defined by NMS binding was found to be approximately 2 times larger in males. Thus the cholinergic innervation of area X in starlings is sexually dimorphic.

223.12

AXONAL CONNECTIONS OF A FOREBRAIN NUCLEUS IN MALE ZEBRA FINCHES. E.F. Foster* & S.W. Bottjer. Dept. Biol., USC, Los Angeles, CA 90089.

The Higher Vocal Center (HVC) is a telencephalic nucleus known to be essential for song production in adult birds. HVC receives afferent inputs from two telencephalic nuclei, mMAN and NIf, and a thalamic nucleus, Uva. Our goal was to map the afferent and efferent projections of one of these nuclei, mMAN, in adult male zebra finches. mMAN was located electrophysiologically by stimulating HVC and recording antidromic multicellular activity in the anterior medial neostriatum. Borders of evoked activity were used to define mMAN and pressure micro-injections of DiI were made in the center of the field of activity. Injections produced light afferent label in HVC, confirming the pathway from mMAN, with fiber label in the LH tract. A small cluster of retrogradely labeled cells were found in the thalamus, directly dorsal to the song nucleus DLM, with fiber label in the FPL tract. Injections dorsal and lateral to mMAN did not label these cells. Results indicate that mMAN receives only one afferent input and makes only one efferent projection. The role of mMAN in vocal behavior is not yet known.

223.13

BLOCKING STEROID HORMONES DURING SONG LEARNING EXTENDS THE SENSITIVE PERIOD FOR LESIONS OF LMAN IN JUVENILE MALE ZEBRA FINCHES. S.D. Brown* and S.W. Bottjer. Univ. of Southern California, Los Angeles CA 90089.

Lesions of the telencephalic nucleus lMAN disrupt song learning in juvenile male zebra finches (≤ 70 days old) but have no effect on stereotyped song produced by older birds. Blocking steroid hormones in juvenile birds also prevents normal development of learned vocal behavior; delayed exposure to testosterone in such birds beyond the normal period of vocal learning permits delayed development of normal song. This finding raises the possibility that low levels of steroid hormones may also extend the period during which lMAN is required for learning to produce specific song patterns.

To test this idea, male zebra finches were castrated at 20 days of age and received continuous exposure to an anti-androgen (flutamide) or an anti-estrogen (tamoxifen) until adulthood (≥ 90 days). lMAN was then electrolytically lesioned, and song behavior was recorded pre-operatively and for six to eight weeks following surgery. Lesions of lMAN resulted in immediate deficits ranging from severely disrupted song consisting of only one or two notes to more subtle changes in which several notes that were consistently produced pre-operatively were deleted from the final song. Normal siblings that received lMAN lesions at matched ages showed no behavioral deficits. Thus, preventing development of stable song behavior by blocking access to sex steroid seems to extend the period during which the neural circuit that includes lMAN is essential for song learning.

223.14

CHEMICAL LESIONS OF A THALAMIC NUCLEUS DISRUPT SONG DEVELOPMENT IN MALE ZEBRA FINCHES. K.A. Halsema* & S.W. Bottjer. Dept. Biol., USC, Los Angeles, CA 90089.

Previous studies indicate that discrete portions of the neural song system in male zebra finches play a critical role in the development of song, but not in the production of the stable adult vocal pattern. Damage to either lMAN or Area X in juvenile males producing an unstable vocal pattern severely disrupts song development, whereas the same type of lesion in adults does not result in any change of the stereotyped song pattern. Area X projects trans-synaptically to lMAN through the thalamic nucleus DLM. In order to evaluate the contribution of DLM to the development of song behavior, we lesioned the dorsal thalamic region containing DLM using ibotenic acid. As in the case of lMAN lesions, damage to DLM disrupted song behavior only in those males producing an unstable vocal pattern at the time of lesion. Chemical ablations of DLM in young males between 50 to 60 days of age producing an unstable song resulted in an abnormal adult vocal pattern consisting of a highly repetitive sequence of 1 to 3 poorly modulated syllable types. The same lesion in 70 to 80 day old males did not interfere with further development of the relatively stable vocal pattern. Interestingly, in contrast to lesions of DLM's target (lMAN), damage to this thalamic nucleus produced no immediate disruption of the vocal pattern. Rather, abnormal song patterns develop gradually following the lesion. This difference in the immediacy of the disruption may reflect a modulatory role for DLM neurons in the motor development of song.

223.15

EXPRESSION OF AN IMMEDIATE EARLY GENE IN SONGBIRD BRAIN: ANATOMY, CONNECTIONS AND EFFECTIVE STIMULI. C. V. Mello, F. Nottebohm and D. F. Clayton*, Lab. of Animal Behavior, Rockefeller Univ., New York, NY 10021 & Dept. of Cell & Structural Biology, Univ. of Illinois, Urbana, Il 61801

ZENK is the canary homolog of an immediate early gene which is highly responsive in neurons to membrane depolarizing signals. We have recently shown that ZENK is rapidly induced in discrete regions of the forebrain of songbirds after presentation of tape-recorded birdsong (PNAS, in press). In particular, a high ZENK induction was observed in the caudo-medial neostriatum (NCM) and the medial hyperstriatum ventrale (HV), with a preference for conspecific over heterospecific song. We show here that ZENK induction in response to birdsong also occurs in more lateral areas of the forebrain, including: a) the neostriatum adjacent to and possibly including subregions of the auditory Field "L"; b) the neostriatum adjacent to HVC (shelf); c) the archistriatum adjacent to RA (cup); d) portions of the caudal paleostriatal complex; e) other forebrain areas whose connections are not yet known. We have established the time-course of this genomic response and are now further studying its specificity by testing the effects of various auditory stimuli, such as bird's own song played forwards and backwards, conspecific and heterospecific songs, and white noise. We are also using neuroanatomical tract-tracing methods to establish the connections of areas where a high ZENK induction occurs and their possible relation to auditory areas and to the song control circuit.

223.16

PROGRESSIVE DEVELOPMENT OF THE PROJECTION FROM HVC TO AREA X IN CANARIES. Alvarez-Buylla*,A., and Mateo, A.
Rockefeller University, New York, N.Y. 10021.

The high vocal center (HVC) in canaries plays an important role in the production of learned song. Two main efferent pathways originate from the HVC. Large HVC neurons that project to area X are born before hatching or soon thereafter. Small projection neurons that connect HVC to RA are born throughout juvenile development and adulthood. Here we studied the development of the projection interconnecting the HVC to area X. Male canaries of 1, 2, 4, 8, and 13 months were injected with the retrograde tracer Fluoro-Gold(FG) in area X. HVC volume, as revealed by FG, doubled between 2 and 4 months ($0.11\pm0.02mm^3$ to $0.24\pm0.02mm^3$). The density of HVC-area X cells was 287± 36, 307±54, and 276±61 cells /mm ^{2}± sd at 1,2 and 4 months and dropped to 161±46 by 8 months. DiI crystals placed into the HVC of canaries of different ages revealed that the size of area X and the density of innervation from HVC increases dramatically also between 2 and 4 months. The projection neurons interconnecting HVC and area X are part of a circuit important for song aquisition. These results indicate that between 2 and 4 months HVC becomes a major afferent to area X, perhaps inducing it to grow and differentiate. While these projection neurons are born early, their axons wait 2-4 months to form a mature projection to area X. The influence of HVC on the circuit required for song aquisition may become more important at later stages in juvenile development.

223.17

YOUNG MALE SONGBIRDS IMITATE THE MALE-TYPICAL VOCALIZATIONS OF SINGING MOTHERS. H.B. Simpson* and D.S. Vicario. The Rockefeller University, New York, NY 10021.

In zebra finches, only the males sing and young males learn their song and long call during a limited period in development by imitating adult male models. Females produce a variety of communication calls; these are acoustically simpler and are not learned. The present work investigated whether it is primarily the gender of the parent or the type of vocalizations produced that determines which parent will be imitated when a young bird is exposed to both a mother and father that sing.

Females with learned, male-like song and long calls were produced by early treatment with estradiol. Two such females were paired with singing males, one of which had a male-typical long call, while the other was chosen because he happened to produce a female-like long call. These pairs were placed in individual breeding cages and given successive cohorts of foster chicks to raise (each cohort consisted of 4 chicks <11d old). The young were removed at 60-80 days of age. Their vocalizations were recorded after 100 days of age. The two pairs raised 7 clutches containing a total of 12 males.

Songs and calls were displayed as sonograms and compared to both foster parents' vocalizations. Analysis showed that young males copied only one parent's song but were as likely to copy the song of their female (n=5) as of their male (n=7) foster parent. Males who copied their mother's song copied her male-like long call as well (5/5). In contrast, only 1 of 7 males that copied their father's song also copied the father's long call; he came from the pair where both parents produced male-like calls. The other males produced calls with unusual features (4/7) or copied the mother's call (2/7). These data suggest that young birds do not select models by their sex or plumage: male and female parents can be equally good tutors, if they produce male-typical vocalizations. (Supported by MH40900 & GM07739).

223.18

DEVELOPMENT OF VOCAL LEARNING IN THE BUDGERIGAR. E. F. Powell, R. J. Dooling*, S. M. Farabaugh. Department of Psychology, University of Maryland, College Park, MD 20742.

Vocalizations of ten budgerigars (*Melopsittacus undulatus*) were sampled from day of hatching to four weeks post-fledging. Food begging calls recorded in the nest box showed changes with age in peak frequency, frequency modulation (FM), and duration. Peak frequency decreased from hatching until asymptotic weight was reached (22 days). FM changed dramatically with age as food-begging calls progressed from nearly pure tones, to simple sinusoidal modulations, to repeated, complex, individually distinctive patterns of modulation. Call duration was invariant with age in birds from two small clutches but increased significantly in birds from a large clutch from 200 ms (at hatching) to average of 500 ms (at 20 days of age). Within a week of fledging, each bird produced a contact call consisting of a shortened version of its final food-begging call recorded in the nest box. These calls were of roughly the same duration across birds (188 ± 36 ms). By three weeks post-fledging, budgerigars were highly social, their contact call repertoire often contained more than one call type, and there was sharing and imitation among the calls of parents, fledglings, and other social companions.

The decrease in peak frequency with age is probably related to the lengthening of the vocal tract while the increase in FM may be related to the maturation of basic syringeal coordination. Changes in duration and FM of begging calls may also reflect differential parental reinforcement of nestling begging behavior. This interaction may mark the earliest stage of vocal learning where an association between the nestling's vocal behavior and a feeding response from its parents are formed and strengthened. These early associations may be precursors of more complicated associations affecting the production, perception, and learning of contact calls in social encounters throughout adulthood.

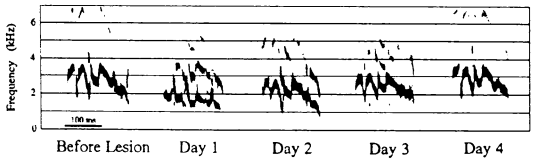
(supported by NIH - DC00198).

223.19

THE EFFECT OF SYRINGEAL DENERVATION ON CALL PRODUCTION IN THE BUDGERIGAR. James T. Heaton, Susan M. Farabaugh, and Steven E. Brauth*. Department of Psychology, Univ. of Maryland, College Park, MD 20742.

In the budgerigar, the left and right tracheosyringeal nerves were sectioned in order to provide insight into the role of the hypoglossal nuclei in the control of call production. Pre- and post-surgical distance or contact calls were examined for quantifiable changes in peak frequency, fundamental frequency, frequency modulation, and duration. After resecting a portion of either the right or left ts nerve above the anastomosis, both peak frequency and fundamental frequency of distance calls dropped markedly on day 1 post-surgery, while call structure itself remained largely unchanged. Peak and fundamental frequencies returned to normal by day 4 (see figure below).

Vocalizations produced after bilateral syringeal denervation were abnormal, consisting entirely of poorly modulated broadband harmonic series. Significantly, individual call durations as well as the rhythm and patterning of vocalizations resembling warble song after both unilateral and bilateral ts nerve resection were remarkably similar to pre-surgical values, thus suggesting that the control of spectral and temporal aspects of budgerigar distance calls are associated with different neural circuits.

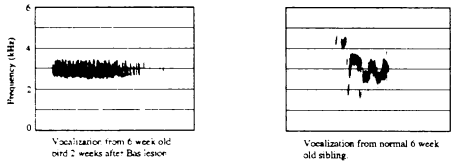


223.20

EFFECT OF FOREBRAIN AUDITORY LESIONS ON CALL DEVELOPMENT IN BUDGERIGARS. William S. Hall*, James T. Heaton, Paul L. Cohen & Steven E. Brauth, Dept. of Psychology, University of Maryland, College Park, MD 20742-4411.

Lesions were placed in forebrain auditory structures including nucleus basalis (Bas), nucleus ovoidalis (Ov) and Field "L" in budgerigars at 3-5 weeks posthatching and as adults. The calls of birds sustaining Bas lesions before fledging or as adults were markedly abnormal in that they showed little frequency modulation and individual distinctiveness. Call durations, however, were similar for lesioned and unlesioned birds (see figure below).

In contrast to the effects of Bas lesions, Ov-lesioned birds produced calls in which temporal as well as spectral features were highly abnormal. Calls of Ov birds were often very long (e.g. 0.5 - 1 sec.), showed little frequency modulation and did not possess any of the distinctive spectral features exhibited by cagemates. Significantly, the calls of Field "L"-lesioned birds were similar to those of siblings and cagemates, although call durations were more variable. This implies that other thalamic auditory projections, either to subpallial fields or to areas adjacent to Field "L", are important for vocal learning.



Supported by NIMH Grant MH40698 (SEB) and a Whitehall Foundation Award (WSH) (J91-17).

INVERTEBRATE LEARNING AND BEHAVIOR II

224.1

ENVIRONMENTAL STIMULI AFFECT MEMORY AFTER LEARNING THAT FOOD IS INEDIBLE IN *APLYSIA FASCIATA*. M. Schwarz* & A.J. Susswein. Dept of Life Sciences, Bar Ilan University, Ramat Gan, Israel 52 900

We have examined whether exposure to a novel stimulus affects memory after learning in *Aplysia fasciata*. Previous work has shown that presence of a conspecific in the medium during training affects the ability to learn that a specific food is inedible. In absence of a conspecific, learning is impaired. We now demonstrate that brief exposure to a medium lacking a conspecific immediately after training erases memory of the learning, even when animals are returned to an environment having a conspecific. Controls that are handled or transferred to a new medium containing a conspecific show normal memory. Exposure as brief as 1 hour to an environment lacking a conspecific is sufficient to abolish memory. After memory is abolished, animals are fully able to re-learn and remember that a food is inedible, but behave like naive animals when re-trained. Thus, the brief experience does not impair the ability to learn, only the memory of a previous learning. We also examined whether lack of a conspecific interferes with memory even when animals receive this experience 24 hrs after the training. This experience affected memory, but not as severely as immediate exposure. To examine whether the effect on memory is caused by exposure to *any* behaviorally-significant new environment, or is specific to exposure to an environment lacking conspecifics, animals were transferred immediately after training to a medium of 120% seawater, or were exposed to an alternate food. These experiences did not affect memory. The effect of a conspecific on memory was also examined in sexually immature *Aplysia*, which presumably are insensitive to pheromones released by conspecifics. Lack of a conspecific did not abolish memory. These data indicate that memory in *Aplysia* is affected by posttraining treatments, as it is in vertebrates.

224.2

PLASTICITY IN A SENSORY-MOTOR PATHWAY OF THE LOBSTER STOMATOGASTRIC NERVOUS SYSTEM. R. Nargeot, M. Moulins and F. Nagy* Lab. Neurobiol. Physiol. Comp. Université de Bordeaux I. CNRS. Arcachon 33120. France.

A fundamental question in neurobiology is understanding how activity of the central nervous system can be durably modified by sensory information. To address this problem we study a well-known "simple" model, the crustacean stomatogastric nervous system.

Recent work on this system in Palinurus has shown that a sensory input pathway conveyed by the ventral postero-lateral nerve (vpln) is able to induce a short-term inactivation (loss of burst-generating oscillatory behaviour) of a motoneuron (VD) belonging to the pyloric network. We find that with repetitive stimulation of the vpln *in vitro*, the duration of VD inactivation remains constant. However the efficacy of this sensory pathway can be durably facilitated by prior conditioning stimulation of the contralateral-vpln.

Several cellular elements concerned in this phenomenon have now been identified. We have a situation in which the cellular mechanisms implicated in the induction and maintenance of change in efficacy of the sensory-motor pathway can be studied.

224.3

CONTRASTING EFFECTS OF KNOWN NEUROTRANSMITTERS ON VISUAL-VESTIBULAR RESPONSES IN *HERMISSENDA*

R.F. Rogers(*) [1], D.M. Fass[1], S.M. Specht[2], and L.D. Matzel[1]
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In the marine mollusk *Hermissenda*, stimulation of vestibular hair cells induces a hyperpolarization in ipsilateral B photoreceptors which is mediated by an outward K^+ conductance that reverses at a membrane potential between -70 to -80mv. We attempted to further characterize this interaction using pressure microapplication of putative neurotransmitters known to exist within this nervous system. GABA, Acetylcholine (ACH), Serotonin (5-HT), Histamine (HIST), and Dopamine (DA) (25μM) were pressure applied to both the terminal branches and the cell bodies of the B photoreceptor. The observed effects were as follows: (1) Both GABA and ACH (but not 5-HT, HIST, or DA) induced a hyperpolarization of the B photoreceptor when applied to the terminal branches. GABA is believed to mediate the visual-vestibular interaction while ACH mediates the interaction between photoreceptors. The GABA response was gradual in onset and prolonged (10-20 sec), while ACH resulted in a rapid hyperpolarization of short duration (1-2 sec). (2) Hair cell-induced hyperpolarization of the B cell was attenuated by 60-90% when stimulation of the hair cells was preceded by GABA or ACH pressure microapplication to the terminal branches, indicating activation of a common conductance. (3) Pressure application of 5-HT (but not GABA or ACH) onto the B cell bodies led to a brief and rapid depolarization (5-10mv) which was accompanied by a transient increase in spike height (1-4mv). This result suggests a possible modulation of the visual-vestibular interaction by 5-HT. (4) Finally, we investigated the possibility that these synaptic events were G-protein mediated. Iontophoretic injection of GDP-βS completely blocked the visual-vestibular interaction following 20-30 min of injection.

224.4

CELLULAR ANALYSIS OF LONG TERM HABITUATION OF TAIL-INDUCED SIPHON WITHDRAWAL IN *APLYSIA CALIFORNICA*. **M. Stopfer*, Y. Tal and T.J. Carew. Departments of Psychology and Biology, Yale University, New Haven, CT.**

Long term memory for habituation in the tail-induced siphon withdrawal reflex (T-SWR) in *Aplysia* can be confined to one side of the body (Stopfer et al, 1991). This lateralization of memory is likely due to a bilaterally symmetric sensory system which mediates the T-SWR. Here we report that long term memory for habituation can also be lateralized in a reduced preparation that is amenable to a cellular analysis, and we provide a preliminary description of some of the cellular components contributing to the T-SWR.

Preparations consisting of the tail and mantle organs connected to the CNS (n=13) first received baseline stimuli delivered via implanted electrodes in the tail, and the duration of T-SWR was measured. Next, habituation training was delivered to one side of the tail: 4 blocks (90 min apart) of 30 stimuli each (ISI=30 sec) were given. Bilateral tests were conducted (blind) 24 hr after training. Trained-side test scores were significantly decremented relative to their own pre-scores ($p<0.01$), whereas control-side scores were not. Thus, this preparation exhibits lateralized long term habituation like intact animals.

As a first step in a cellular analysis we have characterized the responses of tail sensory neurons (SNs) in the pleural ganglia to the identical stimulus employed in behavioral studies. SNs respond to the tail stimulus with an action potential barrage equal in duration to the tail stimulus (100msec). When tail stimuli were delivered at an ISI that habituates the T-SWR, there was no significant change in action potential number or latency. There was, however, a gradual increase in spike width. Motor neurons that receive monosynaptic EPSPs from SNs often fired to stimuli too weak to elicit responses in the SNs and often received input 50-75msec before the SNs, indicating the presence of an additional, lower-threshold, sensory pathway. We are currently examining long term changes in the tail SNs and their synaptic output, as well as the low-threshold pathway, following lateralized long term habituation.

224.5

LEARNED MODIFICATION OF HEAD WAVING BEHAVIOR IN *APLYSIA* WITH AVERSIVE REINFORCEMENT. K. Fitzgerald*, C.A. Takacs and T.J. Carew. Depts. of Psychology and Biology, Yale University, New Haven, CT 06520.

Head waving in *Aplysia* is a spontaneously occurring exploratory behavior consisting in part of alternating lateral flexions of the neck. Animals can learn to modify this behavior in response to stimuli such as light or food. We report here that *Aplysia* can also learn to modify the lateral component of head waving following presentation of local electric shock to one anterior tentacle (AT; the ATs are symmetric structures that project bilaterally from the head).

Animals were suspended in a seawater tank and their baseline head waving behavior was monitored for 10 min. Next, during a 10 min training phase, a series of shocks was delivered to one of the ATs. In a subsequent 10 min test period, animals spent significantly less time flexing toward the side on which the AT had received shock (mean change 85±22 sec, $p<.002$, $n=20$).

We have begun to investigate the neural mechanism of this modulation by examining the effects of AT shock on identified central neurons. Cerebral ganglion mechanoafferent sensory neurons (SNs), which innervate the ATs (Rosen et al., 1979), exhibited significant spike broadening in response to AT shock ($p<.04$, $n=5$). Furthermore, the effect of AT shock was side-specific: SNs ipsilateral to site of shock exhibited significantly greater broadening than contralateral SNs (30 s after shock, mean difference 31±2.2%, $p<.0001$, $n=5$). Since these SNs are likely to participate in the head withdrawal reflex, the modulation that we observe suggests that AT shock could, in addition to its effects on head waving behavior, produce side-specific sensitization of head withdrawal.

We are currently assessing the modulatory effects of AT shock on neural circuitry involved in head waving. For example, AT shock appears to inhibit pedal ganglion MNs that innervate the neck region. In addition, we are exploring the use of AT shock as an aversive reinforcer for operant conditioning of head waving behavior.

224.6

5-HYDROXYTRYPTOPHAN ELICITS SUSTAINED CPG ACTIVITY FOR RHYTHMIC SHELL MOVEMENTS IN LYMNAEA STAGNALIS. E.A. Kabotyanski, W. Winlow, D.A. Sakharov, L. Bauce and Ken Lukowiak*. Neuroscience Research Group, University of Calgary, AB Canada T2N 4N1.

Knowledge regarding the intrinsic and network properties of neurons that form central pattern generators (CPG's) are critical to our understanding of the neural basis of rhythm generation. However, in many in vitro reduced preparations CPG's are strongly inhibited and thus unuseful. Here we report a method to obtain ongoing CPG activity in Lymnaea. The injection of 5 hydroxytryptophan (5HTP), 100 μg/g after a 20-30 min delay induces stereotyped, rhythmic lengthwise shell movements. The effect persists for approximately 2h, and includes an increase of ciliary locomotion velocity as well as the suppression of defensive reflexes. Rhythms possessing similar characteristics were obtained in semi-isolated and isolated CNS preparations. Following 5HTP injection there is a dramatic increase both in serotonin levels (HPLC assay) of the CNS and in firing of pedal 5HTergic cells, that correlate well with the behavioural effects. The CPG responsible for rhythmic shell movements in Lymnaea appears to be 5HT dependent and the application of 5HTP is a useful tool to enable us to investigate further how this rhythm is generated. Supported by ESF and MRC (Canada).

224.7

DISTRIBUTION OF APGWamide-LIKE AND FMRFamide-LIKE IMMUNOREACTIVE NEURONS INNERVATING THE PENIS AND THE DART SAC IN MESOCEREBRUM OF THE SNAIL *HELIX ASPERSA*. G. Li and R. Chase*. Dept. Biology, McGill Univ., Montreal, Quebec, H3A 1B1, Canada.

The penis and the dart sac are innervated by the nervus penis (NP) and the nervus cutaneus pedalis primus dexter (NCPD), respectively. Electrophysiological evidence suggests that right mesocerebrum neurons are capable of commanding penis eversion and dart release. It is also known, from immunohistochemistry, that two major peptides in the mesocerebrum are APGWamide and FMRFamide. The APGWamide-like immunoreactive substance was observed in the penis but not in the dart sac (Griffond et al.). We therefore hypothesized that mesocerebrum cells projecting to the penis contain APGWamide, while cells projecting to the dart sac contain FMRFamide.

A double-label study was conducted. The NP or the NCPD was backfilled with Neurobiotin, later conjugated with Rhodamine-Avidin. Tissue sections were immunoreacted with antisera for APGWamide or FMRFamide, later conjugated with FITC. Primary results indicate that the mesocerebrum neurons backfilled from NP may contain either APGWamide-like peptide or FMRFamide-like peptide, and neurons backfilled from the NCPD may likewise contain either FRMRamide-like peptide or APGWamide-like peptide. A quantitative analysis using confocal laser scanning microscopy is in progress.

224.8

RECOVERY OF TAIL MECHANOSENSORY FUNCTION IN *APLYSIA* FOLLOWING CRUSH OF NERVE P9. I. Steffensen and C.E. Morris*, Loeb Institute (and Biology Dept.) University of Ottawa, OCH, Ottawa, Ontario, K1Y 4E9.

The tail withdrawal reflex in *Aplysia* is mediated by mechanosensory afferents carried in nerve P9. Using a combination of scanning electron microscopy and immunofluorescence against class III ß-tubulin to follow the tail afferents, we concluded that a certain class of epidermal structures - tufts of cilia surrounded by microvilli - probably constitute the sensory terminals. If this interpretation is correct, crushing the nerve carrying the afferents should both abolish the tail withdrawal reflex and destroy the epidermal endings. Moreover, recovery of the reflex should coincide with regeneration of the epidermal endings. We are video-monitoring the post-crush recovery of the tail withdrawal reflex and following the status of the epidermal endings with the above morphological techniques. The two P9 nerves are crushed about 1.5 cm from the pedal ganglia through a single ~1 cm incision which is reclosed with a cyanoacrylate adhesive. Both locomotory functions (P9 also carries tail motor efferents) and the withdrawal response are abolished by this procedure; recovery is evident within 2-3 days. Supported by the NCE for Neural Regeneration, Canada.

224.9

FUNCTIONAL UNCOUPLING OF CHOLINERGIC INHIBITORY INTERNEURONS PLAYS AN IMPORTANT ROLE IN SHORT-TERM SENSITIZATION OF *APLYSIA* GILL AND SIPHON WITHDRAWAL REFLEX (GSWR). L.-E. Trudeau* and V.F. Castellucci. Lab. de Neurobiologie et Comportement. Inst. Rech. Clin. de Montréal, Montréal, (Québec), Canada. H2W 1R7.

Polysynaptic transmission between sensory and motor neurons of the neuronal network mediating the GSWR is important for the expression and plasticity of this simple behavior. We have investigated the importance of cholinergic inhibitory interneurons in this network. Central application of the nicotinic blocker d-tubocurarine (d-TC) (100 μM) potentiates evoked gill contractions (422% increase, n = 5) and siphon nerve-evoked compound EPSPs recorded in motoneurons (189% increase, n = 10). Excitatory interneurons receive excitation followed by powerful inhibitory input upon siphon nerve stimulation. Application of d-TC blocks this rapid inhibition and prolongs the excitation (205% increase in area, n = 5). Heterosynaptic facilitation of compound EPSCs in motoneurons is reduced in preparations already disinhibited by pre-treatment with d-TC. Facilitation of sensory-motor synapses is however unaffected. These data indicate that (1) transmission through the GSW neuronal network is gated by a feedback inhibitory mechanism, and (2) a reduction of cholinergic inhibition onto excitatory interneurons may be a mechanism by which transmission within the GSW network is increased during various forms of learning such as sensitization. They also place new emphasis on the important role of inhibitory interneurons in determining the plastic properties of neuronal networks.

224.10

INTERNEURONAL PLASTICITY CONTRIBUTES SIGNIFICANTLY TO ENHANCED MOTOR NEURON FIRING DURING SENSITIZATION OF THE *APLYSIA* SIPHON-WITHDRAWAL REFLEX. J.R. Lieb Jr.* and W.N. Frost. Dept of Neurobiology & Anatomy, Univ of Texas Medical School, Houston Tx 77225.

Physiological studies have shown that four different circuit modifications occur during sensitization of the *Aplysia* siphon-withdrawal reflex. How much each site contributes to the sensitized response is currently unclear because individual circuit modifications cannot be readily introduced or removed in physiological experiments. In an effort to explore the importance of these sites of plasticity in sensitization, we have constructed a realistic computer simulation of the siphon-withdrawal circuit, consisting of the LE sensory neurons, the L29, L30, and L34 interneurons, and the LFS motor neurons.

In this study, our goal was to evaluate the relative contribution of sensory neuron versus interneuron sites of plasticity. In sensitization, the sensory neuron synapses undergo presynaptic facilitation. Doubling these connections in the simulation produced an enhancement of both the phasic and long-lasting components of the LFS motor neuron firing response to a simulated siphon stimulus. The increase in long-lasting LFS firing was due to increased interneuronal recruitment by the sensory neurons. Sensitization also involves a decrease in the L30-L29 and L30-L34 inhibitory synapses, and an increase in the L29-LFS excitatory synapse. Adding these interneuronal modifications to the simulation resulted in a further enhancement of both the phasic and long-lasting components of the LFS firing response. These results support the idea that the enhancement of siphon withdrawal seen in sensitization is due to the combined action of a distributed set of circuit modifications. While the interneuronal changes reinforce the sensory neuron facilitation, they may also have an additional, specialized role in biasing the sensitized response toward movements produced by LFS neurons, rather than other types of motor neurons.

224.11

INTRACELLULAR AND PATCH-CLAMP RECORDING IN THE NEMATODE *C. ELEGANS*. S. R. Lockery*, C. M. Loer and T. J. Sejnowski. Computational Neurobiology Lab., Salk Institute, La Jolla, CA 92186.

Although a complete anatomical circuit is available for the nematode *C. elegans*, further progress in understanding the neuronal basis of behavior has been hampered by the inability to make electrophysiological recordings. Using quartz sharp electrodes and conventional patch pipettes, we explored the feasibility of recording from identified muscles and neurons in *C. elegans*. Worms were affixed to coverslips, maintained in Ascaris saline, and observed using an inverted microscope with Nomarski optics. Quartz electrodes driven through the cuticle lost 50% of their initial resistance but remained sharp. Dye fills (Lucifer Yellow) of a number of cells were obtained including an amphid sheath cell, individual body muscles, and pharyngeal muscle cells. In some preparations, pharyngeal muscles were dye coupled, while in others they were not. Pharyngeal contractions could sometimes be produced by passing depolarizing current. To facilitate patch recording, cells were exposed by making a slit in the cuticle over the nerve ring. Cells were judged to be neurons on the basis of size, appearance of the nucleus, and presence of one or two processes. As previously shown by D. Raizen and L. Avery (personal communication), gigaohm seals (5-20 G) could readily be formed and single channel currents observed. Seals were stable and lasted over an hour. Thus, it is likely that sharp electrodes can be used to record from muscles and whole-cell recordings can be used to study the intrinsic properties of neurons in *C. elegans*.

Supported by The Howard Hughes Medical Institute.

224.12

ISOLATION OF LEARNING SPECIFIC MUTANTS IN *CAENORHABDITIS ELEGANS*. N. Kumar*, J.Y.M. Wen, G.Morrison, J. Parker and D. van der Kooy, Neurobiology Research Group, Dept. of Anatomy, University of Toronto, Toronto, Ontario, M5S 1A8.

The nematode *Caenorhabditis elegans* is an ideal model system in the search for the molecular and cellular basis for associative learning and memory because of its well characterized genetics, neuroanatomy and development. Using *C. elegans* we developed a classical conditioning paradigm in order to search for learning impaired mutant lines. The assay utilizes the chemotaxic responses to the conditioning stimuli (CS) Na+ (Na CH3COO) and Cl- (NH4Cl), and to *E. coli* (a food source) as an unconditioned stimulus (US). The animals were conditioned by presenting one of the CS ions (CS+) paired with the US followed by an exposure to the other CS ion (CS-) in the absence of the US. Testing consisted of placing approximately 100 of the conditioned animals between point gradients of each CS (in the absence of the US) and allowing them to migrate towards one of the gradient centers. CS levels on testing were balanced such that naive animals displayed equal preferences for either CS ion. Conditioned animals showed a significant preference for the CS+ ion that previously predicted the presence of food.

Wild type animals were mutagenized using the chemical mutagen EMS and then F2 animals were segregated into clonal lines (*C. elegans* is a hermaphrodite). These lines were then assayed for classical conditioning using the procedure described above. To date we have isolated 2 mutant lines of animals that, following conditioning, display only the naive preferences for the CS+ and CS- ions. However, these mutant animals seem to possess normal sensory and motor functions. When the unconditioned preferences for either of the CS ions or for the US are tested in accumulation assays, mutant animals display normal preferences compared to wild type controls. Thus, the 2 lines of mutant worms may be specifically impaired in their ability to make associations between the CS+ and the US.

224.13

LASER ABLATION OF CIRUIT ELEMENTS I N *C. ELEGANS*. S.R. Wicks* and C.H. Rankin. Department of Psychology, University of British Columbia, Vancouver, B.C., Canada. V6T 1Z4.

The nematode *C. elegans* exhibits a broad behavioral repertoire despite possession of rather limited nervous system. The anatomical connectivity of all 302 neurons in the nematode nervous system has been described at the synaptic level by serial section electron microscopy (White et al, 1986). Furthermore, a specific subcircuit of 85 neurons--the touch withdrawal circuit--has been shown to underlie the worm's response to touch (Chalfie et al, 1985). In response to a vibration, the worm will react by either accelerating or reversing. This response has been termed the tap withdrawal reflex and is potentially a more sensitive measure of sensorimotor integration than response to touch. The neural bases of this behavior were investigated by circuit analysis.

In these studies we attempted to define the functional role of each of the neurons in the circuit by systematically laser ablating these identified cells. The response of these worms to tap then was assessed. To determine the function of a cell, in some worms the cell of interest was killed and the effect of its loss on the behavior was assessed; in other worms the cell of interest was left intact but cells in all alternative pathways were killed. An example of our results is that, in the intact circuit, the head-touch cells (ALMs) are required for the reversal response to tap. In the absence of the head-touch cells, the tail-touch cells mediate an acceleration response to tap.

224.14

HABITUATION OF TWO REFLEXES MEDIATED BY A SINGLE CIRCUIT IN *C. ELEGANS* . C.H. Rankin*, B.D.G. Hartnett and S.R. Wicks. Department of Psychology, University of British Columbia, Vancouver, B.C., Canada. V6T 1Z4.

An important question in the analysis of the neural substrates of habituation is whether response decrement is due to habituation of a single cell or at several cells within an identified circuit. *C. elegans* offers both a clearly defined behavior and an experimentally tractable nervous system to investigate this question. In this worm, the tap withdrawal circuit consists of two interconnected subcircuits, head-touch induced backward movement and tail-touch induced forward movement. Although both subcircuits are activated by tap, backward movement is the predominant response of wild-type worms. Using laser ablation techniques and mutants lacking specific cells in the circuit we manipulated input to the tap withdrawal circuit by removing either the head-touch or the tail-touch receptors. We then compared the dynamics of the habituation of accelerations produced in the absence of head-touch receptor input with those of the reversals produced in the absence of tail-touch receptor input. A comparison of these curves with the habituation dynamics of control animals allowed an assessment of the role of each of the subcircuits in habituation in the intact animal. We found that worms lacking functional tail-touch receptors habituate more slowly and to a lesser degree than control worms. Our results suggest that habituation of the tap withdrawal response in *C. elegans* occurs in at least two cells.

224.15

THE RELATIONSHIP BETWEEN DISHABITUATION AND HABITUATION IN *C. ELEGANS* . C.D.O. Beck*, B.S. Broster and C.H. Rankin. Department of Psychology, University of British Columbia, Vancouver, B.C., Canada. V6T 1Z4.

Dishabituation is the facilitation of a previously habituated response. It is not clear whether the mechanism underlying dishabituation reverses the process underlying habituation or whether dishabituation is a separate facilitatory process superimposed on habituation. To investigate the relationship between habituation and dishabituation we compared dishabituation to another process that alleviates habituation, ie. spontaneous recovery.

In one set of experiments we habituated the tap withdrawal response in *C. elegans* at either a 10 s or 60 s interstimulus interval and dishabituated half of the animals with a 60 V shock. When we compared the recovery following dishabituation to normal recovery we found that at a 10 s ISI dishabituation produced a transient increase in recovery, whereas at a 60 s ISI dishabituation resulted in a consistent and longer lasting advantage. In another set of experiments, again using a 10 and 60 s ISI we examined differences in rehabituation between animals dishabituated, or allowed to recover. The dishabituation group rehabituated slightly faster with a 10 s ISI, but was no different from the recovery group with a 60 s ISI. The extent to which dishabituation is similar to recovery seems to vary with ISI, which may be a reflection of ISI dependent differences in the interplay of mechanisms underlying habituation and dishabituation.

224.16

AN EXPLORATION OF LONG-TERM MEMORY IN *C. ELEGANS*. T. M. Marion*, C. D. O. Beck, and C. H. Rankin. Dept. of Psychology, Univ. of British Columbia, Vancouver, BC V6T 1Z4.

The nematode *Caenorhabditis elegans* may be used as a simple systems model of the dynamics of memory. *C. elegans* is capable of long-term retention (over 24 hrs) of habituation training with vibrational stimuli at a 10 s interstimulus interval (ISI) (Rankin et al., 1990). We investigated the dynamics of long-term habituation by varying ISI, number of stimuli and training schedule. The stimulus used was a train of six taps (8.5 Hz, delivered to the plate holding the worm) shown in previous experiments to evoke the reversal response (swimming backward for a short distance) in experimentally naive animals. Worms received habituation training (60 s ISI): a) 40 stimuli (Massed training), b) four 10 stimuli blocks separated by 60 min. (Distributed training), c) 10 stimuli, and d) a single stimulus. All worms received 10 stimuli on day 2 (at least 24 hrs later) as a test of retention of habituation training. A preliminary analysis of the data suggests that both massed and distributed training with 40 stimuli produced long-term retention, whereas training with 10 stimuli or one stimulus did not. Further research will explore the effects of retention interval, interstimulus interval, and context on long-term memory.

225.1

PRESENTATION OF THE STIMULI PREVIOUSLY ASSOCIATED WITH SOCIAL STRESS, INCREASE THE EXPRESSION OF THE CFOS ONCOGENE. C.A.Cohen*, T.S. Zaccheo, K.A. Miczek, and R.M. Kream. Department of Psychology, Tufts University, Research Building, 490 Boston Ave., Medford, MA 02155, Department of Anesthesiology, Tufts University School of Medicine, 136 Harrison Ave., Boston MA 02111 and the Department of Electrical Engineering, Tufts University, Halligan Hall, Medford, MA 02155.

Previously, we have reported that exposure to social stress increases cFos gene expression peaked at one hour after the stress and the density of met-enkephalin stained fibers peaked at three hours after stress in the periaqueductal gray region of the brainstem. In the current experiment, the time course was extended to 24 hours post- stress and in addition we were interested in assessing the effect of re-exposure of the intruder to the salient stimuli associated with the original encounter, but physical contact with the resident did not occur. An intruder male rat was placed into the home cage of a resident and subsequently exposed to attack and threat. Once the animal showed unambiguous signs of submission, the intruder was placed into a protective cage for one hour while being exposed to the threat of an attack. No physical harm could come to the intruder at this time. The intruders were perfused with saline followed by 4% paraformaldehyde, at various time intervals after the initial social stress experience or re-exposure to the salient stimuli associated with the stressful encounter. The brains were removed and placed in fixative for two hours followed by a solution of 20% sucrose and 0.1M phosphate buffer where they remained until sectioning. The control animals were handled in the same manner as mentioned above. All brains were sectioned (32 μm) and prepared for immunohistochemistry using antibodies for Fos and met-enkephalin. Preliminary data suggest that re-exposure to the relevant stimuli associated with the social stress experience is sufficient to increase Fos expression in the brainstem 24 hours after the encounter. These changes are being monitored via radioimmunoassay and molecular biological techniques.

225.2

INDUCTION OF C-FOS mRNA IN THE BRAIN AND ANTERIOR PITUITARY GLAND OF THE RAT FOLLOWING INTRODUCTION TO A NOVEL ENVIRONMENT. R.J.Handa* and K.R.Nunley Dept. of Cell Biol., Neurobiol., and Anat. Loyola Univ., Stritch School of Medicine, Maywood, IL

To determine some of the rapid genomic changes which can occur following novelty stress, we examined the induction of cFOS mRNA in the brain and anterior pituitary gland following the introduction to a novel open field (OF) environment. Adult male Fischer 344 rats (Harlan Inc., Indianapolis IN) were placed into the OF (48"x48") for 20 minutes. Rats were sacrificed following removal from the OF, or were returned to their home cage and sacrificed 30, 45, or 60 min. later. Control rats were sacrificed immediately upon initial removal from their home cage. Brains were frozen in isopentane for subsequent analysis by In Situ Hybridization (ISH) histochemistry, or dissected and homogenized in GITC to obtain total RNA for analysis by Northern blot hybridization. C-FOS mRNA was examined using a radiolabelled oligonucleotide probe complementary to nucleotides 138-185 of rat c-FOS mRNA. Northern blot hybridization of total RNA showed hybridization to a single 2.3 Kb species of mRNA. C-FOS mRNA was low or absent in all brain areas examined from home cage animals. Following the introduction to the open field, large increases in cFOS mRNA were detected in the medial frontal cortex, piriform cortex and hippocampus with greatest levels achieved 30 min after removal from the open field. In the anterior pituitary gland, cFOS was highest after 20min in the open field and declined rapidly. The timecourse c-FOS induction in the ant. pituitary correlates well with the ACTH secretory response to novelty stress. Additional areas of cFOS induction, as demonstrated by ISH were the paraventricular n., septohypothalamic n., anteroventral preoptic n. and layers 2,3 and 5 of the parietal and cingulate cortex. These data demonstrate some of the brain areas which are undergoing rapid genomic activation following behavioral paradigms such as the introduction to the open field environment. NSF BNS9109226

225.3

SELECTIVE INDUCTION OF FOS RELATED ANTIGENS FOLLOWING THE INITIATION OF SOUND STRESS R.P. Dilts* and M.C. Boadle-Biber, Dept. of Physiology, Medical Coll. of Virginia/VCU, Richmond, VA 23298-0551

The immediate early gene products FOS and FOS related antigens (FRA) have been proposed as an index of cellular activity within the CNS (Sagar and Sharp, *Science*; 1988). In an attempt to delineate the neuronal circuitry involved with the activation of serotoninergic neurons within the dorsal raphe following sound stress, FRA immunoreactivity (FRA-IR) was localized. At 0.5, 1 and 2 hours following the initiation of sound stress, rats were anesthetized with equithesin and perfused with 4% buffered paraformaldehyde. Rats placed in sound chambers but not exposed to sound stress served as controls. Frozen sections were processed to detect FRA-IR nuclei using DAB/imidazole reaction product with strepavidin labelled peroxidase (Jackson Immuno Research, West Grove, PA). Five antibodies which recognize different epitopes within FOS proteins have been utilized, each yielding a different distribution of FRA-IR. FRA antisera (1:5,000) directed towards the conserved M2 sequence (amino acids 127-152) in FOS, a gift from M. Iadorola, produced the greatest number of labelled nuclei. Regions in which sound stress produced more FRA-IR nuclei than controls included: the region of the B7 serotonin neurons within the dorsal raphe and surrounding central gray, the central and basolateral nuclei of the amygdala, the lateral habenula and the ventral subiculum as well as the entorhinal cortex within the hippocampal complex. Numerous neurons were found within the hypothalamus which did not appear to be restricted to cytoarchitectural boundaries. Regions with FRA-IR nuclei within the hypothalamus included the lateral hypothalamus, zona incerta, the anterior paraventricular area (magnocellular and parvocellular), arcuate nucleus and medial preoptic area. This data suggest that FRA are differentially expressed within selected nuclei following exposure to sound stress and provide a useful index to identify neuronal populations involved with the activation of the ascending serotoninergic system. NIH Grant NS14090 to M.C.B².

225.4

STRESS INDUCED c-FOS PROTEIN IN THE BRAIN: TEMPORAL-SPATIAL PATTERNING. L. Wetmore*, W. Wan and D.M. Nance, Departments of Physiology and Pathology, Univ. of Manitoba, Winnipeg, MB., R3E 0W3, Canada.

The c-fos oncogene protein was used as an indicator of neuronal activation following exposure of adult male S/D rats to intermittent footshock. Animals were given scrambled footshock (1.6mA) on a variable interval schedule (ITI=3.5min) for 60 min. Each 5 sec shock was preceeded by a 15 sec warning tone which terminated with shock onset. Rats were sacrificed and the brains removed at 0, 2, 6, 12 and 24 hours post shock. Alternate sections were processed for c-fos protein immuno-reactivity (Oncogene Sci) using the PAP technique. The expression was found to be both temporally dependent and regionally specific. Relative to controls, elevated levels of c-fos protein were detected at 0 (maximal) and 2 hours (moderate) post shock, but declined and remained at basal levels at the 6, 12 and 24 hr intervals. In all animals, significantly elevated c-fos expression was detected in the intermediate and ventral divisions of the lateral septal area(LSA), bed nucleus of the stria terminalis, preoptic area(POA), paraventricular nucleus of the hypothalamus, amygdala, locus coeruleus, A1 and A2 cell groups of the brainstem. Other hypothalamic and limbic regions exhibited increased c-fos expression of a more variable nature. Additional experiments were performed to determine the duration of footshock exposure required to induce these changes in c-fos expression. Following 15 minutes of shock, c-fos protein was observed only in the LSA and the POA. After 30 minutes of shock, increases in c-fos protein was detected in all brain regions examined except for the A1 and A2 cell groups. The data indicate that c-fos expression is transient and occurs in distinct neuronal regions which actually constitute a highly interconnected neuronal circuitry involved in an animals adaptive response to a stressor. Given the limbic system involvement in this response and its previous association with psychological phenomenon, future studies will determine the conditionability of stress induced c-fos expression. Additionally, the neurochemical specificities of neurons induced to express c-fos by footshock will be determined. Supported by MRC of Canada.

225.5

EVIDENCE FOR THE ACTIVATION OF ASCENDING SEROTONINERGIC SYSTEMS DURING SOUND STRESS IN RATS M.C. Boadle-Biber*, T. Phan and R.P. Dilts Medical Coll. of Virginia/VCU, Richmond, VA 23298-0551

Tryptophan Hydroxylase (TrpH) is the rate-limiting enzyme in serotonin (5-HT) biosynthesis, requiring tryptophan, free oxygen and tetrahydrobiopterin co-factor to produce 5-hydroxytryptophan (5-HTP). Sound stress increases 5-HT turnover as measured by the ratio of the metabolite 5-HIAA to 5-HT as well as TrpH activity measured *ex vivo*. The purpose of these studies was to determine whether sound stress enhances 5-HTP accumulation *in vivo*. Rats (200-300g) were weighed, given an injection (I.P.) of saline, gepirone (10mg/kg) or tryptophan (100mg/kg) and placed in a sound stress chamber in groups of 4 animals. After 1 hour of sound stress, rats were given the decarboxylase inhibitor NSD-1015 (m-hydroxybenzylhydrazine, 200mg/kg I.P.) and sacrificed 30 minutes later. Brains were dissected and the midbrain, hippocampus, caudate and cortex frozen on dry ice. 5-HTP, as well as tryptophan, 5-HT and 5-HIAA were measured using HPLC-EC. Results were analyzed using ANOVA ($p < 0.01$) and means subsequently compared using Newman-Keul's test. Sound stress significantly increased 5-HTP in the midbrains and hippocampus ($F_{3,12} = 13.04$ & 11.59: 703.4 ± 29.4 & 261.4 ± 26.9 ng/g. wet wt. ± S.E.M., respectively) compared with non-stressed controls (423.5 ± 84.1 & 157.1 ± 12.9). Gepirone, a 5-HT1a agonist , blocked the increase in 5-HTP and had no effect on non-stressed controls (401.2 ± 43.5 & 132.4 ± 10.6). Preliminary results indicate that tryptophan loading 1-hour before the administration of NSD-1015 increases 5-HTP, 5-HT and 5-HIAA. However, sound stress with tryptophan loading further increases 5-HTP accumulation. These results support the hypothesis that sound stress increases TrpH activity *in vivo* and provides further support for the activation of serotoninergic neurons during sound stress. NIH Grant NS14090 to M.C.B².

225.6

NMDA LESION OF AMYGDALA BLOCKS PSYCHOLOGICAL STRESSSR- INDUCED ACTIVATION OF PREFRONTAL DOPAMINE AND SEROTONIN SYSTEMS: A BEHAVIORAL, NEUROHUMORAL, AND NEUROCHEMICAL STUDY OF CONDITIONED FEAR IN THE RAT L.E.Goldstein, A.M.Rasmusson, B.S.Bunney, R.H.Roth*, Yale University School of Medicine, New Haven, CT 06510.

We have characterized a model of fear conditioning in the rat. Prior to conditioning, male Sragues-Dawley rats were bilaterally lesioned with N-methyl-D-aspartate (NMDA); saline was used as control. Lesion and control injections were aimed at the basolateral-central nuclei of the amygdala. After recovery, all animals were tested in our conditioned fear model during the dark phase. On Day 1, the rat is placed in the testing environment for a 30 min habituation phase (PR1). This period is immediately followed by a 30 min conditioning period (PR2) in which 10 pairings of a 5 sec non-startle eliciting white noise tone and a co-terminating 0.5 sec 0.4 mA footshock are delivered. On Day 2, the rat is reexposed to the testing chamber and presented with ten tones over another 30 minute period (PR3). Rats are sacrificed immediately after PR3 and the brain dissected for neurochemical analysis of tissue dopamine (DA), serotonin (5HT), and metabolite levels by HPLC-ED. Animal behaviors are remotely coded for locomotion, grooming, defecation, freezing, and ultrasonic vocalization (UV). Previous studies using this model have shown that these parameters are under strong stimulus control. NMDA lesion of the amygdala blocked the conditioned metabolic activation of both DA and 5HT in the prefrontal cortex, serum corticosterone elevation, and UV. In addition, PR3 freezing behavior was dramatically suppressed. Other lesion abnormalities include hyperkinesis and failure to retrieve a gauze pad placed on top of the home cage (stimulus-response uncoupling). These data suggest that the amygdala plays a role in (1) the metabolic activation of the prefrontal DA and 5HT systems and (2) the coordination of such behaviors as ultrasonic vocalization and freezing in reponse to a psychological stressor. Supported by USPHS grants MH14092 & MH25642, and Tourette Syndrome Association.

225.7

SEROTONERGIC AGONISTS ENHANCED LONG-TERM EFFECTS OF STRESS ON BEHAVIOR IN THE RAT. E. Grauer*, Y. Kapon, E. Segev and B.A. Weissman. Israel Inst. Biol. Res., Ness-Ziona, Israel.

Prior exposure to stress affects performance in various tests, especially in those associated with aversively motivated behavior. One interpretation of this phenomena assumes that anxiety generated by stress generalizes to impair other behaviors. Evidence of serotonergic (5-HT) involvement in anxiety suggest that the phenomena may be modulated by 5-HT agonists. Rats exposed to 2 hrs of immobilization and cold stress were tested 24 hrs later for changes in an open field activity. Stress alone had no effect on this behavior. At doses that had no overt effect on the behavior of naive animals, buspirone (5 mg/kg, ip) and 8-OH-DPAT (0.25 mg/kg, ip) significantly reduced open field activity of animals with prior exposure to stress. Since the behavioral impairment was detected 24 hrs following stress exposure, 5-HT1A receptors were characterized at that time by equilibrium binding study of [3H]8-OH-DPAT in the forebrain. An increase in the density of binding sites observed in stressed animals may account for their behavioral suppression. This atypical behavioral profile is in accord with human data suggesting initial anxiogenic activity of buspiron.

225.8

THE EFFECT OF INESCAPABLE SHOCK ON SHUTTLE-BOX ESCAPE PERFORMANCE, CONDITIONED FEAR, AND ANXIETY IN RATS WITH LESIONS OF THE DORSAL RAPHE NUCLEUS OR CENTRAL NUCLEUS OF THE AMYGDALA. R. E. Grahn*, B.A. Kalman, L. Sutton , L.H. Silbert, E.P. Wiertelak, L.R. Watkins, and S.F. Maier. Dept. of Psychology, University of Colorado, Boulder, CO 80302.

Exposure to inescapable shock (IS) produces behavioral sequelae including poor escape performance, enhanced conditioning of fear and anxiety. We investigated the role of the dorsal raphe nucleus (DRN) and central nucleus of the amygdala (CeA) in producing these phenomena. Sprague-Dawley rats were restrained or given IS one week after receiving electrolytic lesion of either the DRN, the CeA, or sham surgery. Subjects were tested 24 hr after restraint or IS in a shuttle-box escape task. This session began with a 10 min period during which baseline freezing was measured. Freezing is defined as a complete absence of movement except for that required for respiration and is considered to be a measure of fear conditioned to the contextual cues present in the shuttle-box (Fanselow & Lester, 1988). Rats were then exposed to a single shock and freezing was measured for 20 min. Escape from shock in the shuttle-box was subsequently measured. Four days after escape testing subjects were observed on a elevated plus maze for five min. Lesions of the DRN, but not the CeA, eliminated the interference with escape learning that is normally produced by IS. In addition, DRN and CeA lesions reduced freezing behavior, both before and after footshock. Both also reduced anxiety as measured by performance on an elevated plus maze. These findings support a recently proposed hypothesis (Maier, 1992) concerning the role of the DRN and CeA in mediating the effects of exposure to uncontrollable stressors. Current studies address the role of stressor controllability and serotonergic pathways that originate from the DRN.

225.9

CHRONIC STRESS ALTERS DENDRITIC MORPHOLOGY OF HIPPOCAMPAL NEURONS IN THE RAT

Bruce S. McEwen*, Yoshifumi Watanabe, Elizabeth Gould, and Heather A. Cameron Lab. of Neuroendocrinology, Rockefeller University, 1230 York Ave., N.Y., N.Y. 10021

Elevated levels of circulating glucocorticoids for 3 weeks decrease the number of dendritic branch points and the length of dendrites of CA3 pyramidal cells (Woolley et al., Brain Res.,1990); and 3 months of treatment with excess glucocorticoids reduce pyramidal cell number in this region (Sapolsky et al. J.Neurosci.1985). In order to determine whether chronic stress results in similar changes in rat hippocampal neurons, we performed a morphological analysis of Golgi-impregnated hippocampal neurons on the brains of unstressed control rats and rats subjected to short term (7 day) and chronic (21 day) restraint stress. Adult male Sprague Dawley rats were assigned to one of the following groups: 1) 7 days of 6 hours per day stress in wire mesh restrainers, 2) short term unstressed controls, 3) 21 days of 6 hours per day stress in wire mesh restrainers, or 4) chronic unstressed controls. The rats were perfused and the brains were processed for single-section Golgi impregnation as previously described (Woolley et al., 1990). Chronic stress resulted in significant decreases in the number of dendritic branch points and the length of dendrites in the apical dendritic tree in the CA3 pyramidal neurons; no significant differences were noted in the basal dendritic tree of rats subjected to chronic stress. No changes in the apical or basal dendritic trees of CA3 pyramidal cells were observed with 7d restraint stress. Whether these decreases in the number of dendritic branch points and the length of dendrites lead to cell death remains to be determined. Supported by MH41256 and The Health Foundation.

225.10

EFFECTS OF CORTICOSTERONE & STRESS ON SPATIAL LEARNING, HIPPOCAMPAL PLASTICITY & NEURON LOSS.

S.R. Bodnoff[1,2*], A. Humphreys[3], G.M. Rose[3,4] & M.J. Meaney[2]. [1]Concordia Univ. & [2]Douglas Hospital Research Centre, McGill Univ., Montreal, Canada, [3]Dept. of Pharmacology, UCHSC & [4]Medical Research Service, VAMC, Denver, CO, U.S.A.

We have previously demonstrated that chronic exposure to elevated levels of corticosterone (CORT) in mid-aged rats impairs acquisition in a spatial learning task (Bodnoff et al., Neuroscience Abst., 1991). The present studies examined CORT effects upon spatial learning under 2 conditions: (i) long-term pellet implants & (ii) chronic social stress. The pellets produced stable levels of CORT for 3 months (medium dose: 10-15 ug/dl; high dose: 25-30 ug/dl). High-dose rats demonstrated significantly slower acquisition in the Morris swim maze relative to the medium dose and controls. We also studied rats living in a stressful environment (mixed-sex housing in which males were rotated twice weekly), a manipulation which increased a.m. CORT levels approximately 3-fold relative to controls. After 6 months, a subtle but significant acquisition impairment was seen in the stressed rats. Moreover, this deficit was absent in stressed rats that were adrenalectomized and given low CORT replacement. These behavioral impairments will be discussed in the context of markers of synaptic plasticity and hippocampal neuron loss.

225.11

STRESS ALTERS THE NONLINEAR RESPONSE CHARACTERISTICS OF HIPPOCAMPAL DENTATE GRANULE CELLS IN THE ANESTHETIZED RAT. M.J. Mana*, M.J. Zigmond, and T.W. Berger+. *Depts. of Behavioral Neuroscience and Psychiatry, Univ. Pittsburgh, Pgh, PA 15260; Dept. of Biomed. Eng. and NIBS Program, Univ. of Southern California, Los Angeles, CA.*

Chronic stress elevates norepinephrine (NE) synthesis and release in the hippocampus. However, the effect of these changes on the electrophysiological activity of hippocampal neurons is unknown. We used nonlinear systems analytic procedures to study the effect of chronic cold stress on the dynamics of dentate granule cell responses to perforant path stimulation. A train of impulses with random (Poisson) interimpulse intervals (ISI; mean frequency = 2 Hz) was used as the input. First, second, and third order kernels describing the relationship between ISIs and the amplitude of evoked granule cell population spikes were computed.

The average population spike amplitude for all impulses in a train (first order kernel) was not significantly altered in stressed rats. However, chronic stress did alter second order nonlinearities, which reflect the influence of any preceding impulse on the response to the most current impulse as a function of ISI. In unstressed rats, granule cell responses were inhibited for ISI = 0-50 ms, but 0-70 ms in stressed rats. In unstressed rats, facilitation occurred for ISI = 50-300 ms (maximum = 105% of first order kernel; ISI = 90 ms), whereas stressed rats displayed facilitation for ISI = 70-200 ms (maximum = 65% at ISI = 110 ms). And in unstressed rats, suppression occurred for ISI = 300-400 ms, whereas stressed rats displayed suppression for 200-400 ms and 500-700 ms. Stress also changed third order nonlinearities, which reflect the influence of any two preceding impulses that is not described by the lower order kernels. Interestingly, the α-2 antagonist yohimbine (4 mg/kg, ip), which increases hippocampal NE, partially reversed the stress-related changes in second and third order nonlinearities--suggesting that these changes reflect a decrease in basal NE modulation of hippocampal excitability. Supported by MH43947, MH00343, AFOSR, ONR, & MRC of Canada (MJM).

225.12

LESIONS OF THE FASCICULUS RETROFLEXUS (FR) PRODUCE A DEFICIT IN SPONTANEOUS ALTERNATION BEHAVIOR.

A. DiCamillo, M. Murray, and F. Haun*. Dept. of Anatomy & Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

In investigating the functions of the habenula-interpeduncular (Hb-IPN) system, we have found that removing the Hb input to the IPN by making bilateral FR lesions produces a chronic elevation in circulating levels of stress hormones in adult rats. We tested whether a spontaneous behavior of these animals, a tendency to explore alternate arms of a T-maze, might also be affected. Adult female rats meeting a 70% criterion of spontaneous alternation (SA) between two reinforced arms of a T-maze (first trial forced) received either sham lesions, bilateral (BL), or unilateral (UL) lesions of the FR. The sham group (n=6) alternated at a mean rate of 77.3% pre-operatively and 80.7% post-operatively. In contrast, both types of FR lesion significantly decreased post-operative SA to chance levels: SA rate was reduced from 77.8% to 57.3% in the BL group (n=6) and from 86.6% to 56.5% in the UL group (n=6). This elimination of spontaneous alternation was accompanied by the post-operative appearance of a response (side) bias, although the direction of the bias (left or right arm of the maze preferred) was not predictable by the side of the unilateral lesion. These results demonstrate that FR lesions alter a spontaneous behavior in a way that previously has been associated with hippocampal or thalamic damage. We suggest that a deficit in spontaneous alternation may reflect a stress-induced change in exploration strategy. Supported by NIH grants NS28856 (FH) and NS16556 (MM).

225.13

ATTENUATION OF ENDOCRINE AND BEHAVIORAL RESPONSES TO SOCIAL CONFLICT STRESS IN RATS BY MICROINJECTION OF CYTOTOXIC ANTIBODY TO CORTICOTROPIN RELEASING FACTOR AND RICIN A CHAIN TOXIN WITHIN THE PARAVENTRICULAR NUCLEI. E. Menzaghi, S.C. Heinrichs, E. Merlo Pich, F.J.H. Tilders[1] and G.F. Koob*. The Scripps Research Institute, Dept. of Neuropharmacology. CVN7, 10666 N. Torrey Pines Rd., La Jolla CA 92037 and [1]Department of Pharmacology, Free University, 1081 BT Amsterdam, The Netherlands.

Previous work has demonstrated that non-linked cellular toxins (Ricin A chain and monensin) added to a cytotoxic IgG2a monoclonal antibody to Corticotropin Releasing Factor (CRF-MAb) specifically affect some CRF neurons after central injection into the hypothalamic paraventricular nuclei (PVN). Since the CRF neurons of the PVN are involved in mediating the endocrine responses to stress, we attempted here to evaluate the long-term effects of CRF-MAb/toxins injection in the PVN on behavioral and endocrine reactivity to a social stressor. Social interaction stress was induced in naive intruder rat by brief social defeat and non-injurious exposure in the home cage territory of an aggressive resident rat. Social defeat produces a specific CRF-dependent release of plasma ACTH and an increase in emotionality of the intruder rat measured with the Elevated Plus-Maze. Two weeks after bilateral intra-PVN injection of CRF-MAb/toxins intruder rats were exposed for twenty minutes to resident animals. CRF-MAb/toxins caused a significant 63% reduction of the ACTH release induced by the resident/intruder interaction and blocked the anxiogenic effect of stress on open arms exploration in the Elevated Plus-Maze. These data point to the possible involvment of CRF neurons within the PVN in mediating not only the endocrine responses but also some behavioral responses to social interaction stress in the rat. The intracerebral microinjection of CRF-MAb/toxins may prove to be a valuable tool for dissecting the contribution of CRF neurons to the pattern of stress responses (Research grant NIADDK Am 26741 to G.F. Koob).

225.14

IN SITU HYBRIDIZATION OF VASOPRESSIN AND CORTICOTROPIN RELEASING FACTOR IN RAT HYPOTHALAMUS FOLLOWING EXPOSURE TO STRESSORS. R.L.Galli* & H.R.Lieberman. Military Performance and Neuroscience, U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760, & *Boston University, Boston, MA 02215

Vasopressin (VP) and corticotropin releasing factor (CRF) interact to stimulate the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary during the physiological response to stress. VP and CRF synthesizing cells have been anatomically localized to specific subpopulations within nuclei of the hypothalamus. Non-isotopic in situ hybridization was performed to investigate changes in VP and CRF mRNA at the single cell level. Adult male Fisher 344 and Sprague-Dawley rats were stressed by restraint or by swimming. Digoxigenin-11-dUTP (Boehringer Mannheim Biochemicals) labeled oligonucleotide probes were hybridized to 10μ coronal sections cut at the level of the hypothalamus. The pattern of changes in VP and CRF mRNA expression in the different hypothalamic nuclei in response to the two stressors was examined and compared to no-stress control groups. VP mRNA increased in the supraoptic (SON) and paraventricular (PVN) nuclei of Fisher rats and in the PVN of Sprague-Dawley rats following one hour of restraint stress.

225.15

Corticotropin Releasing Factor (CRF) Injected in Region of the Central Nucleus of the Amygdala (CNA) Increases Tryptophan Hydroxylase (TrpH) Activity and 5-HTP Accumulation but not Serotonin Turnover. K.C. Corley*, R. Dilts, T-H. Phan and M.C. Boadle-Biber. Dept. Physiol., Va Commonwealth University, Richmond, VA 23298.

TrpH is the rate-limiting enzyme in 5-HT synthesis that converts tryptophan to 5-HTP. An acute, 1-h exposure to sound stress (SS) produces *ex vivo* an increase in TrpH activity (Brain Res., 482: 306, 1989) which can be mimicked by intracranial injection of CRF into the region of the CNA (Neurochem. Int., 20: 81, 1992). However, unlike SS, CRF does not increase tissue levels of 5-HIAA or the ratio of 5-HIAA/5-HT, both indices of enhanced 5-HT turnover. The present study was undertaken to determine if intracranial injections of CRF enhance the *in vivo* conversion of tryptophan to 5-HTP, as found for SS (Soc. Neurosci. Abstr., 1992). CRF (0.5 μg per side) or vehicle via bilateral guide cannulae implanted 7-10 days earlier under surgical anesthesia was injected into the region of the CNA of rats, and 15 min later they were given a decarboxylase inhibitor (NSD 1015, 200 mg/kg i.p.). The accumulation of 5-HTP over a 30 min period was later assayed in the cortex and midbrain by HPLC-EC. Compared with vehicle controls, significant ($P < 0.01$) increases of 5-HTP were found [cortex: 119.6 ± 0.5 vs. 202.5 ± 3.7 ng 5-HTP/g ± SEM; $t(3) = 17.2$; midbrain: 338.4 ± 3.4 vs 684.8 ± 49.9 ng 5-HTP/g ± SEM; $t(4) = 6.9$]. Questions remain as to how to explain the failure of CRF to mimic the increased 5-HT turnover produced by SS. However, both the enhanced accumulation of 5-HTP *in vivo* and the increased TrpH activity *ex vivo* in response to CRF resemble that of SS and suggest common neural events. *Supported by NIH Grant NS14090 to MCB-B.*

225.16

MUSCARINIC RECEPTORS MEDIATE THE EFFECT OF ACETYLCHOLINE ON NEURONS OF THE BED NUCLEUS OF THE STRIA TERMINALIS. J.H. Casada* and N. Dafny. Dept. Neurobiol. and Anat., The University of Texas Medical School at Houston, TX 77225.

The bed nucleus of the stria terminalis (BNST) is considered to be essential in mediating the effects of the limbic system on ACTH secretion, a hallmark of stress. Electrical stimulation of BNST has also been shown to produce stress-like changes in corticosterone secretion, behavior and autonomic activity. Previous experiments have shown that acetylcholine (Ach) is excitatory to certain BNST neurons, thus suggesting that BNST Ach may play a role in the expression of the stress response. This study was designed to determine the type of receptor mediating the effect of Ach on BNST neurons. Twenty-one rats (200-350 g) were anesthetized with urethane (1.25 g/kg, i.p.), and action potentials were recorded from single BNST neurons using a recording microelectrode mounted on multibarrel microiontophoresis pipettes filled with Ach, atropine (Atr), and hexamethonium (Hex). 45% of BNST neurons showed a dose-dependent monophasic increase in firing rate in response to Ach. Atr, a muscarinic antagonist, blocked the effects of Ach in all neurons tested, while Hex, a nicotinic antagonist, did not alter Ach responses. Since amygdala stimulation (AmygS), like Ach is excitatory to BNST neurons, each of the neurons was also tested for responsiveness to AmygS and for the ability of Atr to block responses to AmygS. Atr had no effect on the responses of BNST neurons to AmygS, and there was no correlation between neurons responsive to Ach and those responsive to AmygS. Thus, this experiment shows that the excitatory effect of Ach on BNST neurons is mediated by muscarinic receptors and is consistent with the possible role of BNST Ach in modulating the endocrine and autonomic effects of stress.

225.17

STRESS-INDUCED RELEASE OF CORTICOTROPIN RELEASING FACTOR IN THE AMYGDALA MEASURED BY IN VIVO MICRODIALYSIS. E. Merlo Pich*, G.F. Koob, S.C. Sattler, F. Menzaghi, M. Heilig, S.C. Heinrichs, [1]W. Vale and F. Weiss. [1]The Salk Institute, and Department of Neuropharmacology, Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA 92037.

The amygdala is a part of the limbic system that mediates behavioral, autonomic and neuroendocrine responses to stressful stimuli. Anatomical studies showed that corticotropin releasing factor (CRF)-containing cell bodies and terminals are densely distributed in the central amygdaloid nucleus (Ce). The observation that ICV administration of CRF induces behavioral and autonomic changes resembling the stress response suggests that the CRF-amygdaloid system may be important in the mediation of some component of this response. To test this hypothesis we used the intracranial microdialysis technique for the measurement of immunoreactive CRF measurement in awake, freely moving rats recently developed in our laboratory (Merlo Pich et al, Soc Neurosci Abstr 542.7, 1991). Male Wistar rats were surgically implanted with a chronic guide cannula one week before the experiment. Microdialysis probes were inserted 12 h prior to sampling. For the experiment the flow rate was 3.0 μl/min, and fractions were collected at 20 min intervals. Basal values of CRF were estimated to be 2.13 ± 0.44 fmol/sample. Immobilization stress (20 min) transiently increased CRF levels to 12.7 ± 3.4 fmol/sample. Perfusion with the depolarizing agent 4-aminopyridine (10 mM) induced a 3-fold increase in CRF, suggesting that the sampled CRF was of synaptic origin of CRF release. Histological verification was performed by immunocytochemistry to visualize the probe traces with respect to CRF neurons in the Ce. The evidence of stress-induced release of CRF in Ce in conjuction with behavioral evidence that local injections of the CRF antagonist α-helical-CRF (250 ng) in the rat Ce attenuate the effects of stress on the Elevated Plus-Maze suggests that the CRF amygdaloid system is involved in the mediation of the stress response.

225.18

AFFERENT AND EFFERENT PROJECTIONS OF BARRINGTON'S NUCLEUS, A CORTICOTROPIN-RELEASING FACTOR (CRF)-CONTAINING PONTINE NUCLEUS. M.E. Page*, P.H. Luppi, G. Aston-Jones, and R.J. Valentino. Dept. of Mental Health Sci., Hahnemann Univ., Philadelphia, PA 19102, U.S.A.

Barrington's nucleus contains a substantial number of CRF-immunoreactive neurons (Suttin and Jacobowitz, 1988), suggesting that in addition to its role in micturition it may be important in stress responses. However, the afferent and efferent connections of this nucleus have not been completely identified. To characterize the anatomic connections of this nucleus in detail the anterograde and retrograde tract tracer, cholera toxin b subunit (CTb) was iontophoresed (0.5 μA, 5 min) into Barrington's nucleus of 3 halothane-anesthetized rats and rats were perfused 10 days later. Visualization of CTb with streptavidin-HRP immunohistochemistry revealed numerous retrogradely labeled cells in the periaqueductal gray (PAG), the lateral hypothalamus and cortical layers 4 and 5. A smaller number of cells were retrogradely labeled in the nucleus of the solitary tract, the nucleus paragigantocellularis, the dorsal motor nucleus of vagus, medial vestibular nucleus and paraventricular nucleus of the hypothalamus. Anterograde labeling indicated that in addition to the previously described descending projections to the sacral spinal cord, Barrington's neurons project to the ventrolateral PAG and the lateral mammillary nucleus in the hypothalamus. Additional possible connections are also being examined. These results demonstrate that Barrington's neurons receive projections from brain regions of diverse function and that these neurons project to supraspinal regions that are involved in functions other than micturition. Exposure of rats to 30 min. inescapable footshock (1.5 mA, 0.5 ms, 0.033 Hz) increased Fos protein immunoreactivity of CRF-immunoreactive neurons of Barrington's nucleus suggesting that these neurons are activated by stress. Taken together with anatomic findings, these results implicate a role for Barrington's nucleus in stress responses. Supported by MH00840, MH40008 and NS 24698.

226.1

EFFECTS OF DORSAL OR MEDIAN RAPHE INJECTIONS OF 8-OH-DPAT ON RESPONSE INHIBITION. P.J. Fletcher*, Sect. Biopsychology, Clarke Inst. Psychiatry, Toronto, Ontario, Canada M5T 1R8.

Although it is well established that brain 5-hydroxytryptamine (5-HT) is important for behavioural inhibition few studies have systematically compared the effects of separately manipulating dorsal raphe (DR) and median raphe (MR) 5-HT activity on response inhibition. These experiments were undertaken to examine further the effects of reducing brain 5-HT on response inhibition, and to assess the relative importance of 5-HT efferents arising from the DR and MR in mediating such inhibition. Adult male rats, equipped with a single stainless steel guide cannula aimed at either the DR or MR, and reduced to 85% free-feeding body weight were used. Rats were tested for their ability to withold responding (bar pressing for 45 mg food pellets) following raphe injection of various doses of 8-OH-DPAT, which suppresses 5-HT raphe neuronal activity. Three operant paradigms were used: extinction of a previously rewarded response (EXT: reward omission), omission training (OT: requiring cessation of responding to receive reward) and performance of a go/nogo discrimination (involving alternate signalled periods of reward, S+, and extinction, S-). Rats injected with 8-OH-DPAT in the MR showed increased responding during EXT (0.2 and 1μg) and OT (1μg); DR injections were ineffective in both paradigms. Injections of 8-OH-DPAT into the DR (0.2,1 and 5μg) or MR (5μg) reduced accuracy of responding in the go/nogo task. In the case of DR injections this was due to reductions in responding during S+ periods, but in the case of MR injections responding was increased during S- periods. The results suggest that suppressing 5-HT function leads to difficulties in restraining behaviour, particularly when rewards are expected (EXT and OT). These effects appear to be mediated by 5-HT neurons arising from the MR, rather than the DR.

226.2

ATTENUATION BY PROPRANOLOL OF 8-OH-DPAT-INDUCED REDUCTION OF LORDOSIS BEHAVIOR. M. Andrade, S. Montanez, M. Caldarola-Pastuszka, J. Hines*, and L. Uphouse. Department of Biology, Texas Woman's University, Denton, Texas, 76204.

After a 10 min pretest for sexual receptivity, proestrous rats were injected i.p. with 0.15 mg/kg 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). Inhibition of lordosis behavior occurred within 15 min. Treatment with 1 or 2 mg/kg (-)propranolol, 30 min before 8-OH-DPAT, significantly attenuated the effects of 8-OH-DPAT. I.p. injection of 2 mg/kg propranolol 30 min before bilateral infusion of 200 ng 8-OH-DPAT into the VMN failed to reduce the inhibition produced by 8-OH-DPAT. However, simultaneous infusion with 1000 ng propranolol and 200 ng 8-OH-DPAT per cannulae site did attenuate the effects of 8-OH-DPAT. These results provide further evidence for the inhibitory effects of VMN 5-HT_{1A} receptors in female lordosis behavior.

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226.3

SPECIES DIFFERENCES IN SENSORIMOTOR REACTIVITY AND LOCOMOTOR ACTIVITY FOLLOWING REPEATED ADMINISTRATION OF THE 5-HYDROXYTRYPTAMINE$_1$ (5-HT_1) AGONISTS (±)-8-HYDROXY-DPAT (8-OH-DPAT) OR L-(M-CHLOROPHENYL) PIPERAZINE (mCPP). M. Schmidt*, D. Helton, C. Murphy-Farmer, and J. Tizzano. Lilly Research Labs, Eli Lilly and Company, Greenfield, IN, 46140.

Single doses of 5-HT_{1a} agonists decrease startle amplitude while 5-HT_{1b} agonists increase startle amplitude in mice. In contrast, single doses of 5-HT_1 agonists produce effects in rats opposite to those seen in mice. In this study, drug-related changes in auditory startle and locomotor activity were compared in rats and mice following repetitive dosing (5 days) with the 5-HT_{1a} receptor agonist, 8-OH-DPAT (1, 3, 10 mg/kg, ip), or the preferential 5-HT_{1b} agonist, mCPP (1.25, 2.5, 5 mg/kg, ip). Auditory startle was assessed on days 1-5, while activity levels were measured on days 1 and 5. Changes in auditory reactivity produced by single doses of 8-OH-DPAT or mCPP in mice were only slightly reduced following repetitive doses. Similarly, increases or decreases in reactivity produced by single doses of 8-OH-DPAT or mCPP in rats were not altered following repetitive dosing.

In contrast, although 8-OH-DPAT or mCPP decreased locomotor activity in both species initially, repetitive dosing with 8-OH-DPAT resulted in activity levels at or above control levels by the fifth day in both species. However, both rats and mice continued to show decreased activity levels on the fifth day of dosing with mCPP. These data indicate that drug-related changes resulting from repetitive dosing with 5-HT_1 agonists may depend on the intrinsic activity of the compound at 5-HT receptors, the species examined, or the type of behavioral assessment.

226.4

NON-RECIPROCAL CROSS-TOLERANCE BETWEEN LSD AND 8-OH-DPAT IN RATS K.M. Krebs and M.A. Geyer*. UCSD Dept Psychiatry, La Jolla, CA 92093.

Like other hallucinogens, LSD (d-lysergic acid diethylamide-25) has characteristic effects on rats' behavior, including decreases in locomotor activity and investigatory holepokes and rearing. Because LSD is an agonist at both 5HT-1A and 5HT-1C/2 receptors, it is unclear which behavioral effects are influenced by actions at which receptor sites. Because there are no selective 5HT-1A antagonists with which to address this question, LSD's specific 5HT-1A effects are difficult to assess. An alternative method is the use of tolerance and cross-tolerance regimens. In the present study, rats were pretreated with either saline, the 5HT-1A-selective agonist, 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT) (0.5 mg/kg sc) or LSD (60 µg/kg sc), every 12 hours for 5 days. 36 hours later, rats were tested in a Behavioral Pattern Monitor (BPM) after either saline, 0.5 mg/kg 8-OH-DPAT, or 60 µg/kg LSD. The BPM is a 30.5 x 61 cm chamber, with 10 holes. Infrared photobeams assess holepokes, quantify movement, and chart the pattern of locomotor behavior. As expected, tolerance to the decreases in locomotor activity produced by acute 8-OH-DPAT occurred when rats were pretreated with 8-OH-DPAT. However, only partial tolerance to LSD was found, perhaps due to the latency between preteatment and behavioral testing. Interestingly, pretreatment with 8-OH-DPAT produced partial cross-tolerance to LSD, but pretreatment with LSD produced no cross-tolerance to 8-OH-DPAT. Our interpretation of these findings is that the LSD pretreatment was less effective than the 8-OH-DPAT pretreatment at producing adaptive changes in 5HT-1A receptor function and therefore failed to produce cross-tolerance with 8-OH-DPAT. However, the 8-OH-DPAT pretreatment was effective in blocking the 5HT-1A component of LSD's action. That only partial cross-tolerance was produced by the 8-OH-DPAT pretreatment supports the hypothesis that LSD's effects in this model reflect a combination of 5HT-1A and 5HT-1C/2 effects.

226.5

8-OH-DPAT IN THE MIDBRAIN CENTRAL GRAY, BUT NOT THE DORSAL RAPHE NUCLEUS, INHIBITS FEMALE LORDOSIS BEHAVIOR. M. Droge*, M. Caldarola-Pastuszka, and L. Uphouse. Department of Biology, Texas Woman's University, Denton, Texas, 76204.

Sexually receptive female rats were infused intracranially with 500-2000 ng 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) into the midbrain central gray (MCG), in the vicinity of the dorsal raphe nucleus (DRN), or directly into the DRN. When cannulae were located within the DRN, there was little evidence of change in lordosis behavior but a decrease in locomotor activity was commonly observed. In contrast, when cannulae were located anterior, ventromedial or lateral to the DRN, inhibition of lordosis behavior was rapid and robust. Both the lordosis-to-mount ratio (L/M) and the quality of the lordosis reflex were reduced following the infusion. The MCG receives lordosis-facilitating input from the ventromedial nucleus of the hypothalamus and from ascending sensory pathways and contributes information to descending motor systems involved in the lordosis response. Thus the MCG is a critical link in the completion of the estrogen-dependent lordosis reflex. The present results suggest that 5-HT_{1A} receptors in the MCG prevent the completion of this reflex.

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226.6

REDUCTION OF FEMALE LORDOSIS BEHAVIOR FOLLOWING VENTROMEDIAL HYPOTHALAMIC INFUSIONS WITH 5-OH-DPAC OR 5-MeO-DPAC. M. Caldarola-Pastuszka, J. Williams* and L. Uphouse. Department of Biology, Texas Woman's University, Denton, Texas, 76204 and Department of Biology, Southeastern Oklahoma State University, Durant, Oklahoma, 74701.

Intact, female rats were implanted with bilateral cannulae directed stereotaxically into the ventromedial nucleus of the hypothalamus (VMN). Following recovery from surgery, rats were tested on proestrous for sexual receptivity. Following a pretest, rats were infused i.c. with 400 ng, 1000 ng or 2000 ng of either 5-OH-3-dipropyl-aminochroman (5-OH-DPAC) or 5-methoxy-3-dipropyl-aminochroman (5-MeO-DPAC). Some inhibition of lordosis behavior and considerable resistance to attempts by the male to mount were evident at 400 ng of the drugs; significant suppressions of lordosis behavior occurred at 2000 ng. The chroman derivatives were less effective than 8-OH-DPAT, but more effective than 5-HT, in suppressing lordosis behavior following VMN infusions. Therefore, these studies suggest that the 5-HT_{1A} sites within the VMN may be responsible for serotonin's inhibition of lordosis behavior.

Supported by NIH RO1 HD28419 and GM08256.

226.7

NEONATAL ACTH 1-24 TREATMENT INCREASES HYPOTHALAMIC SEROTONIN INNERVATION IN THE FEMALE RAT. S.E. Alves*, H.M. Akbari, E.C. Azmitia and F.L. Strand. Department of Biology and Center for Neural Science, New York University, New York, NY 10003.

Administration of ACTH 1-24 to neonatal female rats during the first week postnatal causes delayed reproductive maturation and decreased female sexual behavior among these animals tested as virgins (60 days of age)[1]. This treatment also increases hypothalamic serotonin (5-HT) fiber density as measured by high-affinity specific 5-HT uptake at postnatal day 7 and at adulthood (days 80-90)[1]. As 5-HT is believed to have an inhibitory effect on female sexual behavior through 5-HT_{1A} receptor activation within the medial basal hypothalamus, this current study investigates whether early postnatal ACTH 1-24 alters 5-HT innervation and/or 5-HT_{1A} receptor number within this hypothalamic region. Female Sprague-Dawley rat pups were injected s.c. with either ACTH 1-24 (0.1mg/kg) (donated by Organon, Inc.) or saline vehicle once daily from postnatal day one (day of birth) to day 7. Animals were perfused transcardiacally (n=4 per subgroup) on day 7 and immunohistochemical staining for 5-HT and the 5-HT_{1A} receptor was performed using antibodies against 5-HT and the 5-HT_{1A} receptor. 5-HT fiber density within the medial basal hypothalamus was increased in ACTH treated animals compared to controls. This increase in 5-HT innervation was apparent within the ventromedial nucleus (VMN), the primary region responsible for the control of female sexual behavior in the rat. There does not appear to be a difference in 5-HT_{1A} receptor immunoreactivity at postnatal day 7 between ACTH and saline treated animals. We are currently investigating these parameters in adult animals. Based on this study we suggest that 5-HT innervation into the developing female hypothalamus is susceptible to postnatal manipulation with ACTH 1-24 and that the resulting change in this monoamine fiber density within the VMN during development may be responsible for the observed deficits in reproductive behavior. This study was supported by the Council for Tobacco Research.

[1]Alves. et al., *submitted for publication*, 1992.

226.8

FLESINOXAN, A 5-HT1A AGONIST, IN THE MPOA INCREASED EX COPULA SEMINAL EMISSIONS AND PENILE REFLEXES IN RATS. L.A. Lumley*, J. Moses, R.C. Eaton, and E.M. Hull. Psychology Dept., SUNY at Buffalo, Buffalo, NY 14260.

Initially serotonin was believed to inhibit male sexual behavior. However, systemically administered selective 5-HT1A agonists have been reported to facilitate male sexual behavior by decreasing the ejaculatory latency, the postejaculatory interval, and the number of intromissions before ejaculation (reviewed in Ahlenius, 1991). We recently reported that flesinoxan (FLES) microinjected into the medial preoptic area (MPOA) facilitated male sexual behavior by decreasing ejaculation latency and postejaculatory interval (Lumley et al., 1990).

In a resent experiment on ex copula genital reflexes in restrained supine male rats FLES (10 ug/.5ul) into the MPOA significantly increased the number of seminal emissions ($p<.03$) and reduced the latency to reflex ($p<.001$). In addition, FLES increased the total number of penile reflexes ($p<.04$), in particular E2's ($p<.02$), erections characterized by tumescence of both the base and the tip of the glans.

These data may explain why FLES in the MPOA facilitated copulation. The increase in penile reflexes and seminal emissions may be related to the increased copulatory rate and decreased ejaculatory threshold.

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226.9

FLUOXETINE INDUCED INHIBITION OF MALE RAT SEXUAL BEHAVIOR: POSSIBLE ROLE OF THE NUCLEUS PARAGIGANTOCELLULARIS. D. Yells[1], M. Prendergast[1], S. Hendricks[2]*, M. Nakamura[2], D. Fitzpatrick[2], E. Rosa-Molinar[2]. [1]Department of Psychology, University of Nebraska at Omaha and [2]Department of Psychiatry, University of Nebraska Medical Center, Omaha, NE 68182.

We have previously found that male rats with bilateral lesions of the nucleus paragigantocellularis (PGi) display profound changes in copulatory behavior when tested until sexually exhausted. Other researchers (Marson & McKenna, 1990) have demonstrated that the PGi contains 5HT-immunoreactive neurons and neurons that project to the spinal nucleus of the bulbocavernosus. We hypothesized that inhibition of male rat copulatory behavior following administration of the 5HT reuptake blocker fluoxetine may be mediated by neurons originating in the PGi. In Experiment 1, male rats received systemic injections of either 0, 5, 10, or 20 mg/kg of fluoxetine 45 min prior to being tested for sexual behavior in an exhaustion paradigm. There was a significant reduction in both latency to exhaustion and number of ejaculations to exhaustion for rats receiving the highest dose. Additionally, animals displayed dose dependent increases in mount frequency, ejaculation latency, post-ejaculatory interval, and intercopulatory interval and decreases in copulatory efficiency. These changes were especially noticeable just prior to exhausion. In Experiment 2, we are investigating whether the fluoxetine induced inhibition of copulatory behavior can be reduced or eliminated following bilateral lesions of the PGi.

226.10

REDUCED SENSITIVITY TO 8-OH-DPAT-INDUCED SUPPRESSION OF LORDOSIS BEHAVIOR WITH REPEATED ESTROGEN PLUS PROGESTERONE PRIMING. L. Uphouse*, M. Caldarola-Pastuszka, S. Montanez, and A. Jackson Department of Biology, Texas Woman's University, Denton, Texas, 76204.

Ovariectomized rats were injected with 25 µg estradiol followed 48 hr later by 500 µg progesterone. The rats were tested for sexual receptivity 4-6 hrs later following either i.p. treatment with 0.15 mg/kg 8-hydroxy-2-(di-n-propyl-amino)tetralin (8-OH-DPAT) or a bilateral ventromedial hypothalamic (VMN) infusion of 200 ng 8-OH-DPAT. Either treatment reduced lordosis behavior. Repetition of the treatment one week later produced little suppression of lordosis. When rats were primed for two weeks with estradiol plus progesterone prior to treatment with 8-OH-DPAT, there was still an attenuation of the effects of 8-OH-DPAT, but less than when 8-OH-DPAT accompanied the first week's priming. These findings suggest that repeated treatment with relatively high doses of gonadal hormones can reduce the sensitivity of 5-HT receptors to 8-OH-DPAT. They also suggest that activation of 5-HT_{1A} receptors can lead to a subsensitivity to 8-OH-DPAT, present at least a week later.

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226.11

PHARMACOLOGICAL DEPLETION OF CATECHOLAMINES MODIFIES COVERT ORIENTING IN RHESUS MONKEY. E.A. Witte, M.E. Lickey*, and R. T. Marrocco. Institute of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

Catecholamine depletion in humans using the alpha-2 adrenergic agonist clonidine and the dopaminergic antagonist droperidol affects attentional processing in a covert target detection (CTD) task (1). We sought to replicate these results with Rhesus macaques using a CTD task very similar to that used previously (1). In addition to using i.m. clonidine and droperidol to deplete catecholamine levels, we used guanfacine, another alpha-2 adrenergic agonist, and normal saline controls.

During the CTD task, rhesus monkeys pressed a bar to illuminate a fixation spot in the center of a video monitor. Fixation was monitored with the scleral search coil method. Following a random delay interval, a peripheral cue was presented to one, both hemifields, or omitted on some trials. A target was presented to either the left or right hemifield at intervals of 100, 400, or 700 msec after the cue. The cue was presented to either the same hemifield as the target (valid condition); the opposite hemifield (invalid condition), to both hemifields (neutral condition), or was omitted (no-cue condition). The monkey's reaction times (RTs) to target detection were measured.

The primary effect of catecholamine depletion was a significant reduction in invalid trial RTs compared to those for saline control trials. No general drowsiness or impairment of saccadic eye movement frequency was seen. This result implies that the neural systems underlying human and non-human primates are quite similar in the degree to which they depend on normal levels of central nervous system catecholamines. Supported by the McDonnell-Pew Foundation.

(1) Clark, M, Geffen, GM, & Geffen, LB. Psychopharm. 1989, 27, 131-139.

226.12

BEHAVIORAL AROUSAL POSSIBLE AFTER PROCAINE INJECTION INTO EITHER LOCUS COERULEUS OR NUCLEUS ACCUMBENS, BUT NOT BOTH. B.E.Morton* +, R.M.Chesire, W.E.Winn +, B.E.Digman, and M.K.Peterman, Depts. of + Biochemistry and Psychology, University of Hawaii, Honolulu, HI 96822.

Although the locus coeruleus (LC) is commonly believed to be the organ of arousal, attention, alerting, orienting, vigilance, or sympathetic nervous system activation, it is well known that intense behavioral arousal can originate from either of two different motives: reward or punishment. An alternative and more utilitarian distinction for the function of the LC is the view that it is the organ of alarm (B.E.Morton, Biol. Psychol., submitted). Clearly, while excitation of LC alarm may lead to arousal, etc., this does not mean it is the actual structure producing arousal.

Confirming this view, we found, using rats (Long-Evans, about 400g, mixed sex, n=6), that injection of 0.5 ul 0.733M procaine HCL, pH 7.4, into both precannulated LC did not abolish arousal. Instead, it produced a reversible display of those behaviors usually appearing to maximize attainable reward, but not the alarm-driven behaviors commonly associated with threats to survival (6/6). Conversely, 0.5 ul procaine, injected into both n. accumbi (NA), reversibly inhibited behaviors activated by reward seeking, but not by survival threats (6/6). Furthermore, 0.5-1.0 ul procaine injections into both LC, and both NA, caused temporary (2-10 min) immobilization of most responses characteristic of either alarm or reward (4/6).

Thus, although LC inactivation resulted in temporary reductions in signs of alarm-driven "fear" (thigmotaxis, grooming, and freezing), LC inhibition simultaneously enhanced "fearless" active exploration. In contrast, inhibition of the NA resulted in signs of alarm, such as increased grooming, piloerection, and vocalization, along with increased overall movement that was highly thigmotactic. Yet, temporary inhibition of both pairs of brain structures brought movement almost to a complete halt.

These results suggest that neither the LC nor the NA are required for arousal. Rather, either of these systems, by producing the foundational motives of alarm or reward, can independently stimulate the downstream organs of arousal and behavior.

226.13

ALPHA-2 ADRENOCEPTOR ANTAGONISTS FACILITATE PLAY INDEPENDENT OF ANY ACTION AT NON-ADRENOCEPTOR IMIDAZOLINE BINDING SITES. S.M. Siviy* and A.E. Fleischhauer. Department of Psychology, Gettysburg College, Gettysburg, PA 17325.

Previous work has demonstrated that idazoxan, an alpha-2 adrenergic antagonist, increases play in juvenile rats (Siviy et al., Psychopharmacology, 100:119-123). In addition to acting at alpha-2 adrenoceptors, idazoxan has also recently been reported to bind with high affinity to non-adrenoceptor imidazoline binding sites. Therefore, the extent to which idazoxan facilitates play through it's action as an adrenoceptor antagonist is unclear. RX 821002 is a derivative of idazoxan which has minimal affinity for imidazoline binding sites, while having high affinity for alpha-2 receptors. In the present study, the effects of both idazoxan and RX 821002 on rough-and-tumble play were assessed in juvenile rats. Male and female rats (30-45 days old) were housed individually and given daily 5 minute opportunities to play. One group of rats were injected with either saline or one of four doses of idazoxan (1, 2, 4, 8 mg/kg) 30 minutes prior to a play session. Another group of rats were injected with either saline or one of four doses of RX 821002 (0.05, 0.1, 0.2, 0.4 mg/kg). Frequency of dorsal contacts and pinning were used to assess levels of play. Both idazoxan and RX 821002 increased pinning in a dose-dependent manner, while having no effect on dorsal contacts. For idazoxan, the maximal increase in pinning was observed after 4 mg/kg, while for RX 821002 the maximal increase was after 0.2 mg/kg. These data provide further support for a direct action of alpha-adrenoceptors in the modulation of mammalian playfulness.

226.14

TONIC AND SENSORY-EVOKED ACTIVITIES OF NORADRENERGIC LOCUS COERULEUS (LC) NEURONS IN PRIMATE VARY WITH DISCRIMINATION PERFORMANCE IN A VIGILANCE TASK. P. Kubiak, J. Rajkowski, G. Luthin* and G. Aston-Jones, Div. Behavioral Neurobiol., Dept. Mental Health Sci., Hahnemann University, Philadelphia, PA 19102.

Previous studies indicate that the LC regulates vigilance, or attentiveness to sensory stimuli. Consistent with this idea, we have recently reported that monkey LC neurons typically respond preferentially to target stimuli in a vigilance task (Aston-Jones et al., Prog. Brain Res. 88: 501, 1991). We extended this analysis to include changes in discrimination performance and basal discharge rates of LC neurons.

Individual LC neurons were recorded in 2 cynomolgus monkeys performing a vigilance task which required bar release within 700 msec of a target stimulus (10 % of trials) but no response to non-target stimuli (90% of trials). Stimuli were horizontal or vertical bars presented on a video screen, one of which occurred per trial immediately after foveation of a central fix spot. The mean baseline discharge rates of LC neurons were typically between 1 and 4 spikes/sec. During prolonged task performance (more than 30 min), each of 15 LC neurons analyzed to date alternated between two levels of tonic activity which differed by 0.5 - 1.5 spike/sec; animals were continuously alert throughout the task. These episodic changes in LC activity corresponded to altered task performance such that epochs of elevated discharge were accompanied by decreased discrimination, reflecting lowered attention to task stimuli. In addition, LC neurons appeared to be unresponsive to both target and non-target task stimuli during such periods of elevated discharge. In contrast, when LC activity resumed the lower level of discharge, discrimination performance markedly improved and neurons exhibited the typical phasic activation by target stimuli. Thus, a strong relationship exists among tonic LC discharge rate, sensory responsiveness of LC neurons and vigilance performance. These and other results (see Rajkowski et al., this volume) support a role for the LC in attention and vigilance. Additional work is underway to determine how these changes in LC activity contribute to the accompanying changes in attention. *Supported by AFOSR grant 90-0147.*

226.15

ACTIVITY OF LOCUS COERULEUS (LC) NEURONS IN BEHAVING MONKEYS VARIES WITH CHANGES IN FOCUSED ATTENTION. J. Rajkowski*, P. Kubiak, & G. Aston-Jones. Div. Behavioral Neurobiol., Dept. Mental Health Sci., Hahnemann University, Philadelphia, PA 19102.

Our previous results revealed that sensory responses of LC neurons in behaving monkeys are selectively elicited for attended stimuli in a discrimination task (Aston-Jones et al., Prog. Brain Res. 8: 501, 1991). Here we report that tonic LC discharge also varies in close correspondence with attentiveness.

Discharge of individual LC neurons was recorded from 2 cynomolgus monkeys performing an attentional task (oddball visual discrimination). The results of activity during this task are presented in an accompanying abstract (Kubiak et al., this volume). This task required that the animal foveate a central fix spot to initiate each trial of stimulus presentation; proper response to target stimuli resulted in juice reward. Such foveation is effortful and reflects attentiveness to the task. During drowsiness there was typically no task performance and LC activity was very low (< 0.5 spikes/sec). We observed that during continuous alertness and task performance the frequencies of both LC discharge and foveation fluctuated over short (10-30 sec) and long time intervals (10-30 min). The long-term changes in LC discharge were consistently inversely correlated with task behavior, such that slightly elevated LC activity (by 0.5 to 1 spike/sec) was accompanied by decreased foveation frequency and poorer task performance. Correlation analyses revealed that this relationship was highly significant (of the 6 cells quantitatively analyzed to date, typically $r = -0.5$, $p < 0.001$). In addition, even short-term increases in LC tonic activity often corresponded to marked, short-lasting reductions in foveation frequency. These results suggest that focused attentiveness varies with tonic LC discharge in an inverted U relationship. Very low LC activity is associated with drowsiness and inattentiveness, while high tonic LC discharge corresponds with labile attention and restlessness; optimal focusing of attention occurs with intermediate levels of tonic LC activity. Additional studies are underway to test whether fluctuations in tonic LC activity cause or reflect changes in attentiveness. *Supported by AFOSR grant 90-0147.*

226.16

IS MELATONIN INVOLVED IN NEURAL SIGNALLING OF PHOTO-INDUCED HATCHING OF HALIBUT (HIPPOGLOSSUS HIPPOGLOSSUS) LARVAE ?
A.Bogsnes, J.V.Helvik, B.T.Walther*. Lab. of Marine Mol. Biology, Univ. of Bergen, HiB, N-5020 Bergen, NORWAY.

Hatching of halibut larvae normally takes place after 14 days of development, but may be delayed for at least two weeks by continuous exposure to white light of 50 lux. Synchronous hatching of such photo-arrested larvae may be induced by 20 minutes in darkness <0.1 lux. This signal causes irreversible secretion of the proteolytic hatching enzyme. The vast majority of such larvae hatch within 90-120 minutes. Since light appears to act as a physiological stimulus, we consider this hatching-regulation to be mediated by CNS. Melatonin is an indolic compound known to be influenced by light/dark cycles. In order to investigate the possible role of melatonin in this regulatory pathway, we have established an HPLC method allowing us to measure nine different indolic compounds in the metabolic pathway of tryptophan, including serotonin and melatonin, in the range of 0.1-0.5 pmole pr. sample. Larvae immediately prior to normal hatching show a significant activity in the serotonergic system by turnover to 5-hydroxy indole acetic acid (5-HIAA). Serotonin is present at slightly lower levels during darkness, even if the level of 5-HIAA remains unchanged. Lower amounts of larval serotonin when dark may be accounted for by the synthesis of an additional compound, e.g. melatonin. Larval melatonin is as yet only indicated, but its presence and fluctuation at such minute levels must be validated by further sample purification and concentration. At these developmental stages catecholamines are present, but their turnover is low and not dependent upon photic treatment at hatching. These findings attest to the likelihood of early serotonergic functions in halibut larvae at hatching. As the pineal develops early, such serotonergic function may relate to pineal sensing of light.

226.17

ASPHYXIA-INDUCED LESION OF THE LIMBIC SYSTEM

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Asphyxia to male Sprague-Dawley rat pups was induced by a delayed cesarean section. An immunohistochemical and functional analysis was carried out on the pups at an age of 3 weeks. By means of immunohistochemistry the nigro-striatal dopamine system was studied. It was demonstrated that asphyxia time-dependently produced an increase in the number of tyrosine hydroxylase immunoreactive (TH-IR) nerve cell bodies of the substantia nigra, while the TH-IR nerve terminal levels in the caudata nucleus were unchanged. Following mild asphyxia increases in rearing and locomotion were found, while severe asphyxia resulted in a reversal of these behaviours. Treatment with the glutamate antagonist MK801 on normal animals resulted in an enhanced rearing and locomotion response. Asphyxia abolished this response indicating that asphyxia induced disturbancies in glutamate-innervated neuronal pathways of the limbic system. Increases in rearing and locomotion behaviours may be regarded as an animal analogue for the hyperkinetic syndrom in MBD-children. The observed behavioural changes may thus indicate an important role of neuronal pathways containing glutamate for the development of the hyperkinetic syndrom in MBD-children.

227.1

NERVE GROWTH FACTOR (NGF) TREATMENT REDUCES THE INHIBITORY EFFECTS OF ACUTE ETHANOL ON CALCIUM CHANNELS IN PC12 CELLS. D. Mullikin-Kilpatrick* and S. N. Treistman. Department of Pharmacology, Univ. of Massachusetts Medical Center, Worcester, MA 01655.

To gain insight into the development of alcohol tolerance, we have examined the effects of acute ethanol on voltage-activated calcium channels in undifferentiated (UND) and NGF-treated PC12 cells, using voltage clamp techniques and nystatin-perforated patch recording. Macroscopic currents were evoked from holding potentials (V_h) of -90 mV and -40 mV, using barium as charge carrier. The presence of non-inactivating currents, and the inhibition of the currents by nifedipine, indicated that the currents were carried mainly through L-type channels in both UND and NGF-treated cells. 25 mM ethanol reversibly inhibited currents evoked from V_h, -90 mV, by ~ 10% and from V_h, -40 mV, the inhibition was ~23% and this was significantly less ($P<0.05$) than the inhibition seen in UND cells. NGF-treated cells were not significantly different from UND cells in their steady-state inactivation characteristics in the absence of ethanol. Steady-state inactivation curves were shifted in the hyperpolarizing direction by 25 mM ethanol in UND cells and this may be one of the mechanisms by which acute ethanol acts. In contrast, the lack of this shift in NGF-treated cells may explain why, in comparison to UND cells, currents in these cells are inhibited less by acute ethanol. Supported by ADAMHA grant AA05542.

227.2

INHIBITION OF (Na,K)-ATPase IN RAT BRAIN BY ACUTE ETHANOL TREATMENT. L. H. Farber*, N. Anlar, and G. J. Siegel. Dept. of Neurology, Hines VA Hospital, and Loyola-Stritch Medical School, Hines, IL 60141

Ethanol produces complex effects throughout the nervous system involving neural transmission, metabolism, membrane permeability and ion flux. The acute effects of ethanol, *in vivo*, on (Na,K)-ATPase, the membrane bound enzyme critical to the regulation of ion gradients, membrane potential, and transport of neurotransmitters, amino acids and sugars, are not well understood.

We have investigated the effect of an acute dose of ethanol on (Na,K)-ATPase activity in the brain stem, cerebellum and cerebrum of the rat over time. Adult Sprague-Dawley rats (330-340 gm.) were injected I.P. (1 ml of 30% (approx. 5.15 M) ethanol/80 gm. weight) twice, 24 hours apart, sacrificed at 90 minutes or 3 hours after the second injection, and their brains were rapidly removed, dissected and frozen in liquid nitrogen. At 90 minutes, in complete homogenates, a 20-30% inhibition of (Na,K)-ATPase activity was found from all areas of the brain, being most pronounced in the cerebellum. At 3 hours after injection, the brain stem (Na,K)-ATPase activity returned to control values. A similar inhibition was seen in washed microsomal membrane fractions from the cerebellum and cerebrum at both 90 minutes and 3 hours after injection. No effect was observed at either time point in the brain stem microsomes.

These data suggest that acute effects of *in vivo* ethanol on brain (Na,K)-ATPase are measurable and are different in washed microsomal membranes as compared to homogenates. Additionally, the time course for these effects may vary with the brain region. We plan to expand our study of the time course and dose response for *in vivo* ethanol in different brain areas.

(We are grateful to Nicholas Emanuele, M.D. for providing alcohol injected rats.)

227.3

EFFECTS OF ALCOHOL EXPOSURE ON GENE EXPRESSION DURING SYNAPTOGENESIS IN RAT BRAIN. A.E.Ryabinin, D.M.Gonzalez#, T.Melcer#, E.P.Riley#,M.C.Wilson*. Dept. Neuropharm., The Scripps Research Institute, La Jolla, CA 92037, #Center for Families at Risk,San Diego State Univ., S.D., CA 920120

Artificial rearing of rat pups with alcohol during the first two weeks after birth leads to specific cell loss and behavioral disorders and is regarded as an adequate animal model for human Fetal Alcohol Syndrome (reviewed by *J.R.West, Alcohol Drug Res.,1987* and *E.P.Riley, Alcohol.Clin.Exp.Res., 1990*). The present investigation was carried out using this model in order to find gene targets for alcohol that could mediate abnormal brain development. Sprague-Dawley rat pups were artificially reared with 6 g/kg/day alcohol during postnatal days 6-10. Gene expression was analyzed using Northern blots, RNase protection assays, *In situ* hybridization and Immunohistochemical staining. Changes in c-fos expression were observed after this treatment. Since expression of c-fos is low at this stage of development, preproenkephalin expression was studied as a marker for c-fos targeted gene expression. Preproenkephalin mRNA level increased in cortex of rat pups reared with alcohol, but not in sham operated animals or those artificially reared with maltose, indicating specificity of this response to alcohol. Changes in expression of genes encoding presynaptic proteins SNAP-25 and Synapsin I were also observed after alcohol treatment as well as in expression of "housekeeping" genes cyclophillin and glyceraldehyde phosphate dehydrogenase, whereas β-actin expression did not change after this treatment. These results suggest that alcohol may disrupt normal patterns of gene expression which underlie brain development. (Supported by NIAAA-Narcology Center Exchange Prog.(AER), NIAAA 06420 (MCW) and NIAAA 06902 (DMG,TM,EPR).

227.4

CHRONIC ETHANOL ADMINISTRATION RESULTS IN TOLERANCE DEVELOPMENT IN POLY-PI TURNOVER IN MOUSE BRAIN. T. A. Lin*, J. P. Zhang and G. Y. Sun. Biochemistry Dept., University of Missouri, Columbia, MO 65212

Many neurotransmitters and hormones in brain are known to transduce signals through interaction with receptors that are coupled to the poly-phosphoinositide (poly-PI) pathway. In turn, this leads to the release of inositol trisphosphate, a second messenger for mobilization of intracellular calcium stores. An in vivo procedure to assess the poly-PI turnover in brain by first prelabeling brain inositol metabolites with [^{3}H]inositol and subsequently using lithium (6-8 gm/kg, i.p.) to block inositol phosphatase activity has been developed in this laboratory. Acute ethanol administration caused a dose-dependent inhibition of the poly-PI turnover activity in brain. As compared to pair-fed controls, mice given ethanol (3-4 gm/kg) twice daily by gavage for 3 weeks developed tolerance to the ethanol-induced inhibition of poly-PI turnover. Blood ethanol levels in the two groups were not different. Chronic ethanol mice also showed a more rapid recovery of the poly-PI activity as compared to the pair-fed controls. Furthermore, an increase in poly-PI turnover activity could be observed in the chronic ethanol mice 7-9 hrs after the last dose, correlating to the time of withdrawal hypersensitivity. These results suggest that the poly-PI turnover activity in brain can be a useful measure for assessing ethanol induced tolerance development in mice (Supported by AA-06661 from NIAAA)

227.5

NMR EVIDENCE FOR ETHANOL-INDUCED REDUCTION IN MOBILITY OF WATER MOLECULES FROM FISH BRAIN. W. R. Klemm*, L. K. Misra, Hung Cao, C. F. Hazlewood. Dept. Vet. Anat. Public Health, Texas A&M, College Station, TX 77843 and Baylor College of Medicine and Rice University.

NMR can measure the dynamics of water protons after they have been energized by radio waves. The time needed to dissipate absorbed energy (T1 relaxation time) is longer for "free" water than it is for water that is associated with macromolecular structures of cells. Here we report T1 data from brain tissue of fish. Fish were randomly assigned in one group exposed for 5 min to alcohol (ethanol, 400mM) in their fish-bowl water and in another group having no alcohol added to their water. Brains from 6-8 fish were pooled for each sample, with four samples per group. Alcohol decreased the T1 values of brain an average of 11% (control mean $\pm$ S.D.: 833 $\pm$ 17 msec; intoxicated: 740 $\pm$ 11 msec). The decrease was highly significant statistically (two-tailed $P < 0.002$). The speed at which this occurs suggests that the dehydration is not an indirect consequence of inhibition of anti-diuretic hormone.

This acute reduction in T1 is consistent with the idea that alcohol-induced dehydration is a mechanism of intoxication (Alcohol, 1990, 7: 49-59; Alc. clin. exp. Res. 1992, in press).

227.6

ACUTE EFFECT OF ETHANOL AND MK-801 ON THE EXCITABILITY OF THE HIPPOCAMPAL DENTATE GYRUS IN THE FREELY MOVING RAT. O. Prospéro-García*, D. R. Miller, P. Robledo and S. J. Henriksen. Dept. Neuropharmacology, The Scripps Research Inst. La jolla, CA 92037.

Previous work has shown that ethanol and MK-801 can antagonize the electrophysiological actions of N-methyl-D-aspartic acid (NMDA) in several experimental hippocampus preparations. In this study the effect of ethanol and MK-801 alone, and in combination, was evaluated for their effects on hippocampal field potentials of chronically implanted freely-moving rats. Paired-shocks (20-160 msec interstimulus interval, ISI) were delivered to the perforant pathway. The amplitude of the conditioning population spike (P1) in the dentate gyrus evoked by the primary shock and the test population spike (P2) evoked by the secondary shock was measured. The ratio of these populations spikes (P2/P1*100) was determined and considered as an index of inhibition of P2. This index was determined during wakefulness under the effect of saline, ethanol (1.5 g/kg, i. p.), MK-801 (0.1 mg/ kg, sc) or ethanol+MK-801. Results showed that in the saline group, P2 is inhibited at ISI = 20 msec and disinhibited (potentiated) at ISI = 60 and 80 msec. Ethanol strongly inhibited P2 at every interstimulus interval, whereas MK-801 showed a biphasic effect, disinhibiting at ISI = 20 msec and inhibiting at ISI = 60 and 80 ms. The combination of these drugs resulted in ISI-dependent effects that suggest both antagonistic as well as synergistic actions of ethanol on NMDA pharmacology.

227.7

ETHANOL-INDUCED DEGENERATION OF DENTATE GYRUS, ENTORHINAL CORTEX AND OTHER OLFACTORY RELATED AREAS IN RATS: EFFECTS OF CO-ADMINISTRATION OF MK-801, DNQX, OR NIMODIPINE. **T.D. Corso*, E.J. Neafsey, and M.A. Collins, Biochem. & Anatomy Depts., Loyola Med. School, Maywood IL 60153.**

We previously reported neurodegeneration in rats maintained with high blood ethanol levels (BELs) above 300mg% for 4 days (Corso et al., Abst. Soc. Neurosci. (1991) 17:1458). We visualized the degeneration with the de Olmos cupric-silver staining method. Postulating that the degeneration involves the influx of calcium, we have attempted to prevent the degeneration by simultaneous treatment with nimodipine (a calcium channel blocker), DNQX (AMPA/KA type glutamate receptor antagonist), or MK-801 (an NMDA type glutamate receptor antagonist). Surprisingly, MK801 alone (4 days) caused degeneration in the same areas as ethanol, and when combined with nontoxic BELs (100 mg%), the damage was significantly increased. Nimodipine or DNQX alone did not induce damage, and neither agent significantly altered the amount of ethanol-induced degeneration caused by 300-400 mg% BEL); co-treatment at higher BELs is under study. Support by LUMC Potts Award.

227.8

EFFECT OF ETHANOL ON WORKING MEMORY: INVOLVEMENT OF THE SEPTOHIPPOCAMPAL PATHWAY. B. Givens* and D.S. Olton. Departments of Psychology, Ohio State Univ., Columbus, OH 43210 and Johns Hopkins Univ., Baltimore, MD 21218.

Low doses of ethanol can impair working memory and alter neural activity in the septohippocampal pathway. The present study used a within-subjects analysis to determine the extent to which changes in hippocampal theta rhythm, an electrophysiological measure of septohippocampal function, were correlated with impairments of working memory. Rats were pre-operatively trained to criterion performance on one of two tasks: spatial alternation or conditional discrimination. Post-operatively, rats were tested 10 min after injection of ethanol (0.0, 0.25, 0.5 or 1.0 g/kg i.p.). In both tasks, ethanol had a dose-dependent effect on choice accuracy and on hippocampal theta rhythm. At 0.5 g/kg, ethanol reduced choice accuracy and suppressed hippocampal theta activity. At 1.0 g/kg, ethanol also affected the time required to complete the task and reaction time. The selective effect of ethanol at 0.5 g/kg on working memory is similar to that observed after microinfusion of the GABA agonist muscimol into the medial septal area, the origin of the septohippocampal pathway. The results are discussed in terms of possible interactions of ethanol with GABAergic regulation of the medial septal area.

227.9

CHRONIC ETHANOL EFFECTS ON IMMUNOHISTOCHEMICAL MARKERS OF CHOLINERGIC SEPTOHIPPOCAMPAL INTEGRITY. M.A. King, B.E. Hunter, and D.W. Walker*, Dept. of Neuroscience, University of Florida and VAMC Gainesville, Florida 32610

We have been examining the effects of chronic ethanol consumption on the cholinergic septohippocampal system with emphasis on the possible mechanistic involvement of nerve growth factor. Male rats were treated for 28 wks. with a nutritionally supplemented liquid diet containing ethanol or isocalorically substituted sucrose in a pair feeding paradigm. Eight weeks later the rats were sacrificed and coded brain sections were stained using choline acetyltransferase (CHAT) and nerve growth factor receptor (NGFR) immunohistochemistry. Labelled neurons in the medial septum and diagonal band of Broca were counted and the area and volumes of these regions were measured. Preliminary data indicate that 37% fewer CHAT+ neurons were counted in alcohol animals. Combined with a 20% decrease in area this represents a 27% decrease in neuronal density. NGFR+ neuronal density was decreased less markedly (16%) in the alcohol animals. These results are consistent with the hypothesis that even with adequate nutritional intake chronic alcohol consumption produces a deficiency in nerve growth factor function. As with other treatments that interfere with NGF reception, an initial decrease in chat expression may presage the actual loss of cholinergic neurons. Neuronal morphometry and other neuronal type markers are currently being used to explore this hypothesis further. Supported by the Veterans Administration and NIAAA grant AA00200.

227.10

ETHANOL DECREASES NEUROTRANSMISSION IN RAT NUCLEUS ACCUMBENS *IN VITRO*: NALOXONE REVERSAL. Z. Nie, X. Yuan, S.G. Madamba and G.R. Siggins*. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The nucleus accumbens (NAcc) has been shown to be an important brain region for the rewarding effects of addictive drugs such as opiates and ethanol. We recently showed that opiate peptides selective for μ, δ and κ receptors superfused onto NAcc neurons in a slice preparation reduced both excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs), with naloxone reversal (Yuan et al., Neurosci. Lett. 134: 223, 1992). Therefore, we used intracellular recording in this rat NAcc slice preparation to investigate ethanol actions (methods as described byYuan et al.). We recorded from a total of 50 neurons within the NAcc core, with pipettes usually containing K-acetate. Resting membrane potentials (RMPs) averaged -83 mV; spike amplitudes averaged 95 mV. Ethanol 22 - 66 mM had little effect on resting membrane properties, but reduced EPSPs evoked by stimulation of the peri-tubercle region ventral to NAcc. Ethanol 22 mM (n=10), 44 mM (n=12), and 66 mM (n=8) all significantly (by ANOVA) decreased the EPSPs to 77% ($P < 0.0002$), 65% ($P < 0.0045$) and 72% ($P < 0.0093$) of control, respectively. One cell tested with 11 mM ethanol showed no effect. To confirm a direct ethanol action on EPSPs, 5 cells were tested in the presence of 30 μM bicuculline to block IPSPs. In these cells ethanol 44 mM still decreased the EPSP size, suggesting IPSPs are not involved in this effect. As ethanol mimicks opiates in reducing EPSPs without effect on RMPs in the NAcc, we applied the opiate antagonist naloxone together with ethanol. Naloxone 2 μM (n=9) significantly reversed ethanol (44 mM) reduction of EPSPs ($P < 0.02$ by ANOVA and Newman-Keuls). Thus, our data show a striking similarity between mechanisms of ethanol and opiate effects in NAcc and suggest that ethanol may act indirectly via some opiate mechanism.

Supported by grants from NIAAA (AA06420) and NIDA (DA03665).

227.11

EFFECT OF ETHANOL AND ACETALDEHYDE ON ß-ENDORPHIN SECRETION IN PRIMARY CULTURES OF RAT HYPOTHALAMIC NEURONS. B.V. Reddy, S. Minami and D.K. Sarkar*. Dept of VCAPP, Washington State University, Pullman, WA 99164-6520.

Central opioid peptides are known to be involved in the neurological and behavioral complications of alcoholism. The cellular mechanism involved in ethanol-regulated opioid activity is presently elusive because of a lack of reliable experimental models. We have previously shown that the fetal hypothalamic neurons can be grown viably in long-term primary cultures. The cultured neurons were often electrically excitable, possessed voltage-activated ionic conductance and secreted ß-EP in response to well-defined physiological challenges, suggesting that the culture system can be useful in determining the regulation of hypothalamic ß-EP activity. In this study, we characterize the effect of ethanol and its metabolite acetaldehyde on the hypothalamic ß-endorphin (ß-EP) secretion from the cultured hypothalamic neurons. Incubation of the cultures with a 50 mM dose of ethanol maintained a constant level of ethanol for a minimum period of 6 h. This and other lower doses of ethanol (6, 12.5 and 25 mM) did not produce any significant effect on cell morphology, viability, DNA or protein content. However, these doses of ethanol (6-50 mM) produced a dose-dependent effect on ß-EP release from the cultured neurons for a period of 2 to 3 h. The ethanol metabolite acetaldehyde also produced a concentration-dependent stimulatory effect on ß-EP secretion. Comparison of the ethanol and acetaldehyde dose-response curves revealed that acetaldehyde are more potent than ethanol in inducing ß-EP release from the cultured neurons. These results suggest that ethanol and its metabolite acetaldehyde stimulates ß-EP release from the hypothalamic neurons, and that the neuron culture system can be useful for studying the cellular mechanisms of ethanol action. (Supported by NIAAA grant # RO1-AA08757).

227.12

ENHANCED ALCOHOL INTAKE IN FAWN-HOODED RATS AFTER WITHDRAWAL FROM ALCOHOL. A.H. Rezvani*, P. Garges, and D. Overstreet. Skipper Bowles Center for Alcohol Studies and Dept. of Psychiatry, University of North Carolina School of Medicine, Chapel Hill, N.C. 27599-7175.

Fawn-Hooded (FH) rats have been proposed as a novel animal model of human alcoholism with genetic serotonin (5-HT) impairment. These rats possess a peripheral and central 5-HT abnormality and exhibit a high preference for alcohol in a free choice situation (Rezvani *et al*, 1990). To further investigate their alcohol drinking behavior, the following experiments were carried out. FH rats were given free access to food, water and a solution of 10% alcohol for at least 15 days. Food, water and alcohol intakes were recorded every 24 hr during the experiments. After establishment of a stable baseline, alcohol was withdrawn for 24 hr. This cycle, (alcohol exposure-24 hr alcohol abstinence-alcohol exposure), was repeated several times in the same group of rats. The results indicated that when FH rats have free access to food, alcohol and water, they drink a significant amount of alcohol (>5g/kg). Twenty-four hr forced abstinence from alcohol induced a significant increase in their alcohol intake for at least one day after re-exposure to alcohol. Compared with pre- and post-abstinence, the amount of water intake during abstinence increased significantly, but total fluid intake and food intake did not change. Administration of fluoxetine, a 5-HT uptake inhibitor, and other serotonergic compounds on the day of abstinence or on the day after abstinence significantly affected the amount of alcohol intake. These data support the proposed idea that FH rats have an endogenous preference for alcohol which can be enhanced by forced abstinence and that the serotonergic systems in the brain may be involved in alcohol-seeking behavior in this strain of rat. (Partially supported by Grant no. 9103 from NCARA to Dr. Rezvani).

227.13

CONSUMPTION OF SWEET, SALTY, SOUR AND BITTER FLAVORED SOLUTIONS BY SELECTIVELY-BRED ALCOHOL PREFERRING P AND NONPREFERRING NP LINES OF RATS. RB STEWART*, RN RUSSELL, T-K LI and JM MURPHY, Indiana University Medical School and VAMC; Purdue School of Science Indianapolis, IN 46202

To determine whether selective breeding for high and low oral ethanol consumption is associated with different preferences for non-pharmacological solutions with various flavors, the self-administration of a range of concentrations of sucrose (0.5-64.0 g/100 ml), NaCl (0.025-3.2 g/100 ml), citric acid (0.008-2.048 g/l), and sucrose octaacetate (0.002-0.512 g/l) was studied in P and NP rats. Separate groups of 7-8 rats from each line were used to test each of the four flavors. The flavor solutions were presented continuously with water always available and the concentration was doubled every 48 hr. Although rats from both lines showed a strong preference for the sucrose solutions, P rats consumed greater amounts than NP rats at most of the concentrations tested (0.5-16.0 g/100 ml, $p<0.05$). Rats of the NP line drank more NaCl solution than P rats but the effect was not as robust as that seen with sucrose and the difference was significant only at one of the NaCl concentrations (0.4 g/100 ml, $p<0.05$). The P and NP rats did not differ in citric acid or sucrose octaacetate intake at any of the concentrations tested. Selective breeding for oral ethanol preference and nonpreference in these lines of rats appears to be associated with differences in the self-administration of sweet and salty flavored solutions. (AA08553 and AA07611)

227.14

COMPARISON OF ALCOHOL-PREFERRING (P) AND -NONPREFERRING (NP) RATS IN A FORCED-SWIM STRESS TEST. C.D. Godfrey, S.L. Jing, R.B. Stewart, J.C. Froehlich and J.M. Murphy*. Dept. Psychology, Purdue Sch. Sci., Dept. Med. & Inst. Psychiat. Res., Indiana U. Sch. Med., IUPUI, Indianapolis, IN 46202.

Rats of the P and NP lines have been selectively bred for alcohol preference and nonpreference. P and NP rats were compared in a swim test to determine if differences in response to an inescapable stressor are associated with genetic differences in alcohol preference. Alcohol-naive adult male P and NP rats (n=10/line) were placed in a round tub filled with ambient temperature tap water (21^{o}C). The rats were unable to touch the bottom or escape from the tub. Behavior was videotaped for 10 minutes on each of two consecutive days and scored for time spent actively swimming or immobile. During both daily sessions, rats of the NP line were less active than P rats and spent approximately 50% ($p<0.05$) more time immobile. The effect of desipramine (10 or 20 mg/kg) or saline on behavior in the swim test was investigated in separate groups of P and NP rats. Both doses of desipramine significantly ($p<0.05$) attenuated time spent immobile in the P and NP rats, but the attenuation was greater in rats of the NP line. Hence, desipramine served to eliminate the line difference in time spent immobile. Compared with NP rats, P rats may be more anxious and/or less susceptible to despair in response to an inescapable stressor. (Supported by AA08553, AA08312 and AA07611)

227.15

ETHANOL SELF-ADMINISTRATION IN TRANSGENIC MICE. C.J. Meliska*, A. Bartke, J.L. Vandergriff, and R.A. Jensen, Departments of Psychology and Physiology, Southern Illinois University at Carbondale, Carbondale, IL 62901-6502.

Evidence suggests that central dopaminergic (DA) mechanisms mediate the rewarding effects of ethanol and various other drugs of abuse. Genetic alterations in DA function may also modulate susceptibility to ethanol self-administration. For example, altered hypothalamic DA turnover rates occur in transgenic (T) mice expressing various growth hormone (GH) genes (Steger, R.W. et al., 1991, *Neuroendocrinology, 53,* 365-372). In particular, male MT/bovine GH T mice show elevated DA turnover relative to T females and non-T littermate controls. Using a two-bottle choice paradigm, we presented tap water and an ascending series of ethanol concentrations (2.2, 4.6 and 10.0%,v/v), for 4 to 6 days per concentration, to 90-day-old male and female T mice and matched littermate controls (N=10/group). Ethanol preference ratios [EtOH/(EtOH+H_2O)] of male T mice were higher at 4.6% (0.67 vs. 0.49, p = .035), and at 10.0% (0.71 vs. 0.36, p = .012) than in non-T males, suggesting that T mice with elevated central DA function may be more prone to self-administer ethanol than controls. Thus, T mice expressing GH genes may provide a useful model for studies of the role of DA function in ethanol self-administration and ethanol abuse.

227.16

TRH ANTAGONISM OF ETHANOL-INDUCED SEDATION IN ALCOHOL-PREFERRING (P) AND -NONPREFERRING (NP) RATS. S. Morzorati*, Y. Morrow, M.J. Kubek, Depts. Psych., Med. & Anat. & Regenstrief Inst., Indiana Univ. Sch. Med., Indianapolis, IN 46202

Sleep time (ST) is significantly shorter in P rats compared with NP rats following a single, sedating dose of ethanol. Intracerebroventricular (icv) administration of TRH antagonizes ethanol-induced sedation in a number of species. The present study was undertaken to determine if P and NP rats are differentially sensitive to TRH in reversing sedation produced by ethanol. Adult alcohol-naive male rats were injected ip with ethanol, 3g/kg, and tested for loss of righting reflex. TRH (5, 20, 40ug) or saline was infused icv (10ul) and ST was calculated. TRH dose-dependently decreased ST in both lines of rats (35-80% in NP rats and 40-50% in P rats). All doses of TRH were effective in NP rats, while only the 2 highest doses were effective in P rats. TRH was approximately twice as potent in NP rats compared with P rats.

These data agree with those indicating that TRH reverses the sedating effects of ethanol. NP rats are more sensitive to TRH than P rats. This may be due to an increase in receptor affinity or a decrease in TRH metabolism in the NP rats. (Supported by AA07611).

227.17

ALCOHOL-ILLNESS ASSOCIATIONS IN THE SELECTIVELY BRED HIGH ALCOHOL DRINKING (HAD) AND LOW ALCOHOL DRINKING (LAD) RATS. R.L. Elder*, N.B. Elder, P.J. Bice, S.A. Bailey and S.W. Kiefer. Department of Psychology, Kansas State University, Manhattan, KS 66506-5302.

Selectively bred HAD and LAD rats obtained from Indiana University were used to examine the acquisition and extinction of an alcohol aversion. Each subject was given two pairings of a 6% (v/v) alcohol solution followed by the intubation of a .15 M LiCl solution. On the day following the second acquisition trial, animals were tested for taste reactivity to 6% alcohol. The animals then received seven extinction trials with the alcohol solution, each trial given every other day. Analysis of the taste reactivity data showed that HAD rats produced significantly fewer aversive responses than LAD rats. The groups did not differ in ingestive reactivity. Although both HADs and LADs showed strong alcohol avoidance on the initial extinction test, the HAD rats consumed significantly more alcohol over the course of the seven extinction trials. These results suggest that, while HAD rats acquired an avoidance of alcohol when it was paired with illness, the avoidance did not appear to entail a shift in palatability nor was it as durable as that found in LAD rats.

227.18

TASTE REACTIVITY AND CONSUMPTION AS MEASURES OF ALCOHOL PALATABILITY IN HIGH ALCOHOL DRINKING (HAD) AND LOW ALCOHOL DRINKING (LAD) RATS. N.B. Elder*, P.J. Bice, and S.W. Kiefer. Department of Psychology, Kansas State University, Manhattan, KS 66506-5302.

Selectively bred, alcohol naive HAD rats and LAD rats (obtained from Indiana University) were tested for taste reactivity to five concentrations of alcohol (5%, 10%, 20%, 30%, and 40% v/v), sucrose, and quinine. After the reactivity tests, a two-bottle consumption test with 10% (v/v) alcohol and distilled water was given for 21 days to verify HAD or LAD status. A second taste reactivity test was conducted at the end of the alcohol access period. The initial reactivity results showed no significant differences between naive HADs and LADs in their responses to alcohol, sucrose, or quinine. However, after the three week access period, significant group differences in taste reactivity did appear. The reactivity of LAD rats remained stable relative to the first test but HAD rats showed a significant increase in ingestive responding and a significant decrease in aversive responding. Our results replicated an identical study with alcohol preferring (P) and alcohol nonpreferring (NP) rats in that experience with alcohol increased its palatability for rats that consumed high amounts of alcohol.

227.19

GENETIC SELECTION FOR RAT LINES THAT SHOW A HIGH AND LOW TASTE PREFERENCE FOR ALCOHOL. P.J. Bice* and S.W. Kiefer, Dept. Psychology, Kansas State University, Manhattan KS 66506-5302.

Rat lines were selectively bred to determine if the taste preference for alcohol (as measured by taste reactivity) was heritable and to examine possible correlated responses to taste. In this project, outbred rats were given an intraoral infusion of alcohol and the resulting orofacial responses were videotaped and analyzed. Based on the number of ingestive responses made to alcohol, two male-female pairs were selected to start a high ingestive responding line (HIR), a low ingestive responding line (LIR), and a control line. After one generation of selection, heritability for the total realized response difference between the HIR and LIR lines was estimated to be .43. Statistical analysis of the ingestive responses to alcohol for the S_1 generation revealed a significant Line effect. The HIR line made significantly more ingestive responses to alcohol than the LIR or Control lines. Both the P_1 and S_1 generations were also given two-bottle consumption tests. Although the HIR rats consumed more alcohol than the LIR rats, the relationship between consumption and taste reactivity revealed no significant phenotypic correlations.

DRUGS OF ABUSE: COCAINE—OTHER STUDIES

228.1

BRAIN 125I–RTI–55 AND 3H–CFT BINDING IN NORMAL AND COCAINE–ABUSING HUMAN SUBJECTS

K.Y. Little,* Kirkman J.A., Carroll F.I., Duncan G.E.

Cocaine causes its behavioral and subjective effects by binding to sites on monoaminergic transporters and inhibiting normal synaptic reuptake. Both RTI–55 and CFT are cocaine congeners which bind to the cocaine binding site and can be radiolabeled. Initial studies with 125I–RTI–55 indicate two binding sites (K_D = 58 pM, 55% binding; K_D = 1.9 nM, 45% binding) which are displaced by other dopamine–selective transporter–binding ligands, including CFT and GBR 12909, but with a 10–fold shift to the right. 125I–RTI–55 binds with high affinity to apparent serotonergic sites in the midbrain, but is only slightly displaced by fluoxetine in the striatum. In addition high affinity 125I–RTI–55 binding (5–500 pM) was found unaltered in striatum from six cocaine–abusers, but there was a trend to increased binding at 2.5 nM. Conversely, 3H–CFT demonstrated significantly increased binding in the striatum of cocaine–abusers at 5 and 10 nM concentrations. Further evidence exploring the hypothesis that RTI–55 and CFT selectively label distinct cocaine binding sites will be presented. Evidence of altered binding in other brain regions of cocaine–abusing subjects will also be presented.

228.2

[^{125}I]RTI-55 BINDING TO THE DOPAMINE TRANSPORTER IN COCAINE OVERDOSE DEATHS. J. Staley, R. Toiba, A. J. Ruttenber, C. V. Wetli, W. Lee Hearn, D.D. Flynn* and D. C. Mash. Depts. Neurology, Pharmacology, Biochemistry and Pathology, Univ. of Miami School of Medicine and the Dade County Medical Examiner Dept., Miami, FL. 33101 and the Univ. of Colorado School of Medicine, Denver, CO, 80262.

Fatal excited delirium has been reported in recreational cocaine users having significantly lower blood levels of cocaine than those usually associated with cocaine overdose deaths. Although the neural mechanisms underlying cocaine-induced psychosis and sudden death are not known, they may be related to the differential regulation by cocaine of neuroamine transporters and receptors. The cocaine analog [^{125}I]RTI-55 is a potent ligand for the dopamine transporter (Boja et al., Eur.J. Pharm. 194:133, 1991). We have characterized the binding of [^{125}I]RTI-55 to the dopamine transporter assayed in human brain membranes. Saturation analysis reveals multiple sites in the putamen with KD values of 0.33 nM (BMAX = 165 pmol/g tissue) and 1.85 nM (BMAX = 473 pmols/g tissue). Competiton studies demonstrate the following rank order of potency: GBR 12935 > mazindol ≥ cocaethylene ≥ (-)-cocaine > fluoxetine ≥ desipramine > (+)-pentazocine > (+)-cocaine. Cocaine competed for [^{125}I]RTI-55 binding sites in control putamen in a biphasic manner with 56% of the sites diplaying high affinity (IC50 = 60 nM) and the remaining sites with low affinity IC50 = 530 nM). Preliminary studies demonstrate that the density of [^{125}I]RTI-55 binding to the high affinity site in the putamen was reduced in the excited delirium subgroup of cocaine overdose deaths as compared to control values ($p < 0.025$). Homologous competiton revealed no alteration in the affinity of the binding sites assayed in the frontal cortex and putamen. Autoradiographic studies are underway to visualize the neural distribution and density of [^{125}I]RTI-55 binding sites in cocaine overdose deaths. (Supported by DA06227)

228.3

DOPAMINERGIC RECEPTOR SUBTYPE REGULATION IN COCAINE-INDUCED PSYCHOSIS AND SUDDEN DEATH: AN AUTORADIOGRAPHIC STUDY. R. Toiba, A. J. Ruttenber, C. V. Wetli, W. Lee Hearn, J. Staley and D. C. Mash*. Depts. of Neurology, Pharmacology, Biochemistry and Pathology, University of Miami School of Medicine and the Dade County Medical Examiner Dept., Miami, FL., 33101 and the University of Colorado School of Medicine, Denver, CO, 80262.

Fatal cocaine intoxication presenting as an excited delirium has been reported in recreational users. Prodromal signs and symptoms of the cocaine intoxication include bizarre and violent behavior and hyperthermia. The cocaine overdose cases selected for receptor autoradiographic analysis were ones in which cocaine users presented with a psychotic reaction, died suddenly with respiratory arrest, and had low toxic levels of cocaine in blood. For quantitative autoradiographic studies, the D1 receptor subtype was labeled with 1 nM [^{3}H]-SCH23390 and the D2 receptor subtype was labeled with 3 nM [^{3}H]-raclopride. The density of the D1 receptors was decreased significantly in the anterior sectors of the caudate and the putamen in the cocaine overdose cases as compared to controls cases. Significant decreases in D1 receptor numbers were observed also over the entorhinal cortex (Brodmann area 28) and within the cortical nucleus of the amygdala. No alteration in the density of D1 or D2 dopaminergic receptors were detected over the nucleus accumbens. The number (BMAX) and affinity (KD) of D2 dopaminergic receptors was unchanged throughout the anterior to posterior extent of the striatum. Within the hypothalamus, no group differences were seen in the density of the D1 receptor sites. In contrast, a significant decrease in the Bmax was determined for the D2 dopaminergic receptor within the anterior and preoptic nuclei of the hypothalamus. These results may be relevant to an understanding of the contribution of selective alterations in D1 and D2 receptor subtypes in central dopaminergic temperature regulation. D1 and D2 dopaminergic receptors mediate opposite effects on thermoregulation, with the D1 receptor mediating a prevailing increase, while the D2 receptor mediates an opposing decrease in core body temperature. The selective down-regulation in the density of the D2 dopaminergic receptor subtype within the hypothalamus may explain the hyperthermia associated with cocaine-induced excited delirium and sudden death. (DA06227)

228.4

mRNA ALTERATIONS IN THE BASAL GANGLIA OF HUMAN COCAINE ADDICTS. Y.L. Hurd*, M. Herman, J. Kleinman and M. Herkenham. NIMH, Clinical Brain Disorder Branch, Neuroscience Center at St. Elizabeth's Hospital, Washington, D.C. 20032 and Section on Functional Neuroanatomy, Bethesda, MD 20892.

Molecular changes in the basal ganglia of human subjects with recent cocaine use were revealed in discrete neuronal populations by *in situ* hybridization histochemistry. Cocaine subjects had a history of repeated cocaine use and had cocaine and/or cocaine metabolites on board at the time of death. Cocaine subjects were compared to control subjects that had both a negative history and toxicology of cocaine use. Synthetic oligodeoxyribonucleotide probes as well as cDNA riboprobes were used to selectively label cells expressing the transcript for enkephalin, dynorphin, glutamic acid decarboxylase, somatostatin, substance P, neuropeptide Y, tyrosine hydroxylase, and subtypes of dopamine and glutamate receptors. In the human striatum dynorphin-containing cells were predominantly localized to the patch compartment, enkephalin-containing cells were predominantly localized to the matrix compartment, while substance P-containing cells were localized to both compartments. In cocaine users enkephalin mRNA levels were found to be decreased in the caudate and putamen while dynorphin mRNA levels were found to be increased (primarily in the patch compartment of the putamen). No significant alterations were observed in the gene expression of substance P, somatostatin, neuropeptide Y, or the dopamine D1 receptor. Selective alterations in other transmitter systems in the basal ganglia were also apparent. Disruption of systems altering sensorimotor and cognitive function as well as neurochemical evidence of "craving" is hypothesized.

228.5

MAZINDOL TREATMENT OF COCAINE ABUSERS

Susan Stine, MD, PhD*

We have studied Mazindol , a dopamine uptake blocker, in the treatment of cocaine addicts. It is hypothesized that mazindol a commonly available anorectic agent may compete with cocaine at the active site and inhibit cocaine use and craving.

There is preliminary evidence from a pilot study that mazindol is effective in decreasing cocaine use in cocaine abusers. The present study is a 12 week double-blind, randomized placebo-controlled treatment protocol. All subjects meet DSM III R criteria for current cocaine dependence, are in good health, between the ages of 20 and 60 and report minimum of 14 gms of total cocaine use during the 12 weeks prior to seeking to treatment. Patients who meet DSM III R criteria for substance dependence disorder other than cocaine or meet criteria for major psychiatric disorder other than depression and anxiety disorder are excluded. Subjects receive 2 mg/day of mazindol (or matching placebo tablets) and receive weekly relapse prevention therapy.

The primary outcome measures are cocaine use as determined by urine toxicology and self report, and "desire to use" measured by a standard scale. Other psychological symptoms as well as serum indicators of dopaminergic function (plasma homovanillic acid and prolactin levels) are obtained as hypothesis generating exploratory analyses as well as indicators of imbalance between groups. 24 subjects have been recruited to date and 11 of those have completed the study (active mazindol n=5, placebo n=6). No significant effect has been observed on cocaine use (urine toxicology or self-report) or desire to use. No difference between groups has been observed for depression or anxiety and mazindol has made no effect on these parameters. This study is continuing and data will be presented with a larger number of subjects (goal is 60 subjects).

228.6

TREATMENT OF NEGATIVE SYMPTOMS AND CRAVING IN COCAINE-ABUSING SCHIZOPHRENICS WITH MAZINDOL. R. M. Johnson, J. J. Erdos*, J. P. Seibyl, D. Miles, D. S. Charney, J. H. Krystal, Schizophrenia Biological Research Center, West Haven VA Med. Center and Dept. of Psychiatry, Yale Univ., New Haven, CT 06510.

Despite the high prevalence and significant morbidity associated with cocaine abuse in schizophrenic patients, there has been little study or effective pharmacotherapies for this population. This study evaluates the efficacy of the dopamine reuptake blocker, mazindol, in the treatment of cocaine-abusing schizophrenics. METHODS: Outpatients meeting DSM-III-R criteria for schizophrenia and concomitant cocaine abuse on a stable dose of neuroleptics were enrolled in a 4 week baseline and 8 week double blind placebo-controlled augmentation trial of oral mazindol at a dose of 2 mg/d. The patients were rated with the following outcome measures on a weekly basis; PANSS, AIMS, Webster Scale for Extrapyramidal Symptoms, Cocaine-Craving Scale, and self-reported cocaine use. Additionally, twice weekly urine toxicology screens were done. RESULTS: In an ongoing study, 4 subjects showed an 11% reduction in negative symptoms on placebo and a 37% reduction in negative symptoms on active mazindol as measured by the PANSS. Although none of the 4 subjects completely stopped cocaine use, mazindol appeared to have an advantage over placebo in decreasing the amount of craving and frequency of cocaine use. IMPLICATIONS: If effective in a larger sample, mazindol may be useful in the treatment this refractory subset of schizophrenic patients. It also may provide clues to neurobiological aspects of cocaine use in this population.

228.7

COCAINE SENSITIZATION IN ROMAN HIGH AND LOW AVOIDANCE RATS IS MODULATED BY SEX AND GONADAL HORMONE STATUS M. Haney*, N. Castanon, M. Cador, M. Le Moal and P. Mormède, INSERM U 259, INRA, Université de Bordeaux II, Bordeaux FRANCE

Roman high avoidance (RHA) and Roman low avoidance (RLA) male rats differ in reactivity to stressful stimuli and sensitivity to amphetamine. The following experiment compares the interaction between sex and gonadal hormone status on the acute and chronic effects of cocaine in the two strains. Adult male and female, RHA and RLA rats were housed in same-sex groups of three. Half the animals were gonadectomized (GDX) while the others were left intact. Sixteen days after gonadectomy, all rats were habituated to an activity cage (1 hr) and then administered cocaine hydrochloride (10 mg/kg IP) during the dark phase of the light cycle. Activity was measured for 2 hrs. This procedure was repeated twice/week for 5 weeks. Overall, RHA rats had higher baseline activity levels and were more sensitive to acute cocaine-induced locomotor enhancement than RLA rats. Following repeated administration, sensitivity to cocaine increased 100-500% above the response to the first injection; the per cent change was greatest in the RLA strain, such that sensitized RLA rats no longer differed from RHA rats in their response to cocaine. Within each strain, females were more sensitive to acute cocaine than males, particularly during proestrus. Females also became more sensitized to repeated cocaine administration than males. GDX did not modulate the effects of acute cocaine, but did significantly attenuate cocaine sensitization in males and females of both strains. Therefore, the factors of sex and gonadal hormone status similarly modulated cocaine sensitization in RHA and RLA rats. In addition, strain differences in acute cocaine sensitivity diminished with repeated drug administration, due to the greater degree of sensitization in RLA rats.

228.8

COCAINE-INDUCED TURNING BEHAVIOR IN RATS WITH UNILATERAL LESION OF THE DOPAMINE PROJECTION: EFFECT OF FETAL MESENCEPHALIC TRANSPLANTS

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Cocaine elicits strong asymmetrical locomotor activity in rats with unilateral 6-hydroxydopamine lesion of the dopaminergic (DA) nigrostriatal pathway. This rotational behavior is directed toward the lesioned side (i.e. ipsiversive). In the present study, fetal brain tissue containing DA cells, was grafted over the DA-denervated striatum in order to provide a partial reinnervation of the striatum, and the transplanted rats were subsequently evaluated for their response to cocaine. After transplantation, a strong contraversive rotational behavior in response to cocaine was observed; this was never observed in lesioned rats without transplant or with sham transplant.

We also examined the effect of repeated exposure to cocaine on the turning behavior in lesioned rats with and without transplants. Following daily administration of cocaine (25 mg/kg per day for 5 days) marked "sensitization" to cocaine was observed: We found an increase of 3-4 fold in a) the rate of ipsiversive turning in lesioned rats without transplants and b) in the rate of contraversive turning in rats with transplants. These data suggest that the transplant-reinnervated striatum responds in an exaggerated fashion not only to the acute effects of cocaine but also to the actions of cocaine responsible for the development of sensitization.

228.9

ACUTE COCAINE TREATMENT DECREASES OXYTOCIN LEVELS IN THE HIPPOCAMPUS. C.H. Walker, J.M. Johns, J.D. Caldwell, A.J. Prange* and C.A. Pedersen. Dept. of Psychiatry, Univ. of North Carolina, Chapel Hill, N.C. 27599.

Oxytocin (OXT) has been suggested as an endogenous mediator of the effects of cocaine and estrogen. We studied the effects of acute cocaine treatment on OXT using twenty female Sprague-Dawley rats (250-275g) that were ovariectomized and one week later received either two consecutive days of s.c. injections b.i.d. of saline or 15 mg/kg of cocaine HCL in conjunction with 10μg of estradiol benzoate i.m.. On the third day the animals were killed and tissues taken for measurement of oxytocin by RIA. The whole hippocampus, ventral tegmental area (VTA) and amygdala were removed from each animal and analyzed for OXT immunoreactive content. Mean picograms per area and per mg tissue were compared for the two groups using an analysis of variance. Acute cocaine treatment significantly reduced oxytocin levels in the hippocampus both in picograms/area ($1,17-F=7.49$, $p<.01$) and in picograms/mg ($1,17-F=4.89$, $p<.04$). Levels in the VTA and amygdala were not significantly different between groups.

228.10

COCAINE ANALGESIA: STRUCTURE-ACTIVITY RELATIONSHIPS. J.Rice, G.Scheulke, K.Weir, R. Powers, Z.J.Bosnjak , L.C.Terry, Dept. of Neurology, Medical College of Wisconsin, Milwaukee, WI 53226.

Cocaine produces supraspinal, non-opiate analgesia in rats that is believed to be mediated, at least in part, by dopamine (Lin et al., Brain Res. 479:306, 1989). The purpose of this study was to determine the analgesic properties of several cocaine metabolites and analogs. Also, brain cocaine (COC) levels were measured using GCMS. Adult rats (N=6-12 per group) were injected intracerebroventricularly (icv) with COC, cocaethylene (COCE), benzoylecgonine (BE), norcocaine (NORCOC), ecgonine (EC), tropaine (TROP), tropacocaine (TRPCOC), and ecgonine methyl ester (EME). Analgesia was assessed using a standard hot plate method (Lin et al., 1989). Cocaine (.370 mcM, potency=1.00) and COCE (.075 mcM) both induced short-term analgesia (10 min); COCE was approx. 5 times more potent. Brain COC levels decreased rapidly (30 min) from 70 to 5 ug/g tissue. EC, TRPCOC, and TROP produced analgesia but were less potent than cocaine (.18, .62, & .11 relative potency, respectively). Both BE and NORCOC produced longer acting analgesia (approx. 60 min.) with potencies similar to COC (1.06 & .860, respectively). EME did not cause analgesia in doses ranging from .3 to 3.7 mcM. The results indicate that: (1) N-demethylation, hydrolysis of the C2 methyl ester group and addition of a carbon molecule at the C2 methyl group of cocaine increase its analgesic properties and/or the duration of analgesia; (2) loss of the C3 benzoxy group attenuates analgesic activity; (3) cocaethylene is a fast-acting and potent analgesic; and (4) brain cocaine levels decline rapidly and correlate with the loss of analgesic activity.

228.11

INHIBITION OF COCAINE-INDUCED SEIZURES AND ANALGESIA BY ECGONINE METHYL ESTER. G.Schuelke, K.Weir, L.C.Terry, Dept. of Neurology, Medical College of Wisconsin, Milwaukee, WI 53226.

Previous studies in rats showed that cocaine-related tropanoid compounds cause thermal (hot plate) analgesia and seizures following intracerebroventricular (icv) injection. These studies showed that several cocaine analogs cause analgesia for up to 60 minutes. Of the tropanoids tested to date, only ecgonine methyl ester (EME) failed to cause seizures, analgesia, or other psychomotor/behavioral effects. The purpose of this study was to determine if EME inhibited cocaine-induced analgesia and seizures. Rats were injected icv with 3.7mcM EME, rested 5, 10, or 15 minutes, given 0.44mcM cocaine icv, and tested for hot plate analgesia. EME caused significant inhibition of cocaine analgesia. Inhibition was not seen when cocaine was injected simultaneously with EME or $\geq$15 minutes after EME. A similar protocol was used to investigate EME inhibition of cocaine-induced tonic/clonic seizures. In this experiment, 2.37, 1.78, or 1.18mcM of cocaine was injected 2 minutes after EME 3.7mcM icv. EME decreased the duration of seizures induced by 2.37mcM of cocaine icv but did not prevent them. Treatment with 1.78mcM of cocaine produced seizures in 33% (4 of 12) of EME-treated rats vs. 88% (8 of 9) control rats (P=.03) with the average time of seizures being 1.6 minutes in the EME-treated rats and 7.8 in controls. Administration of 1.18mcM cocaine produced seizures in 15% of EME treated rats (N=20) and 58% of saline controls (N=19; P=0.01). The data show that EME inhibits cocaine-induced analgesia and seizures. Time- and concentration-dependency of this inhibition suggest that EME binds competitively to CNS cocaine receptors and blocks at least some of it's central effects. These findings may be of possible clinical significance in the treatment by EME or related compounds of cocaine-induced seizures and other clinical effects.

228.12

PHYSIOLOGICAL ACTIONS OF COCAINE IN SENSORY CIRCUITS: I. ENHANCEMENT OF RAT SOMATOSENSORY CORTICAL NEURON RESPONSIVENESS TO VIBRISSAE STIMULATION. I. Bekavac* and B.D. Waterhouse, Dept. of Physiol. and Biophys., Hahnemann U., Phila. PA 19102

Prominent among cocaine's psychostimulant actions are its ability to heighten awareness of the sensory surround and induce sensory hallucinations. While many studies have examined the cellular actions of cocaine in "reward" circuits of the brain, few have investigated the impact of cocaine on neuronal function in primary sensory circuits. The goal of this study was to characterize the effects of cocaine on somatosensory cortical neuronal responsiveness to peripheral activation of afferent synaptic pathways. Extracellular recordings were obtained from spontaneously active single units in the barrelfield cortex of halothane-anesthetized rats. Spontaneous firing rate and cellular responses to mechanical displacement of a single whisker were monitored before and after systemic administration of cocaine (0.25, 0.5, 1.0 and 2.0 mg/kg i.v.). Control responses to vibrissae stimulation consisted of an initial excitatory burst (E1), a post excitatory suppression of activity (I1) and a secondary excitatory discharge (E2). Cocaine effects on spontaneous discharge were variable but generally slight increases in firing rate were noted with low doses, up to 1.0 mg/kg; and marked suppression of activity observed at 2.0 mg/kg. After cocaine injection, E1 responses were unchanged or within (+/-) 20% of control; however, in 9 of 10 cells E2 responses were increased from 50 - 600% above control levels. Such facilitation was observed at doses as low as 0.25 mg/kg but most consistently at 0.5 - 1.0 mg/kg; whereas suppression of both evoked responses was seen at 2.0 mg/kg. Cocaine's effects on spontaneous and evoked discharge were rapid in onset with peak effects occurring at 6 min post-injection and recovery to control patterns of discharge observed by 20 min. These results indicate that cocaine consistently exerts a facilitating effect on a specific late component of cortical neuron responses to sensory stimuli. While the specific neural substrates responsible for this effect have not been identified, such findings demonstrate a clear impact of cocaine on sensory signal transmission at dosages which are capable of supporting behavioral reinforcement. (Supported by NIDA DA 05117)

228.13

PHYSIOLOGICAL ACTIONS OF COCAINE IN SENSORY CIRCUITS: II. DRUG- INDUCED ALTERATIONS IN RECEPTIVE FIELD PROPERTIES OF RAT SOMATOSENSORY CORTICAL NEURONS. B.D. Waterhouse* and I. Bekavac, Dept. of Physiol. and Biophys., Hahnemann U., Phila., PA 19102

In human subjects cocaine has been reported to produce alterations in sensory perception as well as a generalized state of well being. Although considerable effort has been focused on identifying the neural basis of cocaine's euphorogenic properties, there have been few studies aimed at investigating the drug's influence on sensory signal processing. In a companion study we have reported that sytstemically administered cocaine (0.25-1.0 mg/kg, i.v.) can enhance rat somatosensory cortical neuronal responsiveness to mechanical displacement of mystacial vibrissae. The goal of the present experiments was to determine if cocaine exerts a uniform facilitating effect on cortical cell responsivensess to stimulation of different portions of its receptive field (RF). Extracellular recordings were obtained from single units in the barrelfield cortex of halothane-anesthetized rats. Cellular responses to mechanical displacement of "central" and "peripheral" whiskers were monitored before and after systemic administration of cocaine (0.25,0.5,1.0 and 2.0 mg/kg i.v.). Control responses to vibrissae stimulation consisted of an initial excitatory burst (E1) sometimes followed by a post excitatory suppression of activity (I1) and a secondary excitatory discharge (E2). For a given cell, displacement of the "central" whisker produced the most robust response whereas equivalent stimulation of an adjacent "peripheral" whisker produced a lesser response. After injection of cocaine at 0.25, 0.5 or 1.0 mg/kg the magnitude of responses to central whisker stimulation were increased or unchanged from control. Over the same dose range responses to peripheral whisker displacement were progressively reduced, sometimes to the point of complete suppression at 1.0 mg/kg. These results suggest that a net action of systemic cocaine on sensory cortical neurons is to differentially suppress responses from the periphery of the RF while preserving or increasing responses to central RF stimulation. Such drug-induced alterations in the functional dimensions of sensory neuron RF's could lead to changes in sensory perception such as those reported after cocaine self- administration. (NIDA DA05117)

228.14

PHYSIOLOGICAL ACTIONS OF COCAINE IN SENSORY CIRCUITS: III. EFFECTS ON INTRINSIC MEMBRANE PROPERTIES OF RAT SOMATOSENSORY CORTICAL NEURONS. F.M. Sessler,* R.D. Mouradian, C.-S. Lin and B.D. Waterhouse, Dept. of Physiol. and Biophys., Hahnemann Univ., Philadelphia, PA 19102.

The somatosensory cortex, a major target area for monoaminergic projections, contains an heterogeneous neuronal population which is believed to express different complements of membrane receptors, signal transduction mechanisms and ions channels. These neurons can be classified according to unique morphology, electrophysiology and synaptic connectivity. This heterogeneity suggest that classes of neurons may be more sensitive to the action of cocaine than others. Thus, it may be necessary to obtain specific information concerning the influences of cocaine on individual cellular components of the cortical circuitry in order to fully comprehend the potential impact of this drug in the signal processing capabilities of a sensory cortical network. To address this question, experiments were conducted using an in vitro brain slice preparation from rat somatosensory cortex. Microelectrodes (3% neurobiotin in 1 M KCl) were used for electrophysiological and morphological characterization of individual layer V cortical neurons. Excitatory postsynaptic potentials (EPSP) were evoked by stimulating the cortical white matter (WM). Cocaine was applied at various concentrations (0.3-100uM) to examine the full spectrum of the drug's effects. Bath application of cocaine did not produce significant changes in membrane potential and input resistance in most of the neurons recorded, whereas cell excitability, as measured by depolarizing current pulses, was increased by drug application in several cases. Cocaine (30- 100uM) reversibly decreased the amplitude and duration of EPSPs and also reduced the number of evoked action potentials. At lower doses (1-10uM), cocaine produced enhancement of neuronal excitability in response to both, current pulses and stimulation of WM. Preliminary data indicate that cocaine-induced effects may vary across different types of somatosensory neurons. These results support the hypothesis that cocaine actions on primary sensory cortices may involve differential effects of the drug on different electrophysiologically- or morphologically- characterized subpopulations of neocortical neurons. (Supported by NIDA DA05117).

228.15

EVOKED POTENTIAL EXCITABILITY CYCLES DURING EARLY COCAINE ABSTINENCE Richard A. Roemer* and Charles Shagass
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We report on somatosensory evoked potential (SEP) excitability cycles, using right median nerve stimulation, recorded in 22 males who were early abstinent chronic cocaine abusers (median 11 days abstinent). They are compared to age-matched male controls in terms of amplitude and topography of 8 SEP events occurring the first 65 msec poststimulus to the conditioning stimulus alone and to pairs of stimuli at seven ISIs. The ISIs ranged from 5 to 90 msec.

Group by Lead differences were found at 10 msec ISI for P14, N19, N31, and P45; at 55 msec ISI for P28, P39, P45, and N65; and at 90 msec ISI for P28, N31, and N65.

These data indicate differences in SEP recovery between anterior and posterior scalp regions in relation to exposure to cocaine. The data support the viability of SEP recovery cycle studies in demonstrating CNS alterations subsequent to chronic cocaine use.

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228.16

METABOLIC RECOVERY DURING COCAINE WITHDRAWAL, B.B. Young*, S.J. Lee, W.S. Pires, E.S. Cooke and R.P. Hammer, Jr., Lab. of Cellular & Molecular Neuropharmacology, Dept. Anatomy & Reprod. Biology, Univ. Hawaii Sch. Med., Honolulu, HI 96822

Withdrawal following chronic cocaine treatment is known to produce short-term reduction of brain metabolic activity (Clow and Hammer, *Neuropsychopharmacol.*, 4: 71-75, 1991). The present study examined metabolic recovery over time during cocaine withdrawal, and following pharmacotherapy with BMY 14802. Adult male rats were treated for 14 days with saline vehicle or cocaine HCl (10 mg/kg) followed by 3 or 7 days of water vehicle or BMY 14802 (10 mg/kg). Regional cerebral metabolic rate (rCMR) was measured using the quantitative method of Sokoloff; autoradiographic analysis was used to determine rCMR values in 61 brain regions. rCMR was reduced in the infralimbic medial prefrontal cortex after 3 and 7 days of withdrawal. The 3 day withdrawal group showed significant reductions in the nucleus accumbens core and shell, olfactory tubercle, dorsolateral ventral pallidum, globus pallidus, entopeduncular nucleus, central amygdala, lateral hypothalamus, substantia nigra, pars reticulata and central gray compared to the control group, while decreases were observed only in the globus pallidus, entopeduncular nucleus, medial habenula, and entorhinal cortex after 7 days. Treatment with BMY 14802 enhanced metabolic recovery at an earlier time point in most regions. The results suggest that recovery from the metabolic effects of cocaine withdrawal occurs in the mesolimbic system in as little as one week, while sustained deficits are still present in extrapyramidal efferent zones. Treatment with an indirect dopamine agonist can further enhance metabolic recovery. Supported by USPHS awards DA06645, RR08125, and HD01161.

228.17

CYTOTOXICITY OF THE COCAINE METABOLITE BENZOYLECGONINE.
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We have previously reported that the major metabolite of cocaine, benzoylecgonine (BE), increased glycosphingolipid synthesis by glial cells (C6) but not neuronal cells (NG108-15) in culture. This was observed at low BE concentrations (10 μM or less). At higher BE concentrations glycosphingolipid synthesis appeared to decrease, but it was found that this was due to a loss of cells from the substratum.

We have recently found that this loss is not due to BE altering substratum interactions of C6 or NG108-15 cells, but that BE exerts cytotoxic effects. This was demonstrated by decreased cell viability, as determined by the activity of mitochondrial dehydrogenases, with increasing BE concentrations (from 0 to 100 μM). Also, detached C6 and NG108-15 cells were not viable, as determined by Trypan Blue inclusion and determination of DNA synthesis using ^{3}H-thymidine.

Early events in these interactions were studied by examining cells cultivated on the stage of an inverted microscope using differential interference contrast (Nomarski) optics. Retraction of cellular processes within 15 minutes could be observed when both cell types were cultured in the presence of 50 μM BE. Eventual loss of cells could be observed within 3 hours. When the media concentration of BE was reduced to 10 μM, retraction of processes could again be observed, but the time course was slightly longer (30 minutes or more). Cultures of primary astrocytes exhibited similar morphologic reactions. Under these experimental conditions control cells remained viable and extended processes.

These results demonstrate that benzoylecgonine is cytotoxic to both neuronal and glial cells at concentrations near those found in fetal brain following prenatal cocaine exposure.

228.18

GENDER DIFFERENCES IN PLACE PREFERENCE INDUCED BY ICV INJECTIONS OF COCAINE. S. Wang* and G. A. Barr. Biopsychology Doctoral Program, Dept. of Psychology, Hunter College-CUNY, NY, NY 10021 and Dept. Develop. Psychobiology, New York State Psychiatric Institute, NY, NY 10032.

Conditioned place preference (CPP) has been used to study the reinforcing properties of cocaine and other abused drugs. Most investigators have used male rats even though it has been reported that many of the behavioral responses to psychostimulants are gender dependent. In the present experiment, male or female adult rats were injected with cocaine HCl (0, 10, 30, or 100 μg) or saline into the lateral ventricle through indwelling cannulas. Immediately following injection, animals were individually confined into one of two distinguishable sides of a chamber for 30 minutes. Eight hours later, rats were confined to the opposite side and injected with the opposite treatment (cocaine or saline). Training lasted 4 days. On the 5th day, rats were tested for a side preference in a 20 minute test using a three chamber test apparatus. Females demonstrated a dose-dependent preference for the side paired with cocaine, preferring the two highest doses. In contrast, male rats displayed a strong aversion to the side paired with the drug. These effects lasted for several extinction trials. This result was not found when cocaine was injected peripherally. The results indicate a distinct gender difference in the reinforcing properties of cocaine and indicate a central site of action. Further investigation is needed to explain the mechanisms of this result. (Supported in part by DA-06600)

DRUGS OF ABUSE: NICOTINE, COCAINE, ET AL.

229.1

THE SELECTIVE 5-HYDROXYTRYPTAMINE1a AGONIST LY274600 EXACERBATES INCREASES IN SENSORIMOTOR REACTIVITY RESULTING FROM WITHDRAWAL OF CHRONIC NICOTINE. D. Helton*, K. Rasmussen, D. Modlin, J. Barrett, and J. Tizzano. Lilly Research Labs, Eli Lilly and Company, Greenfield, IN, 46130.

Cessation of chronic nicotine (NI) in rats leads to increased startle responding during the first 5 days of nicotine withdrawal. The present study evaluated the effects of pretreatment with 1) the known anxiolytic diazepam, 2) the 5-HT$_{1a}$ agonist LY274600, and 3) NI replacement on auditory startle responding following NI withdrawal. NI (6 mg/kg/day) was continuously administered for 12 days in rats by surgically implanting osmotic pumps (sc). Diazepam (3 mg/kg, ip), LY274600 (.3, 1, 3 mg/kg, sc), and NI (.01, .03, .1, .5 mg/kg, ip) were given 15 minutes before daily evaluation of startle responding for 5 days following cessation of NI exposure. NI replacement reversed the increased reactivity following the termination of chronic NI for 5 days. In addition, diazepam attenuated the increased reactivity observed for 5 days at a dose which did not alter startle responding in naive rats. In contrast, the selective 5-HT$_{1a}$ agonist LY274600 exacerbated withdrawal at doses which did not alter startle responding in naive rats. These results suggest that 5-HT$_{1a}$ agonists may not be useful for attenuating the withdrawal symptoms associated with the cessation of chronic NI exposure.

229.2

NEUROPHYSIOLOGICAL EFFECTS OF SYSTEMIC NICOTINE ON NEURONS IN THE NUCLEUS ACCUMBENS. C. Hart, A. Strauss, and R.L Hakan*. Dept. Psychology, UNC-W, Wilmington, NC 28403

Extracellular recordings of single neurons within the nucleus accumbens (NAS) of halothane anesthetized rats have revealed that systemic nicotine injections (1.0 mg/kg) inhibit the action potentials evoked from normally inactive NAS neurons by fimbria stimulation (Fimbria-driven responses, n= 18/20). These nicotine inhibitions of fimbria-driven NAS action potentials appear to be centrally mediated because they were reversed by subsequent injections of mecamylamine (1.0 mg/kg, s.c., n=6/6) but not by hexamethonium (2.0 mg/kg, s.c., n=0/6). Nicotine induced inhibitions of NAS fimbria-driven units were followed in some experiments by halperidol (0.5 mg/kg, s.c.), in attempts to reveal the possible role of dopamine in these effects. Halperidol was successful at reversing nicotine inhibitions in only a few cases (n= 2/6). In contrast to the fimbria-driven responses, spontaneously active NAS neurons were not affected by nicotine injections. Iontophoretic studies to further localize the site of this nicotine action on NAS neurons are in progress.

229.3

NALOXONE PRECIPITATES NICOTINE ABSTINENCE SYNDROME IN RAT. D.H. Malin, V.A. Carter, J.R. Lake, J.S. Cunningham and O.B. Wilson*[1]. Univ. of Houston-Clear Lake, Houston, TX 77058 and [1]Baylor College of Medicine, Houston, TX 77030.

At last year's meeting this laboratory reported a rapid and convenient rodent model of nicotine abstinence syndrome based primarily on frequency of spontaneous behavioral signs resembling those seen in opiate abstinence syndrome. Might there be an endogenous opioid component in nicotine dependence? The present study determined whether a moderate dose of naloxone s.c. could precipitate an abstinence syndrome in nicotine-dependent rats. Fourteen male Sprague-Dawley rats were continuously infused s.c. for 7 days with 9 mg/kg/day nicotine tartrate in saline via one Alzet osmotic minipump. Fourteen rats were sham-operated and remained nicotine-naive. Half of each group received 4.5 mg/kg naloxone s.c. immediately before a 15 minute observation. The remaining rats received saline s.c. All observations were performed under "blind" conditions. Anova revealed significant drug infusion, drug injection and interaction effects. Post hoc analysis (Dunnett's) revealed that the nicotine-dependent + naloxone group exhibited significantly more overall abstinence signs than all other groups, $p<.01$. This group differed significantly from all others on writhes/gasps, chews, genital licks, and shakes/tremors. (Supported by Neuromedical Technology, Inc.)

229.4

EFFECTS OF PRENATAL COCAINE ON ADULT SEXUAL BEHAVIOR AND ON BRAIN CATECHOLAMINES IN RATS. Ilona Vathy* and Livia Katay. Dept. Psychiatry, Albert Einstein Coll. Medicine, Bronx, NY 10461.

Female rats exposed to cocaine hydrochloride (C) in utero (10 mg/kg twice a day on days of 11-18 of gestation) were significantly inhibited in their adult sexual behavior when compared to saline-exposed (S) controls. In contrast, males exposed prenatally to C exhibited increased mounting and intromitting activity and had shorter post-ejaculatory intromission intervals relative to controls. Examination of the catecholamine content in the hypothalamus, preoptic area (POA), striatum, cortex and cerebellum revealed that prenatal C treatment affects males and females differently. Prenatally C-exposed male rats had significantly higher norepinephrine (NE) and dopamine (DA) levels when compared to S-treated controls. In contrast, NE and DA content in the POA of S and C-treated females were essentially identical. Neither NE nor DA content in other brain regions of C-treated male and female rats were different from S-treated controls. These results suggest that prenatal C exposure, which differentially affects adult male and female sexual behavior, also alters the content of catecholamines in the POA in a sexually dimorphic fashion. Supported by NIDA DA 05833.

229.5

EFFECTS OF CONTINUOUS COCAINE ADMINISTRATION ON OPIOID-REGULATED ADENYLYL CYCLASE ACTIVITY IN RAT NUCLEUS ACCUMBENS. S. Izenwasser* and B.M. Cox. Department of Pharmacology, Uniformed Services University, Bethesda, MD and *Psychobiology Laboratory, NIDA Addiction Research Center, Baltimore, MD.

Cocaine is a psychomotor stimulant that inhibits the reuptake of dopamine into presynaptic dopaminergic terminals. Pretreatment with cocaine has been shown to alter opioid receptor densities in rat brain. Opioid receptor activation leads to inhibition of adenylyl cyclase activity. To investigate the functional consequences of cocaine-induced opioid receptor changes, adenylyl cyclase activity was measured in rat nucleus accumbens following continuous cocaine administration. Male Sprague-Dawley rats were chronically treated with either cocaine (50 mg/kg/day, expressed as free base), or saline (0.9% sodium chloride; 24 µl/day) via subcutaneously implanted osmotic minipumps. After 7 days, the effects of DAMGO, the selective µ-opioid receptor agonist, and the selective δ-opioid receptor agonists DPDPE and DSLET were examined on adenylyl cyclase activity in crude membrane preparations of the nucleus accumbens. Adenylyl cyclase activity was measured with a cAMP radioligand binding assay. There was no change in basal adenylyl cyclase activity in the nucleus accumbens of the animals treated with cocaine as compared to those receiving saline. Treatment with cocaine, however, led to an increase in the maximal inhibition of adenylyl cyclase by DAMGO but had no effect on DPDPE or DSLET inhibition in the nucleus accumbens. These findings suggest that continuous cocaine administration for 7 days results in a selective increase in µ-opioid receptor mediated effector function in the nucleus accumbens. (Supported in part by a grant from NIDA to BMC).

229.6

CHANGES IN BASAL AND OPIOID-REGULATED ADENYLYL CYCLASE ACTIVITY IN RAT NUCLEUS ACCUMBENS AND CAUDATE-PUTAMEN FOLLOWING REPEATED, DAILY COCAINE ADMINISTRATION. E. M. Unterwald*, S. Izenwasser, T.E. Cote, M.J. Kreek, and B.M. Cox. The Rockefeller University, New York, NY and Uniformed Services University, Bethesda, MD.

Cocaine binds to the dopamine transporter and prevents the reuptake of dopamine into presynaptic dopaminergic terminals. Repeated daily cocaine injections have been shown to alter opioid receptor densities in rat brain (Unterwald et al., Brain Res., in press). Adenylyl cyclase activity was measured in rat accumbens and caudate following repeated cocaine administration to determine the functional consequences of cocaine-induced opioid receptor changes. Male Fischer (CDF) rats were injected daily with saline or cocaine HCl (30 mg/kg/day i.p.) in three equal doses over a two hour period for 14 days. The effects of DAMGO (a selective µ-opioid receptor agonist) and DPDPE (a selective δ-opioid receptor agonist) on adenylyl cyclase activity in the nucleus accumbens and the rostral portion of the caudate-putamen were examined using a cAMP radioligand binding assay in crude membrane preparations. There was a 50% decrease in basal adenylyl cyclase activity in the caudate of animals treated with cocaine as compared to those receiving saline injections. In the nucleus accumbens, however, basal adenylyl cyclase activity was unchanged following the cocaine treatment. DAMGO and DPDPE maximally inhibited approximately 25% and 30%, respectively, of basal adenylyl cyclase in saline-treated animals. Treatment with cocaine attenuated the ability of DPDPE to inhibit adenylyl cyclase in both brain regions, but had no effect on the efficacy or potency of DAMGO for inhibiting adenylyl cyclase activity. These results suggest that chronic, repeated cocaine administration results in a selective impairment of δ-opioid receptor mediated effector function in nucleus accumbens and caudate-putamen. [Supported by grants from NIDA (BMC, MJK) and Aaron Diamond Foundation (MJK)].

229.7

COCAINE'S BEHAVIORAL AND LETHAL EFFECTS UPON THE DEVELOPING CHICKEN FETUS ARE BLOCKED BY TREATMENT WITH THE 5-HT1c/5-HT2 ANTAGONIST RITANSERIN. S.B.Sparber*, D.G.Kim and E. Kostarczyk, Dept. of Pharmacology, Univ. of Minn., Mpls. MN 55455.

Cocaine, injected late during development of domestic chick fetuses(i.e. day 19 of incubation) reduces motility shortly after injection, reduces hatchability and causes behavioral changes in chicks for up to 2 weeks after hatching(FASEB J. 5:A1588,1991). This laboratory has published numerous reports on the efficacy of 5-HT1c/5-HT2 antagonists against the expression of opiate and quaisi-opiate withdrawal. Because of similarities between acute effects of cocaine and withdrawal from opiates (e.g. autonomic activation, aroused CNS) we examined the therapeutic potential of a 5-HT1c/5-HT2 antagonist for its capacity to block one or more of cocaine's effects upon the developing chick fetus. In the P.M. of day 17 of incubation ritanserin(0.4 mg/kg egg) or tartrate (0.05M) was injected(20 µl) into chick eggs 2 cm below the air cell. In the A.M. of day 18, starting 10 hr after ritanserin, cocaine HCl(22.5 or 67.5 mg/kg egg) was injected and fetal motility was monitored. Moreover, additional eggs were injected and allowed to hatch. This dose of ritanserin blocked the significant reduction in motility 15-20 min after the low dose of cocaine only. It also blocked the reduction in hatchability of both doses of cocaine. These data confirm our hypothesis that some of the acute toxic effects of cocaine in the chick fetus are mediated indirectly by activation of 5-HT1c/5-HT2 receptors. It remains to be determined if potential functional teratogenic effects of cocaine can also be attenuated or prevented by this class of 5-HT antagonists in this species and in mammalian species as well.

Supported in part by USPHS grant DA04979.

229.8

EFFECT OF A CHIRAL 4-ALKYL SUBSTITUENT IN HALLUCINOGENIC AMPHETAMINES. Robert Oberlender*, P. V. Ramachandran, Michael P. Johnson, Xuemei Huang and David E. Nichols. Departments of Medicinal Chemistry and Pharmacognosy, Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, and H. C. Brown and R. B. Wetherill Laboratories of Chemistry, Purdue University, West Lafayette, IN 47907.

The hallucinogenic amphetamines have recently attracted wide-spread interest, as members of this drug class have become increasingly useful as selective and potent agonists at the 5-HT_2 subtype of serotonin receptors. This investigation was directed toward evaluating the effects of chirality in a branched alkyl group at the 4-position of a 2,5-dimethoxy-substituted hallucinogenic amphetamine analogue. A pair of novel compounds related to the hallucinogen 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) was prepared containing either the *R*- or *S*-2-butyl substituent in place of the 4-methyl group. The synthetic method included an asymmetric hydroboration reaction. No significant difference in the activity of these analogues was observed in male rats for either LSD-like dicriminative stimulus properties or affinity for the agonist-labeled serotonin 5-HT_2 binding site. However, both diastereomers had high affinity for the serotonin 5-HT_2 receptor, but lower behavioral potency than DOM, suggesting that the 2-butyl homologues might have decreased agonist efficacy.

229.9

REGULATION OF ANDROGEN RECEPTOR IMMUNOREACTIVITY BY ANABOLIC STEROIDS IN INTACT AND CASTRATED MALE RAT BRAINS. C. S. Menard* and R. E. Harlan, Dept. of Anatomy, Tulane Med. School, New Orleans, LA 70112.

In an attempt to characterize changes in the central nervous system that occur with anabolic steroid abuse in humans, immunocytochemical localization of androgen receptors in the rostral brains of male rats was conducted after the administration of high levels of anabolic steroids. Specifically, 12 castrated and 12 intact male rats received 14 daily injections of sesame oil vehicle or a cocktail of 2mg/kg testosterone cypionate, 2 mg/kg nandrolone decanoate, and 1 mg/kg boldenone undecylenate. Immunocytochemistry was performed with 1 µg/ml IgG-purified polyclonal rabbit 1° antibody (provided by Gail Prins). In intact males treated with sesame oil only, intense-moderate nuclear immunoreactivity indicated the presence of androgen receptors in many brain regions, including the cortex, septum, bed nucleus of the stria terminalis, preoptic area, hypothalamus, hippocampus, amygdala, midbrain central gray, substantia nigra, ventral tegmental area, and locus coeruleus. However, in castrated males treated with vehicle, immunoreactivity was reduced or absent, suggesting that endogenous androgen levels are necessary for the normal immunoreactivity of these receptors. In castrated and intact males treated with anabolic steroids, the distribution and number of immunoreactive cells were equivalent to that found in intact, oil-treated males. However, the intensity of staining of immunoreactive cells in all anabolic steroid-treated rats was dramatically increased over that seen in intact, oil-treated rats. Therefore, these high levels of anabolic steroids appear to increase androgen receptor immunoreactivity. These results may suggest that androgen receptors in the brain are up-regulated by anabolic steroids. However, regulation of androgen-receptor gene expression must be examined before this relationship can be established. Nevertheless, these results identify the distribution of one central nervous system mechanism modified by anabolic steroids. In addition, the increase in androgen-receptor immunoreactivity induced by anabolic steroids begins to characterize modifications in central nervous system functioning which underlie the psychotic, addictive, motoric, and sexual abnormalities reported when athletes abuse anabolic steroids at these high levels. Supported by NIDA Grant # DA-06194.

229.10

EFFECTS OF POLYAMINES ON PCP-INDUCED EEG AND BEHAVIOR IN THE RAT. D.F. Sisson*, L.R. King and J.E. Moreton, University of Maryland School of Pharmacy, Baltimore, MD 21201.

Phencyclidine (PCP) and PCP-like compounds bind with high affinity to a receptor within the ion channel of the NMDA-receptor complex blocking the channel. Recent studies have shown that the NMDA complex also contains a polyamine binding site. Agonists for the polyamine site, eg. spermine, enhance binding to PCP receptors; inverse agonists, eg. 1,10-diaminodecane (DA-10), decrease binding to PCP receptors; antagonists, eg. diethylenetriamine (DET), block the effects of either agonists or inverse agonists.

We tested the ability of DA-10 (250 nMole) and the polyamine antagonist arcaine (50 nMole), delivered intracerebroventricularly (icv), to attenuate the acute effects of intravenous (iv) PCP administration (1.25 and 2.5 mg/kg). Effects on behavior (locomotion, ataxia and stereotypy) were quantified with a rating scale (0-5). Effects of PCP on EEG power spectrum were measured with a Nicolet Pathfinder II.

Behaviorally, both DA-10 and arcaine significantly reduced PCP-induced ataxia and stereotypy while locomotor response was not affected. Neurally, these agents did not clearly affect PCP-induced augmentation of spectral power in the delta and theta bands of EEG. This dissociation of behavioral and neural effects could indicate that reduced ataxia and stereotypy are produced through mechanisms not specifically related to the PCP receptor. A more sensitive analysis of EEG power spectrum is in progress to determine if subtle neural changes were produced by DA-10 and arcaine.

This work is supported by NIDA grant DA03173.

229.11

EFFECTS OF CHRONIC EXPOSURE TO HIGH LEVELS OF TESTOSTERONE ON AGGRESSION AND SEXUAL BEHAVIOR IN MALE RATS. M.Y. McGinnis*, K.T. Thorner and A.R. Lumia. Dept. Cell Biol/Anat, Mount Sinai Sch. Med. New York, NY 10029 and Skidmore Coll., Biopsych. Prog., Saratoga Springs, NY 12866.

The substantial rise in reported abuse of anabolic steroids in humans has revived interest in the behavioral consequences of chronic exposure to androgens. In this experiment, the effects of chronic testosterone propionate (TP) treatment on intermale aggression and sexual behavior was examined in gonadally intact, male, Long-Evans rats. Sexually experienced males received three weekly injections of 1 mg TP, or propylene glycol (PG: control) for ten weeks. Animals were tested weekly for aggressive and sexual behaviors. Results indicate that TP-treated males displayed more dominance postures and fewer subordinate postures toward opponents than did PG-treated controls. The TP-treated rats also showed increases in the display of threats and initiated fewer attacks. There was no difference between groups in the frequency of approaches toward the opponent. These data suggest that the increase in dominance postures by TP-treated males is not due to increased contact with the opponent male (ie. approaches and attacks), but was due to an increase in threats directed toward the opponent. No effect of chronic TP administration on sexual behavior was found. The results suggest that anabolic steroids exert differential effects on aggressive and sexual behavior in gonadally intact male rats.

PSYCHOTHERAPEUTIC DRUGS: SIGMA RECEPTORS AND ANTIPSYCHOTICS

230.1

SIGMA BINDING IN RAT BRAIN IS DIMINISHED AFTER TREATMENT WITH HALOPERIDOL OR BMY 14802. Diana Marrero,[1] Doris M. Grimes,[2] R. Francis Schlemmer, Jr.,[2] and Duncan P. Taylor*.[1] [1] Biophysics and Molecular Biology, Bristol-Myers Squibb Co., Wallingford, CT 06492-7660; [2]Pharmacodynamics, University of Illinois, Chicago, IL 60612.

It has been proposed that agents with affinity for the σ binding site may be useful in the treatment of schizophrenia and psychosis. Rats were dosed intraperitoneally with haloperidol (2 mg/kg), the putative sigma antipsychotic BMY 14802 (10 mg/kg) or saline. Statistically significant decreases in the density of σ sites (B_{max}) were observed for the binding of (+)-[^{3}H]-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine [(+)-[^{3}H]-3-PPP] in the cortex of rats receiving either drug for 2 weeks or BMY 14802 for 3 weeks. In the cerebellum a trend was observed for a decrease in the B_{max} value for σ binding following 2 weeks' treatment with BMY 14802, while no statistical difference was observed in the B_{max} values following 3 weeks' treatment with either haloperidol or BMY 14802. No changes were seen in the affinity (K_D) of σ sites for (+)-[^{3}H]3-PPP with any treatment. These data confirm the work of others on the effect of chronic administration of haloperidol on σ binding and suggest that BMY 14802 may act at σ sites in the cortex and cerebellum in a similar manner after chronic administration.

230.2

IS THE RAT BRAIN *SIGMA* RECEPTOR THE SAME AS CYTOCHROME P_{450}? HALOPERIDOL DOWN-REGULATES *SIGMA* RECEPTORS BUT NOT CYTOCHROME P_{450}. S. W. Tam*, D. Rominger, S. Diamond and G.N. Lam. Central Nervous System Diseases Research and Drug Metabolism and Pharmacokinetics Section, The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880-0400

Studies were performed to investigate the relationship of sigma receptors with cytochrome P_{450}s in rat brain. Rats were chronically treated with haloperidol (5 mg/kg, s.c. daily) for two weeks, then the *sigma* receptor binding and cytochrome P_{450} activities were measured *in vitro*. The cytochrome P_{450} activities in rat cerebral cortical microsomal fractions were labile and were rapidly converted to P_{420}. Chronic treatment with haloperidol induced significant down-regulation (73-79%) of *sigma* receptor binding in cerebral cortex using either [^{3}H](+)-SKF 10,047, [^{3}H](+)-3-PPP or [^{3}H]DuP 734 as *sigma* ligands, but no significant change in the cytochrome P_{420} activities were detected in these tissues. The results suggest that the sigma receptor and cytochrome P_{450} may be different entities.

230.3

IN VIVO BINDING OF THE *SIGMA*/5-HT_2 RECEPTOR ANTAGONIST DuP 734 TO MOUSE BRAIN. M. Watanabe*, D. Rominger, E.B. De Souza and S. W. Tam. Central Nervous System Diseases Research, The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880-0400

In vivo receptor binding studies with DuP 734 have been performed to relate the behavioral effects to occupation of brain *sigma* receptors. DuP 734 administered orally to mice 30 min before i.v. injection of the *sigma* receptor ligand [^{3}H](+)-SKF 10,047 potently antagonized the binding of [^{3}H](+)-SKF 10,047 to mouse brain *sigma* receptors *in vivo* with an ED_{50} of 0.07 mg/kg, p.o.. Near maximal antagonism of [^{3}H](+)-SKF 10,047 binding was obtained at 0.3 - 1 mg/kg, p.o. DuP 734 which corresponds well to the ED_{50} dose of 0.35 mg/kg, p.o. in the mouse mescaline test. Preliminary *in vivo* binding studies demonstrated specific binding of [^{3}H]DuP 734 (56 - 72 %) to several brain regions in mice. [^{3}H]DuP 734 binding to cerebral cortex and cerebellum peaked around 45 min after i.v. injection and significant levels of specific binding were maintained when measured up to 4 hr post dose. Results on the pharmacokinetics and pharmacology of *in vivo* [^{3}H]DuP 734 receptor binding in mouse brain regions will be presented.

230.4

Administration of XJ448, a Novel Selective Sigma Receptor Ligand, Causes Regional Differences in Dopamine Turnover in the Rat. C.M. Krause, C.M. Rominger*, S.W.Tam and R.Zaczek, The DuPont Merck Pharmaceutical Co., Wilmington, Delaware.

The selective sigma receptor ligand XJ448 has been shown to antagonize the inhibition of dopamine neuronal firing induced by (+)-3-PPP. The effects of acute subcutaneous administration of XJ448 on the levels of dopamine, serotonin and their principal metabolites in rats were examined 1 hr after dosing. No changes in serotonin or 5-HIAA were observed after 1,3,9 or 27 mg/kg XJ448. Increases in dopamine turnover as indicated by elevations in the levels of dopamine metabolites DOPAC (16% above control) and HVA (32% above control) were observed in the medial frontal cortex after 3 mg/kg XJ448. Increasing the dose of the drug to 9 mg/kg produced a further increase in dopamine turnover in this region (increases of 40% and 57% above control for DOPAC and HVA , respectively). In other regions of the brain, higher doses of the drug were required to increase dopamine turnover. No increase in the level of DOPAC was observed in striatum, nucleus accumbens, hypothalamus, substantia nigra or ventral tegmental area at doses as high as 9 mg/kg of XJ448. In these regions, HVA was only marginally increased (less than 20% over control) at 9 mg/kg of the drug. XJ448 at a dose of 27 mg/kg produced increases in the levels of both dopamine metabolites in all regions studied. In contrast to the effect of XJ448 in preferentially increasing dopamine turnover in the frontal cortex over the striatum, haloperidol (0.1 mg/kg) induced an equal or greater increase in dopamine turnover in the straitum than in the frontal cortex. These results suggest that sigma receptors have a modulatory function on dopamine neuronal activity with a preferential effect in the frontal cortex.

230.5

SIGMA-SELECTIVE ANTIPSYCHOTIC COMPOUNDS: COMPARATIVE *IN-VIVO* PROFILES. J.F. McElroy*, K.A. Amy K.L. Zeller, J.F. Cawley, W.G. Carey, P.J. Gilligan, and G.F. Steinfels. CNS Diseases Research, The Du Pont Merck Pharmaceutical Co., Experimental Station, P.O. Box 80400, Wilmington, DE 19880-0400

Sigma receptors have been implicated in the etiology of psychosis and this receptor may provide a novel target for the discovery and development of antipsychotic agents. XJ448 (4-(2'-(4"-Cyanophenyl)-2'-oxoethyl))-1-(cyclopropylmethyl) piperidine, is a high affinity (Ki=9 nM) and selective ligand for the *sigma* binding site in rodent brain. *Sigma* receptor antagonists have also been discovered in the laboratories of Bristol Myers-Squibb (BMY14802), Burroughs Wellcome (Rimcazole), Merck (L687,384), and Nova (NPC16377). The present study compares XJ448 to these *sigma* antagonists in a battery of behavioral animal models that have predictive value for antipsychotic therapeutic activity and side effect liability. XJ448 is orally active in the Mouse Mescaline Scratch (ED_{50}=0.8 mg/kg) and Mouse Isolation-Induced Aggression (ED_{50}=1.5 mg/kg) tests and is weakly active in the Mouse Apomorphine-Induced Climbing test (ED_{50}=29.2 mg/kg). XJ448 reverses the inhibition of DA cell firing induced by the *sigma* agonist (+)-3PPP, consistent with the hypothesis that *sigma* receptors may modulate DA neuronal cell firing. The order of potency in these efficacy models predictive of antipsychotic therapeutic activity is XJ448 >> Rimcazole & BMY14802 > L687,384 > NPC16377. None of these *sigma* compounds produces catalepsy in rats, an animal model predictive of side effect liability. These results suggest that XJ448, might represent a novel antipsychotic drug with little propensity for the motor side effects (e.g., Tardive Dyskinesia) typically associated with antipsychotic drug treatment.

230.6

DuP 734 blocks the Electroencephalographic (EEG) *beta-2* increase induced by (+)-pentazocine in the Long Evans Rat. G. P. ALBERICI*, W. G. Carey, and G. F. STEINFELS. The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880-0400 USA.

DuP 734 a potent *sigma* and $5HT_2$ receptor ligand, with low affinity for the dopamine D2 receptor, is effective in behavioral tests indicating a possible antipsychotic use. DuP 734 was tested in the rat cortical background spectral EEG model. In these studies we used the change in *beta-2* (25-50 Hz) relative power (RP) as our measure of drug activity. (+)-Pentazocine (a potent *sigma* ligand) at 21.3 mg/kg sc induces an increase in *beta-2* RP activity. DuP 734 was administered alone or prior to (+)-pentazocine. DuP 734 by itself shows no significant change in *beta-2* RP at behaviorally active doses. However, DuP 734 dose dependantly blocks the (+)-pentazocine induced *beta-2* increase. Ketanserin (a $5HT_2$ antagonist), at 0.3 mg/kg sc, does not block the (+)-pentazocine induced *beta-2* RP increase. Based on this evidence, the rat EEG model *beta-2* RP measure may have utility as a measure of in vivo *sigma* receptor activity.

230.7

DUP 734 DIFFERENTIALLY ANTAGONIZES THE LOCOMOTOR STIMULANT EFFECTS OF COCAINE, D-AMPHETAMINE AND APOMORPHINE IN MICE AND RATS. L. Cook, J. F. McElroy and K. W. Rohrbach*, Central Nervous System Diseases Research, The DuPont Merck Pharmaceutical Company, Wilmington, DE 19880-0400

DuP 734 modifies the activity of many agents which are hallucinogens and stimulants. The evidence suggests that DuP 734 does this by modulating the presynaptic dopamine system, possibly through sigma receptors (Tam etal., JPET, submitted; Cook etal., JPET submitted). In the present studies, we tested DuP 734 in comparison with haloperidol and ketanserin for ability to modify the increases in locomotor activity induced by cocaine, D-amphetamine and apomorphine. DuP 734 significantly antagonized the locomotor stimulant effects of cocaine in both mice and rats. It did this at doses which did not effect baseline locomotor activity. DuP 734 selectively antagonized cocaine induced stimulation of locomotor activity in mice at 0.2 mg/kg p.o. and in rats at 2.3 mg/kg p.o. whereas the doses that decreased spontaneous baseline locomotor activity in mice and rats were 2.3 and >7.7 mg/kg p.o. respectively. DuP 734 antagonized the locomotor stimulant properties of D-amphetamine in mice at 0.2 mg/kg p.o., whereas in rats, it only antagonized the locomotor stimulant properties of D-amphetamine at doses which decreased baseline spontaneous locomotor activity. Neither haloperidol nor ketanserin antagonized the locomotor stimulant properties of cocaine or D-amphetamine in mice or rats at doses that did not also antagonize spontaneous baseline locomotor activity. DuP 734 did not selectively antagonize the locomotor stimulant effects of apomorphine in either mice or rats. Haloperidol selectively antagonized the locomotor stimulant effects of apomorphine in rats but not in mice. Ketanserin selectively antagonized the locomotor stimulant effects of apomorphine in mice but not in rats. These results suggest a potential use for DuP 734 in the treatment of cocaine abuse which is supported by the observation that DuP 734 (7.5 and 10 mg/kg p.o.) blocked generalization to cocaine in drug discrimination.

230.8

PHARMACOLOGICAL PROFILE OF THE ATYPICAL NEUROLEPTIC DRUG EMD 57445 **G.D. Bartoszyk*, H.E. Greiner, J. Harting, M. Stohrer, C.A. Seyfried. E. Merck, Biol. Res., Dept. of CNS-Res., POB 4119, 6100 Darmstadt, Germany.**

EMD 57445 ((S)-(-)-5-[4-hydroxy-4-(1,3-benzodioxol-5-yl)-piperidin-1-ylmethyl]-3-(4-methoxyphenyl)-oxazolidin-2-one HCl) exhibited the following binding profile in vitro (receptor and IC_{50}, resp.): σ: 6 nM; D_2: 5200 nM. Affinity to D_1, $5HT_{1A}$, $5HT_{1B}$, $5HT_{1C}$, $5HT_{1D}$, $5HT_2$, $5HT_3$, BDZ, α_1, α_2, NMDA, μ, κ, δ, M_1/M_2 was ≥ 1 μM. EMD 57445 stimulated rat striatal dopamine synthesis with ED_{50}-values of 3.4 mg/kg po and 2.1 mg/kg sc; striatal levels of DOPAC and HVA were increased with threshold doses of 1 and 3 mg/kg sc, resp., but 3MT levels were unaffected. Avoidance behavior in rats was inhibited with ED_{50}-values of 5 mg/kg both po and sc. EMD 57445 reversed dopamine-agonist (apomorphine) induced stereotyped behavior with ED_{50}-values of 1.2 mg/kg po and 0.4 mg/kg sc (climbing mice) and 2.1 mg/kg po and 1.0 mg/kg sc (stereotypies rats), resp.. Only marginal cataleptic activity was observed in rats up to 1000 mg/kg po and 300 mg/kg sc. Chronic application of EMD 57445 for 19 days to rats did not result in supersensitized dopamine receptors. The results characterize EMD 57445 as a potent D_2-dopamine antagonist in vivo with high affinity to the σ- and comparably low affinity to the D_2-receptor in vitro, a profile shared with some other atypical neuroleptic drugs.

230.9

CHRONIC FLUPHENAZINE TREATMENT INCREASES GAD_{67} mRNA LEVELS IN SPECIFIC REGIONS OF THE RAT BRAIN. A.E.Johnson*, U.Liminga, N.Lindefors¤, L.Gunne, F.-A.Wiesel. Dept. of Psychiatry, Uppsala University, Uppsala, Sweden and ¤Dept. of Pharmacology, Karolinska Institute, Stockholm, Sweden.

Chronic neuroleptic treatment is associated with the development of severe motor disturbances collectively known as tardive dyskinesia. Long-term treatment of rats with the neuroleptic fluphenazine decanoate (FLU) increases vacuous chewing movements (VCM), a model of dyskinesia. One transmitter system known to be affected by neuroleptics is the GABA system. The purpose of this experiment was to more closely examine the effects of chronic FLU treatment on GABAergic neurotransmission. Adult female Sprague-Dawley rats received 6 monthly injections of FLU (30mg/kg/month i.m.) or vehicle (N=7/group). Animals were killed 60 days after the last injection. The brains were processed for *in situ* hybridization histochemistry with an oligodeoxynucleotide probe directed against glutamic acid decarboxylase$_{67}$ messenger RNA (GAD_{67} mRNA). The results of behavioral studies showed that throughout the treatment period and one week prior to sacrifice, rats given FLU exhibited significantly more VCM than controls. Autoradiographic analysis indicated that FLU treatment increased GAD_{67} mRNA levels in the core (101%) and dorsal shell regions (30%) of n. accumbens, caudate putamen (121%) and in the entopeduncular n. (92%). No differences were found in other brain regions including the ventral shell of the accumbens, globus pallidus, cortex or in substantia nigra. These results indicate that chronic neuroleptic exposure can alter GABAergic transmission. Whether these changes are related to the development and expression of VCM remains to be demonstrated. The effect of FLU treatment on GABA-A receptor binding is currently under investigation. These experiments were funded by grants awarded to N.L, L.G. and to F.-A.W. from the Swedish Medical Research Council.

230.10

ATYPICAL ANTIPSYCHOTIC-LIKE EFFECTS OF NEUROTENSIN ON MIDBRAIN DOPAMINE CELLS. Jeffrey M. Goldstein* and Linda C. Litwin. Department of Pharmacology, ICI Pharmaceuticals Group, A Business Unit of ICI Americas Inc., Wilmington, DE 19897.

The acute administration of most antipsychotic (AP) drugs increases the number of spontaneously firing midbrain DA cells located in substantia nigra (A9) or ventral tegmentum (A10), whereas atypical (clozapine-like) APs exhibit a dose-related selectivity for A10. The present study tested the hypothesis that neurotensin (NT) would have a clozapine-like profile in this paradigm. Since NT does not readily cross the blood-brain barrier, it was injected intraventricularly, and compared to haloperidol and clozapine injected via the same route. Male Sprague-Dawley rats were anesthetized with chloral hydrate and stereotaxically injected with either NT (1-100 μg), haloperidol (10-30 μg), or clozapine (1-100 μg), and the number of spontaneously firing DA cells determined using standard extracellular recording techniques. NT was found to increase both A9 and A10 DA cells at the 100 μg dose, was selective for A10 at lower doses (3-30 μg), and was inactive at 1 μg. Clozapine had a similar profile of activity, producing selective increases in A10 at doses that were lower then those required to produce equivalent increases in A9. In contrast, haloperidol produced equally extensive dose-related increases in both A9 and A10 at 17.5-30 μg, with no apparent selectivity for either area, and was inactive at 10 μg. It was of interest that both NT and clozapine were found to be more potent than haloperidol after intraventricular administration. The results of this study provide the first evidence that NT itself has an atypical clozapine-like profile. Testing of a more potent analog of NT is now in progress.

230.11

SERTINDOLE - A LIMBIC SELECTIVE NEUROLEPTIC WITH POTENT ANXIOLYTIC EFFECTS.
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Sertindole is a putative neuroleptic with high limbic selectivity [1]. We have assessed the anxiolytic potential of sertindole in various animal models. Sertindole shows extremely potent effects in social interaction test (unfamiliar and bright light test conditions) in rats (Minimal effective dose (MED)= 0.000023 nanomol/kg) and in test of explorative behaviour in black and white test box in mice (MED=0.00023 nanomol/kg). Potent effects are obtained in human threat test in marmosets (MED=2.3 nanomol/kg) and test of explorative behaviour in black and white test box in rats (MED=11 nanomol/kg). Sertindole exerts a moderate inhibition of isolation-induced aggressive behaviour in mice (ED50=4200 nanomol/kg), and has no effect on footshock-induced ultrasonic vocalization (rat) and shock-induced suppresion of drinking (rat). In conclusion, sertindole exerts potent anxiolytic-like effects both in rodents and in the marmoset. The profile of sertindole is different from that of benzodiazepines, which together with a lack of sedative effects may indicate clinical advantages.

1) Sánchez, C. et al., Drug Devel.Res., **22**, 1991, 239-250.

230.12

EVALUATION OF Wy-47,791, A GAMMA-CARBOLINE ANTIPSYCHOTIC CANDIDATE, IN A PRIMATE SOCIAL COLONY MODEL OF PSYCHOSIS.
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Wy-47,791 (Wy), 8-fluoro-2,3,4,5-tetrahydro-2-[2-(4-pyridinyl) ethyl]-1H-pyrido[4,3-b]indole HCl, has been identified as an antipsychotic candidate in rodent screens. The present study was designed to assess the antipsychotic activity and side effect profile of Wy in a non-human primate social colony model of psychosis. Gevotroline, a gamma-carboline, demonstrated activity in this model similar to known antipsychotics and with minimal movement disturbances. Wy was tested alone and in combination with the dopamine agonist apomorphine (APO) which was used to induce the model psychosis. Four females from a stable social colony of 5 adult stumptail macaque (Macaca arctoides) received one of 6 doses of Wy, 1-16 mg/kg, for 2 days in a cross-over design. At least 5 days separated each dose regimen. Wy was given n.g. at 0800 and 1630 on Day 1 and at 0800 on Day 2 of treatment. APO, 1 mg/kg, was given i.m. at 1015. Two 1 hr observation sessions were conducted daily by a "blind" observer at 0900 and 1030. Observation of baseline behavior and APO-induced behavioral changes preceded Wy treatment. At doses $\geq$ 8 mg/kg, Wy significantly antagonized APO-induced increased submissiveness and increased checking (visual scanning), behaviors that model paranoia and hypervigilance respectively. Wy only weakly antagonized APO-induced stereotypy. Wy alone significantly increased resting time at higher doses. Wy induced low levels of movement abnormalities in all treated monkeys at 16 mg/kg. The results demonstrate that Wy has an antipsychotic profile similar to known antipsychotics and gevotroline in this model at doses which produce low levels of movement abnormalities and sedation. (This research was supported by Wyeth-Ayerst Laboratories, Princeton, NJ).

230.13

EFFECTS OF CHRONIC TREATMENT WITH SM-9018, A POTENTIAL ATYPICAL ANTIPSYCHOTIC, ON BEHAVIORAL DOPAMINERGIC AND SEROTONERGIC SENSITIVITY IN RATS.
Y. Ohno*, K. Okada, K. Ishida, T. Kato and M. Nakamura. Res. Lab., Sumitomo Pharm., Konohana-ku, Osaka 554, Japan.

SM-9018 is a novel 5-HT_2/D_2 receptor antagonist which has antipsychotic efficacy with few extrapyramidal side-effects. To examine whether chronic SM-9018 induces dopaminergic and/or serotonergic supersensitivity, we studied the behavioral responses to dopamine and 5-HT agonists in rats after 2 weeks of chronic treatment with SM-9018 or with haloperidol (HAL) at a dose sufficient to block D_2 receptors (0.1 and 0.3 mg/kg/day s.c., respectively). Four days after withdrawal of the drug, no significant change in apomorphine-induced stereotyped behaviors was observed in the SM-9018-treated group. However, the response to apomorphine was markedly enhanced by HAL treatment. Increase of the dose of SM-9018 to 0.3 mg/kg/day for 2 weeks resulted in a small increase of AP-induced stereotypy, but this action was still weaker than that of HAL. On the other hand, serotonergic behaviors, such as 5-hydroxytryptophan-induced wet dog shakes and 8-OH-DPAT-induced flat body posture, were unaffected by treatment with either SM-9018 or HAL. These results suggest that SM-9018 is weaker than haloperidol in inducing dopaminergic supersensitivity after chronic administration and has low potential for induction of tardive dyskinesia.

230.14

TARDIVE DYSKINESIA AND NEUROLEPTIC INDUCED IMMUNOLOGIC ANOMALIES.
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Neuroleptics such as haloperidol decanoate (HAL) increas the likelihood of free-radical formation which may result in neuronal changes in brain areas involved in tardive dyskinesia (TD). We examined the effects of chronic HAL alone and in combination with the anti-oxidant, free radical scavenger, Coenzyme Q10 (CoQ), on immunological markers in rats. Rats were treated with either vehicle or HAL (21mg/kg every 3 wks) alone or in combination with CoQ (50mg/kg/day) for 6 months. Rats were divided into TD and no TD groups based on ratings for vacuous chewing movements.

Using flow cytometry in peripheral blood lymphocytes, number of cells staining for CD4 was decreased in the HAL-CoQ/no TD group, but increased in the HAL-CoQ/TD group versus controls. Class II MHC staining of a secondary cell population was eliminated in the HAL-CoQ/TD rats. IL-2r staining was increased 4-fold in rats treated with HAL-CoQ /no TD versus controls. In splenic lymphocytes, CD4 staining intensity was increased in the HAL/TD group and cell number decreased in CD8 and Class II MHC stains in the HAL-CoQ/no TD group.

Our findings indicate significant differences in lymphocyte populations following HAL alone versus HAL-CoQ, and in TD versus no TD rats, suggesting immunologic effects of these drugs as covariates of TD.

230.15

POSSIBLE LOSS OF DOPAMINE TERMINALS FOLLOWING SHORT TERM NEUROLEPTIC TREATMENT.
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Prolonged medication with typical neuroleptics leads to several types of neurological disorders. Some of the symptoms of motor dysfunction, such as the extrapyramidal syndrome, appear after treatments of short duration. Others, such as tardive dyskinesia, typically appear after years of medication. In this context, rats were administered haloperidol (Hal) daily (0.5 mg/kg; ip) for 21 days. Endogenous striatal dopamine (Da) levels and numbers of presynaptic uptake sites were assessed by, respectively, HPLC and [^{3}H] mazindol (Maz) binding. Rats that received Hal had reduced Da levels and reduced number of Maz binding sites. MK-801 (0.5 mg/kg,,ip) or ganglioside (30 mg/kg, ip) co-administered with Hal in other groups of rats failed to attenuate these effects. These results indicate that short term Hal treatment may cause loss of Da cells and/or terminals. This damage, unlike that caused by other modulators of Da activity, is not mediated by interactions with excitatory amino acids.

230.16

COMBINATIONS OF SUB-THRESHOLD DOSES OF HALOPERIDOL AND 5-HT1A AGONISTS AND PARTIAL AGONISTS SUPPRESS CONDITIONED AVOIDANCE RESPONDING IN RATS.
A.T. Shropshire* and K.L. Marquis. Wyeth-Ayerst Research, CN8000, Princeton, NJ 08543

Previous work by Wadenberg and Ahlenius (J Neural Transm [GenSect] 83:43-53, 1991) demonstrated strong interaction between a 5-HT_{1A} agonist (8-OH-DPAT) and a DA D_2 antagonist (raclopride) on some critical tests for antipsychotic-like actions and extrapyramidal motor effects. They suggested such a 5-HT_{1A}/DA D_2 combination might offer new antipsychotic drugs with higher clinical efficacy and fewer extrapyramidal side effects (EPS). In the present study, we examined the effects of doses of haloperidol (0.06 and 0.125 mg/kg sc), which do not suppress conditioned avoidance responding (CAR) in rats, in combination with sub-threshold doses of the 5-HT_{1A} agonist 8-OH-DPAT (0.1 mg/kg sc), the 5-HT_{1A} partial agonists gepirone (5.0 mg/kg ip) and ipsapirone (2.5 and 5.0 mg/kg ip) and the 5-HT_{1A} weak partial agonist BMY 7378 (1.0 mg/kg ip) on CAR in the rat. When given alone, only the 5.0 mg/kg dose of ipsapirone significantly affected CAR when compared to control data obtained previously. Combination treatments produced significant suppression of CAR in all cases. These findings extend the report of Wadenberg and Ahlenius to the observation that 5-HT_{1A} ligands ranging in levels of agonism can enhance apparent antipsychotic effects of D_2 antagonists.

230.17

THE ROLE OF SEROTONIN RECEPTOR SUBTYPES IN THE EFFECTS OF NEUROLEPTIC DRUGS B.A.Ellenbroek*, E.P.M. Prinssen & A.R. Cools, Psychoneuropharmacol. Res. Unit, P.O. Box 9101, 6500 HB Nijmegen, the Netherlands

Despite the fact that the concept of atypical neuroleptics as well as the first true atypical neuroleptic clozapine was introduced some fifteen years ago, few advances have been made in elucidating the mechanisms underlying such neuroleptic drugs. In the present study we have investigated the role of serotonin receptors in the effects of neuroleptic drugs in the **PAW TEST**. This test has been extensively validated as an animal model with predictive validity for schizophrenia and it models both the therapeutic efficacy (TE) as well as the extrapyramidal side effects (EPS) of neuroleptic drugs.

The results show that adding a 5-HT_2 antagonist (ketanserin) to the classical neuroleptic haloperidol reduced the EPS without affecting TE. Adding a 5-HT_{1A} agonist (8-OHDPAT) on the other hand reduced both the TE and the EPS. Conversely adding a 5-HT_{1C}/5-HT_2 agonist (DOI) to the atypical neuroleptic risperidone increased EPS and to a lesser extent TE. However, adding DOI to clozapine did not affect EPS, but rather led to a strong decrease in TE. These data suggest that (1) distinct serotonin receptors differentially affect the effects of neuroleptic drugs (2) distinct neuroleptics can influence EPS and TE via different working mechanisms.

EPILEPSY: HUMAN STUDIES AND ANIMAL MODELS II

231.1

LEVELS OF S100β PROTEIN IN POST-OPERATIVE BRAIN TISSUE FROM EPILEPTIC PATIENTS. O. Yeralan, C. Rovnaghi, F.A. Boop, +L.J. Van Eldik, P.E. Bean and W.S.T. Griffin.* Depts. of Pediatrics, Anatomy, and Neurosurgery, UAMS, Little Rock, AR 72205; +Dept. of Pharmacology, Vanderbilt U., Nashville, TN 37232.

Gliosis in and around epileptic foci is the most common neuropathological finding in epilepsy. S100β, a calcium binding protein synthesized and secreted by astrocytes, can stimulate elevation of intracellular calcium in neurons. The levels of S100β are increased in activated astrocytes such as those characteristic of the gliosis observed in some neurodegenerative diseases. S100β expression was studied by immunohistochemistry, Western analysis, and Enzyme Linked Immunosorbent Assay (ELISA) in post-operative brain tissue samples from 11 epileptics and in analogous samples taken post-mortem from 5 age-matched controls. Compared to control sections, there was an elevated number of S100β immunoreactive astrocytes in tissue sections from epileptics; these astrocytes were activated, *i.e.*, enlarged with prominent processes. By ELISA, the levels of S100β were 500-2,000 ng/mg protein in 7 of 11 patients compared to 100-500 ng/mg protein in the 5 controls. Western analysis of the same tissue yielded similar results. The levels of S100β were found to correlate with the degree of gliosis, the age of the individual, and/or the severity and duration of the disease. Further work is underway to determine if elevated levels of this calcium-binding neurite extension factor might contribute to (*i*) synaptic reorganization and (*ii*) neuronal loss characteristic of epilepsy.

231.2

SELECTIVE REGULATION OF HIPPOCAMPAL NPY AND Tα1 α-TUBULIN mRNA's IN A RAT MODEL OF EPILEPTOGENESIS.
W.F. Colmers[1]*, K. Makus[1], Y.L. Ma[2] and F.D. Miller[2]
Dept of [1]Pharmacology and [2]Anatomy and Cell Biology, Univ. Alberta, Edmonton, Canada, T6G 2H7

Recent reports suggest that numerous neuronal gene products are induced following experimentally-induced seizure. We have tested the hypothesis that neuronal phenotype is altered by stimuli well before seizure itself is induced. Male Sprague-Dawley rats were anesthetized with subcutaneous urethane, placed in a stereotaxic apparatus, and stimulating and recording electrodes placed in the angular bundle and granule cell layer of dentate gyrus, respectively. Paired test stimuli (2 Hz, 30 V, 100 μs 40 msec ISI) elicited granule cell population EPSP and population spike responses that exhibited paired-pulse inhibition on the second pulse. Trains of stimuli (30V, 100 μs, 20 Hz, 10s, 1/min) were superimposed on test stimuli for 2-24h. Animals were allowed to recover for a time after stimulation ceased, then brains were prepared for cryotomy and *in situ* hybridization (ISH) with riboprobes for NPY and Tα1 α-tubulin mRNA's.

Stimulus trains induced brief (1-2s) afterdischarges. 4h stimulation resulted in smaller responses to both pulses of test stimuli, and briefer afterdischarges. 24h stimulation resulted in massive disinhibition. After 4 h stimulation, ISH showed NPY mRNA to be elevated in ipsilateral, and some contralateral hilar and CA1/CA3 interneurons, while Tα1 α-tubulin mRNA was elevated in ipsilateral (and some contralateral) dentate granule cells; some elevation in CA3 and CA1 pyramids was seen. After 24h stimulation, levels of NPY and Tα1 α-tubulin mRNA's increased in the same populations of neurons, and the contralateral increases were much greater than after 4h.

Stimuli that do not lead to permanent alterations in the electrical behavior of hippocampus appear to be able to markedly alter neuronal phenotype in specific neurons in hippocampus, suggesting that neurons may normally compensate for elevated levels of excitation.

Supported by MRC (Canada). WFC and FDM are AHFMR Scholars; YLM is supported by the Alberta Paraplegic Association; KM was an AHFMR summer student.

231.3

CHANGES IN VASOPRESSIN MRNA IN THE PARAVENTRICULAR NUCLEUS AFTER AMYGDALA KINDLING. **R.S. Greenwood*, R.B. Meeker, A. Abdou and J.N. Hayward. Dept. of Neurology and Pediatrics and Neurobiology Curriculum, Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27599.**

We have previously shown that resting plasma vasopressin (VP) is elevated and VP mRNA expression is chronically higher in the magnocellular neuroendocrine system after amygdala kindled seizures. In the supraoptic nucleus we have found a two fold increase in VP mRNA in magnocellular neurons 1 to 4 months after kindling was completed. In the present study we sought to determine if all subpopulations of VP-containing neurons in the paraventricular nucleus (PVN) show chronic elevation of VP mRNA. Sprague Dawley rats with amygdala electrodes received daily kindling stimuli until they reached class 4 or 5 kindled seizures. For VP *in situ* hybridization, 20 um brain sections from kindled and control animals were hybridized with a 30mer oligonucleotide probe specific for vasopressin. Three regions of the PVN approximating the posterolateral posterior magnocellular (pml), the ventral tail of the medial parvicelluar (mpv, mpdv), and the dorsal parvicellular and the dorsal part of the medial parvicellular (dp, mpdd) PVN subdivisions were sampled for analysis of autoradiographic silver grain density using an image analysis system (Bioquant IV). The magnocellular regions of the PVN in the kindled rats had more VP mRNA than control rats. We were unable to detect a change in VP hybridization in the predominantly parvicellular region in kindled rats.

We conclude that kindling results in vasopressin up-regulation in PVN magnocellular neurons but not in dorsal parvicellular neurons.

Supported by NIH Javits Award NS 13411.

231.4

ENHANCED EXPRESSION OF NEUROPEPTIDE Y IN GRANULE CELLS AND IN INTERNEURONS OF THE DENTATE GYRUS AFTER KAINIC ACID-INDUCED SEIZURES IN THE RAT. G. Sperk, B. Gruber, R. Bellmann, S. Greber. (SPON: European Neuroscience Association). Dept. of Pharmacology, University of Innsbruck, 6020-Innsbruck, Austria.

Distribution of neuropeptide Y (NPY) mRNA has been investigated at various time intervals after seizures induced by i.p. injection kainic acid (10 mg/kg). In rats which had experienced a full limbic seizure syndrome high concentetions of NPY mRNA were found in the granule cell layer of the dentate gyrus 24 hours after injection of the toxin. No NPY message was found there in untreated rats. The increase in NPY mRNA declined at later intervals (2 to 30 d after kainic acid). At the same time a loss of NPY expressing cells was observed in the deep hilus of the dentate gyrus. Pyramidal shaped basket cells laying closely to the inner surface of the granule cell layer were spared and exhibited increasing concentrations of NPY message which could be seen even 4 months later. In animals which had received the same treatment with kainic acid without developing motor seizures no NPY mRNA was observed in granule cells after 24 hours. At this time, however, a marked induction of the peptide message was detected in interneurons throughout the hilus.

These data suggest an early activation of presumably inhibitory NPY containing neurons of the hilus. In animals exposing motor seizures this activation affects granule cells. Although damage of hilar interneurons can be seen presumably GABA-ergic basket cells become long lasting activated. Supported by the Austrian Scientific Reaserch Funds (P8128).

231.5

C-FOS-LIKE IMMUNOREACTIVITY AFTER SPONTANEOUS SEIZURES IN RATS. L.E.A.M. Mello*, A.M. Tan, E.A. Cavalheiro and D.M. Finch Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024, and Departamento de Neurologia, de Fisiologia, Escola Paulista de Medicina, 04023 São Paulo, Brazil.

Systemic injections of pilocarpine (320-350 mg/kg, i.p., 30 min after 1 mg/kg scopolamine) were used to induce status epilepticus in adult, male Sprague-Dawley rats (150-250 g, seizures controlled after 1 h with 4.0 mg/kg diazepam). After a variable period (4-34 days), the rats entered a phase of spontaneous recurrent seizures (SRS) that lasted for as long as the animals were allowed to survive. SRS were similar to stage 5 kindled seizures, lasting an average of 30-60 sec. To assess fos-like-immunoreactivity (FLI) after SRS, animals were sacrificed 1 h after an SRS was observed, and processed with anti c-fos (Cambridge).

Light microscopic examination of coronal brain sections revealed no neuronal FLI, except in 2 animals. One of these was processed within 10 days of SE induction. The other was processed after an unusual flurry of 5 seizures that occurred in the span of 1 hour. The latter animal showed staining in the piriform and perirhinal cortices, and in scattered locations throughout neocortex. SE was induced in naive animals or reinduced in chronic animals (100 mg/kg pilocarpine dose for the chronic animals), and only the naive animals showed FLI. These results indicate that severe *spontaneous* seizures can induce FLI. However, they also suggest (1) that most single spontaneous seizures are subthreshold for this technique or (2) that a state of chronic seizures down-regulates c-fos induction. Supported by NIH Grants NS 23074 and NS 16721, The NIH Fogarty Center; and FAPESP and CNPq (Brazil).

231.6

PENTYLENETETRAZOL KINDLING INCREASES VASOPRESSIN MRNA IN THE BNST AND MA OF RATS. J.N. Bicknell*, P. Szot and D.M. Dorsa. GRECC, Seattle VAMC, WA 98108 and Dept. of Pharmacology, Univ. Washington, Seattle, WA 98195.

Kindling is an animal model of epilepsy that resembles partial complex seizures observed in humans. Kindling is produced in rats with a periodic administration of a subconvulsant stimuli (chemical or electrical) until clonic/tonic convulsions are observed. The present study was performed to determine if chemical kindling with pentylenetetrazol (PTZ) altered the level of vasopressin mRNA containing neurons of the limbic system including the bed nucleus of the stria terminalis (BNST) and medial amygdala (MA). Chemical kindling was performed by administering PTZ intraperitoneally at a subconvulsant dose (30 mg/kg) every other day for about 4 weeks until stage 5 tonic/clonic seizures were observed. Once kindled the animals were sacrificed 24 hours following the last convulsion. In situ hybridization was performed in control and kindled rats using a 48 base oligonucleotide to the glycopeptide portion of the vasopressin (VP) gene. VP mRNA was not significantly different in hypothalamic VP neurons of the paraventricular, suprachiasmatic and supraoptic nucleus, though the supraoptic nucleus showed a tendency to be higher in the kindled rats compared to the control. In contrast, VP mRNA levels in the BNST and MA of kindled rats was significantly higher ($p<0.05$ and $p<0.10$) compared to controls, suggesting a possible role for VP in kindled seizures. Interestingly, VP gene expression in these neurons is regulated by testosterone. Preliminary data has suggested a sex difference in c-fos gene expression in the hippocampus following a PTZ seizure. A possible involvement of VP neuronal systems in gender specific differences in seizure-related c-fos expression is under investigation. (Supported by NS 20311 and the VA)

231.7

KINDLED SEIZURES FAIL TO INDUCE FOS PROTEIN IN SUBSTANTIA NIGRA PARS RETICULATA C.D. Applegate*, D. Piekut and S. Pretel, University of Rochester School of Medicine, Rochester, NY 14642.

FOS immunolabeling is being widely used to identify activated neuronal networks following seizures induced using a variety of experimental models. Despite the demonstrated involvement of the substantia nigra pars reticulata (SNpr) in seizure expression in virtually all seizure models, this structure has not been reported to express FOS protein following seizures suggesting that the networks defined by FOS immunolabeling may be incomplete. In this study we examined the SNpr for FOS labeling at various time points following kindled seizures to establish that the lack of reported labeling is not due to differences in the time course of FOS induction in SNpr. Male rats were kindled from the entorhinal cortex and sacrificed at 1-12h following seizure. Tissue was processed for FOS labeling using standard protocols. No FOS immunolabeling was observed at any time point following seizure in SNpr neurons. The pattern of labeling of other structures was consistent with previous reports and was maximum at 1-3h, decreased at 4-6h and virtually absent at 12h. In a second study, immunolabeling of serum response factor (SRF), a suggested necessary nuclear protein for FOS induction, was examined. All SNpr cells showed nuclear labeling for SRF. Together, results suggest SNpr neurons have at least some of the nuclear machinery for FOS induction but that seizures are not effective stimuli for inducing FOS in these cells.

231.8

EXPRESSION OF PRODYNORPHIN mRNA IN FOS ACTIVATED CELLS IN RAT NUCLEUS TRACTUS SOLITARIUS AFTER INDUCED SEIZURES. Robert K Kanter*, Jeffery T Erickson, David E Millhorn. University of North Carolina, Chapel Hill, NC 27599

Activation of the c-fos gene occurs in rat hippocampus (**Science** 1987; 237:192) and nucleus tractus solitarius (NTS) (**Ped Res** 1992;31:349A) after pentylenetetrazole (Ptz) induced seizures. Fos protein appears to act as a transcription regulatory factor for the proenkephalin (proENK) gene in hippocampus (**Science** 1989; 246:1622), but other seizure associated Fos target genes and their tissue locations have not been reported. We performed an immunohistochemical and in situ hybridization study to identify neuropeptide genes potentially regulated by Fos in NTS after Ptz (i.p. 45 mg/kg plus 10 mg/kg every 5 minutes until motor seizures occurred). Fos immunoreactive (IR) cells were identified with the avidin-biotin method after incubation with anti-Fos antibody. Cells containing prodynorphin (proDYN), proENK, or c-fos mRNA were identified by autoradiography after in situ hybridization with ^{35}S labeled oligonucleotides. Levels of c-fos mRNA peaked at 1-2 hours, while the number of Fos IR cells peaked at 2-4 hours after seizures in NTS. Control and sham treated (i.p. saline) animals had negligible c-fos mRNA or Fos IR cells in NTS. At 4 hours after seizures, proENK and proDYN mRNA were colocalized with Fos IR in many cells of the NTS. While proENK and proDYN mRNA were detectable in NTS under control conditions, proDYN mRNA level was mildly enhanced by 7 hours, and strongly increased 24 hours postictally. These observations suggest a potential role of the c-fos gene in regulation of proDYN expression in rat brainstem after Ptz induced seizures.

231.9

EXCITATORY AMINO ACID ANTAGONISTS PREVENT SEIZURES AND *C-FOS* INDUCTION IN A RAT MODEL OF PERINATAL HYPOXIA. I.R. Firkusny*, H.K. Blume, and F.E. Jensen. Dept. of Neurology, Children's Hospital and Harvard Medical School, Boston, MA 02115.

We have previously shown that hypoxia is acutely epileptogenic in the immature rat (P5-17), and that *c-fos* immunoreactivity (IR) is present predominantly in neocortex at 4 hrs post-hypoxia. Furthermore, long term seizure susceptibility is increased after hypoxia-induced seizures at P10. This age window corresponds to a period of development when various regions of the brain exhibit a transient increase in NMDA and non-NMDA receptors, while the GABA system is still immature compared to the adult. To determine whether activity at these excitatory amino acid (EAA) receptors might underlie the age-dependent epileptogenicity of hypoxia, the relative efficacy of the systemic pretreatment with the NMDA antagonist MK-801 and the AMPA antagonist NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline) were compared to the GABA agonist lorazepam on seizure frequency and *c-fos* IR in immature rats. Long Evans rats aged P10 were rendered hypoxic in 4%O_2 after i.p. pretreatment with saline ($n=20$), MK-801 (1mg/kg, $n=11$), NBQX (20mg/kg, $n=10$), or lorazepam (1mg/kg, $n=10$). Compared to saline, pretreatment with MK-801 or NBQX significantly reduced seizures ($p<0.001$), while lorazepam did not. In addition, *c-fos* IR was reduced compared to control hypoxic pups in 63% of MK-801 treated animals and 56% of NBQX treated animals, compared to 44% of lorazepam treated animals. These results suggest that EAA receptors may be involved in the genesis of neonatal hypoxia-induced seizures, and that EAA antagonists may be superior to conventional benzodiazepine anticonvulsants in neonatal seizures associated with hypoxia.

231.10

SUPPRESSION OF BASAL LEVELS OF *ZIF268* mRNA IN A CHRONIC SEIZURE MODEL. A.J. Cole* and J.M. Baraban. Depts. of Neurology and Neuroscience, Johns Hopkins University Sch. of Med., Baltimore, MD 21205.

In contrast to the transient expression of several transcription factors seen in brain after stimulation, *zif268* displays significant basal expression in cortex. The functional role of this basal expression is unknown, however it may serve as a marker of normal cortical physiology as it is dependent on afferent synaptic activity (Worley et al., *PNAS* 1991). We used the maximal electroconvulsive seizure (MES) model to examine the effects of repeated seizures on the basal expression of *zif268*. Rats were sacrificed at various time points after 10 daily MES and compared to animals that received a single MES. Brains were processed for *in situ* hybridization, immunohistochemistry, northern blot analysis, and gel shift analysis of transcription factor binding to DNA. Basal levels of *zif268* mRNA assayed by *in situ* or Northern blot were markedly reduced 4-36h after the tenth seizure, but returned to near baseline by 48h. A single seizure caused no supression. Surprisingly, after chronic seizures there was no suppression of *zif268* protein levels assessed immunohistochemically or of binding activity to the *zif268* consensus sequence in nuclear extracts. This dissociation between mRNA and protein responses suggests that repeated stimulation may affect *zif268* expression at both transcriptional and post-transcriptional levels.

231.11

DIFFERENCES IN THE PATTERNS OF FOS EXPRESSION FOLLOWING ELECTROCONVULSIVE SHOCK(ECS)-INDUCED GENERALIZED TONIC AND GENERALIZED CLONIC SEIZURES IN C57BL/6J MICE. G.M. Samoriski*, C.D. Applegate and D.T. Piekut. University of Rochester Medical Center, Rochester, NY 14642.

Studies suggest that the expression of generalized tonic vs generalized clonic seizures may be mediated by separate brain systems. The goal of this study was to define anatomical pathways that participate in the expression of these two seizure phenotypes using FOS immunocytochemistry (ICC). Seizures were induced in adult C57BL/6J male mice by transcorneal ECS (0.2 sec, variable current). Animals were perfused at 3 hrs and brain sections were incubated in primary antiserum against both FOS and FOS-related antigens. Neurons of the dorsal central gray, deep layers of the superior colliculus and the area surrounding the ventral aspect of the inferior colliculus express FOS after elicitation of tonic hindlimb extension (THE, n = 8) but not after clonic seizures (n = 3). Other brain regions expressed FOS in a graded fashion. The peripeduncular area, substantia nigra pars lateralis, lemniscal/paralemniscal region and ventromedial hypothalamus exhibited robust FOS staining following THE but were noticeably less labeled after a clonic seizure. Forebrain structures were labeled following seizures of either phenotype but more neurons of the anterior piriform cortex expressed FOS following a clonic seizure than after THE. The pattern of labeling was dependent on the seizure phenotype expressed and independent of stimulus intensity. Results suggest both qualitative and quantitative differences in FOS labeling as a function of the seizure phenotype expressed.

231.12

FUNCTIONAL MAPPING OF BICUCULLINE-INDUCED "FOREBRAIN" AND "BRAINSTEM" SEIZURES: A ^{14}C-2-DEOXYGLUCOSE STUDY A. Handforth, R. Peters, B.E. Swartz* & D.M. Treiman, VAMC Wadsworth and Dept. of Neurology, UCLA, Los Angeles, California 90024.

Studies have suggested that forebrain seizures result in face and forelimb clonus, brainstem seizures in tonic and running-bouncing clonus (Browning and Nelson, Exp Neurol 93:546-556, 1986). We studied the anatomic basis of "forebrain" and "brainstem" seizures, utilizing the ^{14}C-2-deoxyglucose (2DG) method in rat. IV bicuculline was infused at 0.25 ug/sec, the rate slowly increased until seizures occurred, at which time 2DG, 30 uCi, was administered as a bolus. Controls (n=5) received vehicle. Following sacrifice at 10 min post-2DG, cut cerebral sections were apposed to X-ray film to form autoradiographs; these were analyzed semiquantitatively with an image analyzer.

Rats with "forebrain" type seizures (n=6) showed metabolic activation of forebrain (cortex, thalamus, hippocampus) and of substantia nigra. Of 8 rats with "brainstem" seizure behaviors, 3 displayed activation of mainly brainstem structures, particularly inferior colliculus, periaqueductal gray, substantia nigra, and midbrain-pons reticular formation. Five displayed activation within brainstem plus widespread forebrain areas. These results directly support the concept of "forebrain" and "brainstem" seizures, and also indicate that both areas of cerebrum can simultaneously engage in seizure activity.

231.13

LIDOCAINE KINDLING AND REPEATED COCAINE ADMINISTRATION DO NOT ALTER SODIUM CHANNEL-LINKED PHOSPHOINOSITIDE (PI) TURNOVER IN RAT CORTICAL AND HIPPOCAMPAL SLICES. R.L. Margolis*, D.-M. Chuang, S.R.B. Weiss and R.M. Post. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892

Repeated administration of a subconvulsant dose of a local anesthetic eventually produces seizures, a phenomenon similar to electrical kindling. Since local anesthetics acutely alter sodium channel conductance, we investigated the possibility that local anesthetic kindling also modulates the function of these channels. Rats received daily injections of lidocaine until seizures were observed. Kindled rats were compared with non-kindled rats receiving lidocaine or saline. Basal-, batrachotoxin (BTX, a voltage-sensitive sodium channel activator)-, and ibotenic acid (IBO, a glutamate receptor agonist)-stimulated PI turnover did not differ among the three groups in slices of either hippocampus (HC) or piriform cortex (PC), though IBO-stimulated PI turnover was much greater in the HC than in the PC. In a second experiment, rats were injected with cocaine or saline daily for six days, and PI turnover was assayed in sliced frontal cortex. Cocaine treatment had no effect on BTX-induced PI turnover, while in vitro cocaine blocked the BTX effect. In vitro addition or one week pretreatment in vivo with carbamazepine did not alter the effect of cocaine on BTX-induced PI turnover. These results demonstrate that local anesthetic kindling does not alter PI turnover coupled to sodium channel activation, and imply that this type of kindling does not influence the function of the BTX binding site of the sodium channel or the coupling of the PI system to this site.

231.14

ENDURING INCREASE IN MEMBRANE-ASSOCIATED PROTEIN KINASE C ACTIVITY IN THE HIPPOCAMPAL-KINDLED RAT. K. Akiyama[1]*, I. Kohira[2] and A. Daigen[1]. [1]Dept. of Neuropsychiatry and [2]Dept. of Neurology, Okayama University Medical School, Okayama 700, JAPAN.

In a previous study (A. Daigen et al. Brain Research 545:131-136, 1991), we demonstrated that the membrane-associated protein kinase C (PKC) activity in the amygdala/pyriform cortex (AM/PC) and both the right and left hippocampus (HIPP) of rats kindled from the left HIPP increased significantly four weeks after the occurrence of the last seizure compared with control rats. In the present study, we examined whether the effect of HIPP-kindling on membrane-associated PKC activity would be more enduring, and could be seen after the last partial (stage 1-3) seizure. 15 to 16 weeks after the final kindled full seizure, the membrane-associated PKC activity which was expressed as nmol/min/mg protein increased significantly in the AM/PC and left HIPP compared with control rats. When expressed as pmol/min/mg wet tissue weight, it increased significantly in the right HIPP as well as the AM/PC and left HIPP. The cytosolic PKC activity did not differ in any brain region examined. One week after the final partial seizure, neither the membrane-associated nor the cytosolic PKC activities in any brain region examined in the partially kindled and control groups differed significantly. These results suggest that activation of membrane-associated PKC may be involved in the long-lasting seizure susceptibility induced by kindling.

231.15

PILOCARPINE-INDUCED SEIZURES: EFFECT ON EXTRACELLULAR EXCITATORY AMINO ACID LEVELS IN RAT HIPPOCAMPUS WITH AND WITHOUT GLUTAMATE UPTAKE INHIBITOR. M.H. Millan, A.G. Chapman, B.S. Meldrum*. Institute of Psychiatry, Denmark Hill, London SE5 8AF, U.K.

Microdialysis was performed in the dorsal hippocampus of the freely moving rats during pilocarpine-induced seizures. Dialysis probes were implanted 24h before perfusion with Ringer solution (3µl/min). 15µl samples were collected and analyzed by HPLC. Extracellular levels of GLU, ASP and GLN were slightly lowered after the i.p. pilocarpine injection (400mg/kg) and during consecutive motor limbic seizures. The GLU uptake inhibitor L-trans-pyrrolidine-2,4 dicarboxylate produced significant increases in GLU (by 67%) and ASP (by 276%) levels. Concentrations of these amino acids were further slightly increased after pilocarpine injection (by 13 and 17% respectively) prior to seizures. After development of seizures ASP and GLU levels were slightly reduced. These results indicate that uptake mechanisms may prevent any increase in extracellular concentration of GLU and ASP associated with enhanced release during the development of seizures.

231.16

DEPRESSION OF AMINO ACID AND DOPAMINE CONTENT OF STRIATUM OF AUDIOGENIC-SEIZURE SUSCEPTIBLE (AGS) BALB/C MICE. J. P. Vriend* and N. A. M. Alexiuk. Dept. of Anatomy, University of Manitoba, Winnipeg, MB, Canada, R3E 0W3.

Nigrostriatal connections have been shown to provide a neuroanatomical substrate for regulating propagation of audiogenic seizures. Hence, in this study, concentrations of amino acids, monoamines, and monoamine metabolites were determined in extracts of tissue punches of striatum of audiogenic-seizure susceptible (AGS) and resistant (AGR) substrains of Balb/c mice. In situ activity of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) were determined by measuring the accumulation of L-dopa and 5-HTP after administration of the decarboxylase inhibitor, NSD-1015. Concentrations of amino acids and monoamines were detected by a coulometric electrode array system. Highly significant decreases in both excitatory (glutamate, aspartate) and inhibitory (GABA, taurine, glycine) amino acids were observed in tissue extracts of caudate nucleus of AGS mice compared to that of AGR controls. Highly significant decreases in DA content, in content of DA metabolites, and in activity of TH (but not of TPH) were observed in AGS Balb/c mice compared to AGR controls. These data suggest that AGS mice have a defect in the ability to retain amino acids in the intracellular compartment. The data also raise the issue of the role of GABAergic and dopaminergic neurons in regulation of audiogenic seizures.

231.17

THE ONTOGENY OF BINDING SITES TO γ-HYDROXYBUTYRIC ACID IN RAT BRAIN. O. Carter Snead III,* C.C. Liu, P.K. Banerjee, Div. Neurol. Childrens Hospital Los Angeles, Dept. Neurol. Univ. Southern California, School of Medicine, Los Angeles, CA 90027

γ-Hydroxybutyric acid (GHB) is a naturally occurring compound which has the ability to induce generalized absence seizures when given to animals. Since generalized absence seizures have a unique developmental profile, we sought to further validate the GHB model of absence seizures in rat by characterizing the ontogeny of the GHB receptor in rat brain, determining the exact time point when the characteristic GHB absence seizure appears during development, and correlating these two ontogenic phenomena.

Rat pups were sacrificed at ages ranging from the first postnatal day to adulthood, the brains removed, and subjected to [^{3}H] GHB autoradiography (Snead et al, Epilepsy Res. 1990;7:121). In another group of experiments animals over the same age range were implanted with electrodes and an attempt made to induce GHB absence seizures. Binding to [^{3}H]GHB was not detected in brain slices until the 18th postnatal day. At this age the regional distribution of [^{3}H]GHB binding was characteristic of that seen in adult animals, being maximal in cortex and hippocampus. GHB absence seizures did not appear until 18-21 days of postnatal age. This developmental specificity confirms the validity of the GHB animal model of absence seizure. The data also suggest that the GHB receptor itself may be involved in the mechanism of absence seizures in this animal model.

231.18

RESISTANCE TO SEIZURE INDUCED HIPPOCAMPAL DAMAGE FOLLOWING KAINIC ACID SEIZURES IN 5 DAY OLD RATS. E.F. Sperber * and K.Z. Haas. Departments of Neurology & Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

We have previously demonstrated that kainic acid seizures produce age-related neuropathologic and neurophysiologic hippocampal changes in rats. In adult rats, kainic acid seizures result in cell loss in the CA3/CA4 hipppocampal subfields, sprouting of mossy fibers to the dentate supragranular layer and enhanced paired-pulse inhibition in the dentate gyrus. In contrast, 15-16 day old rats appear to be resistant to these types of seizure induced hippocampal changes.

In this study, we examined the potentially detrimental effect of kainic acid seizures on the hippocampus of 5 day old rats. Five day old rats were administered kainic acid (2.5 mg/kg, IP). They were remained in status epilepticus for at least 30 min. Two to four weeks following the seizure, the brains were examined for hippocampal neuronal loss (cresyl violet stain) and synaptic reorganization of the mossy fibers in the dentate gyrus (Timm histochemistry). The present results indicate that kainic acid induced seizures did not produce hippocampal neuronal loss or synaptic reorganization. These findings extend our previous findings and indicate that immature 5 and 15 day old rats are more resistant to the development of seizure induced hippocampal damage than adult rats.

Supported by Epilepsy Foundation of America

231.19

AMINOOXYACETIC ACID CAUSES SELECTIVE NEURONAL LOSS IN LAYER III OF THE RAT MEDIAL ENTORHINAL CORTEX. F. Du* and R. Schwarcz, Maryland Psychiatric Research Center, Baltimore, MD 21228.

Focal injection of aminooxyacetic acid (AOAA), a non-specific yet powerful inhibitor of kynurenic acid synthesis, causes NMDA receptor-mediated seizures and selective neuronal damage in the rat hippocampus (Exp. Neurol. 113: 378, 1991). We have now examined the possible neurodegenerative effects of AOAA in another part of the seizure circuit, the entorhinal cortex (EC). Assessed 7 days following the injection of 75 μg/0.75 μl AOAA into the medial EC, neuronal loss was consistently observed in layer III of the medial EC in Nissl-stained horizontal sections. Selective neuronal degeneration of the medial EC was observed even when AOAA was injected more laterally. Higher doses of AOAA also killed neurons in layer II and in deep layers. The extent of the lesion along both the medio-lateral and the dorso-ventral axes varied slightly from case to case. Behavioral seizures were noticed between 2-4 hours after AOAA injection in all rats whose brains subsequently showed lesions. Since neuropathological changes in layer III of the EC have been described in neuropsychiatric diseases such as schizophrenia and Alzheimer's disease and may be involved in seizure disorders, the selective ablation of EC neurons described here may provide an experimental model for those pathological conditions.

Supported by USPHS grants NS 16102 and MH 44211.

EPILEPSY: BASIC MECHANISMS II

232.1

NEURONAL CELL DENSITY CHANGES IN THE HIPPOCAMPUS OF AN EPILEPTIC MUTANT, STARGAZER X. Qiao* and J.L. Noebels. Developmental Neurogenetics Laboratory, Section of Neurophysiology, Department of Neurology and Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

We have discovered hippocampal mossy fiber sprouting in the molecular layer of the dentate gyrus and CA3 pyramidal cell body region following the onset of inherited spike-wave seizures in the *stg* mutant. To test the hypothesis that neuronal loss in this region may play an important role in the synaptic reorganization, quantitative measurements of neuronal density and morphology were performed in the adult +/+ and *stg/stg* hippocampus. Using computerized image analysis, the perimeter measurements (3 mice, 20-24 sections per group) of the dentate gyrus (+/+ 17230.30±377.27 μm^2, *stg/stg* 18937.37±1049.25 μm^2) and CA3 regions (+/+ 24933.33±708.09 μm^2, *stg/stg* 27712,12±1801.22 μm^2) showed no significant difference in area between genotypes ($P > 0.05$). The density of granule cells (+/+ 11246.03±292.43/mm^2, *stg/stg* 11411.77±312.53/mm^2) and CA3 pyramidal cells (+/+ 6253.32± 268.51/mm^2, *stg/stg* 5666.75±202.60/mm^2) were also similar in both genotypes ($P > 0.05$), as were the cell volumes of the granule cells (+/+ 43.31±1.26 μm^2, *stg/stg* 45.95±1.04 μm^2) and CA3 pyramidal cells (+/+ 88.95±3.92 μm^2, *stg/stg* 98.41±4.00 μm^2). In contrast, hilar polymorphic cells are significantly reduced ($P < 0.05$) in density (28%) and increased ($P < 0.05$) in volume (23%) in the *stg* mutant (density 3943.22± 169.24/mm^2, volume 57.39±2.18 μm^2) compared to +/+ (density 5457.84±220.60/mm^2, volume 46.78±2.29 μm^2). Preliminary immunocytochemical staining of glial fibrillary acidic protein did not reveal any obvious difference in the hilar astrocyte population between the mutant and control sections. These data suggest that there is a selective reduction of the hilar cell population in the *stg* mutant, which may be related to mossy fiber sprouting and spike-wave seizure activity.

232.2

Structural impairment in hippocampal neurons following a single epileptic afterdischarge. Z. Horváth*, M. Hsu, E. Pierre, D. Vadi, F. Gallyas and G. Buzsáki. Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, 197 University Avenue, Newark, NJ 07102.

A highly sensitive silver-impregnation procedure was used to reveal whether lasting structural changes can be induced by a brief epileptic afterdischarge. In groups of rats, implanted with perforant path or entorhinal cortex stimulating electrodes and epidural recording electrodes, a single afterdischarge (< 2 min) was induced and the animals were perfused 1 to 16 hour after the electrical seizure. Most silver-impregnated "dark" neurons were present in the strata radiatum-lucidum, pyramidale and oriens of CA3, followed by the hilus and strata oriens and pyramidale of CA1. "Dark" cells were present at all time points in both hippocampi, but not in other structures. The findings suggest that even short epileptic epochs are capable of triggering long-lasting morphological changes and interneurons are more vulnerable than principal cells. We hypothesize that the observed structural changes might explain the cumulative damaging effects of successive epileptic episodes.

232.3

EEG PATTERN RECOGNITION BY BACKPROPAGATION NETWORK G. Jando, R. M. Siegel*, Z. Horvath and G. Buzsaki. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

A backpropagation network was trained to recognize the high voltage spike-and-wave spindle (HVS) pattern in the rat, a rodent model of human *petit mal* epilepsy. The spontaneously occurring HVS were examined in 250 rats of the Fisher 344 and Brown Norway strains and their F1, F2 and backcross hybrids (Vadasz et al., this meeting). Neocortical EEG and movement of the rat were recorded for twelve night hours in each animal and analog data were filtered (low cut: 1 Hz; high cut: 50 Hz) and sampled at 100 Hz with 12 bit precision. A training data set was generated by manually marking durations of HVS epochs in 16 representative animals selected from each group. Training data were presented to backpropagation networks with variable numbers of input, hidden and output cells. The performance of different types of networks were first examined with the training samples and then the best configuration was tested on novel sets of the EEG data. FFT transformation of EEG significantly improved the pattern recognition ability of the network. With the most effective configuration (16 input; 19 hidden; 1 output cells) the summed squared error dropped by 80% as compared with that of the initial random weights. When testing the network with new patterns the manual and automatic evaluations were compared quantitatively. HVSs which were detected properly by the network reached 91-99% of the manually marked HVS patterns, while falsely detected events (non-HVS, artefacts) varied between 18% and 40%. These findings demonstrate the utility of backpropagation networks in automatic recognition of EEG patterns.

232.4

UNIT RECORDINGS FROM THE CEREBELLUM CORRELATE WITH SPIKE AND WAVE CORTICAL EEG PATTERNS IN THE RAT. Adam Kandel* and György Buzsáki. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

High voltage spike and wave spindles (HVS) in rodents are regarded as a model of petit mal generalized non-convulsive seizures in humans. In previous studies the ventral anterior (VA) and the ventral lateral (VL) have been implicated as important initiators for neuronal oscillations underlying neocortical HVS. The deep cerebellar nuclei (the only output from the cerebellum) have a heavy projection to the thalamus, mainly to the VA/VL. Spike and wave patterns recorded from the frontal cortex of the rat were observed to correlate with unit recordings in the cerebella of rats. In this study the EEG was recorded with screw electrodes implanted in the calvarian bone over the frontal cortex. A microelectrode drive was placed over the cerebellum above the deep cerebellar nuclei (AP= 11.50-13.50; ML=0.00-1.50) and recordings were attained from 0-8 mm vertical and from 4-6 tracks per animal. Tremor was recorded with a magnet-coil type accelero-meter. Single or multiple units were recorded with 1-3 MΩ tungsten microelectrodes. Units were found to occur in correlation with HVS with the majority of neurons recorded discharged during the wave component, whereas a smaller portion fired in association with the spike component of the HVS. From our data we conclude that cerebellar neurons may contribute to the emergence and/or maintenance of HVS.

232.5

DEGENERATION AND REINNERVATION INTERVALS AS MECHANISMS FOR THE LATENT PERIOD BETWEEN FOCAL DAMAGE AND LATER FOCAL CHRONIC SEIZURES. T. Babb*, J. Pretorius, F. Cifuentes and G. Mathern. Dept. of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

One of the most puzzling phenomena in epilepsy is the well-known latent period between damage-induced acute seizures and the establishment of chronic seizures. Historically, the rat kainate model of epileptic hippocampus (HC) has demonstrated rapidly occurring (7-30 days) reactive synaptogenesis of intrinsic circuits (both "excitatory" and "inhibitory"). However, the typical latent period (90-120 days) before chronic HC seizures far exceeds the initial time course of reactive synaptogenesis (as demonstrated by Mathern, et al., 1992, this meeting). We propose that markers for degeneration and reinnervation might suggest mechanisms that would be impermissive or growth-promoting for functional synaptic reorganization. Results show that at 21 days post-kainate, the bilaterally-dependent spiking was associated with dense dentate supragranular proliferating astrocytes (GFAP) and sprouting (GAP-43) that was related to sprouting by mossy fibers (neo-Timm). At 180 days, when HC EEG seizures and behavioral partial complex seizures occurred, there was a subnormal GFAP band in the supragranular layer which was overlapped by an increased supragranular band of GAP-43 staining. These results suggest that early in reactive synaptogenesis GFAP marks the factors such as degenerating axons, microglia and activated astrocytes that are impermissive to functional synaptogenesis. The GAP-43 may mark neuritic processes that may progress into new supragranular synapses. By 180 days, the absence of supragranular GFAP may mean the impermissive factors were cleared and functional synaptogenesis established, as indicated by the dense GAP-43 band and the HC EEG focal and behavioral seizures. These results suggest that changes in glial functions at deafferented zones may contribute to the latent period by interacting to inhibit or promote functional neoinnervations. Supported by NIH Grant NS02808 and an EFA Fellowship to G.M. GAP-43 was a gift from Dr. Larry Benowitz.

232.6

PROGRESSIVE HIPPOCAMPAL ELECTROGRAPHIC HYPEREXCITABILITY CORRELATES WITH REACTIVE SYNAPTOGENESIS IN THE RAT KAINATE SEIZURE MODEL. G. Mathern, J. Pretorius, F. Cifuentes, M. Nuwer* and T. Babb. Dept. of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

Previously, Mathern et al., (Dendron, 1992, vol. 1) showed that bilateral intrahippocampal kainate in rats induced progressive cell loss and mossy fiber sprouting in hippocampi and eventually to subiculum at 4 months. Partial complex seizures were observed at 6 months. This study recorded EEG bilaterally from hippocampus and frontal cortex to relate critical stages of EEG changes to cytologic and circuit alterations in the hippocampi. EEGs were recorded at the time of kainate injection (0.1 μg/μl bilaterally), every 4-6 days using both fast (to resolve interictal spikes) and slow paper speeds (to detect EEG seizures, many of which were observed behaviorally). Minor brain sections were alternately stained for: 1) recent cell death (silver stain), 2) reactive astrocytes (GFAP), 3) cell counts (CV), 4) reactive synaptogenesis (GAP-43), 5) "excitatory" sprouting (neo-Timms, AChE), and 6) "inhibitory" sprouting (GAD). Results indicate the pattern of hippocampal EEG changes predicts reactive synaptogenesis. Acutely, kainate induced a consistent pattern of partial hippocampal status lasting only 4-8 hours. For the next 5-7 days there was diffuse background slowing with occasional synchronous spikes. From 7-21 days there was a second period of diffuse, bilaterally synchronous hyperexcitability, and generalized seizures. After day 21 a silent period occurred lasting 90 to 120 days, then bilaterally-independent hippocampal spikes began. At 120 to 180 days, these developed into independent hippocampal polyspikes and paroxymal fast activity. This independent hippocampal EEG activity caused complex partial seizures in these animals. Synaptic reorganization paralleled the EEG changes, showing more sprouting as independent EEG activity was seen. Supported by NIH Grant NS02808 and an EFA Fellowship to G.M.

232.7

A METHOD FOR 3-D RECONSTRUCTION OF THE CELL-ORGANIZATION IN CORTEX CEREBRI - DEVELOPED FOR THE ANALYSES OF EPILEPTIC CORTEX (WORK IN PROGRESS). T.Skoglund*, R. Pascher, M. Rydmark, C-H. Berthold, T. Gustavssson. Dept. of Anatomy and MedNet-laboratory, University of Göteborg, Medicinarg. 3, 413 90 GÖTEBORG, SWEDEN.

The hypothesis that a developmental disturbance underlies epilepsy was put forward in the early 1900's by Alzheimer and Ranke. In order to study if this disturbance could be found in cortex cerebri we developed a system for 3-D reconstruction of the cellorganization in nervous tissue.

During the development of the system we used cortex cerebri from cat. Light microscopical images of consecutive semithin (0.5 um) serial sections of cat motor cortex were digitized into a image analyses system (Teragon 4000) via a CCD-camera attached to a microscope. The image-stack in the computer, containing 914 photographic images, were aligned and analyzed and the coordinates and a classification of the neurons and the glial-cells were saved in a database. The database (i.e. the cortical prism containing about 20.000 cells) could be visualized in a 3-D graphic system (Megatek 9300) where the cells were represented by semi-realistic models. Parameters as celldensity, clustering and lamellar organization could be computed.

We are now applying the same procedure on cortex cerebri from human epileptic cortex. The cortex (in this case area 21) has been removed in the neurosurgical treatment of the patients epilepsy. Instead of Vestopal W we have used LR Gold which makes it possible to do immunohistological classification of the cells. At the conference we will show the results from our studies comparing the cellorganization in epileptic cortex and in normal, non-epileptic human cortex.

232.8

MK-801 BLOCKED SEIZURE-RELATED HIPPOCAMPAL DAMAGE INDUCED BY ELECTRICAL STIMULATION OF THE DEEP PREPYRIFORM CORTEX. K.Inoue, K.Morimoto* and S.Otsuki. Dept.Neuropsychiat. Okayama Univ.Med.Sch. Okayama, 700 Japan.

A model of status epilepticus (SE), which was induced by intermittent electrical stimulation of the deep prepyriform cortex, has been developed in the conscious rat. SE was induced in 9 of 16 rats in the drug-free group. Significant cell loss was observed in the hippocampal CA3 ipsilateral to the stimulation site and bilateral CA1 areas in the status subgroup compared with the sham operation group. In addition, there was a significant correlation between the duration of seizure activity subsequent to the stimulation session and the total number of intact pyramidal neurons observed in the bilateral CA1 and ipsilateral CA3 subfields of the status subgroup. There were significant differences between the status and non-status subgroup with respect to the number of afterdischarges (ADs) and the total AD duration during the stimulation session. Pretreatment with phenobarbital (30mg/kg) prevented the development of SE and hippocampal cell loss completely. Pretreatment with MK-801 (0.25 or 1mg/kg) also prevented hippocampal cell loss, although it did not block SE generation completely, which suggests dissociation of the mechanisms underlying the development of SE and hippocampal damage. These results indicate that prolonged seizure activity actually causes hippocampal damage and it is critically dependent upon NMDA receptor participation.

232.9

GENERALIZED TONIC-CLONIC SEIZURES INDUCED IN RATS BY INTRAVENTRICULAR ADMINISTRATION OF OKADAIC ACID. J.T. Slevin*, G.R. Barnes, Y.J. Farrar, R.M. Alcala, and T.C. Vanaman. VA and the University of Kentucky Medical Centers, Lexington, KY 40536.

We have shown that rat hippocampal protein kinase C [PKC] and phosphoprotein phosphatase 2A [pp2A] apparently redistribute from cytosol to synaptic plasma membrane [SPM], either following an ECS *in vivo* or K^+ depolarization of hippocampal slices *in vitro*. In slices, PKC and pp2A activity crest in SPM at 0.5 and 2 min, respectively, and both return to control values by 5 min. PKC activity is amplified and pp2A redistribution is attenuated in fully kindled, epileptic rats. We report here that rats administered 7.5 nmoles of the pp2A inhibitor, okadaic acid, through an intraventricular cannula experienced generalized tonic-clonic seizures. These were observed, both by electroencephalographic [EEG] recording and behavioral measures, within 5 min of dosing. Dephosphorylation of the synthetic peptide, ^{32}p-RRATVA, using pp2A partially purified from either hippocampi of animals given okadaic acid or vehicle alone, was no different suggesting, among other possibilities, the inhibitor is rapidly dispersed. These data indicate phosphorylation-dephosphorylation events associated with neuronal depolarization may play a central role in epilepsy. [Support: Dept. of Veterans Affairs & NS 21868, NINDS]

232.10

INHERITANCE OF NEOCORTICAL HIGH VOLTAGE SPIKE-AND-WAVE (HVS) PATTERNS IN RATS. D. Carpi[1], G. Jando[1], E. Pierre[1], D. Vadi[1], A. Fleischer[2], A. Lajtha[2], C. Vadasz[2], and G. Buzsaki[1]. Center for Neuroscience[1], Rutgers University, Newark, NJ 07102; Nathan Kline Institute for Psychiatric Research[2], Orangeburg, NY 10962.

Our previous studies on Fisher 344 (F344) and Brown Norway (BN) rats indicated that genetically controlled, spontaneously occurring HVS in rats may serve as an animal model of absence epilepsy. To further investigate the inheritance of HVS patterns, $(F344XBN)F_1$ hybrids were mated to produce segregating $(F344XBN)F_2$ rats (N=82). Neocortical activity was recorded in 6-month-old F344, BN, F_1, and F_2 rats for 12 night hours by a computer assisted system. HVS episodes were detected by a backpropagation neural network (Jando et al., this meeting). 74% of the F_2 animals expressed HVS, a proportion that fit well the Mendelian 3:1 ratio expected for monogenic inheritance with complete dominance. However, since occurrence of HVS in F344, BN and F_1 groups did not fit this simple Mendelian model, and significant sex differences were also detected in BN, F_1, and F_2 animals, we suggest that HVS is a treshold trait whose expression is affected by one or more quantitative trait loci, and sex-linked gene effects.

232.11

INCREASED NUMBER OF $GABA_b$ RECEPTORS IN THE LETHARGIC (*lh/lh*) MOUSE MODEL OF ABSENCE EPILEPSY. F-H Lin, Z. Cao & D. Hosford*. Duke & VA Medical Centers, Durham, N.C. 27705.

Lh/lh mice have a single-locus defect on chromosome 2 and spontaneous absence seizures. Pharmacologic studies appear to indicate a requirement for $GABA_b$ receptors in the expression of these seizures (Hosford et. al., Soc. Neurosci. 17:170, 1991); electrophysiologic studies show enhanced synaptic activation of $GABA_b$ receptors in slices from *lh/lh* mice (Clark et. al., Soc. Neurosci. 17:170, 1991). We used a radioligand binding assay to test the hypothesis that an increase in the affinity (K_d) or number (B_{max}) of $GABA_B$ receptors underlies enhanced synaptic activation of these receptors.

We measured [^{3}H]-baclofen binding sites to neocortical plasma membranes prepared from *lh/lh* and wild (+/+) age-matched congenic mice. The B_{max} was significantly greater (20%) in *lh/lh* (4.2 pmol/mg protein, n = 43 pairs, $p < 0.02$) than +/+ mice (3.3 pmol/mg protein) in an age-independent manner. The K_d (50 nM) was unchanged. Interestingly, the subset of *lh/lh* mice with greater seizure frequency (40-70 seizures/15 min, measured by bipolar electrodes implanted into neocortex; n = 11) had a significantly greater B_{max} ($p < 0.003$) than the subset with lower seizure frequency (1-10 seizures/15 min; n = 11). The increased number of $GABA_b$ receptors was selective, because binding to NMDA receptors (NMDA-displaceable [^{3}H]glutamate binding) and to $GABA_A$ receptors ([^{3}H]muscimol binding) was not significantly different in the two strains.

These data support our hypothesis that the increased number of $GABA_b$ receptors in *lh/lh* mice underlie enhanced synaptic activation of these receptors. Together with evidence that $GABA_b$ receptor activation can produce disinhibition (Ogata, Gen. Pharmacol. 2:395-402, 1990), our data also support a role for $GABA_b$ receptors in the expression of absence seizures in *lh/lh* mice.

232.12

INCREASED HIPPOCAMPAL NMDA RECEPTOR DENSITY IN GENETICALLY EPILEPSY-PRONE RATS. K.A. Ortiz and D.D. Savage* Dept. Pharmacology, University of New Mexico School of Medicine, Albuquerque, NM, 87131-5316.

Previous *in vitro* autoradiographic studies indicated that [3H]-glutamate receptor binding site density is increased in the apical dendritic field regions of hippocampal formation in Genetically Epilepsy-Prone (GEPR-9) rats compared to non-epileptic Sprague-Dawley controls. Using two different radioligands that selectively bind to the NMDA receptor-operated ion channel complex, we investigated whether the NMDA subtype of glutamate receptors is one of the subtypes elevated in GEPR-9 rat hippocampal formation.

Eight-μm-thick histological sections of brain containing either dorsal or ventral hippocampal formation were incubated with either [3H]-MK-801 (12 nM) or [3H]-CGS 19755 (33 nM). Specific [3H]-MK 801 binding was defined as the difference between [3H]-MK 801 binding in the absence and presence of 200 μM unlabelled ketamine. Specific [3H]-CGS 19755 binding was defined as the difference between [3H]-CGS 19755 binding in the absence and presence of 10 μM unlabelled CGS 19755. Binding was measured in three apical dendritic field regions of hippocampal formation: 1) dentate gyrus *s. moleculare*, 2) hippocampal CA_1 *s. radiatum* and 3) subiculum *s. radiatum*. Specific [3H]-MK 801 binding was increased by 33% to 47% dorsal hippocampal formation of GEPR-9 rats compared to controls. Lesser elevations, ranging from 11 to 17% above control, were noted in ventral hippocampal formation of GEPR-9 rats. Specific [3H]-CGS 19755 binding was increased 20-57% in dorsal hippocampal formation of GEPR-9 rats compared to controls. Lesser elevations, ranging from 14 to 23% above control, were noted in ventral hippocampal formation of GEPR-9 rats.

These results indicate elevated NMDA receptor density occurs primarily in the dorsal hippocampal formation of GEPR-9 rats. This increased NMDA receptor density may contribute to elevated hippocampal glutamatergic neurotransmission and enhanced seizure susceptibility in GEPR-9 rats. Since these GEPR-9 rats were not deliberately convulsed, an alternative interpretation of the binding data is that the GEPR-9 rats may have experienced spontaneous seizures which consequently elevated NMDA receptor density. (Supported by RR08139 and RR05583).

232.13

LOSS OF GAD mRNA-CONTAINING NEURONS IN THE HILUS OF THE DENTATE GYRUS FOLLOWING PILOCARPINE-INDUCED SEIZURES. A. Obenaus*, M. Esclapez and C.R. Houser. Dept. of Anatomy and Cell Biology and Brain Research Institute, UCLA, and VA Medical Center, Los Angeles, CA 90024.

In situ hybridization methods were used to determine if glutamic acid decarboxylase (GAD) mRNA-containing neurons within the dentate hilus are vulnerable to seizure-induced damage in the pilocarpine model of chronic seizures (Turski et al., Synapse 3:154, 1989). Sprague-Dawley rats (150-200 gm) were injected with pilocarpine (325-350 mg/kg, i.p.) to induce severe behavioral seizures and were studied histologically at 1, 2 and 4 week intervals. *In situ* hybridization histochemistry, using a digoxigenin labeled GAD cRNA probe, demonstrated a substantial decrease in the number of GAD mRNA-labeled neurons within the hilus of the pilocarpine-treated rats as compared to controls. Supplemental studies, utilizing cresyl-violet staining, neuronal degeneration methods and GFAP immunohistochemistry, suggested that the decrease in GAD mRNA-labeled neurons was related to neuronal loss rather than to a decrease in GAD mRNA levels. The loss of GAD mRNA-containing hilar neurons contrasted with the relative preservation of labeled putative basket cells along the inner border of the granule cell layer. The results suggest that, in this model, a subpopulation of GABA neurons within the dentate gyrus is selectively vulnerable to seizure-induced damage. Supported by VA Medical Research Funds and NS21908.

232.14

ENHANCED ASPARTATE RELEASE RELATED TO SEIZURES IN EPILEPTIC (EL) MICE. H.J. Flavin* and T.N. Seyfried. Biology Dept., Boston College, Chestnut Hill, Ma. 02167.

The release of the putative neurotransmitters aspartate, glutamate, and gamma-aminobutyric acid (GABA) was studied in hippocampal slices from epileptic (EL) and non-seizure control C57BL/6J (B6) and ddy mice. The EL mouse is a genetic model of temporal lobe epilepsy. We previously showed that the endogenous release of aspartate was significantly higher in slices from adult EL mice (many seizures) than B6 mice (Flavin et al., 1991 *J. Neurochem.* **56**, 1007-1011). In the present study, we used young (38 ± 4 days) mice prior to seizure expression in EL mice. The calcium-dependent potassium-induced release was compared between strains. Release of endogenous amino acids was measured using liquid chromatography with fluorescence detection and was expressed as pmol amino acid released/min incubation/slice ± SEM. No significant differences were found between the B6, ddy and EL mice for the release of glutamate (22.95 ± 2.0; 32.28 ± 5.8; and 25.86 ± 2.6, respectively) or GABA (23.52 ± 0.7; 19.51 ± 3.2; and 21.80 ± 3.2, respectively). Aspartate release, however, was significantly higher in the EL mice (15.79 ± 0.8) than in either the B6 mice or the ddy mice (8.54 ± 1.4 and 8.39 ± 1.7, respectively). Thus, enhanced aspartate release in EL slices is expressed both before and after seizure onset. Further, the enhanced aspartate release may be related to the cause rather than to the effects of seizure activity. (Supported by NIH Grant 23355 and the Michael P. Walsh Fellowship.)

232.15

SINGLE GENE CONTROL OF ATP AND GTP HYDROLYSIS IN MOUSE BRAIN K.M. Ding-Allen*, and T.N. Seyfried
Dept. of Biology, Boston College, Chestnut Hill, MA 02167.

In order to discriminate between catalysis by one enzyme or two different enzymes, both ATP and GTP were used as substrates in the Ca^{2+}- or Mg^{2+}-stimulated nucleotide triphosphatase assay in brain microsomes from C57/BL6 (B6) and DBA/2J (D2) mice. The total substrate concentration was kept constant while varying the relative concentrations of ATP and GTP. The activity was constant and linear which is consistent with a single enzyme model. Furthermore, competitive inhibition experiments, using non-hydrolyzable ATP or GTP analogs, suggest that the same enzyme hydrolyses both ATP and GTP. Previous studies showed that ATPase and GTPase activities are significantly lower in the audiogenic seizure (AGS) susceptible D2 mice than in the resistant B6 mice. B6D2F1 hybrids had ATPase and GTPase activities that were intermediate to those of the parental strains. Analysis of eight BXD recombinant inbred (RI) strains showed that ATPase and GTPase activities were inherited together and displayed a bimodal distribution, suggesting single gene control. Hence, the data suggest that a single enzyme, under single gene control, is responsible for the Ca^{2+}- or Mg^{2+}-stimulated hydrolysis of either ATP or GTP in mouse brain. (Supported by NIH Grant 23355 and NIMH NRSA MH10138.)

232.16

ENHANCED RELEASE OF SOMATOSTATIN (SLI) IN THE RAT HIPPOCAMPUS IN CHRONIC MODELS OF LIMBIC EPILEPSY:COMPARISON WITH NEUROPEPTIDE Y (NPY). A.Monno, M. Rizzi, A. Galli, M.T. Tacconi*, R. Samanin and A. Vezzani. Lab. of Neuropharmacology, Mario Negri Institute for Pharmacological Research, Milan, Italy.

Spontaneous efflux of SLI from hippocampal slices of rats (3.5±0.48 fmol/ml every 10 min) was increased 1.5 times in the electrically kindled hippocampus (HP) 48 h after stage 2 and 2 times on average bilaterally one week after stage 5 ($p<0.05$). Ca^{2+}-dependent 50 mM KCl-induced release was enhanced (1.8 times on average, $p<0.01$) bilaterally at both stages compared to shams (17±1.6 times baseline, $p<0.01$). The release did not differ 48 h after a single afterdischarge. 50 mM KCl-induced NPY release was enhanced bilaterally at both stages of kindling (4.1± 0.7 times sham value, $p<0.01$). One month after 10 mg/kg kainic acid, KCl-induced SLI release was similar to shams in dentate gyrus (DG) slices in rats with behavioral seizures and a two-fold increase in NPY release was found in the DG of the same animals ($p<0.05$). These peptides' role in hippocampal epileptogenesis is worth further investigation.

232.17

NORADRENERGIC ABNORMALITIES IN THE CENTRAL NERVOUS SYSTEM OF GENETICALLY EPILEPSY-PRONE RATS: STUDIES WITH MICRODIALYSIS. Q-S. Yan, P. C. Jobe and J. W. Dailey.* Department of Basic Sciences, University of Illinois College of Medicine at Peoria, Peoria, Illinois 61656.

An expanding body of data supports the concept that the genetically epilepsy-prone rat (GEPR) brain is characterized by a deficiency in the number of noradrenergic terminals, in the amount of norepinephrine (NE) released per terminal and in the capacity of the postsynaptic receptors to compensate for the presynaptic deficits. The present study was designed to provide a direct demonstration of abnormalities in the central noradrenergic transmission of GEPR by using intracerebromicrodialysis. Under anesthesia, guide cannulae were stereotaxically placed over thalami of both severe seizure GEPRs (GEPR-9s) and seizure-resistant control rats. After recovery from surgery, dialysis probes were inserted and the animals were placed individually into a plexiglass chamber where they were allowed to move about freely. Artificial CSF was perfused at 1 μL/min and samples were collected for analysis on HPLC with electrochemical detection. After 2-3 hours of sampling, a stable baseline for NE was established and three basal release measures were made. Either desipramine (5 μM in artificial CSF) or yohimbine (5 μM in artificial CSF) was administered through the dialysis probe for 2 hours and dialysis was continued for another 4 hours. Significantly diminished in vivo NE release from the thalamus was seen after the treatment with either drug in GEPR-9s when compared with seizure-resistant controls. These findings are consistent with the hypothesis that NE deficiencies exist within some brain areas of GEPR and serve as a partial determinant of the abnormal state of seizure predisposition exhibited by these animals. (Supported by UICOM-P Internal Research Grant and NIH NS22672)

232.18

A SPECIFIC 70-KDA PROTEIN FOUND IN EPILEPTIC RAT CORTEX: A POSSIBLE LINKAGE TO EPILEPTOGENESIS. M. Onozuka[1], S. Imai[2], S. Ozono[3], K. Nagata[4], M. Tsuchiya[4], M. Tsujitani[4] and Y. Fukami[5]*. [1]Dept. of Anatomy, Gifu Univ. Sch. of Med., Gifu 500, Japan; [2]Research Center for Biological Function, The Kitasato Institute, Tokyo 108, Japan; [3]Dept. of Pathology, Kanagawa Dent. Col. Yokosuka 238, Japan; [4]POLA Pharmaceutical R & D Laboratory, Yokohama 244, Japan; and [5]Dept. of Physiology, Asahi Univ. Sch. Dent., Gifu 501-02, Japan.

In order to evaluate a protein-related mechanism underlying epileptogenesis, we quantitatively analyzed the 70-kDa protein (namely, P70), a specific protein found in the cobalt-induced epileptic focus of rat cerebrum, and examined its effect on the electrocorticogram (ECoG) and cortical neurons in cerebral slices and its immunocytochemical localization in rat cerebrum. Cobalt-induced epileptogenic cortex exhibited a marked induction of P70. Its initiation time was ahead of the generation of epileptogenic activities. The anticonvulsant phenytoin (PHT) attenuated the epileptogenic activities, but failed to suppress protein induction. Injection of this protein into the motor region of normal rat cerebral cortex elicited an epileptiform ECoG and behavioral seizures. It also caused epileptiform activity with paroxysmal depolarization shifts in cortical neurons. These epileptogenic phenomena elicited by P70 were abolished by prior treatment with PHT or phenobarbital. Immunocytochemical analysis with an antiserum against P70 revealed that the reactivity was confined to pyramidal cells only in the region of the focus and was mainly localized on somatic, dendritic, and nuclear membranes and microtubles. These findings suggest the possibility that P70 may be linked to epileptogenesis.

232.19

NEUROPROTECTION FROM NMDA AND NON-NMDA MEDIATED DAMAGE OF SOMATOSTATIN AND NPY CELLS IN A RAT MODEL OF STATUS EPILEPTICUS. L.P. Penix*[1], and C.G.Wasterlain[2]. [1]Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892, and [2]Brain Research Institute, UCLA.

Somatostatin positive interneurons are selectively lost in the dentate hilus of patients with long standing temporal lobe epilepsy. Somatostatin neurons have also been shown to be vulnerable to non-NMDA but not to NMDA mediated neurotoxicity in cell culture. However, selective mechanisms of hilar neuronal death have not been demonstrated in in-vivo models of epileptic brain damage. We administered the non-competitive NMDA antagonist MK-801 and the competitive non-NMDA antagonist NBQX to rats during a 24 hour perforant path stimulation paradigm described by Sloviter in order to prevent the damage to somatostatin positive hilar interneurons. MK-801 and NBQX were administered i.v. at 100 μg/kg/hr and 1.5 mg/kg/hr, respectively. Paired pulse inhibition was lost in each of the three groups. Control animals had 64.7 ± 2.9 % and 50.3 ± 1.5 % loss of SS- and NPY-like immunoreactive interneurons, respectively. Animals treated with MK-801 had 37 ± 5.0 % ($p<0.05$) and 38 ± 4.4 % (N.S.) loss. Animals treated with NBQX had 30.7 ± 10.3 % ($p<0.01$) and 18 ± 4.8 % ($p<0.05$) loss. These data show that antagonism of both NMDA and non-NMDA receptors significantly protect against damage of SS-immunoreactive hilar interneurons yet only non-NMDA antagonism significantly protects against damage of NPY-immunoreactive cells. In addition, lack of protection of paired pulse inhibition suggests the possibility that SS- and NPY-immunoreactive interneurons may not be responsible for this particular type of inhibition.

233.1

GALANIN INNERVATION WITHIN THE NUCLEUS BASALIS IN DOWN SYNDROME. E.J. Mufson*, E.J. Cochran, R. Casanova and J.H. Kordower. Dept. of Neurol. Sci. and Rush Alzheimer's Dis. Ctr., Rush Presbyterian St. Luke's Med. Ctr., Chicago, IL. 60612.

Galaninergic fibers have been reported to hypertrophy in response to cholinergic cell loss within the nucleus basalis (nbM) in Alzheimer's disease (AD). Since brains of patients with Down syndrome exhibit neuropathologic changes similar to AD, we investigated the pattern of galanin innervation within the nbM of these individuals. Paraformaldehyde (4%) immersion fixed 40μ thick sections throughout the entire extent of the nbM of Down patients (ages 46, 47 and 47 years) were singly or concurrently immunohistochemically stained for galanin (GAL), nerve growth factor receptor (NGFR; a marker for basal forebrain cholinergic neurons) and the cytoskeletal markers thioflavin-S, paired helical filament (PHF) and Tau. All cases exhibited cytoskeletal positive neuritic plaques, neurofibrillary tangles and neurites within neo and limbic cortex. The severity of nbM NGFR containing neuron loss ranged from moderate to severe. Numerous cytoskeletal extracellular globose shaped tangles and neurites, but virtually no neuritic plaques, were found within the nbM. GAL immunoreactive (ir) sections revealed positive fibers and only a few small oval shaped atrophic GAL-ir neurons distributed mainly within the anterior regions of the nbM. The extent of the GAL-ir fiber innervation exceeded the levels expected from the number of nbM GAL-ir neurons. In the Down cases, hypoinnervation of GAL-ir fibers was seen in regions containing clusters of NGFR-ir neurons relative to AD and age-matched controls. GAL-ir fibers within the Down nbM were observed mainly in areas devoid or adjacent to clusters of NGFR-ir perikarya. Interestingly, AD and age matched controls exhibited similar GAL-ir fiber innervation in close apposition to NGFR-ir neurons. Support: AG10668, AG09466, AG10161 and IDPHS.

233.2

DISTRIBUTION OF CYTOCHROME OXIDASE (COX) ACTIVITY AND mRNA IN MONKEY AND HUMAN BRAIN. COX mRNA DISTRIBUTION CORRELATES WITH NEURONS VULNERABLE TO ALZHEIMER PATHOLOGY. K.Chandrasekaran*, J. Stoll, D.R. Brady and S.I. Rapoport. Laboratory of Neurosciences, NIA, Bethesda, MD 20892.

Differential screening of a frontal pole cDNA library from monkey (*Macaca mulatta*) brain revealed 3 cDNA clones whose cognate mRNAs are expressed more in frontal pole than in primary visual cortex. The cDNA clones were identified as mitochondrial (mit) DNA encoded cytochrome oxidase (COX) subunits I, II and III. Each clone showed higher expression of mRNA in association neocortices than in the primary sensory or motor cortices. COX mRNA and COX activity were localized, by *in situ* hybridization and by enzyme histochemistry, respectively, in regions of monkey and human brain. In monkey cerebral cortex, COX enzyme activity was highest in dendrite-rich neuropil. In contrast, COX mRNA was detected mainly in cell bodies and apical dendrites of medium to large projection neurons. Thus, in the entorhinal cortex, the highest levels of COX mRNA were localized in neuronal cell bodies of layers II and IV, neurons involved in the input and output pathways of the hippocampus, respectively. In superior temporal sulcus, perirhinal and prefrontal cortices, highest levels were detected in cell bodies of layers III and V-VI, presumably neurons involved in corticocortical connections. In normal human brain, similar distributions of COX mRNA were observed. The laminar distribution of COX mRNA corresponded with neurons vulnerable to Alzheimer disease (AD) pathology. In AD brains, there was a significant decrease in COX mRNA in vulnerable brain regions. Preliminary results also showed deletions in mitDNA. These results indicate high levels of a mitochondrial oxidative enzyme, and of the mRNA for its subunit, in brain regions vulnerable to AD pathology. This neuropathology is accompanied by deletions in mitDNA and reduced COX mRNA.

233.3

THE FRONTAL CORTEX IN NEURODEGENERATIVE DISORDERS: CELLULAR AND REGIONAL PATTERNS OF VULNERABILITY. E.A. Nimchinsky*, P.R. Hof, D.P. Perl and J.H. Morrison. Fishberg Res Ctr for Neurobiology, Mount Sinai Sch of Med, New York, NY 10029.

We have conducted an analysis of the primary motor, premotor and prefrontal regions (areas 4, 6, and 9) in normal human brain as well as in Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and ALS/Parkinsonism-dementia complex of Guam (ALS/PD). SMI32, an antibody that recognizes nonphosphorylated neurofilament protein stains virtually all of the giant pyramidal (Betz) cells in layer V of area 4, and exhibits a bilaminar staining pattern in areas 6 and 9. Interestingly, the largest of the Betz cells are labeled with an antibody to the calcium-binding protein calretinin (CR). CR has not been found in any other pyramidal cell population. In AD, area 9 shows a reduction of SMI32-immunoreactive (ir) cells and the presence of NFT, while in area 4 SMI32-ir neurons including Betz cells are spared. In ALS, there is a dramatic reduction in the number of SMI32- and CR-ir Betz cells in area 4, without the formation of neurofibrillary tangles (NFT), whereas areas 6 and 9 show no such loss of SMI32-ir cells. In ALS/PD, where NFT preferentially occur in superficial cortical layers, ALS-predominant cases show a loss of cells also in the deep cortical layers of area 4. PD-predominant cases show alterations mostly in superficial layers of area 9 and have intact populations of SMI32- and CR-ir giant cells in area 4. These observations suggest that SMI32-ir pyramidal cells may react to insult with or without NFT formation and express specific regional vulnerability in various neurodegenerative diseases depending upon the particular cortical efferent systems involved as reflected in the relative dominance of cognitive or motor symptoms.

233.4

DISTRIBUTION AND CELLULAR ANALYSIS OF mRNA FOR COMPLEMENT COMPONENTS C1Q AND C4 IN ALZHEIMER'S AND NORMAL BRAIN. M. Lampert-Etchells, S.A. Johnson, G.M. Pasinetti and C.E. Finch*. Andrus Gerontology Center and Dept. of Biol. Sci., Univ. of Southern California, L.A., CA 90089.

Complement mRNAs for components C4 and C1qB are present in AD (Alzheimer's disease) and normal human brain. In a group of 7 AD and 6 control individuals average C1qB and C4 mRNA concentration (as measured by *in situ* grain density) in AD frontal cortex is increased 2-3 fold. In addition the average number of C1qB and C4 mRNA expressing cells per total cells counted is 3 times higher in the AD group than in the control group. This data along with localization of complement proteins in plaques and tangles, (McGeer, et al. Can. J. Neurol. Sci. 16:516) suggest possible involvement of brain derived complement in the Alzheimer's neurodegeneration. The cellular distribution of C4 and C1qB mRNA in the frontal cortex is stratified. Grain density and the number of labeled cells are highest in layers 1-2 then gradually diminish from layers 3 to 6. No hybridization is seen in white matter. There is no correlation between the frequency of C4 and C1qB mRNA expressing cells and the frequency of plaques in these 7 AD individuals. There is a positive correlation of C4 and C1qB mRNA levels in the same specimen, suggesting coordinate regulation. In contrast, the average grain density of mRNA for the AD/control non-changing clone 17-3, is not positively correlated with that of C4 or of C1qB. Ongoing studies address identification of the cell type responsible for the increased mRNA for C1qB and C4 in AD and whether C1q and C4 mRNAs are made in the same individual cell. Supported by NIH AG-07909 and AG-10673.

233.5

PATHOLOGY OF EPINEPHRINE NEURONS IN ALZHEIMER'S. W.J. Burke*, N.J. Galvin, H.D. Chung, K.N. Gillespie, A.M. Cataldo, R.A. Nixon. Dept. Neurology, St. Louis Univ. Med. Sch., St. Louis VAMC, St. Louis, MO 63110; McLean Hosp., Mailman Res. Ctr., Belmont, MA 02178

We reported chemical changes but no cell loss in the epinephrine (Epi) C-1 neurons in Alzheimer's disease (AD) (Ann. Neurol.24:532,1988). To examine for histopathological changes in Epi neurons in AD we performed the following: 1) Computer assisted analysis of area distribution of 1000 control and 2500 AD neurons from 2 controls 58±15 yrs. and 4 AD 76.7±2.3 yrs. Mean areas and distributions were compared using t and Kalmogorov-Smirnov z tests respectively. 2) Immunohistochemical analysis of: a) Tau-2 and Alz-50 positive C-1 neurons, b) Staining density of cathepsin D in AD and control C-1 neurons. The mean area of AD C-1 neurons was 21% less than controls (AD:328±4 mm^2 vs control: 416±7; $p<0.001$). The size distributions differed significantly ($p<0.001$) with the peak of AD distribution shifted toward smaller neurons. Both Tau-2 and Alz-50 positive neurons were found in C-1. Cathepsin staining density of some AD C-1 neurons appeared increased compared to control and adjacent normal neurons. We conclude that characteristic pathological changes found in other parts of the brain in AD also occur in C-1 neurons.

233.6

SYNAPSE NUMBERS IN THE POSTERIOR CINGULATE CORTEX (AREA 23) IN PATIENTS WITH ALZHEIMER'S DISEASE. S.W. Scheff* and D. A. Price. Center on Aging, Dept. Anatomy & Neurobiology, Univ. Kentucky, Lexington, KY 40536.

The cingulate gyrus, a key limbic structure, undergoes neuron loss in Alzheimer's disease (AD) beyond that observed in normal aging. This cortical area plays an important role in emotion, motivation, attention and memory; behaviors known to decline in AD. Several key cortical structures demonstrate a substantial decrement in connectivity in AD as evidenced by a significant loss of cortical synapses. There is a significant enlargement of synaptic apposition length, which correlates with the declines in synaptic numbers; as synapse density declined, synapse size increased. A major exception is the entorhinal cortex (area 28) which receives a major input from the posterior cingulate cortex but fails to show any change in synapse numbers. Because of the extensive connections of the posterior cingulate with other limbic structures and its known involvement in AD, it is important to quantitatively assess its functional connectivity (synaptic density) and to determine the degree of neuropathological changes in AD.

Human brains were obtained at postmortem examination from 10 patients who met the NINCDS-NIA criteria for AD and from 10 age-matched controls. All tissues were obtained within 11 hours postmortem. Both lamina III and V were quantitatively assessed for synapse number and size as well as possible correlations to other AD related pathologies. Supported by Alzheimer Association and NIA AG05144.

233.7

THE ASSOCIATION OF KERATAN SULFATE PROTEOGLYCAN AND ALZHEIMER'S DISEASE. E. E. Geisert, Jr.* and R.E. Powers. Department of Cell Biology and UAB Brain Resource Program, University of Alabama at Birmingham, Birmingham, Alabama 35294.

A large keratan sulfate proteoglycan that migrates at over 440 kDa on polyacrylamide gels is present in autopsy specimens of human subjects with Alzheimer type damage. A monoclonal antibody (TED 15) that specifically recognizes keratan sulfate glycosaminoglycan side chain stained sections of temporal lobe (superior temporal gyrus and hippocampus) from young controls (n = 10) aged controls (n = 8) and subjects with AD (n = 8). Immunostaining was completely abolished by pretreatment of hippocampal tissue with keratanase.

Normal appearing astrocytes were stained in all cases. TED 15 immunostained selected neuronal populations that appeared morphologically normal, some damaged neurons and a subpopulation of "soft or immature senile plaques were stained in tissue from aged controls and AD subjects. Neurofibrillary tangles were infrequently stained with TED 15 in AD cases. Amyloid cores, neuritic plaques, dystrophic neurites, granulovacuolar degeneration and hirano bodies were not stained with TED 15.

These findings document the presence of proteoglycans in the brains of aged humans and subjects with AD suggesting a role for keratan sulfate in age and disease related neuronal damage.

233.8

TWO DISTINCT SYNAPTIC VESICLE PROTEINS ARE DECREASED IN ALZHEIMER'S DISEASE. A. Pruchnicki*, J. Gleeson, W.G. Honer and P. Davies. Departments of Medicine and Pathology, Albert Einstein College of Medicine, New York, NY 10461.

Recent reports have indicated that declining synapse density may contribute to the development of dementia, especially of the Alzheimer's disease type. These studies have employed a limited number of monoclonal antibodies raised to synaptophysin or synapsin (E. Masliah et al. 1991, Am. J. Pathol. 138:235-246). The study reported here examined this issue with both qualitative and quantitative methods. Three monoclonal antibodies believed to be reacting with two different synaptic proteins, were produced, and used to examine four brain regions (W. Honer and P. Davies, 1991, Proc. 3rd IBRO World Congress of Neurosci). Samples were obtained from autopsy specimens from both Alzheimer's disease patients and age-matched controls, confirmed by pathology.

Western blot analysis indicated that one antibody (EP10) reacted with a 38Kd antigen and two others (SP12 and 14) with a distinct lower molecular weight protein. Immunocytochemistry showed a synaptic-like reticular pattern of staining. Neither Western blotting nor immunocytochemistry gave quantitative data. Elisa analysis, however, was able to show that there were statistically significant decreases, in the range of 25-75%, in synaptic density in hippocampus and temporal cortex, using all three antibodies. Only one of the three antibodies showed a similar significant decrease in occipital cortex and caudate.

These results are consistent with the theory that synapse loss, as measured by several antibody reactivities, occurs in those brain regions most closely associated with Alzheimer's disease.

233.9

IMMUNOCYTOCHEMICAL EVIDENCE OF ACCELERATED SPECTRIN PROTEOLYSIS IN HIPPOCAMPUS FROM ALZHEIMER DISEASE PATIENTS. M-C de Lacoste*, C.E. Lewis, C.L. White, J.S. Morrow. Depts. OB/GYN & Pathol., Yale University, New Haven CT & Dept. Pathol., Southwestern Medical Center, Dallas, Texas

It has been hypothesized that accelerated calpain-I mediated proteolysis of specific isoforms of the spectrin-based neuronal cytoskeleton may serve as a mechanism by which neurons are irreversibly damaged in Alzheimer disease (AD). This study was undertaken to test this hypothesis using immunocytochemical techniques and the RA150 antibody that recognizes only calpain-cleaved alpha fodrin. RA150 is made against a synthetic peptide characterizing the C-terminus of the calpain-I cleavage site of the 11th repeat of alpha fodrin (i.e. antigen 150N).

Methods: Standard avidin-biotin immunoperoxidase techniques were used on cryostat-sectioned formalin-fixed blocks of hippocampal tissue from AD and normal brains. Results:In the AD brains, the RA150 immunodecorated a large subset of pyramidal neurons in the CA1 subfield as well as neuritic plaques throughout the hippocampal formation. In contrast, there was considerably less immunostaining in the normal brains. Moreover, in AD brains liquid phase preabsorption of RA150 with the synthetic peptide to the RA150N antigen resulted in a marked decrease in immunoreactivity. In addition, double-labelling techniques using either Thioflavin-S in conjunction with RA150 or RA150 simultaneously with an anti-PHF antibody evidenced colocalization of neurofibrillary tangles and spectrin breakdown products. In brief, data obtained in this study provide evidence of accelerated spectrin proteolysis in AD hippocampus. (Supported by HD 21711 (MCL & CLW)).

233.10

DECREASED NEURONAL DENSITY IN CA1-2 AND CA3 REGIONS OF ALZHEIMER'S HIPPOCAMPUS. K.A. Young*, D. Kuhl, C. Conley, Dennis Merrill, Claire Harrison and D. Strete. Department of Medical Pharmacology & Toxicology, Texas A&M College of Medicine; Department of Biology, McLennan Community College; Laboratory Services, Waco VAMC and Olin E. Teague VAMC; and Department of Psychiatry, Scott and White Clinic, Temple, Texas 76508.

A combination of classical and stereological neuron counting techniques was used to obtain a preliminary estimate of the relative density of neurons in the hippocampus of 23 histologically-verified male Alzheimer's patients and 22 age-matched controls with no history of dementia. Parrafin-embedded cresyl violet-stained sections from portions of hippocampus removed during routine brain autopsies from 1983-1988 were analyzed with the optical dissector sampling technique. The results suggest that the number of neurons but not the standard cross-sectional volume of the hippocampus was decreased in the CA1-2 and CA3 fields of patients with Alzheimer's dementia. The density of neurons in the CA1-2 area of Alzheimer's patients was significantly decreased by 31% compared to controls, while the density of neurons in the CA3 region was decreased by 25%. The density of neurons in the CA4 field of Alzheimer's patients was not significantly different from controls. These results are consistent with previous reports of relatively focal CA1-2 and CA3 hippocampal pathology in patients with Alzheimer's dementia. The minimalist technique used in this study may be useful in obtaining preliminary estimates of neuron density in other regions of the brain routinely sampled in brain autopsies.

233.11

Latent Type IV Collagenases in the Hippocampus From Alzheimer and Control Specimens. Jon R Backstrom, Giselle P Lim, Michael J Cullen, and Zoltan A Tokes*. Departments of Biochemistry, Anatomy and Cell Biology, University of Southern California, School of Med., Los Angeles, CA 90033.

Earlier, we examined the proteinases from hippocampal specimens which could be detected in SDS-polyacrylamide gels containing gelatin (substrate gels). We found an increase in metalloproteinases (MPs) with masses of 280, 130, and 100 kD from the AD specimens relative to the control specimens and no significant difference in the 70 kD MP (J Neurochem. 58: 983, 1992). Therefore, experiments were designed to further characterize the three larger MPs. To this end, we purified MPs from brain extracts and cell cultures. The cells included HL-60 cells which resemble macrophages upon stimulation with phorbol esters and the glioblastoma cell line U-87. The brain and HL-60 MPs cross-reacted with antisera raised against neutrophil metalloproteinases. The Tris-soluble brain fraction and purified MPs were latent and could be activated with an organomercurial compound, which caused a >50-fold increase in gelatinase activity and fragmentation of the enzymes. Endogenous MP-280 in the Triton-insoluble brain fraction has a higher avidity for the matrix than MP-130 and MP-100 which had similar properties. This finding correlated with the results from gelatin-agarose purification in that a higher concentration of DMSO was necessary to efficiently elute MP-280 from the matrix. Immunohistology revealed that the distribution of MPs and the tissue inhibitor of metalloproteinases (TIMP-1,-2) differed between AD and control tissues. Since serine proteinases are known to activate MPs, we postulate that the accumulation of latent MPs in AD tissues relative to control tissues may be due to an excess of serine proteinase inhibitors which could block their activation. Supported by NIA R01-AG 09681.

233.12

HIPPOCAMPAL SCLEROSIS AS A CAUSE OF DEMENTIA: CLINICOPATHOLOGICAL STUDY OF PROSPECTIVELY STUDIED ELDERLY SUBJECTS. **D.W. Dickson*, H.A. Crystal, M.K. Aronson and E. Grober. Neuropathology and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.**

Of 90 brains obtained from prospectively studied elderly subjects, 13 had hippocampal sclerosis (HpScl) affecting the CA-1 region of the hippocampus, including 5 men and 7 women. The average age was 86.8 ± 6.2 years. Four cases had co-existing pathology of senile dementia of the Alzheimer type (SDAT), including many senile plaques and neurofibrillary tangles in neocortex. Two cases had substantia nigra and cortical Lewy bodies (LBD), including one case with co-existing SDAT and another with pathological aging. The average Blessed score for the 5 with either SDAT or LBD, or both, was 29.2 ± 4.1, while the remaining 7 cases had a Blessed score of 16.7 ± 5.6. Of the 7 cases with neither SDAT nor LBD, 4 cases had no other explanation for cognitive impairment besides HpScl. The remaining 3 had ischemic lesions in cortex or cerebral white matter that may have contributed to cognitive impairment. Clinical features included risk factors for cerebrovascular disease, including hypertension (6/12), atherosclerotic vascular disease (6/12), EKG abnormalities (6/12), cardiomegaly (4/12), myocardial infarction by either history, EKG or pathologic confirmation (4/12), congestive heart failure (2/12) and diabetes mellitus (2/12). Memory disturbances were prominent in at least 7 cases. In two cases the onset of memory problems was considered abrupt; one patient was treated with phenytoin for "transient global amnesia." Another patient had a nearly pure amnestic syndrome, before developing global cognitive decline later in his disease course. None of the cases had seizures. CT findings were usually only diffuse cortical atrophy, but 3 had lacunes, 1 had leukoencephalopathy and 1 had severe basal ganglia mineralization. The results show that HpScl is common in elderly demented subjects and is sometimes the best pathological explanation for dementia. It may account for increased risk of dementia in elderly subjects with history of myocardial infarction noted in other studies.

234.1

INCREASED CORTICAL NGF-LIKE ACTIVITY IN ALZHEIMER'S DISEASE: ELISA AND BIOASSAY RESULTS. S.A. Scott*, J.A. Weingartner, S. Liang, W. Everson[1] and K.A. Crutcher. *Departments of Neurosurgery and [1]OB/GYN, University of Cincinnati, Cincinnati OH 45267.*

NGF promotes survival of basal forebrain cholinergic neurons in vivo and in vitro, leading to the suggestion that declines in NGF underlie AD-related basal forebrain neuronal degeneration. To test this hypothesis, NGF-like protein levels were measured in AD using a 2-site ELISA. Samples of frontal and occipital cortex from 11 AD and 14 control brains were run using recombinant human NGF (rhNGF; provided by Genentech) as the standard. Neither rhNT-3 nor rhBDNF (provided by Amgen) were detected by this assay; antibody binding specificity was confirmed using Western blots. A 2-fold AD-related increase in NGF-like activity ($p<.001$) was found in each brain region whether or not the values were corrected for recovery. This increase was unrelated to age or postmortem interval. NGF-like biological activity was also measured in the same tissue samples using E9 chick sympathetic ganglia. Explants were exposed to either rhNGF (detectable as low as 10pg/ml) or brain extracts (1:50 dilution) with or without anti-NGF antibodies at a concentration (20ug/ml) that completely inhibited the effect of 1 ng/ml of rhNGF. Both AD and control brain extracts stimulated neurite outgrowth and the inhibition of neurite outgrowth by anti-NGF antibodies was significantly greater with AD extracts (>60% growth reduction) than with control extracts (25% reduction).

These data suggest that reduced NGF does not underlie basal forebrain neuronal degeneration in AD. Rather, increased NGF-like activity may represent a response to atrophy of basal forebrain neurons that are affected by a more global neurodegenerative process in this disease. (This work was supported by the Samuel A. Blank Research Fund of the Alzheimer's Association.)

234.2

FIBROBLAST GROWTH FACTOR RECEPTOR 4-LIKE IMMUNOREACTIVITY IN SENILE PLAQUES. O. Yasuhara, A. Matsuo[1], Y. Hara[1], I. Tooyama[1], K. Hanai[1], P.L. McGeer, H. Kimura[1] and S. C. Sung*. Kinsmen Lab. of Neurol. Res., U. of British Columbia, Vancouver, Canada, V6T1Z3; [1]Institute of Mol. Neurobiology, Shiga U. of Med. Sci., Otsu, Shiga, Japan

We have previously reported that acidic fibroblast growth factor (aFGF) is expressed in reactive astrocytes surrounding senile plaques in Alzheimer's disease (AD) (Dementia 1991;2:64-70). We now report on the immunohistochemical localization in AD and control brain tissue of FGF receptor 4 (FGFR4) which is specific for aFGF. A polyclonal antibody to the 125-141 FGFR4 amino acid sequence (Partanen et al., EMBO J 1991;10:1347-1356) was prepared in rabbits by immunizing with the synthetic haptenic antigen conjugated to carrier poly-L-glutamate. In cerebral cortices of both AD cases and age-matched controls, FGFR4-like immunoreactivity was observed in capillaries. In addition, senile plaques were positively stained in AD cases. The FGFR4-like immunoreactivity was localized mainly to consolidated plaques. The present study indicates that astroglial aFGF may be involved in plaque formation by binding to both high and low affinity receptors (FGFR4 and heparan sulfate proteoglycan) in senile plaques.

234.3

CHARACTERIZATION OF GAP-43 IN LAN-1 NEUROBLASTOMA CELLS. M. R. Martzen*, S. Sullivan, J. Cheetham, M. K. O'Banion and P. D. Coleman. University of Rochester School of Medicine and Dentistry, Depts. of Neurobiology and Anatomy and Neurology, Rochester, NY 14642.

Growth associated protein-43 (GAP-43) is a phosphoprotein expressed in neurons at high levels during development and regeneration which persists at lower levels in the normal adult brain. In post-mortem samples from Alzheimer's disease (AD) cases with high neurofibrillary tangle (NFT) density we have observed decreased phosphorylation of membrane-associated GAP-43 and a 6 fold decrease in GAP-43 mRNA. In an attempt to investigate the relationship between GAP-43 and NFT's, we have initiated studies on the effects of cytoskeletal disruption with the human neuroblastoma cell line LAN-1. Retinoic acid (RA) treatment of LAN-1 cells increases GAP-43 message and causes the cells to extend neurites that possess growth cones. Radiolabeling of these chemically differentiated cells with ^{32}P-orthophosphate and with ^{35}S-cysteine/methionine, followed by immunoprecipitation of labeled GAP-43 protein and ultra high-resolution 2D gel electrophoresis of the immunoprecipitate, indicate a minimum of 5 GAP-43 isoforms, three of which are phosphorylated. Preliminary studies also indicate that exposing chemically differentiated cultures to colchicine or cytochalasin B for 24 hours also decreases GAP-43 mRNA. Experiments to define further these GAP-43 isoforms and their modulation by cytoskeletal disruption are currently in progress. Supported by grants AG 01121, AG 03644 and AG 09016 (PDC) from the National Institute on Aging.

234.4

TRANSFORMING GROWTH FACTOR-β1 IS PRESENT IN PLAQUES IN ALZHEIMER'S DISEASE AND DOWN'S SYNDROME. E.A. van der Wal*, F. Gómez-Pinilla and C.W. Cotman. Irvine Research Unit in Brain Aging, *University of California, Irvine, CA 92717*

Transforming growth factor-β1 (TGF-β1), a cytokine synthesized by various tissues, acts as a regulator of the extracellular matrix and has growth related functions. Recent evidence suggests that Alzheimer's disease (AD) and its hallmark, plaques, may be partially caused by an imbalance in trophic support. Plaque-biogenesis may involve growth factors that induce sprouting of neurites towards plaques and/or may modify the extracellular matrix. Accordingly, we examined the distribution of TGF-β1 in the hippocampus and in the entorhinal cortex (EC) from AD and Down's syndrome (DS) human brain tissue. TGF-β1 immunoreactivity was investigated in controls, AD and DS by ABC immunohistochemistry with two anti-TGF-β1 antibodies. The control tissue showed light staining only in fibers. The AD cases displayed TGF-β1 positive immunoreactivity in fibers as well as in plaques. Plaque identity was confirmed by immunostaining with an anti-βA4-antibody. The TGF-β1 plaques in AD were mainly located in the dentate gyrus (DG) of the hippocampus. The TGF-β1 immunostained plaques in DS, however, showed a preference for the EC. The TGF-β1 staining pattern within plaques was identical for both AD and DS; strong immunoreactive aggregates intermingled with weak staining. TGF-β1 may play a role in the cascade of events leading to plaque-biogenesis, possibly by directly modulating the extracellular matrix or coordinating the actions of bFGF (also found in plaques).

234.5

CLONING OF HUMAN SCG10: ITS DEDUCED AMINO ACID SEQUENCE AND ABNORMAL EXPRESSION IN ALZHEIMER'S DISEASE. T.Okazaki, H.Wang, C.W.Wuenschell*, L.-C.Lo#, E.Masliah‡, T.Saitoh‡ and N.Mori. Div. Neurogerontology, Ethel Percy Andrus Gerontology Center, *Med. Center, Univ. Southern California, Los Angeles, CA90089. #Howard Hughes Med. Inst., Div. Biol., Caltech, Pasadena, CA91125. ‡Dept. Neurosciences, School of Med., UCSD, La Jolla, CA92093-0624.

SCG10 is a neural-specific, growth-associated protein that is broadly expressed in embryonic central and peripheral neurons in rats. The SCG10 expression in brain is high during prenatal and postnatal development, and low but significant expression persists into adulthood (see the abstract by T. Himi). As a way of studying SCG10 expression in normal and deseased human brains, we cloned and analyzed human SCG10 cDNA. By screening a human fetal brain cDNA library with rat SCG10 cDNA probe, we isolated a human SCG10 cDNA clone. In comparison with rat SCG10, the order of only two amino acids was exchanged in the two species with no apparent change in the amino acid composition and probably in the tertiary structure of the protein. It reveals that SCG10 is highly conserved in mammalian evolution, suggesting that it has an important function in neurons. The human SCG10 sequence was highly homologous to the human stathmin (also called p19), which was recently isolated by Sobel and his co-workers. Stathmin/p19 contains at least three serine residues which are phosphorylated presumably by protein kinase A. Amino acid sequences surrounding these three serine residues were conserved in the human SCG10 sequence, suggesting that SCG10 also is a phosphoprotein. Immunohistochemistry using the rat antibody has shown that SCG10 is expressed at different levels in several regions of the nomal human brain, and that levels of SCG10 expression have changed in the brains of Alzheimer's disease. The result implies that abberant expression of SCG10 may be involved in the pathogenesis of Alzheimer's disease, as in the case of other neuronal growth-associated proteins such as GAP-43 and tau.

235.1

FAMILIAL INCIDENCE OF DIFFUSE PLAQUES IN AGED DOGS.

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Aged dogs develop diffuse and possibly neuritic plaques and represent an animal model of plaque formation in Alzheimer's disease. We examined the distribution and incidence of plaques in 38 aged beagle and 22 aged St. Bernard dogs. The results provide evidence for a familial, possibly genetic, influence on the incidence of diffuse plaques.

The dogs lived in controlled laboratory colonies in a study conducted by the United States Department of Energy and did not receive any experimental treatment. At the time of their natural deaths, autopsies were performed and the brains were removed and stored in 10% formalin. The parahippocampal region was embedded in paraffin, sectioned at 8 μm and stained using thioflavin-S, the Gallyas method, and the modified Bielschowsky method. Twenty-two (58%) of the beagles had diffuse plaques in the parahippocampal region whereas only 2 (9%) of the St. Bernard's had plaques. Neuritic plaques, neurofibrillary tangles, and neuropil threads were not observed and neuron loss was not evident. The density of plaques was greatest in inferior temporal cortex>perirhinal cortex>entorhinal cortex ≈subiculum ≈dentate gyrus>CA1. In beagles from 10 of 11 litters there was congruence ($\chi 2$= 5.6, P=0.025). That is, if one animal had plaques, its littermate also had plaques. The results demonstrate breed differences in the incidence of diffuse plaques in aged dogs and a clear familial association of plaques among littermates in aged beagle dogs.

235.2

βA_4 ACCUMULATION IN AGED CANINE BRAIN: AN ANIMAL MODEL OF EARLY PLAQUE FORMATION IN ALZHEIMER'S DISEASE.

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Department of Psychobiology*, U.C. Irvine, CA 92717 USA and Depts. of Anesthesiology† and LEHR†, U.C. Davis, CA 95817 USA.

The aged canine brain has long been suggested as an animal model of Alzheimer's disease (AD); yet little is known about the components of canine plaques compared to AD plaques. We characterized the subtypes and molecular components of plaques in 8 aged canines (maintained from birth in a laboratory colony) with a variety of techniques: Bielschowsky's, Thioflavin and Congo red staining, as well as with antibodies to βA_4 peptide, APP, heparan sulfate (HS), bFGF, C3d, GFAP, Tau-1 and SMI-31. In agreement with earlier reports, we find that βA_4 reveals the greatest number of plaques. These "diffuse" plaques were not Congo red nor Thioflavin positive. βA_4-positive plaques were seen in 4 of the 8 dogs. In 3 of the 4 dogs, plaques were embedded in a diffuse linear zone of βA_4 immunoreactivity running the length of the mid-molecular layer of the dentate gyrus, suggesting that diffuse βA_4 peptide aggregated into plaques. Within the cortex and hippocampus, the majority of βA_4-positive plaques engulfed Nissl-positive neurons. Fewer plaques were detected with Bielschowsky's staining. These were faint, diffuse-brown and rarely contained curly fibers. Neither Tau-1 or SMI-31 revealed dystrophic fibers and plaques did not show bFGF or HS-positive staining, although positively stained neurons similar to those seen in humans were detected. Our data suggest that the trophic and substrate factors (i.e. bFGF and HS) which could help induce neuritic growth are not present in canine plaques. Consequently, little if any neuritic involvement occurs within these canine plaques.

235.3

CHARACTERIZATION OF ALZ-50 IMMUNOREACTIVE NEURONS IN THE NORMAL SHEEP STRIATUM.

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Alz-50 is a monoclonal antibody that recognizes the N-terminus of tau molecules in cells undergoing the early stages of neurofibrillary degeneration, and in a limited population of neurons in the brains of normal humans and other mammals. The process that leads to the exposure of the epitope that Alz-50 binds to in normal brain is not well understood, but its elucidation may provide an insight into both normal tau processing and the pathophysiology of Alzheimer's disease (AD). To better characterize the Alz-50 staining of neurons in the normal brain, we examined the Alz-50 immunoreactive neurons in the normal sheep striatum using immunoblotting as well as immunocytochemistry at the light and electron microscopic levels, and double-staining with Alz-50 and other antisera.

Alz-50 stained a population of medium aspiny neurons in the sheep striatum, as it does in humans. These cells also expressed neuropeptide Y and somatostatin immunoreactivity, as well as NADPH diaphorase activity, but did not stain with antisera against tau, β-amyloid protein, ubiquitin or heat shock proteins. Alz-50 stained only tau bands on immunoblots, and immunocytochemical staining was blocked by preadsorption with a synthetic peptide corresponding to the N-terminal of tau. The presence of the Alz-50 epitope, which many neurons only express in AD, in a discrete and well-characterized normal cell population may help identify a cellular pathway that is aberrantly triggered during AD.

235.4

EVALUATION OF MOUSE TRISOMY 16 HIPPOCAMPAL TRANSPLANTS AS A MODEL OF ALZHEIMER'S DISEASE PATHOLOGY.

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Human trisomy 21 (TS21) is associated with the age-related development of Alzheimer's-like pathology. In an attempt to develop an animal model of this pathology, we used TS16 mice, which have both phenotypic and genotypic features in common with human TS21. Many genes of human chromosome 21 have homologues located on mouse chromosome 16, including the β-amyloid precusor protein gene. Proteolytic processing of this protein in Alzheimer's disease (AD) leads to formation of the β/A4 protein, the major component of amyloid plaques. If the genes responsible for the AD-like pathology in human TS21 are found on mouse chromosome 16, the TS16 mice might develop similar pathology.

Hippocampal explants from TS16 mice or their euploid littermates at E17 were transplanted into the striatum or lateral ventricle of wild-type neonatal hosts. Transplantation of the hippocampal grafts was necessary since trisomy mice die in utero at about E18. Hosts receiving transplants were allowed to survive from 1-13 months. Brains from these animals were examined by immunohistochemistry for deposits of β/A4 protein (using antibodies raised against β1-28 or β3-22) or for neurofibrillary tangles (using anti-Alz 50 or anti-tau 1). Transplants became well-vascularized, and both neuron- and glial-specific stains revealed healthy cells. Methods which readily stained amyloid deposits and neurofibrillary tangles in AD brain failed to detect similiar pathologies in the trisomy transplants at any age, although neurons in both types of transplants expressed the beta-amyloid precursor protein. Intracellular inclusions similiar to those reported recently (Jucker et al., Science (1992) *255*:1443) were seen after staining with anti-β/A4 in both trisomy and euploid hippocampal transplants at long survival times. We conclude that TS16 mouse hippocampal transplants do not develop AD-like amyloid deposits or neurofibrillary tangles by 13 months of age.

235.5

IMMUNOCYTOCHEMICAL EVIDENCE OF INCREASED SPECTRIN PROTEOLYSIS IN COLCHICINE-TREATED RATS.

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This study was undertaken to determine the effects of colchicine (C), an axoplasmic flow inhibitor, on the spectrin-based neuronal cytoskeleton. Post-translational modification, i.e., calpain-cleavage of spectrin has been associated with a number of pathogenic conditions including exposure to neurotoxins such as colchicine. We hypothesized that colchicine-treated (CT) animals would exhibit an increased level of spectrin proteolysis. Experimental: C was administered in a single dose (80μg/20μl in saline) into the lateral ventricles of adult female Sprague-Dawley rats 24 hours prior to sacrifice. Standard ABC techniques on vibratome-sectioned (40μm) tissue were utilized using the RA150 antibody. RA150, an antibody made to a synthetic peptide characterizing the C-terminus of the calpain-1 cleavage site of alpha fodrin, serves as a specific marker of spectrin proteolysis. Results: Little or no RA150 immunostaining was observed in untreated controls. In contrast, intense staining was visualized in the CT animals. Comment: These results suggest an increase in spectrin proteolysis in CT animals, further clarifying the mechanisms of C action. They are significant in light of 1) recent evidence that some of the pathological changes found in CT animals mimic those found in Alzheimer disease (AD) (Shigematsu & McGeer, 1992) and 2) data indicating that accelerated calpain cleavage of cytoskeletal proteins may accompany certain neuropathological conditions such as AD. Thus C-induced spectrin proteolysis may assist in studies of these conditions. (Supported by HD13587(FN) & HD21711(MCL). The RA150 antibody was kindly provided by Dr. Jon Morrow).

236.1

NEURONAL CULTURES: A MEANS TO CHARACTERIZE CEREBROSPINAL FLUID ANTIBODIES OF DEMENTIA PATIENTS.
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Antibodies in the cerebrospinal fluid (CSF) of Alzheimer's disease (AD) patients recognize distinctly different structures in the developing rat CNS compared to CSF antibodies from other dementia groups. To rapidly screen and characterize embryonic cell types recognized by CSF antibodies a cell culture system was developed. Cells dissected from E-18 medial septum were dispersed, plated on poly-L-lysine coated sterile micro-16-well modules affixed to a standard laboratory slide and maintained in sterile conditions for 10 days. CSF from 90 AD, 21 multi-infarct dementia (MID), 83 non-specified dementia (NSD) patients and 35 controls were incubated in this 4% PF-fixed cell culture system. Immunocytochemical results revealed that CSF from 30 AD, 1 MID, 4 NSD and 0 controls stained microglial cells in culture. Furthermore, an immuno-EM study revealed that other AD-CSF samples specifically stained glioblasts containing a nucleus with patchy chromatin material. The scanty cytoplasm was characterized by abundant free and poly-ribosomes, isolated cisternae of rough endoplasmic reticulum and a small Golgi apparatus with diluted saccules. The AD-CSF immunoreactivity was specifically localized in the cytoplasmic matrix. These observations suggest that this cell culture system can significantly enhance differential diagnosis of the dementias and as well to further subgroup AD patients according to whether the CSF contains antibodies directed against, *e.g.*, microglia or glioblasts. Thus, it seems that immune responses play a role in neurodegenerative processes and that antibodies may reflect abnormal proteins, which when identified could improve diagnostic and treatment strategies for AD.

236.2

AN ELEVATED LEVEL OF NEURAL THREAD PROTEIN (NTP) IN HUMAN CULTURED OLFACTORY EPITHELIAL NEUROBLAST OF ALZHEIMER'S PATIENTS.
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An elevated level of NTP was reported in brains of patients with Alzheimer's disease (AD). As part of our clinical study we have measured NTP level in antemortem cerebrospinal fluid (CSF) in a large patient population (n>400) of AD, normal, psychiatric, neurological, and other dementia patients in order to study the clinical utility of NTP as a potential antemortem marker for AD. The clinically diagnosed AD CSF specimens had much higher NTP levels than those of non-AD. In this communication we report the NTP measurement in human cultured olfactory epithelial neuroblast (B.L. Wolozin et al. J. Mol Neurosci 3:137-146, 1992). The concentration of NTP in the cell homogenates was assayed by microparticle enzyme immunoassay (MEIA). Olfactory neuroblast (ON) from normal and Parkinson's disease patients showed no detectable NTP level while ON from patients with Alzheimer's disease showed elevated NTP level (60-1700 pg/ml of neuroblast homogenates).

236.3

DETECTION OF GLUTAMINE SYNTHETASE IN THE CSF OF ALZHEIMER'S DISEASED PATIENTS: A POTENTIAL DIAGNOSTIC BIOCHEMICAL MARKER
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Photoaffinity probes have shown changes in specific nucleotide binding proteins in Alzheimer's Diseased (AD) brain (Khatoon,S. et al. 1989, *Ann Neurol*). If such changes occured in the CSF of AD patients, this could be used as a diagnostic procedure. Testing of CSF with nucleotide photoaffinity analogs identified a 42kD ATP binding protein in the CSF of AD patients that is not observed in CSF from normal patients or other neurological controls. The photolabeling with $[\gamma^{32}P]2N_3ATP$ shows saturation at 30μM and it is specifically abolished by the addition of 25μM ATP. Photoinsertion of $2N_3ATP$ into the 42kD protein is only weakly protected by ADP, AMP, or Adenosine. This data indicates that this is a specific ATP binding protein. This protein was observed in 23 out of 24 AD CSFs examined and absent in 42 of 42 control samples examined. This 42kD protein was identified as glutamine synthetase by the following: similar nucleotide binding properties, co-migration on 2D gels, reaction with a polyclonal α-GS antibody, and elevated of glutamine synthetase enzyme activity only in the AD CSF.
Supported by Eli Lilly and Co. & NIH grant GM-35766-07.

236.4

CERULOPLASMIN, TRANSFERRIN, AND FERRITIN IN CEREBROSPINAL FLUID IN ALZHEIMER DISEASE AND PARKINSON DISEASE.
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Lipid peroxidation is increased in frontal cortex in Alzheimer disease (AD) and in substantia nigra in Parkinson disease (PD), suggesting impaired CNS anti-oxidative mechanisms in these diseases. Ceruloplasmin (CP), transferrin (TF), and ferritin (FT) interact to prevent iron-catalyzed production of free radicals. We evaluated CP, TF, and FT in CSF from individuals with AD (n=10), PD (n=14), and normal controls (n=10). CP was significantly increased in AD (p=0.019) but not PD CSF. TF was increased by 39% in AD CSF (26.0 $\pm$ 10.3 vs. 18.8 $\pm$ 4.0 ug/ml), but this increase was not statistically significant (p = 0.10). FT was not elevated in AD or PD CSF. This study provides no evidence for reduced CNS extracellular anti-oxidative mechanisms in AD or PD. Elevated CP concentrations in AD CSF suggest that extracellular anti-oxidant capacity may be increased, perhaps as a secondary event, in this disease.

DEGENERATIVE DISEASE: ALZHEIMER'S—TAU

237.1

PK40, AN ATP-REGULATED MEMBER OF THE ERK-FAMILY OF KINASES, CONVERTS TAU PROTEIN INTO PHF-TAU AS FOUND IN ALZHEIMER'S DISEASE.
P.A. Eden, H. Roder#, W. Schroeder# and V.M. Ingram*. Dept. Biology, M.I.T. Cambridge, MA 02139 and #Bayer AG, Wuppertal FRG.

The bovine protein kinase PK40 phosphorylates bovine and human TAU at sites including Lys-Ser-Pro motifs. PK40 phosphorylation of TAU is inhibited by a physiologically relevant excess of ATP over magnesium. The hyperphosphorylation of TAU induces electrophoretic and antigenic changes similar to the abnormally phosphorylated form of TAU in the tangles of Alzheimer's Disease (AD). PK40 likely plays a major role in the chronic hyperphosphorylation of TAU in AD and in the formation of neurofibrillary tangles.

We identify PK40 as a member of a family of extracellular signal-regulated kinases (ERKs). Like ERKs, PK40 autophosphorylates at a Tyr residue resulting in an increase in activity. Several ERK proteins similar in size to PK40 were detected in human neuroblastoma cells and are discussed. The significance for AD of TAU hyperphosphorylation and of PK40 regulation is also discussed. We present a model in which somatic mitochondrial DNA mutations provide a mechanism for age-related neurodegeneration.

237.2

LACK OF THE CARBOXYL TERMINAL SEQUENCE OF TAU IN GHOST TANGLES OF ALZHEIMER'S DISEASE.
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Using seven independent antibodies against the amino terminal to the carboxyl terminal sequence of tau, we biochemically analyzed and compared the neuropathogenesis of two Alzheimer's disease (AD) brains from the viewpoint of the abnormal processing on tau, the major constituent of paired helical filaments (PHF). One showed typical AD with senile plaques and intracellular neurofibrillary tangles. The other showed advanced AD with senile plaques and virtually the sole of ghost tangles. We confirmed the previous observation that the carboxyl-thirds of tau are tightly associated with PHF isolated in the presence of SDS (Kondo et al, Neuron 1, 827-834,1988). We found the biochemical nature of ghost tangles that lacked the final carboxyl terminal sequence as well as the amino half of tau, unlike intracellular tangles. From these biochemical results taken together with the current evidence for ubiquitin in ghost tangles, we concluded that ghost tangles were extensively processed and irreversibly tranformed into highly insoluble extracellular deposits in AD brains.

237.3

DISTRIBUTION AND FUNCTION OF FLUORESCENTLY LABELED BOVINE TAU PROTEIN IN LIVING CELLS. Q.LU * AND J.G.WOOD. Dept.of Anatomy and Cell Biology, Emory Univ. Sch. of Med., Atlanta, GA 30322

Bovine tau protein was tagged with fluorescent dye 5 (and 6)-carboxy-x-rhodamine-succinimidyl ester and microinjected into cultured Chinese Hamster Ovary (CHO) cells to study the intracellular distribution and function of the fluorescent analog. X-rhodamine tau incorporated rapidly into centrosomes within seconds after microinjection. It labeled distinctly the microtubule network as early as 5~10 minutes following microinjection. In addition, X-rhodamine tau was transported into the nucleus and labeled the nucleolus. Double labeling of the injected cells with DiC6(3) indicated that fluorescent tau can bind to endoplasmic reticulum, most evidently in the cells where injected tau failed to label microtubules strongly. The concentrations of injected X-rhodamine tau ranged from 1.68~5 mg/ml, yet bundling of microtubules was not observed. Studies of nocodazole effects on the microtubules established that X-rhodamine tau stabilized microtubules against depolymerization conditions. Together with the earlier reports that X-rhodamine tau promotes microtubule assembly in vitro (JCB, 115: 384a), we conclude that this fluorescent analog of tau is associated with microtubules and their related structures in living cells, and is competent to stabilize microtubules against microtubule depolymerizing drug treatment. AG 06383; NS 17731.

237.4

INHIBITION OF PROTEIN KINASE C INDUCED ALZ-50 IN THE CULTURE OF HUMAN NEUROBLASTOMA CELLS. L. Zhang, B.L. Wolozin, C.Y. Chen, and T. Sunderland* Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892

Aberrant protein phosphorylation has been previously documented in Alzheimer's disease (AD). Phosphorylation of Tau protein and amyloid precursor protein may play pivotal roles in the pathogenesis of the illness. Both of these processes are thought to involve protein kinase C (PKC) directly and indirectly. To investigate the role of PKC in the pathophysiological processes of AD, we have studied the effects of kinase inhibitors on the induction of Alz-50 immunoreactivity in human NSH neuroblastoma cells. Our preliminary results show that H-7, an inhibitor of PKC, protein kinase A (PKA) and protein kinase G (PKG), induces Alz-50 immunoreactivity, while HA-1004, an inhibitor of PKA and PKG, but not PKC, has no such an effect. Down regulation of cellular PKC by long term incubation with phorbol 12-myristate 13-acetate, gives a similar result as H-7. Together, these data emphasize the importance of PKC in the development of markers of AD pathology.

237.5

UNIQUE TAU AND NEUROFIBRILLARY TANGLE DISTRIBUTION IN THE HIPPOCAMPAL FORMATION OF PATIENTS WITH FAMILIAL "TANGLE ONLY" DEMENTIA. J. Leverenz, T. Bird, D. Nochlin, S.G. Greenberg*, C.B. Saper. Depts. of Pharm. & Physiol. Sci. and Neurology, Univ. of Chicago, Chicago, IL 60637, Depts. of Neurology and Pathology, University of Washington, Seattle, WA 98195 and Burke Med. Res. Inst., White Plains, NY 10605.

The relationship of post-translational modifications of tau protein with the formation of neurofibrillary tangles (NFT) and neuronal death in Alzheimer's disease (AD) remains controversial. It may be possible to obtain insights into the process of NFT formation from the study of unique familial dementing illnesses that demonstrate patterns of tau modification and NFT formation that differ substantially from AD. We have identified such a disorder, characterized by onset in young adulthood of psychotic behavior and dementia (Neurology 42:120, 1992). Classical neuropathological methods reveal moderate numbers of NFT in the cerebral cortex, subiculum, amygdala and brainstem, but no plaque formation. NFT are not seen in the hippocampal CA fields, but cell loss occurs in this location in long standing cases. Immunostaining with antibodies against pathological taus, such as Alz-50 or PHF-1, demonstrated granular neuronal staining in the hippocampus only in the cases with symptoms for > 20 yrs. There appeared to be a progression from Alz-50 staining, to PHF-1 staining, to cell loss, involving first the CA1 field, then CA2-3, and finally the dentate granule cells. Antisera against amyloid or ubiquitin stained NFT in the cortex, but not neurons containing granular Alz-50- or PHF-1-positive material in the hippocampus. Our observations suggest that pathological tau formation, indicated by progression from Alz-50 to PHF-1 staining, may contribute to cell loss, even in the absence of classical NFT formation, but that staining with amyloid or ubiquitin antibodies may occur only after actual NFT formation.

237.6

THE ABNORMAL PHOSPHORYLATION OF TAU AT SER396 IN ALZHEIMER'S DISEASE RECAPITULATES PHOSPHORYLATION DURING DEVELOPMENT AND CONTRIBUTES TO REDUCED MICROTUBULE BINDING SE Merrick, GT Bramblett, M Goedert, R Jakes, JQ Trojanowski, and VM-Y Lee*. Dept of Path and Instit of Neurosci, University of PA, Philadelphia, PA 19104, USA and Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.

Paired helical filaments (PHFs), the major building block of Alzheimer's disease (AD) neurofibrillary tangles (NFTs) has been shown recently to be comprised of the microtubule associated protein, tau. However the tau in PHFs differs from normal adult tau by the extent and sites of phosphorylation. One such site, Ser396 (located within a KSPV sequence), was shown to be phosphorylated in PHF tau but not in normal adult tau. To determine whether phosphorylation at Ser396 affects the binding of PHF tau to microtubules (MTs), we performed MT binding assays on 1) isolated and dephosphorylated PHF tau and 2) Chinese Hamster Ovarian (CHO) cells that were transfected with either a normal or mutant tau construct at Ser396. We showed that PHF tau is functionally impaired with respect to its ability to bind to MTs and that this defect correlates with abnormal phosphorylation, including the presence of phosphate on Ser396. We further examined the regulation of phosphorylation at Ser396 and showed that it is phosphorylated in fetal tau isolated from fetal brain but not postnatal human brain. This suggests that phosphorylation of tau at Ser396 occurs in fetal human tau and is developmentally regulated. Finally, we showed that protein kinases exist in the CHO cells transfected with the tau construct that can phosphorylate tau at Ser396 and that phosphorylation at this site can retard the migration of tau. These results support the notion that phosphorylation of tau in AD may recapitulate development when axons extend to their targets, MTs are more flexible, and MT stabilizing proteins such as tau bind to MT with less affinity than in mature neurons.

237.7

CASEIN KINASE II PHOSPHORYLATION OF TAU AND MAP-2. L.A. Greenwood* and G.V.W. Johnson. Department of Psychiatry, University of Alabama at Birmingham, Birmingham, AL 35294-0017.

Casein kinase II (CKII) is a cyclic nucleotide- and calcium-independent protein kinase directed by the presence of acidic or phosphorylated amino acid residues immediately C-terminal to the phosphoacceptor serine/threonine. Recently, CKII has been proposed to play a role in the formation of paired helical filaments, composed largely of excessively or inappropriately phosphorylated tau, found in Alzheimer's Disease brain. In this study, we examined the in vitro CKII phosphorylation of purified bovine tau and MAP-2, and the individual human tau isoform containing three microtubule-binding repeats in the C-terminal half of the molecule (T3).

Tau, MAP-2 and T3, were phosphorylated by CKII to a stoichiometry of 0.10, 0.80, and 0.05 mol phosphate/mol protein, respectively. The presence of polyethylenimine enhanced CKII catalyzed phosphate incorporation into these substrates approximately 5-fold. Two-dimensional phosphopeptide mapping demonstrated that tau was phosphorylated by CKII primarily on one peptide. Cleavage of phosphorylated tau at cysteine residues revealed that CKII phosphorylated tau predominately on the C-terminal half of the molecule. Two-dimensional phosphopeptide mapping of T3 displayed a different pattern of phosphopeptides compared to purified tau suggesting that endogenous phosphorylation may influence CKII phosphorylation of purified tau. However, cleavage of CKII phosphorylated T3 at cysteine residues produced a profile similar to purified tau, in that the phosphate was incorporated predominantly in the C-terminal half of the molecule. Two-dimensional phosphopeptide mapping of MAP-2 phosphorylated by CKII also demonstrated phosphorylation predominately on a single peptide. Site-specific phosphorylation of tau and MAP-2 by CKII may be important for the regulation of their function or alteration in disease states.

Supported by NIH grants NS27538 and AG06569, and a grant from the Alzheimer's Association.

237.8

PHOSPHORYLATION, CALPAIN HYDROLYSIS AND TUBULIN BINDING OF THE RECOMBINANT HUMAN TAU ISOFORM, T3. J.M. Litersky*, J.A. Greenwood, C.W. Scott† and G.V.W. Johnson. Dept. of Psychiatry, University of Alabama at Birmingham, Birmingham, AL 35294-0017 and †ICI Americas Inc., Wilmington, DE 19897.

Tau is an integral component of the paired helical filaments (PHFs) in Alzheimer's disease brain. All six isoforms of tau are present in soluble PHFs and appear to be abnormally phosphorylated. In this study we examined the phosphorylation, calpain degradation and tubulin binding of the recombinant human tau isoform, T3 which contains three tandem microtubule binding repeats in the C-terminal region, and no inserts in the N-terminal half of the molecule.

T3 was phosphorylated by cAMP-dependent protein kinase (cAMP-PK) and Ca^{++}/calmodulin-dependent protein kinase II (CaMKII) more extensively than bovine tau. In contrast, casein kinase II (CKII) phosphorylated T3 to a significantly lesser extent than bovine tau. Phosphorylation of T3 with each of the kinases in the presence of ^{32}P-ATP and subsequent cleavage at the only cysteine residue within the molecule revealed that CaMKII and CKII phosphorylate residues in the C-terminal of the molecule, while cAMP-PK phosphorylates sites in the N-terminal region, as well as in the C-terminal. Phosphopeptide mapping demonstrated that cAMP-PK and CaMKII phosphorylate the same sites in the C-terminal, while CKII phosphorylates a unique site. T3, which contains no detectable phosphate, was proteolyzed by calpain at a significantly faster rate than bovine or human tau. However, phosphorylation of T3 with cAMP-PK did not alter its susceptibility to calpain hydrolysis in contrast to what was previously observed with bovine tau. Binding of T3 to immobilized bovine tubulin showed that phosphorylation by cAMP-PK significantly reduced T3-tubulin interactions, while phosphorylation of T3 with CaMKII did not alter tubulin binding.

Supported by NIH grants NS27538 and AG06569 and grants from the American Health Assistance Foundation and the Alzheimer's Association.

237.9

PHOSPHORYLATION STATES OF BOVINE TAU, PORCINE TAU, AND A TAU FRAGMENT BOUND TO ALZHEIMER PHFs. C.W Scott*, L. Poulter, D. Barratt, C. Wischik and C.B. Caputo. ICI Americas Inc., Wilmington, DE 19897 and MRC Laboratory of Molecular Biology, Cambridge, UK.

PHFs contain modified tau protein (τ) and are present in both of the major histopathologic lesions of Alzheimer's disease, neuritic plaques and neurofibrillary tangles. We investigated the nature and specificity of τPHF modifications by comparing τ extracted from pronase-treated PHFs to τ extracted from bovine and porcine microtubules. Each preparation was digested with proteases, fractionated by RPHPLC, and analyzed by mass spectrometry and Edman sequencing. Peptides spanning aa 299-391of the longest human tau isoform (τ4L) were identified in the τPHF sample, although none contained any post-translational modifications. One phospho peptide was identified in the porcine τ digest; the phosphorylated residue was Ser404. Three phospho peptides were identified in the bovine τ digest representing aa 40-53, 193-251, 402-417 of τ4L. Nonphosphorylated peptides encompassing the KSPV motif were identified from both porcine and bovine τ suggesting that this site was not phosphorylated in vivo. However, phosphate-dependent antibodies T3P (kindly provided by Dr. V. Lee) and SMI-34 recognized the porcine and bovine τ samples in a phosphate-dependent manner by immunoblot and ELISA. Thus these antibodies may react with phosphate groups outside the KSPV site. In conclusion, no evidence of post-translational modifications of aa 299-391of τPHF was observed. Direct chemical analysis rather than antibody reactivity is needed to map the locations of aberrant phosphorylation that occur N- and C-terminal to aa 299-391 of τPHF.

237.10

INDUCTION OF AMYLOID FIBRIL FORMATION BY TAU PROTEIN. C.B. Caputo*, C.W Scott, I.R.E. Sobel, and L.A. Sygowski. ICI Pharmaceuticals Group, ICI Americas, Wilmington, DE 19897.

Paired helical filaments (PHFs) possess amyloid-like properties, contain tau protein (τ) and form intracellularly in neurons in Alzheimer's disease. We compared the properties of PHFs with those of the amyloid fibrils (Caputo et al. Arch. Biochm. Biophys. 292:199,1992) formed from C-APP, a synthetic peptide of the last 20 amino acids of β-amyloid precursor protein. C-APP fibrils resembled PHFs morphologically after C-APP was heated at 60° C for 1 h. τ considerably enhanced C-APP fibril formation, without influencing its morphology. Bovine τ, phosphorylated at 3 sites, was as effective as the shortest, longest, and 4-repeat human τ isoforms expressed in E. coli or a fragment containing the tubulin-binding region. τ remained bound to C-APP fibrils, as determined by τ immunoreactivity of the fibrils upon centrifugation. C-APP fibrils were insoluble in solvents that also do not dissolve PHFs such as SDS, guanidine-HCl, triton X-100, and CAPS, whereas both PHFs and C-APP fibrils became soluble using a 6 M guanidine-SCN procedure. We conclude that C-APP fibrils share morphological and solubility properties with PHFs and both bind τ tightly.

237.11

THE ALZHEIMER-LIKE STATE OF TAU PROTEIN: KINASES AND PHOSPHORYLATION SITES. **E.-M.Mandelkow*, J.Biernat, B.Lichtenberg-Kraag, B.Steiner, H.Wille, G.Drewes, N.Gustke, H.Meyer (a), M.Goedert (b), E.Mandelkow. Max-Planck-Unit for Struct. Mol. Biol., D-2000 Hamburg 52; (a) Inst. physiol. Chem., Univ. of Bochum, D-4630 Bochum; (b) MRC-LMB, Cambridge CB2 2QH, U.K.**

Tau is a major component of the paired helical filaments (PHFs) of Alzheimer's disease where it is abnormally phosphorylated. Our aim is to analyze the structure and function of tau in normal and pathological conditions. (1) We prepared a kinase from brain capable of transforming normal or recombinant tau into the Alzheimer state, as judged by several criteria: (i) a reduced electrophoretic mobility in an SDS gel; (ii) reaction with PHF-specific monoclonal antibodies; (iii) alterations in microtubule binding. (2) We determined several abnormal phosphorylation sites in tau, including Ser199 and Ser202. Most sites are Ser followed by Pro, indicating a Pro-directed kinase. (3) We determined epitopes of antibodies sensitive to the PHF-like state of tau. Two of the antibodies tested have epitopes including Ser199 and Ser202; they are phosphorylated when tau is in a PHF-like state. The phosphorylation sites and antibody epitopes were confirmed by site-directed mutagenesis. (4) We probed the function of recombinant tau proteins in vitro, with or without phosphorylation, in terms of microtubule binding or assembly into filaments closely resembling PHFs. - Supported by BMFT and DFG.

237.12

OKADAIC ACID INDUCES HYPERPHOSPHORYLATED FORMS OF TAU PROTEIN IN HUMAN BRAIN SLICES. K.A. Harris, G.A. Oyler, and M.L. Billingsley*, Dept. Pharmacology, Penn State University College of Medicine, Hershey, PA 17033

Hyperphosphorylated forms of the microtubule-associated protein tau (tau-P) form paired helical filaments seen in Alzheimer's disease. In fresh tissue slices of human temporal lobe, tau phosphorylation was increased in a dose-dependent manner by treatment with the phosphatase inhibitor okadaic acid (5-20 μM). Lower concentrations of okadaic acid (15-125 nM) did not alter tau electrophoretic mobility, suggesting that inhibition of protein phosphatase 2B (calcineurin) was important in generation of tau-P. Treatment with NMDA, quisqualate, or kainate failed to alter tau-P. Immunoreactivity to Alz-50, a monoclonal antibody which recognizes the A68-tau epitope was increased following okadaic acid treatment and comigrated with tau-A68 purified from Alzheimer's brain. In vitro incubation with purified bovine calcineurin dephosphorylated the tau-A68 isoform formed following okadaic acid treatment. Inhibition of calmodulin-dependent protein kinase II using the specific inhibitor KN-62 prevented formation of okadaic acid-induced tau-A68 immunoreactivity in slices. These data suggest that the tau-A68 phosphorylation site(s) is regulated by calmodulin kinase II and okadaic acid-sensitive protein phosphatases; alterations in these enzyme activities may be implicated in the pathogenesis of Alzheimer's disease.

237.13

POSTMORTEM CHANGES IN CYTOSKELETAL PROTEINS RESEMBLE ALTERATIONS IN ALZHEIMER'S DISEASE. C. Schwab* and J.W. Geddes. Sanders-Brown Center on Aging and Dept. Anatomy & Neurobiology, Univ. Kentucky, Lexington, KY 40536.

Alzheimer's disease (AD) is characterized by disruptions in the structure and localization of microtubule-associated proteins (MAPs), particularly the tau proteins. Loss of tau from axons and accumulation in somatodendritic compartments, as detected by immunocytochemistry, is one of the earliest identified abnormalities in AD. The mechanisms which lead to the accumulation of tau in the somatodendritic compartment are uncertain.

We examined the distribution of tau and other cytoskeletal proteins in the rat hippocampus at various times postmortem. As early as 30 min postmortem, there was a decrease in tau immunoreactivity in axons and increase in neuronal cell bodies. Similarly, MAP1, MAP2, and MAP5/1B accumulated in the cell body and proximal portion of the apical dendrites and were lost from the distal dendrites. In contrast, the cellular localization of phosphorylated neurofilaments was similar in control and postmortem animals. In Western blots, a loss of MAP1, MAP2, and MAP5 was evident by 4h postmortem, whereas the levels of tau were relatively stable. Postmortem accumulation of microtubules, but not of neurofilaments, in the soma and proximal dendrites was also evident using electron microscopy.

These results suggest that: i) the cytoskeletal alterations observed in AD are not causally related to, but result from, neuronal death; ii) the prominence of tau in AD pathology may reflect its postmortem stability in contrast to other MAPs; and iii) results regarding the levels and distribution of cytoskeletal proteins obtained from postmortem human tissue must be interpreted with caution. (Supported by NIA grant AG10678 to JWG).

238.1

AN INVESTIGATION INTO THE ORIGIN OF NEUROPIL THREADS IN ALZHEIMER'S DISEASE CORTEX USING CONFOCAL LASER MICROSCOPY. M.L.Schmidt,J.Murray,& J.Q.Trojanowski*. Dept.of Path.& Lab.Med.,U.of Penn, Sch.of Med. PA 19104

Neuropil threads (NTs) are abnormal processes found in tangle rich grey matter areas of the brains of patients with Alzheimer's disease (AD). Although NTs contain paired helical filaments (PHFs) and share multiple tau epitopes with neurofibrillary tangles (NFTs), the relationship between NTs and tangle bearing neurons is unclear. For this reason, we investigated the origin of NTs using histochemical and immunochemical methods with the aid of a confocal laser scanning microscope. Only a small fraction of NTs (<1%) in 3.5 X 10^6 μm^3 of cortex could be traced to local cortical neurons with NFTs or to neurons that did not contain NFTs, and no NTs were observed to originate from astrocytes. These results indicate that only a very small percentage of NTs occur in the proximal parts of dendrites that emanate from neurons located within NT-rich grey matter, while the majority of NTs appear to reside in the distal parts of dendrites and/or the terminal arborizations of axons. Hence, NTs could disrupt local and long distance neuronal circuitry and thereby contribute to the cognitive impairments seen in AD patients.

238.2

DOES BRAIN MICROVESSEL PATHOLOGY PROVOKE ALZHEIMER'S DISEASE?.

J.C. de la Torre*. University of Ottawa, Fac.of Medicine, Ottawa, Ontario Canada KlH 8M5.

Previous light microscopic findings and recent ultrastructural studies have demonstrated extensive structural distortions of cerebral capillaries in Alzheimer's brains. Alzheimer's disease (AD) subjects additionally show reduced cerebral blood flow (CBF), glucose metabolism and oxygen utilization which appear inversely proportional to increased disease severity. Other work supports the notion that congophilic angiopathy and glial fibrillary acidic protein (GFAP) in AD brains precede plaques and tangles formation. These and other findings, including our experimental work in rats[1], have led us to develop a hypothetical model which appears consistent with the pathogenesis and progression of AD.

During aging, brain capillaries (site of the blood-brain barrier) can degenerate progressively from amyloid deposits, thickened basement membrane, cerebral atrophy, reduced vessel elasticity, genetic predisposition, etc. When structural abnormalities of brain microvasculature begin to interfere with basic laws of fluid dynamics, hemorheological compromise develops. The product of hemorheological compromise results in higher cerebral capillary resistance, blood viscosity, abnormal flow patterns, and shear stress of vessel walls. The net effect is chronic disturbed blood flow to the brain that impairs the delivery of essential nutrients (particularly oxygen and glucose) to cerebral neurons. As ischemic-sensitive neurons lower their oxidative phosphorylation and ATP production to sub-functional levels, they release a diffusible glial mitogen that directly stimulates reactive astrocytosis. These GFAP- reactive glia proliferate quickly in response to brain injury and can spread to unaffected tissue.

It has been suggested that β/ amyloid precursor protein (APP), may be expressed from reactive glia and microglia following neuronal injury thus providing the nidus of neuritic plaque formation. As brain tissue space is invaded by reactive glia, neuronal cytoskeletal damage results in neurofibrillary tangles.

Evolving microvascular insufficiency also limits the availability of proteases responsible for the removal of APP and the products of cell injury involved in plaque formation. The linear outcome of this process is slow and progressive neuronal damage, transmission failure and brain tissue death.

[1] de la Torre JC, Park G, Fortin T et al: Abstr. Soc. Neurosci. 17:1066, 1991.

238.3

MICROGLIA ARE NOT EXCLUSIVELY ASSOCIATED WITH PLAQUE-RICH REGIONS OF THE DENTATE GYRUS IN ALZHEIMER'S DISEASE. M.T. Roe[1], C.M. Hulette[1], G. Einstein[2], B.J. Crain[1,2]* Depts. of Pathology[1] and Neurobiology[2], Duke Univ. School of Medicine, Durham, NC 27710.

The role of microglial cells in plaque formation in Alzheimer's Disease (AD) remains uncertain. We studied the relative spatial distributions of plaques and reactive microglia in the molecular layer of the dentate gyrus (DG) of the hippocampus to determine if reactive microglia are confined to the same area as plaques or are more broadly distributed. Tissue from ten AD cases with numerous plaques in the DG (Group 1) was compared with tissue from five age-matched AD cases without plaques in the DG (Group 2) and five age-matched control cases (Group 3). Serial sections were immunostained with antibodies to beta-amyloid (for plaques) and L-ferritin (for reactive microglia). Selected cases were immunostained with antibody to glial fibrillary acidic protein (for reactive astrocytes) to ensure that antibody to L-ferritin was not staining astrocytes. Relative spatial distributions of plaques and microglia were determined in the three groups by measuring the distances of plaques and microglia from the top of the granule cell layer (GCL). In plaque-rich regions, reactive microglia were most numerous in the same area as plaques (0.05-0.20 mm from the GCL), but were distributed broadly within the molecular layer (up to 0.45 mm from the GCL). In plaque-free regions in all three groups, reactive microglia were less common and were distributed uniformly (up to 0.50 mm from the GCL). Therefore, reactive microglia were not exclusively confined to plaque-rich regions since they were distributed more broadly than plaques and were common in plaque-free regions. Reactive microglia thus appear to be associated with both plaque formation and more generalized brain injury in AD. Supported by AG 09216.

238.4

STRUCTURAL ANALYSIS OF α1-ANTICHYMOTRYPSIN IN ALZHEIMER'S DISEASE. Kelly Johnson-Wood, Cheryl Blomquist, Robin Barbour, Russell Blacher, Fred Esch, Ivan Lieberburg, Lawrence Fritz*, and Dale Schenk. Athena Neurosciences, South San Francisco, CA. 94080.

Multiple studies have identified α1-antichymotrypsin (α1-ACT) as a major component of neuritic plaques. Its presence frequently co-localizes with β-peptide deposition and its synthesis appears to be increased in the brain tissue of Alzheimer's disease patients. Several groups have used this observation to see if total levels of α1-ACT in either cerebrospinal fluid or plasma are specifically increased in AD patients. While some investigators have identified an increase, others have not. In an attempt to more fully understand the role of the α1-ACT in Alzheimer's disease, we have developed several monoclonal antibodies to it and have used these antibodies to aid in the purification of α1-ACT from plasma and brain tissue of both Alzheimer's disease and control patients. The results of this analysis suggestSu heterogeneity in the molecule that might have relevance to the disease process in Alzheimer's disease.

238.5

THREE DIFFERENT HEPARAN SULFATE PROTEOGLYCAN PROTEIN CORES ARE PRESENT IN THE AMYLOID PLAQUES OF ALZHEIMER'S DISEASE. J. Eisler,[1,2]* L.Buee,[2] W. Ding,[2] R. Kisilevsky,[3] S. Narindrasorasak,[3] H. Fillit, M.D.[1,2] [1]Fishberg Center for Neurobiology, [2]Ritter Department of Geriatrics, Mount Sinai Medical Center, Box 1070, New York, NY 10029, and [3]Department of Pathology, Queens University, Kingston, Canada k71 3n6.

Heparan Sulfate Proteoglycan (HSPG) may play a role in amyloidosis, including Alzheimer's Disease (AD). Both polyclonal antibodies to the basement membrane HSPG named perlecan (MW~ 600 k Da, protein core ~ 400 kDa), and monoclonal antibodies (mAbs) 7E12 to another basement membrane HSPG (M.W. ~ 250 kDa, protein core ~ 110 kDa) identify HSPG in amyloid plaques, and also stain occasional neurons in AD as well as normal and abnormal cerebrovasculature. Immunoblot analysis employing these two purified HSPGs was performed to determine whether these HSPGs share common epitopes or are actually distinct molecules. Results indicate that the mAbs reactive with 110 kDa HSPG protein core do not recognize perlecan, and polyclonal antibodies to perlecan react minimally with the 110 kDa HSPG protein core possibly due to recognition of the heparan sulfate side chains which both molecules have in common. HSPG was also purified from the supernatant of human neuroblastoma cells (SKNSH), and immunoblot analysis revealed an intermediate weight HSPG (M.W. protein core ~ 150 kDa) recognized by the mAbs to the 110 kDa HSPG but not by the polyclonal antibodies perlecan. In conclusion, while the three proteoglycans do in fact share common heparan sulfate side chains, they probably have distinct protein cores. Therefore, there are at least two types, and possibly three of HSPG in amyloid plaques. In addition, these HSPGs are derived from different cellular sources, particularly vascular endothelial cells and neurons.

238.6

RESTRICTION ENDONUCLEASE ANALYSIS OF AMYLOID PRECURSOR PROTEIN GENE IN GUAMANIAN PARKINSNONISM-DEMENTIA AND AMYOTROPHIC LATERAL SCLEROSIS.

D.C. Guiroy*, V. R. Nerurkar, I. Wakayama, R.M. Garruto, R. Yanagihara, D.C. Gajdusek. Laboratory of Central Nervous System Studies, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD 20892.

Guamanian parkinsonism-dementia (PD) and amyotrophic lateral sclerosis (ALS) are neurological disorders characterized neuropathologically by abundant neurofibrillary tangles. Codon altering point mutations in the amyloid precursor protein gene, leading to altered tertiary configuration, may explain the predominant intracellular amyloid deposition in these disorders. To determine if point mutations are present in the amyloid protein precursor gene, we studied high molecular weight DNA extracted from brain and lymphocytes of 12 cases of Guamanian PD and 9 cases of Guamanian ALS by polymerase chain reaction using primers 5'GCCTAATTCTCTCATAGTCTTAATTCCCAC 3' and 5'GAGCCGATAACGCGTCCATCG 3' of the APP gene. Restriction enzyme analysis of the 319 bp enzymatically amplified product using Bcl I, which is specific for T/GATCA (Valine>Isoleucine), did not reveal a point mutation at codon 717 in Guamanian PD and ALS. Further restriction enzyme analyses and direct sequencing of the amplified amyloid precursor protein gene is underway to determine the presence of point mutations.

238.7

LEUPEPTIN INDUCES AN ACCUMULATION OF UBIQUITIN-CONJUGATED PROTEINS AND AMYLOID PRECURSOR PROTEIN (APP) FRAGMENT IN RAT BRAIN. I. Hajimohammadreza, V.E.R. Anderson, J.B. Cavanagh, B.H. Anderton and P.N. Leigh **(SPON: Brain Research Association).** Institute of Psychiatry, London SE5 8AF, UK.

The processing of APP to β-amyloid may be an early feature of Alzheimer's disease pathology. Little is known of mechanisms by which β-amyloid is formed but lysosomal proteolysis may be involved. Leupeptin is a potent serine and thiol protease inhibitor which blocks several lysosomal proteases. Infusion of leupeptin into rat cerebral ventricle causes a rapid accumulation of lysosomes appearing in hippocampal and cerebellar neurones first and later in glia. We have demonstrated increased lysosomal immunoreactivity for both ubiquitin and APP following leupeptin infusion, indicating altered proteolytic processing. SDS-PAGE analysis of proteins followed by Western blotting showed an increase in ubiquitin immunoreactivity and a strong APP immunoreactive band at 19 kDa in leupeptin intoxication. This in-vivo model demonstrates that neurones can process APP via the lysosomes. It may indicate that lysosomes are transported from the neuronal perikarya via astrocytes on the way to the vascular bed and pia-arachnoid.

238.8

SOLUBILIZATION AND ANALYSIS OF ALZHEIMER DISEASE PAIRED HELICAL FILAMENTS. D.R. Sparkman*, W.J. Goux, C.L. White III, and S.J. Hill. U.T. Southwestern Med. Ctr., Dallas, TX 75235 and U.T. at Dallas, Richardson, TX 75083.

Alzheimer disease is a neurodegenerative disorder that is characterized by neurofibrillary degeneration. The paired helical filaments (PHF) of the neurofibrillary tangles are SDS-insoluble structures, in contrast to the non-tangle SDS-soluble PHF. An abnormal form of tau has been found to be associated with both types of PHF, but does not account for their differences in solubilities. The insoluble PHF core structures have been isolated by SDS extraction and pronase digestion, and solubilized by heating in dimethyl sulfoxide. Analysis by proton nuclear magnetic resonance has demonstrated the presence of a glycolipid component associated with the insoluble PHF. Similar studies of the soluble, non-tangle PHF also detected a glycolipid component, in addition to protein. X-ray microprobe analysis of the two types of PHF support the PHF core structures being predominately nonproteinaceous, in contrast to the non-tangle PHF. The associated of a glycolipid component with the abnormal tau may give rise to soluble PHF, which over time becomes covalently modified to give rise to an insoluble, glycolipid PHF core structure with tau forming the outer fuzzy coat.

238.9

ANTIBODY MAPPING OF ALZHEIMER DISEASE PAIRED HELICAL FILAMENTS USING FREEZE-DRYING/DEEP-ETCH TECHNIQUES. D.M. Appelt* and B.J. Balin+, Medical College of Pennsylvania, *Dept. of Anatomy and Neurobiology, +Dept. of Pathology & Laboratory Medicine, 3200 Henry Ave., Phila., PA 19129.

Paired helical filaments (PHFs) are the major constituents of neurofibrillary tangles (NFTs), one of the hallmarks of Alzheimer's Disease (AD). Recent evidence suggests that PHFs are comprised of highly phosphorylated tau proteins known as A68 (Lee et al., Science 251:672-678, 1991). The formation of insoluble paired structures from these modified tau proteins into individual PHFs and macromolecular complexes, such as the NFTs, remains to be determined. To clarify these issues, we have utilized freeze-drying/deep-etch electron microscopy techniques of isolated PHFs to obtain stereoscopic images. These images allow us to view the individual filaments as they relate to one another in three dimensions. PHFs were extracted and isolated from AD brains using well-established biochemical techniques. Affinity purification of these PHFs was employed to obtain a contaminant-free population. Monoclonal antibodies to tau and PHF (Tau 1, Tau 14, Tau 46, Tau 60, Alz 50) were used to examine the arrangement of tau and A68 epitopes on each filament. Their labeling patterns suggest that specific epitopes of these proteins are discretely arranged along the PHFs. The arrangement of these epitopes, in particular the amino and carboxyl termini, provides an indication of the structural relationships of tau molecules both on and between the helical strands. This alignment of tau proteins (e.g., parallel/anti-parallel) along the filaments may allow us to determine the potential sites on the tau molecules that interact to form insoluble PHFs and, ultimately, the NFTs. Supported by PHS grant AG10160.

238.10

ALZHEIMER PAIRED HELICAL FILAMENTS: SHARED EPITOPES WITH TAU AND BETA-AMYLOID. L. McLaughlin*, G.E. Dean, & F.P. Zemlan Dept. of Molec. Genetics, Biochem. and Microbiol., and the Alzheimer's Research Center, Univ. of Cincinnati, Cincinnati, OH 45627.

Neurofibrillary tangles (NFT) are one of the characteristic neuropathological lesions seen in Alzheimer's diseased brains. These intracellular lesions are seen to consist chiefly of tenaciously insoluble structures called Paired Helical Filaments (PHF). On the basis of limited peptide sequenation and immunological reactivity, PHF is thought to contain abnormal forms of the microtubule-associated protein tau (TAU). This conclusion is based, however, on studies performed with either 1) highly impure preparations of NFT's or 2) a group of soluble proteins known as the A68 proteins whose relationship with PHF has not clearly been established. A purification procedure previously developed in this laboratory has enabled the isolation of the PHF structural proteins resulting in several bands of approximately 56-66 kD (AD 66 proteins) as seen on silver stained PAGE. To determine which regions of TAU are present in PHF we have performed more detailed epitope-mapping studies of these highly purified PHF-derived proteins with antibodies specifically raised against defined regions of the TAU protein. The data from the specific TAU antibodies suggests that only some TAU isoforms are present and that these appear to be abnormally phosphorylated. Immunological studies have also suggested the presence of the amyloid protein (the major proteinacious component of plaques) in PHF. Similar immunological studies with antibodies raised against defined regions of the amyloid precursor protein (APP) were therefore carried out. Results from these experiments show reactivity of the AD 66 proteins with antibodies raised against the carboxy-terminal and the beta-amyloid region of the APP. To rule out the presence of amyloid protein in the PHF-derived proteins will require further investigation.

238.11

DISTRIBUTION OF INTERMEDIATE FILAMENTS IN THE RETINAS OF NORMAL AND ALZHEIMER'S DISEASE DONOR EYES. Kathryn V. Porrello, David R. Hinton and Janet C. Blanks*. Doheny Eye Institute and Departments of Pathology and Ophthalmology, USC School of Medicine, Los Angeles, CA 90033.

The retinas of patients with Alzheimer's disease (AD) exhibit a selective degeneration of the ganglion cell layer (GCL) and the nerve fiber layer (NFL). This retinal degeneration is not accompanied by the characteristic neurofibrillary tangles (NFT) and neuritic plaques that are associated with neuronal degeneration in AD brains. Evidence suggests that these lesions represent abnormalities in the neuronal cytoskeleton. In particular, NFT contain small segments of neurofilaments (NF) that appear to be abnormally phosphorylated and distributed within the neuron. AD brains also express increased levels of glial fibrillary acidic protein (GFAP), which appears to be related to the astrocytic gliosis that occurs near degenerating neurons. To investigate whether similar cytoskeletal abnormalities exist in AD retinas, the distribution of NF and GFAP was examined in 10 AD and 11 age-matched normal control eyes. Monoclonal antibodies specific for phosphorylated (SMI 31) and non-phosphorylated (SMI 32) epitopes of the 200 kD NF demonstrated a differential distribution of NF within the normal retina. SMI 31 labeled the NFL intensely and produced a laminar pattern in the inner plexiform layer (IPL). SMI 32 labeling was greatly reduced in the NFL and IPL, but sporadically labeled cell somata in the GCL and NFL. In AD retinas, the distribution of SMI 31 labeling of the NFL was greatly reduced compared with controls, and appeared proportional to the degree of optic nerve degeneration. SMI 32 labeling of AD retinas was similar to that of controls. In normal retinas, anti-GFAP labeling was usually restricted to the perivascular astrocytes of the GCL and NFL. In most AD retinas, anti-GFAP labeling appeared to be more extensive compared with controls, and included labeling of the Müller cell processes. These results suggest that the retinal degeneration in AD patients involves cytoskeletal abnormalities.

238.12

DISTRIBUTION OF NEUROFIBRILLARY TANGLES AND AMYLOID DEPOSITS IN THE HIPPOCAMPUS AND THE TEMPORAL NEOCORTEX: A STUDY OF ONE YEAR AUTOPSY POPULATION FROM A GERIATRIC HOSPITAL. C. Bouras[1*], P. Giannakopoulos[2], P.R. Hof[3], N.K. Robakis[3,4], M. Surini[1], J.P. Michel[2]. [1]Dept. of Psychiatry, Univ. of Geneva, CH-1225, Switzerland, [2]Institutions Universitaires de Gériatrie, CH-1226 Geneva, Switzerland, and [3]Fishberg Research Center for Neurobiology and [4]Psychiatry Mt Sinai Sch. Med., New York, NY 10029.

The brains of 114 cases (68-101 years old) were obtained at autopsy from the Geriatric Hospital of Geneva in 1989. The patients included 47 men (41.2%, 83±7 years old) and 67 women (58.8%, 87±6 years old). The clinical diagnosis was established according to the DSM-IIIR definition of dementia and four clinical groups were retained. Twelve patients presented with degenerative dementia, 18 with other types of dementia (vascular, traumatic or mixed), 22 with slight memory impairment and disorientation, and 62 cases without known neurologic or psychiatric disorders. We assessed quantitatively the distribution of neurofibrillary tangles (NFT) and amyloid deposits (AMLD) using anti-A4 and anti-Tau antibodies in the CA1 field of the hippocampus, in layers II and V of the entorhinal cortex and in layers II-III and V-VI of the inferior temporal cortex. These cortical areas exhibited various degrees of NFT formation and AMLD was present only in 27% to 62% of the cases depending on the areas investigated. For instance, NFT were present in 83% of the cases in the CA1, 92% in the subiculum, 100% in the entorhinal cortex and 91% in the inferior temporal cortex. AMLD were found in 76% of all cases in CA1, 42% in subiculum, 50% in the entorhinal cortex and 62% in the inferior temporal cortex. Moreover, memory impairment and disorientation were correlated with the number of NFT in layer II of the entorhinal cortex ($p<0.05$), whereas dementia was correlated with the involvement of hippocampal formation and inferior temporal neocortex ($p<0.05$) suggesting that dementia is linked to the presence of neocortical damage.

238.13

LAMINAR AND REGIONAL DISTRIBUTION OF NEUROFIBRILLARY TANGLES AND PICK BODIES IN PICK'S DISEASE: COMPARISON WITH ALZHEIMER'S DISEASE.

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The regional and laminar distribution of Pick bodies were analyzed in fourteen patients with Pick's disease (72.6±8.4 year-old, PD) showing the classical fronto-temporal cortical atrophy. Results were compared to the distribution of neurofibrillary tangles (NFT) from thirteen neuropathologically confirmed Alzheimer's disease (AD) cases (87.3±8.0 year-old) with no Pick bodies. Pick bodies and NFT were stained with an antibody to tau proteins.

In PD patients, Pick bodies were observed in high densities in the fascia dentata, the CA1 pyramidal layers, layers II to V of the entorhinal cortex and in layers II-IIIa and VI of the inferior temporal and superior frontal cortex. A quantitative assessment revealed the highest densities of Pick bodies in fascia dentata, CA1, and inferior temporal layers II-IIIa (500±89, 151±24, 184±71 respectively).

Interestingly, most PD cases showed the presence of NFT in this areas suggesting that PD and AD are likely to coexist more frequently than is usually thought. In addition, NFT exhibited a complementary distribution to that of Pick bodies in neocortical areas, in that they were mostly located in layers III and V.

This suggests that the typical lesions of PD and AD involve different sets of neurons and that specific elements of cortical circuitry are differentially affected in these conditions. Furthermore, these differences in laminar distribution of lesions in PD and AD may account for some of the clinical features typical of each of these dementing illnesses.

238.14

DYSTROPHIC NEURITES ARE COMPOSED OF BOTH DENDRITES AND AXONS, AND MAY BE ASSOCIATED WITH EARLY STAGE NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASE

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Dystrophic neurites are one of the pathological characteristics of AD. The term "neurite", however, does not identify a fiber's origin. It has been suggested that tau-immunoreactive dystrophic neurites are of dendritic origin (Ihara, 1988; Mckee *et al.*, 1989). Additionally, Weghe *et al.* 1991 and Yamaguchi *et al.* 1991 showed that tau-positive neurites were only associated with extracelluar neurofibrillary tangles (NFTs). In the present study, a tau-1 monoclonal antibody (Boerhinger) was used to examine the hippocampal formation in AD tissue. We confirm these previous observations and report two new findings. The tau-1 antibody stained dystrophic neurites of both dendritic and axonal origin. The morphological appearance of these axonal dystrophic neurites was diverse, and was related to the perikaryl origin of the axon. Axonal dystrophic neurites arising from NFTs were readily detected in CA3 and CA1. Axonal neurites were also seen in some axonal fiber tracts and terminal regions. In addition, dystrophic neurites of unknown origin were found not only within extracellular NTFs as suggested above, but also associated with the proximal dendrites of some "pretangle" neurons and intracellular NFTs. We have not determined if the neurites associated with the earliest stages of tangle formation are axonal or dendritic. This study suggests that dystrophic neurites are composed of both dendritic and axonal components and may be associated with "pretangle" neurons and intracellular NFTs prior to the formation of extracellular NFTs. These neuritic changes may contribute to neuronal dysfunction in Alzheimer's disease.

238.15

CHARACTERIZATION OF THE ALZ50 EPITOPE IN CEREBRAL CORTEX.

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The neurofibrillary tangles (NFTs) characteristic of Alzheimer's disease (AD) appear to be composed of abnormal forms of tau. ALZ50 is a monoclonal antibody that stains NFTs in AD brains. ALZ50 binds to both the abnormal tau in NFTs and normal tau yet does not stain normal brain sections. The ALZ50 epitope has variously been identified as a phosphorylated epitope near the C-terminus of tau, a conformational epitope within 3-4kD of the N-terminus and an 8 residue peptide at the N-terminus. Identification of the ALZ50 epitope may provide insight into the changes in tau that occur in AD.

Tau-enriched extracts were prepared from normal aged and AD cerebral cortex and used for western blotting. Fixation of the blots prior to antibody staining did not reduce ALZ50 staining. This result suggests that the lack of ALZ50 staining of normal brains is not a fixation artefact. Digestion with either acid or alkaline phosphatases changed the mobility of tau bands but there was no effect on ALZ50 staining. These results show that the binding of ALZ50 to its epitope on tau is not phosphorylation-dependent.

ALZ50 did not recognize tau extracted from the brains of non-mammalian species while tau from all mammalian species was stained to a similar extent. Both rat and mouse tau were stained as strongly as bovine, monkey and human tau. These results suggest that the ALZ50 epitope may not be at the N-terminus of tau since this is where there is most sequence divergence between species. Further work is needed to localize the ALZ50 epitope in tau and explain why this antibody stains AD but not normal brains.

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238.16

LIPOFUSCIN CONTENT IN THE NEUROFIBRILLARY BEARING NEURONS OF ALZHEIMER DISEASE.

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Lipofuscin is an autofluorescent cellular inclusion in the neuronal perikaryon resulting from the accumulation of metabolic by-products which cannot be further degraded. Thus, it may represent a beneficial cellular mechanism for storing toxic waste. By using computer enhanced morphometry and fluorometric analysis, the amount of lipofuscin was measured in 500 hippocampal neurons (regions CA2 and CA3) with and without neurofibrillary tangles(NFT) in brains of 10 patients with Alzheimer disease(AD), as well as in age matched controls.The mean values for cellular area demonstrate that the lipofuscin content in those cells carrying NFT is only 10% of the total cellular area, whereas in the AD neurons free of NFT and in the age matched controls, lipofuscin amounted to 31% and 33% respectively. The intrinsic autofluorescence of lipofuscin confirmed these inclusions to be three times more abundant in AD neurons without NFT and control cells than in those neurons containing NFT.We propose a breakdown in the capacity for making lipofuscin may result in the neuronal inability to store toxic waste. Such a defect could be responsible for the generation of NFT and ultimately may contribute to neuronal death.(NIH#AG08017.)

DEGENERATIVE DISEASE: ALZHEIMER'S—CLINICAL OBSERVATIONS

239.1

EARLY DETECTION OF HUNTINGTON'S DISEASE.

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Huntington's Disease (HD) is an autosomal dominant, genetic condition with 100% penetrance, representing a phenotypic expression of DNA located on the short arm of chromosome number 4, yet the actual gene identity remains obscure, most likely representing a deletion of a peptide directly or indirectly involved in the maintenance of extrapyramidal control of motor activity within the basal ganglia. The early detection of at risk members of HD families often takes on special meaning because the clinical syndrome is usually adult onset and at risk individuals need to clearly establish their genetic risk to facilitate decisions concerning vocational and family planning as well as a resolution of deep seated emotional conflicts.

Genetic certainty is established in our at risk family members using several different DNA probes applied to tissue samples from at least the index case, 2 afflicted and 1 non-afflicted family members. We have enhanced our prediction of clinical risk in ambivalent cases by performing Spect (Ceretec technicium) scanning. The selective lack of perfusion of the caudate appears to be a highly sensitive and very early sign of degenerative changes in this nucleus. Scans of caudate anatomy performed with Magnetic Resonance Imaging (MRI) or Computerized Axial Tomography (CAT) procedures can be falsely negative if caudate mass is maintained by astrocytic proliferation.

239.2

ELECTROPHYSIOLOGIC ANALYSIS OF EXTRAPYRAMIDAL MOTOR SIGNS (EPS) IN ALZHEIMER DISEASE (AD) COMPARED TO CONTROL SUBJECTS.

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Reports of EPS prevalence in AD are based on subjective clinical ratings and vary greatly. We performed clinical examinations and then employed sensitive, objective measures of rigidity (strain gauge tone arm), bradykinesia (computer determined reaction and movement time; RT & MT), and resting tremor (accelerometers) on 50 AD patients and 40 age and gender matched control subjects. No AD patients had other neurologic diagnoses and none was taking medications that would contaminate our measures. Dementia severity was determined by the Blessed Dementia Scale. Clinically, EPS were not present in control subjects and were present in only 2 AD patients. Objective measures, however, demonstrated significant differences between AD and control groups for tone (.096 ± .008 vs .042 ± .004 Nm/degree, p<.001; AD vs Controls, Mean ± SEM), MT (349 ± 23 vs 286 ± 21 msec, p<.03) and RT (350 ± 17 vs 317 ± 20 msec, p<.01). Resting tremor was absent in all subjects. Tone was significantly increased even in a subset of 24 "mild" AD patients (p<.05 from controls) and increased with severity of dementia (p<.05, ANOVA). We conclude that subclinical EPS are present in a large percentage of "pure" AD patients and EPS progress with the severity of disease.

239.3

Olfactory Dysfunction in Dementia Claire Murphy*, Jill Razani, Rani Nijjar, Alicia Garcia and Samuel Jinich (San Diego State University and UCSD Medical Center, San Diego, CA)

Patients with Probable Alzheimer's disease have significant impairments in olfactory function (Murphy et al, Neurobiology of Aging, 1990; Society for Neuroscience Abstracts, 1987, 1989). Patients with Down's Syndrome who live into their 30's and 40's develop plaques and tangles in the same areas of the brain that Alzheimer's patients do and these patients exhibit dementia. In an effort to investigate whether these patients show olfactory dysfunction, we tested Down's Syndrome patients 20-24 years old using standardized psychophysical testing procedures: two-alternative, forced-choice threshold testing for butanol, odor identification using a scratch and sniff test, and a recognition memory test. We found impairment in olfactory sensitivity and odor identification. We also found impairment in odor memory which was highly related to dementia. Some 25-40 % of patients who are HIV+ eventually develop dementia. In order to investigate whether these patients also show olfactory dysfunction, we tested male patients 20-40 years old for butanol threshold as described above. Results suggest that dementia in HIV+ patients is accompanied by diminished olfactory sensitivity. Given the proximity of olfactory projections to limbic and temporal lobe structures involved in memory, further investigation of the performance of patients with various dementias on a variety of olfactory tasks may prove to be informative.

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239.4

Sleep and computerized EEG in Alzheimer's disease: Effects of THA.

D Petit, J Montplaisir*, D Lorrain & S Gauthier

Introduction: Patients with Alzheimer's disease (AD) present a modified sleep architecture and a diffuse slowing of the awake EEG. Since the cholinergic system, which is greatly impaired in AD, is crucial for REM sleep generation, this study aims to determine whether slowing of the EEG is more pronounced during REM sleep than during wakefulness. Another goal is to determine whether the anticholinesterase THA corrects sleep deficits and diminishes EEG slowing in AD patients.

Method: Ten mild to moderate AD patients (mean age: 61 yrs) and ten controls matched for age and sex were studied in the sleep lab for two nights and the intervening day. The following montage was used: F3/C3, F4/C4, T3/T5, T4/T6, P3/O1, P4/O2. Artifact-free samples collected during REM sleep and wakefulness were subjected to spectral analysis. AD patients were recorded again after each of two 2-week treatments with THA and placebo (up to 100 mg/day).

Results: AD patients showed EEG slowing in both states but it was more marked during REM sleep than during wakefulness ($F(1,10)=6.91$, $p<.025$). Spectral analysis of the REM sleep EEG allowed correct classification of 100% of the subjects. It also revealed differences in the degree of slowing in different cortical regions that did not appear in wakefulness. Temporal regions were the most impaired and presented a strong asymmetry in favor of a more impaired left side. THA reduced only REM sleep EEG slowing in frontal ($t=5.03$, $p<.01$) and parieto-occipital ($t=4.22$, $p<.01$) regions, when compared to baseline (THA vs placebo= NS). THA produced no change in the sleep variables studied.

Conclusion: Spectral analysis of the REM sleep EEG is a powerful diagnostic tool for AD. It is a sensitive gauge of cholinergic treatment efficacy. The lack of effect of THA was probably due to the low dosages given to avoid hepatotoxicity.

239.5

VOLUMETRIC MAGNETIC RESONANCE IMAGING IN ALZHEIMER'S DISEASE: CORRELATIONS WITH DISEASE SEVERITY. Daly E, DeCarli CD, Haxby JV, Soncrant T*, Schapiro MB, Rapoport SI, Horwitz B, Murphy DGM

We related cognitive decline in males with Alzheimer's disease (DAT) to brain morphometric changes. Cognitive dysfunction was evaluated by the Folstein Mini-Mental State Examination (MMS) and Wechsler Adult Intelligence Scale. Brain MRI studies were performed on a Picker 0.5 Tesla scanner. We analyzed 7-mm thick, contiguous slices axial slices (TR 2000 msec, TE 20 msec), and 5mm thick coronal slices. Mean ($\pm$ S.D) MMS of DAT men was 16 ± 7; mean ($\pm$ S.D) age of DAT men and controls (NC) was 68 ± 9 yr and 70 ± 8 yr respectively. DAT men had significantly smaller cerebral brain matter and temporal lobe volumes ($p< 0.05$), and significantly larger volumes in every CSF measure (except right and left cerebral hemispheric peripheral CSF) than controls. The volume of the subcortical nuclei did not differ significantly between groups. Men with mild DAT had significantly smaller cerebral brain matter and temporal lobe volumes and significantly larger lateral ventricles than controls. Total temporal lobe volume discriminated between groups with a sensitivity and specificity of greater than 85%. When peripheral and central CSF volumes were analyzed as a function of dementia severity, both increased significantly (central CSF, $r=0.68$, $p < 0.0001$; peripheral CSF, $r=0.41$, $p < 0.02$). The slope of ventricular CSF regression (1.6% per point on MMS) was , however, 3 times that of the peripheral CSF slope [0.67% per point on MMS ($z= 3.37$, $p <0.0002$)] Neuropsychological measures of disease severity in DAT patients were significantly ($p< 0.05$) and appropriately correlated to the normalized volumes of cerebral brain matter and right lateral ventricle. We conclude that in DAT: (i) there is no effect of the disease process over and above the healthy aging process on volumes of caudate, lenticular and thalamic nuclei, (ii) the differential expansion of peripheral and central CSF represent different atrophic processes, and (iii) measurement of temporal lobe brain matter volume provides a relatively sensitive and specific means of discriminating between DAT and NC.

239.6

BLOOD-BRAIN BARRIER INTEGRITY IN AGING AND ALZHEIMER'S DISEASE. M. T. Caserta*, C. Allen, G. D. Lapin, & D. R. Groothuis, Depts. of Psychiatry, Neurology, Biomedical Engineering and Neurobiology, Northwestern Univ. Med. School, Evanston Hosp., Evanston, IL 60201

Alzheimer's disease (AD) is characterized by increased senile plaques and neurofibrillary tangles in brain. It is not clear if the β-amyloid protein is generated by synthetic processes in the brain or crosses the blood-brain barrier (BBB). Previous studies about BBB function in AD have been inconclusive. We studied the BBB in 11 elderly patients with a clinical diagnosis of AD; all had no medical or neurological illness. We studied BBB function with a computed tomographic (CT) method (Groothuis et al., Ann Neurol 30: 581,1991) that measures transcapillary transport of meglumine iothalamate. We studied influx (K_1) in hippocampus, temporal and frontal cortex. In all regions, K_1 was between 1-3 μl/g/min, which is within the normal range. We conclude that there is no generalized abnormality of the BBB in AD. This does not exclude the possibility of a specific BBB defect with regard to β-amyloid protein. (Supp. by Washington Square Health Foundation and NIH Grant TO1 MH19151.)

239.7

IN VIVO BRAIN GLUCOSE AND PHOSPHORUS METABOLISM IN ALZHEIMER'S DISEASE. Murphy DGM, Bottomley PA, Salerno J, Williams W*, Schapiro MB, Rapoport SI, Alger J, Horwitz B.NIA,Bldg 10, Rm 6C 414, Bethesda.

Positron emission tomography (PET), magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) were used to study brain glucose and phosphorous metabolism in dementia of the Alzheimer type (DAT) to determine if there are differences in concentrations or ratios of phosphorus metabolites between DAT patients and age/sex-matched healthy controls (NC), if severity of DAT is correlated with phosphorus metabolite concentrations/ratios, and if glucose flux and phosphorus metabolism are related. We studied 9 drug-free, otherwise healthy DAT patients (6 M/3 F) and 8 NC (5 M/3F). Mean ages ($\pm$ SD) of the patients and NC were 60 ± 10 yr and 64 ± 16, respectively. PET, MRI, and MRS were all carried out on the same volume of brain matter (ROI), from 3.5 - 6.5 cm above the inferior orbitomeatal line. MRI and MRS was performed on a 1.5 Tesla scanner (General Electric, Milwaukee, WI). ROI volume was determined by quantitative MRI, so concentrations of phosphorus metabolites could be expressed per kg of brain tissue. In MRS, phosphorus concentrations were measured relative to reference vials of phosphonitrilic chloride trimer around the head. Resting regional metabolic rates for glucose (rCMRglc) were obtained using PET (Scanditronix PC-1024-7B, Uppsala, Sweden) with (18F)-2-fluoro-2-deoxy-D-glucose (FDG) in 7 of the DAT patients (5 male, 2 female) and 7 of the NC (5 male, 2 female). Mean rCMRglc was significantly lower in the DAT group than controls ($p < 0.01$), 5.01 ± 0.9 vs 6.8 ± 0.6 mg/100g/min. However, we found no significant group difference in any phosphorus metabolite concentration/ratio, and no significant correlation between any phosphorus metabolite concentration/ratio and severity of dementia, or glucose metabolism. We suggest that glucose metabolism is reduced early in DAT (reflecting decreased basal synaptic functioning), and is unrelated to a rate-limitation in glucose delivery, abnormal glucose metabolism, or abnormal coupling between oxidation and phosphorylation. Normal or near normal levels of phosphorus metabolites are maintained at all stages of DAT. Therefore, altered high-energy phosphate levels are not a consequence of reduced glucose metabolism in DAT, and do not play a major role in the pathophysiology of the disorder

239.8

CEREBRAL GLUCOSE METABOLISM IS REDUCED IN PRIMARY VISUAL CORTEX OF ALZHEIMER'S DISEASE (AD) PATIENTS WITH PROMINENT VISUAL IMPAIRMENT: A PET LONGITUDINAL STUDY. E. De Micheli *, P. Pietrini, C. L. Grady, J. V. Haxby, J. Salerno, A. Gonzales-Aviles, S. I. Rapoport and M. B. Schapiro. Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda MD 20892.

Prominent visual symptoms (VS) are noted early in the course of AD in a subgroup of pts. To further investigate VS in AD, we yearly determined regional cerebral metabolic rates for glucose (rCMRglc) with PET and neuropsychological functions over a 3 yr period in two AD pts (58; 64 y.o) with visual disorientation, optic ataxia, and ocular apraxia (AD+VS). Using Mini-Mental Status Examination (MMSE), the two pts. were mildly-demented (MMSE>20) first evaluation, and became moderately-demented during follow-up (MMSE scores: 23, 22, 15 and 22, 19, 13, respectively). PET examinations (Scanditronix PC1024-7B; 6mm FWHM) were performed in the "resting state" with ^{18}FDG. Results were compared with sex- and age-matched healthy normal volunteers (NV; mean age 58 ± 5 SD, range 52-66) and with a group of 16 sex- and age-matched mildly-moderately AD patients (AD; mean age 52 ± 5 SD, range 52-68). Absolute rCMRglc values were measured in mg/100g tissue/min. To reduce inter-subject variability, rCMRglc were "normalized" to mean cerebellar rCMRglc (a region relatively preserved in AD). Compared to NV, the AD+VS pts. showed significant bilateral reductions in absolute rCMRglc (>2 SD, Z-score analysis) in parietal (PA) and occipital association (OCA) areas from the first PET scan and, in the last two PET scans in the calcarine cortex (Cal), too; normalized rCMRglc values were significantly reduced in PA and OCA as well as in Cal at every examination in the AD+VS pts. Compared to AD pts, the two AD+VS pts. showed bilateral rCMRglc reductions in PA and OCA from the first PET scan; one pt. also in Cal at his last PET scan. Neuropsychological testing confirmed visual-spatial deficit along with a global cognitive deterioration. These results extend previous findings, of reduction in OCA in AD+VS by showing that Cal, usually spared in mild/moderate AD pts, also becomes involved in AD+VS pts., consistent with pathological reports.

239.9

IN VIVO ^{31}P NMR SPECTROSCOPY REVEALS ELEVATED PHOSPHOMONOESTERS AND ALTERED ENERGY METABOLISM IN EARLY ALZHEIMER'S DISEASE

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Previous *in vitro* and *in vivo* ^{31}P NMR spectroscopic studies of Alzheimer's disease (AD) brain have revealed changes in phospholipid and energy metabolism in this dementing illness. In this ongoing, longitudinal study of AD patients at various levels of severity and age-matched normal controls, we have completed 55 cognitive and spectroscopic studies on 21 controls and 26 assessments on 12 AD patients. The results showed an elevation of phosphomonoesters (PME) ($p=0.005$) in the mildly but not moderately demented AD patients as compared to controls. The levels of PME were inversely correlated with measures of severity, suggesting that PME levels in AD may peak even before patients present with an early clinical dementia. The levels of energy metabolites, including phosphocreatine and ADP, appeared to be decreased early in the dementia, suggesting an increased utilization of ATP. The latter may signify increased activity at the synaptic membrane, since this is thought to be the major site of ATP utilization in neurons. *In vivo* ^{31}P NMR spectroscopy is a non-invasive technique that may provide metabolic insights into the pathophysiology of AD and provide a useful tool to monitor the progression and response to experimental therapies in this dementia.

239.10

PERIPHERAL AND CIRCULATING IMMUNE COMPLEXES IN PATIENTS WITH DEMENTIA.

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An immunohistochemical study was carried out on the skin biopsies of 44 patients with Alzheimer's disease (AD), 15 with multi-infarct dementia (MID), 24 with Down's syndrome (DS) and 19 elderly controls, to study β /A4 expression in skin in coexistence with IgG, IgM and complement factor C3c. Immunopositivity for these proteins was found either separately or colocalized in several localizations in skin significantly more frequently in the AD, MID and DS patients than in controls. These data prompted us to analyse the presence of circulating immune complexes (CIC) in sera of patients with dementia and DS and aged-matched controls using both C1q and conglutinin binding ELISA tests. The probable AD and MID patients had more frequently CIC in their sera as compared to elderly nondemented subjects (ANOVA/Duncan; $p< 0.05$). The CIC were detected with highest frequency in the series of 10 DS patients, as contrasted to young controls, only 1/10 of which showed C1q binding-positivity. In the AD patients, the cognitive decline (Mini-Mental Status test) correlated significantly with the CIC values of both tests. The study supports the view that systemic autoimmune mechanisms may be involved, at least partly, in a variety of dementing processes.

239.11

TETRAHYDROAMINOACRIDINE SHOWS A TROPHIC EFFECT ON DIFFERENTIATING CEREBELLAR GRANULE CELLS.

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9-Amino-1,2,3,4-tetrahydroacridine (THA), an inhibitor of acetylcholinesterase, has gained interest during recent years as a therapeutic agent for senile dementia of the Alzheimer type. In this report, we examined whether or not THA has a trophic effect on differentiating cerebellar granule cells using the method of [^{3}H]inositol incorporation into inositol-containing phospholipid. We found that supplementation with THA (30 μM) of cultures grown in 15 mM K^+-containing medium markedly increased survival and promoted the morphological maturation of cerebellar neurons. We have also characterized this THA-induced trophic effect by the assessments of phosphoinositide responses and saturation binding studies and presented evidence that THA selectively increases the expression of muscarinic cholinergic receptor function in cultured cerebellar granule cells. The present observations may offer a new avenue to investigate the mechanisms of action of THA and related therapeutic agents.

DEGENERATIVE DISEASE: ALZHEIMER'S—ETIOLOGIC TOXINS

240.1

THE EFFECTS OF SELENIUM AND VITAMIN E DEPRIVATION IN THE MOUSE.

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Nutritional myopathy, congestive cardiomyopathy have been reported as a consequence of selenium deficiency. Vitamin E deficiency can result in degeneration of axons and myelin degeneration. Selenium deficiency results in decreased activity of glutathione peroxidase activity. This is because selenium is a mandatory element in the formation of glutathione peroxidase. Inhibition of vitamin E and selenium activity may result in observable morphologic changes. The present study histochemically analyzes these morphologic changes. Animals were deprived of either compound or both and were examined at three-month intervals. We then examined them for evidence of neuropathological conditions. In the animals deprived of both selenium and vitamin E, some gliosis of the cortex and striatum was noted, but no neural cell loss. Examination by Bielschowsky stain revealed no inclusion bodies, plaques or tangles. Thioflavin stain did not reveal any plaques. Immunocytochemistry to ubiquitin found occasional positive neurons near the site of vascular changes. Vascular changes were noted and these were accompanied by cuffing. Immunocytochemistry to neurofibrils have not revealed any changes in animals examined at three months.

240.2

EFFECT OF ALUMINUM ON CULTURED RAT MICROGLIA.

J. Goodwin, E. Uemura and W.G. VanMeter*. Dept. of Vet. Anat., Iowa State Univ., Ames, IA 50011.

The significance of aluminum and microglial cell changes in Alzheimer's disease (AD) has not yet been determined. One way in which aluminum and microglia may affect the progression of AD is through the effects of Interleukin-1 (Il-1). Il-1, a cytokine secreted by microglia, is reported to be significantly elevated in the brains of AD patients. We have cultured microglia isolated from the hippocampi of 3-5 day old rat pups. These microglia were exposed to various aluminum compounds in order to elicit the release of Il-1. After 24 h, Il-1 levels were measured using the mouse thymocyte assay. We have found that aluminum silicate (AlSi) causes a significant increase in Il-1 release from cultured rat microglia. Other aluminum compounds such as aluminum hydroxide, aluminum potassium sulfate and aluminum lactate also caused increases in Il-1 but to a lesser degree. Since AlSi is found in the cores of AD plaques, elevated Il-1 in AD may be due to AlSi-activated microglia.

240.3

BEHAVIORAL CONSEQUENCES OF ALF_3 CONSUMPTION. R. L. Isaacson* and J. Varner, Dept. Psychology, SUNY Binghamton, Binghamton, NY 13902-6000.

Excessive aluminum (Al) in the brain has been associated with Alzheimer's disease. Many investigators have attempted to associate high levels of (Al) intake with signs of this disease in animal models. In this study a monomeric fluoroaluminum complex, AlF_3, was added to the daily water supply of 40 Long-Evans rats 4-5 months of age. They were divided into 4 groups based on the water made available daily for longer than 8 months. The control group received distilled, deionized water. The others received water to which AlF_3 had been added in the following concentrations: 0.5, 5.0, or 50 ppm. Body weights were recorded throughout the study. Testing in an open field for activity levels were assessed twice during the exposure period as were examination of walking patterns based on footprints of the animals. "Spatial memory" was assessed in a Morris water maze. Other tests were administered to determine possible changes in emotionality and/or motoric coordination. In general the lowest concentration dose group and the group receiving only water appeared to be similar in performances, and in almost every task these groups appeared to differ from the two higher concentration groups. However, as expected (based on previous reports of individual differences in absorption and retention of Al), considerable variability occurred in the data. Analyses of the Al content by chemical and histologic means are underway. One prominent and potentially significant result is that the group receiving the lowest concentration of AlF_3, was most prone to succumbing to diseases during the course of the experiment, more so than the rats receiving the higher concentrations. This may suggest a differential effect on the physiology of the animals at low levels.

240.4

A WIDESPREAD INCREASE IN THE ACTIVATED ISOFORM OF uCANP IN BRAIN IN ALZHEIMER DISEASE. K.-I. Saito, J. Hamos and R.A. Nixon*. McLean Hospital and Harvard Medical School, Belmont, MA 02178

Calcium-activated neutral proteinases (CANP) are key enzymes in intracellular signaling cascades and are potential mediators of calcium-induced cell injury. The class of CANP requiring micromolar levels of calcium for maximal activity (uCANP or calpain I) is enriched in neurons and exists in tissues in both a precursor form and one or more autolytically activated forms. In this study, we identified three isoforms of uCANP in postmortem brain regions from 22 Alzheimer patients and 17 controls. As an index of changes in the *in vivo* activity of the enzyme in Alzheimer disease, we quantified the relative proportions of activated and precursor isoforms of uCANP by immunoassay. In prefrontal cortex, the ratio of k76 kDa activated to 84 kDa precursor isoforms was increased 3-fold. The 84 Kda form in AD brains was decreased (AD, 22.6% vs. control, 37.2%, $p < 0.001$) and 76 Kda isoform was increased (AD, 41.4% vs. control, 26.6%, $p < 0.001$). The severity of these abnormalities did not correlate with the postmortem interval or with age of the individual. The ratio of activated to precursor uCANP isoforms was also increased 50% in both cerebellum and putamen ($p < 0.001$), where degeneration of neurons is considered minimal. Persistent activation of uCANP, which accords with evidence for disrupted calcium homeostasis in AD, could contribute to the altered protein processing and abnormal phosphorylation associated with neuronal degeneration in AD.

ISCHEMIA: NEUROTRANSMITTERS

241.1

CSF MONOAMINES REFLECT BRAIN BIOCHEMISTRY DURING CEREBRAL ISCHEMIA P. Wester,* R. Busto, W. D. Dietrich, M.D. Ginsberg, M.Y.-T. Globus. CVD Research Center, University of Miami, School of Medicine, Miami, FL 33101.

We have tested the hypothesis that cerebrospinal fluid (CSF) monoamines reflect changes in brain extracellular fluid during cerebral ischemia. Male Wistar rats (n=7) were subjected to permanent (120 min) 2-vessel occlusion plus hypotension under brain hyperthermic (39.0 °C) conditions. Dopamine (DA), norepinephrine (NE), serotonin (5HT) and their metabolites were measured in striatum, lateral ventricle and cisterna magna by microdialysis and HPLC. In striatum, ischemia induced an immediate significant increase of all monoamines (DA 430 x baseline values (BL); NE 6 x BL and 5HT 35 x BL, $p<0.05$ by ANOVA). The increased levels gradually decreased but remained significantly elevated during the ischemic period ($p<0.05$). The metabolites DOPAC, HVA and 5HIAA showed a significant instant and sustained decrease to 17 - 20 % compared to BL values during ischemia ($p<0.05$). In the lateral ventricle, significantly increased levels of DA (5 x BL), NE (11 x BL) and 5HT (19 x BL) occured immediately after the onset of ischemia ($p<0.05$) and remained elevated whereas decreased levels of DOPAC (60% of BL), HVA (31% of BL) and 5HIAA (26 % of BL) were monitored ($p<0.05$). Similarily, in cisterna magna, significantly elevated levels of DA (7 x BL), NE (2 x BL) and 5HT (5 x BL) ($p<0.05$) and decreased concentrations of DOPAC (65% of BL), HVA (65% of BL) and 5HIAA (71% of BL) were recorded. These data suggest that CSF monoamines and their metabolites reflect changes in striatal extracellular fluid during brain hyperthermic cerebral ischemia. CSF neurotransmitters may thus be important as specific neuronal damage markers during ischemic stroke.

241.2

ATTENUATION OF POTASSIUM CYANIDE MEDIATED NEURONAL CELL DEATH BY ADENOSINE. C.D. Sturm and K.-W. Yoon*. Division of Neurosurgery and Surgical Research Institute, St. Louis University School of Medicine, St. Louis, MO 63110-0250.

Glutamate has been shown to play a role in the delayed neuronal cell death seen with ischemia. Attenuation of synaptically released glutamate can be accomplished by modulators such as adenosine and baclofen. We have studied adenosine's ability to attenuate in vitro cell death after exposure to potassium cyanide <KCN> in hippocampal neuronal cell cultures.

Using hippocampal cell cultures obtained from 1 d old rats and Trypan blue staining for cell viability counting, we have found that the NMDA specific antagonist MK801 10 μM attenuates the neuronal cell death secondary to exposure to KCN 1 mM for 60 min. Adenosine 10-1000 μM also decreases the neuronal cell death secondary to the same concentration of KCN in a dose dependent manner. This same neuroprotective effect is mimicked using the A1 specific agonist N6-Cyclopentyladenosine 10-100 nM. The A1 specific receptor antagonist 8-Cyclopentyl-1,3-dimethylxanthine 10-1000 nM blocks the adenosine neuroprotective effect in a dose dependent manner.

We conclude that the neuronal cell death produced by KCN in our experimental model is mediated by glutamate. This neuronal cell death is attenuated by adenosine via the A1 specific receptor. Our results also strongly suggest that adenosine produces this neuroprotective effect against KCN exposure by attenuating endogenous glutamate release. Supported by K08NS01547-01.

241.3

CALCIUM DEPENDENCE OF AMINO ACID RELEASE IN THE ISCHEMIC RAT HIPPOCAMPUS

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Cerebral ischemia causes increases in extracellular levels of amino acid (AA) neurotransmitters in the hippocampus. The degree of calcium (Ca) dependence of this release is controversial[1,2,3]. The present study sought to determine the Ca dependence of release of the AAs glutamate (GLU), glycine (GLY), aspartate (ASP), taurine (TAU), serine (SER) and glutamine (GLN) in the presence of 20 min of ischemia (hypoxia +hypoglycemia) in the rat hippocampal slice. Slices were perfused with either Kreb's with Ca, or Kreb's without Ca, with $MgCl_2$, bubbled with 95%O_2/5%CO_2 at 200μl/min at 38°C for a 45 min washout period. After 10 min baseline samples were collected, 20 min of ischemia was induced with either dextrose-free Kreb's or dextrose-free, Ca-free, Kreb's with $MgCl_2$ bubbled with 95%N_2/5%CO_2. Control groups received sham perfusate changes After two 10 min ischemia samples were collected, all perfusates were replaced with the initial solutions and five 10 min reperfusion samples collected. AA content was analyzed by HPLC. Twenty min of ischemia caused increases in levels of GLU, GLY, TAU and SER, but not ASP, as compared to control in the presence of Ca. Ischemia-evoked peak release was Ca dependent for the AA's GLU, GLY, TAU and SER. GLN did not rise in response to ischemia and was lower than control. In this model, a substantial portion of ischemia-evoked AA release is Ca dependent. This supports the hypothesis that regulation of excitatory AA release during ischemia may be acheived by treatments altering nerve terminal Ca flux.

[1] J. Neurochem. 45: 145-51, 1985. [2] Neurosci. Lett. 96: 202-6, 1989. [3] J. Neurochem. 57: 1159-64, 1991.

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241.4

DOPAMINE IS INVOLVED IN ISCHEMIA-INDUCED INCREASES OF EXTRACELLULAR cAMP IN THE STRIATUM. R. Prado, R. Busto, E. Martinez, I. Valdés, and M.Y.-T. Globus,* CVD Research Center, Univ. of Miami, Sch. of Med., Miami, FL, 33101.

Dopamine has been demonstrated to be involved in the development of ischemic neuronal damage in the striatum. The detrimental effect of dopamine may involve activation of second messenger systems, such as the cAMP cascade, which may enhance the susceptibility of striatal neurons to ischemia. In the present study we have evaluated the relationship between ischemia-induced changes in cAMP and dopamine neurotransmission. Microdialysis probes were implanted in both striata, a D-1 antagonist (SCH-23390, 100uM) was administered through one probe and modified Ringer solution through the other. After a stabilization period, rats were subjected to 20 min of ischemia by 2-vessel occlusion plus hypotension. Extracellular samples were collected, from both striata, before during and after ischemia and analyzed for cAMP by radioimmunoassay. Ischemia induced a significant increase in extracellular cAMP (peak levels mean±SD, fmol/min; 13.6±2.7), which was also observed during 4h of recirculation. Treatment with a D-1 antagonist significantly reduced the magnitude of cAMP release during ischemia (1.8±1.3, $p<0.01$) and completely inhibited the rise during recirculation. These results indicate that ischemia-induced surge in dopamine and activation of D-1 receptors are involved in generation of cAMP during ischemia and recirculation. Further studies will evaluate the importance of this pathway in the detrimental effect of dopamine during ischemia.

241.5

IN VIVO MICRODIALYSIS OF THE BORDER ZONE IN A RAT MODEL OF REVERSIBLE FOCAL ISCHEMIA. W.D. Lust*, J.F. Nash[1], S. Pundik, C.M. Jenkins, S.U. Bhatti and W.R. Selman, Depts. of Neurosurgery and Psychiatry[1], Case Western Reserve Univ. Cleveland, OH

Previous studies have shown that the border zone (BZ) surrounding the ischemic core is metabolically at risk even if reflow is initiated within 2 h of middle cerebral artery occlusion (MCA). Since the BZ is destined for infarction after only 2 h of ischemia, we examined the possibility that the release of cellular contents including neurotransmitters may contribute to the deterioration of this tissue. A dialysis probe was placed in the cerebral cortex 4 mm lateral to the midline and 1 mm anterior to the Bregma and the tissue was perfused at a rate of 2 μl/min for 2 h prior to ischemia. The ipsilateral MCA was occluded and samples were collected at 20 min intervals during 2 h of ischemia and during 1 h of reflow. The brain was frozen in situ, sectioned and the proper positioning of the probe confirmed. Tissue lactate adjacent to the probe site was elevated by 5.8-fold, while ATP levels were depressed to 70% of control. During the ischemic period, lactate levels in the perfusate increased 27%, glucose decreased by 50%, and glutamate increased by over 2-fold. These changes were significantly different from the preischemic values and suggested that the occlusion of the MCA elicited a response typical of that observed after a mild metabolic stress. Despite this relatively mild energy imbalance, the dopamine levels in the perfusate increased more than 16-fold during ischemia, while those for DOPAC and HVA decreased by 50% of more. Serotonin levels did not change despite a marked reduction in its metabolite, 5HIAA. It would appear that brain homeostasis was markedly compromised in the border zone both during and after focal ischemia and these changes could be the triggering events leading to infarction.

241.6

ISCHEMIC DAMAGE TO CA1 HIPPOCAMPUS CORRELATES POORLY WITH GLUTAMATE RELEASE DURING ISCHEMIA S. Cho*, Y. Takeda, and W. Pulsinelli Dept. of Neurology and Neuroscience, Cornell University Medical School, New York, NY 10021

Excessive excitation of postsynaptic neurons by ischemia-induced release of excitatory neurotransmitters (EN) has been proposed as a principal cause of injury to selectively vulnerable neurons. In prior work (Brain Res., 1990), we showed that transection of fimbria/fornix (F/F) but not of either the perforant (P/P) or Schaffer collateral (S/C) pathways, significantly reduced ischemic damage to CA1 neurons. In the present study we examined whether these same pathway lesions altered the release of EN in the CA1 hippocampus of rats subjected to forebrain ischemia. Ten to 14 days after lesioning the afferent pathways, microdialysis probes were inserted into the CA1 hippocampus and the animals were subjected to 20 min of 4-VO ischemia. The dialysates, collected during ischemia and for 20 min after cerebral reperfusion, were analyzed by HPLC. In hippocampi with F/F lesions previously shown to protect against CA1 damage, the EN concentrations were not altered. In hippocampi with P/P or S/C pathway lesions previously shown not to protect against CA1 injury in this model, the release of extracellular glutamate was significantly reduced. The lack of correlation between CA1 glutamate concentrations and CA1 damage in these pathway-lesioned animals suggests that CA1 injury is largely independent of the extracellular glutamate concentration during ischemia and shortly after reperfusion.

	F/F		P/P		S/C	
	Cont(5)	Lesn(12)	Cont(4)	Lesn(5)	Cont(5)	Lesn(6)
ASP	2.7±0.4	2.7±0.8	2.2±0.2	1.7±0.1*	1.7±0.3	0.9±0.2
GLU	18.9±3.0	13.2±2.8	20.1±2.6	9.5±1.5*	8.4±1.5	3.4±1.3*

Values are expressed as μmoles±sem. Student's t-Test, *p<0.05 vs Cont.

241.7

PURINE METABOLITE RELEASE DURING CEREBRAL ISCHEMIA AS ASSESSED BY CHRONICALLY IMPLANTED MICRODIALYSIS PROBES. M.C. Grabb, V.M. Sciotti and D.G.L. Van Wylen*, Department of Physiology, SUNY-Buffalo School of Medicine and Biomedical Sciences, Buffalo, NY 14215.

The purpose of this study was to determine the interstitial fluid (ISF) purine metabolite profile during cerebral ischemia (ISC). Vertebral arteries were cauterized and microdialysis probes were implanted in the caudate nucleus of rats (n=4). After 24 hours, animals were anesthetized with halothane and dialysate samples were collected before (BASE) and during 90 min of ISC, induced by bilateral carotid artery occlusion. Cerebral blood flow (CBF; H_2 clearance) was reduced from 103.1 to 8.8 ml/min/100g during ISC. Dialysate concentrations of adenosine (ADO), inosine (INO), hypoxanthine (HYP), xanthine (XAN), and uric acid (UA) are shown below (mean ± SEM; *p<0.05 vs. BASE):

	ADO(μM)	INO(μM)	HYP(μM)	XAN(μM)	UA(μM)
BASE	0.06±0.01	0.3±0.1	1.1± 0.1	2.0±0.4	9.2±2.7
ISC 10'	3.9±1.8*	3.3±1.1*	13.5± 1.5*	4.6±1.2	7.6±2.4
ISC 30'	5.1±2.4*	8.4±3.0*	34.7± 7.8*	9.9±2.1*	5.1±1.5*
ISC 60'	3.6±1.7	10.3±3.9*	45.5±13.5*	11.5±1.4*	5.0±1.8*
ISC 90'	2.1±1.2	9.7±4.1	52.2±15.0*	11.1±2.7*	4.6±1.6*

ISF levels of all purine metabolites except UA increased progressively during ISC, with HYP being the predominate metabolite. These data indicate that chronically implanted microdialysis probes can be used to assess ISF metabolite and CBF changes during ISC. Supported by NIH HL-40878.

241.8

SPINAL CORD ISCHEMIA INDUCES RELEASE OF AMINO ACIDS AND CONCURRENT NEURONAL DAMAGE. M. Marsala*, L.S. Sorkin and T.L. Yaksh, UCSD, Anesthesiol. Res. Lab., La Jolla, CA 92093

Ischemic induced excitatory amino acid release is postulated to play a causal role in irreversible neuronal damage. Extracellular changes in amino acid concentrations were measured using a microdialysis fiber inserted transversely through the dorsal lumbar enlargement of halothane anesthetized rats; samples (30 min) were assayed using HPLC with UV detection. After washout and a 1 hr control period, 20 min of reversible spinal cord ischemia was induced by the inflation of a Fogarty catheter passed through the femoral artery to the descending thoracic aorta. This was followed by 2 hr of reperfusion. Rats were perfused with saline followed by buffered formalin, and the spinal cord tissue removed and processed for modified Nauta staining and light level microscopy.

Glutamate concentrations were elevated during the ischemia and returned to control after 2 h of reperfusion. Taurine increased 0.5 h postocclusion, peaked at 1.5 h and remained elevated for the full 2 h. Although not significant, mean glycine concentrations increased within 1 h of recirculation and remained high. No consistent changes were detected in aspartate, asparagine or glutamine concentrations. Apparent interneuronal damage, affecting small and medium sized cells, was found predominantly in laminae II-V. Only occasional small areas of focal necrosis, located in the dorsolateral dorsal horn and anterolateral ventral horn, were detected.

These results are consistent with a role for glutamate in ischemic spinal cord interneuronal damage and suggest that changes in taurine concentration detected during the early postischemic period can serve as an important indicator of neuronal damage.

SCHIZOPHRENIA

242.1

SMOOTH PURSUIT TRACKING IN ADOLESCENT PSYCHOTICS: EFFECTS OF BACKGROUND ILLUMINATION R.T. Pivik, Ph.D.*, University of Ottawa, Ottawa General Hospital, Ottawa, Canada, K1H 8L6

Aberrant smooth pursuit tracking performance in adult psychotics shows marked improvement when patients are recorded under dark-adapting conditions. The present investigation assesses the comparability of eye tracking performance in adolescent psychotics with published data in adult patients.

Eleven Ss with psychotic symptomatology (DSM-III-R, RDC criteria) and 8 normal controls were studied. Ss, matched for age (patients:17.4±1.1 yrs; controls:16.1±0.1 yrs), were free from organicity or medications known to affect eye tracking. Control Ss were free from psychiatric disorders. EOGs were recorded on magnetic tape under light and dark-adapting conditions, while Ss tracked a light oscillating sinusoidally at .45 Hz (10 cycles). The root-mean-square (RMS) error measure was calculated from artifact-free, phase and amplitude adjusted target and EOG signals. Data were analyzed using ANOVA procedures.

In the light condition, psychotics showed higher mean RMS and greater variability than controls (M=11.8±6.5% & 8.8±0.67%,respectively). Relative to light-testing data, psychotics' tracking under dark-adapting conditions showed a mean improvement of 18% and decreased variability (M=9.7±3.8%), and controls slight (3%) increases in mean RMS and variability (M=9.1±1.5%).

The absence of statistically significant group differences in these data may relate to the small sample sizes or heterogeneity of the psychotic group. Nine of these Ss experienced their first psychotic episode within an average of 3.2 months (.5-6 mos. range) of the recordings, and the results may, therefore, also reflect early stages of physiological dysfunction which intensifies with exacerbation and persistence of psychotic symptomatology.

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242.2

Force Control Fatigability as a Pathognomonic Sign in Tardive Dyskinesia. P.B. Vrtunski*, K.L. Lewis and H.Y. Meltzer, Microbehavior Lab. VA Medical Center and Case Western Reserve University, Cleveland, OH 44141.

The hypothesis was that response output (button-press) with a target force X at time t would be measurably lower if it were preceded (at time t-1) by a target output of $X+Y$ (a higher force) than if it were preceded by a target output of X-Y (a lower force). Over 150 subjects participated in this study. In addition to schizophrenics with and without tardive dyskinesia, three control groups were employed: normal controls, elderly and drug addicts. The test utilized was a target-matching task (90 6-second trials, 10 targets) with force output ranging from 5 to 560 cN. Results indicated that precedent output at t-1 significantly alters force at t, but in the opposite direction from that expected, i.e., $X+Y$ increased the force while X-Y reduced it. Thus, the fatigability effect results in a threshold change rather than an attenuation of output. In group comparisons, the $X+Y$ precedent separates the groups significantly better than the X-Y precedent. Since normal controls and TD schizophrenics were on the opposite ends of the continuum, with non-dyskinetic schizophrenics, drug addicts and elderly (in that order) ranging between them, the $X+Y$ precedent at t-1 may have a higher clinical value in the assessment of motor impairment.

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242.3

TASK SENSITIVE ABNORMALITIES IN PROCESSING NEGATIVITY AND P3 ACTIVITY IN SCHIZOPHRENIC CHILDREN. R.J. Strandburg*, J.T. Marsh, W.S. Brown, R.F. Asarnow, D. Guthrie and J. Higa. Dept. of Psychiatry, UCLA, Los Angeles, CA 90024

The Continuous Performance Task (CPT) has been shown to be sensitive to schizophrenic impairments. Multi-channel ERP data were recorded from 16 schizophrenic and 16 normal children (SCH & NCH) during performance of easy (detect "8") and hard (detect sequentially recurring digits) versions of the CPT to examine when in the course of CPT processing SCH exhibit deficits. SCH were impaired on both versions of the CPT producing fewer hits, more false alarms, and longer reaction times. Their poorer performance was associated with the absence of a normal increase in amplitude for early processing-related negativities (Np) from non-target to target stimuli suggesting inefficiencies in the allocation of attentional resources for stimulus discrimination and categorization. While P3 amplitude was not significantly smaller in SCH, the pattern of P3 amplitudes obtained for target versus non-target stimuli in easy versus hard versions of the CPT was significantly different in normal and schizophrenic subjects suggestive of SCH deficits during later stages of CPT processing associated with context updating and the evaluation of stimulus significance. No group differences were seen in CNV amplitude, which increased with task demand for both groups.

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242.4

ERYTHROCYTE TRANSKETOLASE ABNORMALITIES IN PATIENTS WITH SCHIZOPHRENIA. M.F. Casanova, B.I. Diamond*, A.P. Pathiraja and T.H. Nguyen. Med. Coll. of Georgia, Augusta, GA 30912-3800.

Several authors have reported the presence of periventricular gliosis in patients with schizophrenia (SC). Astrocytosis in these patients occurs regardless of other subcortical pathology. Although some investigators have interpreted these findings as suggesting a previous encephalitis, we believe they are specific for thiamine deficiency. This study examines the probable presence of thiamine deficiency in patients with SC and in addition attempts to elucidate whether the same is secondary to nutritional factors or a comorbid expression of psychosis. Fourteen patients fulfilling DSM IIIR criteria for SC and 13 age-matched controls were used in the study. A nutritional questionnaire, physical examination, and laboratory evaluation excluded the presence of patients with nutritional abnormalities. The NADH-dependent transketolase assay was measured in erythrocyte hemolysates both in the presence (TPP) and absence (TK) of thiamine pyrophosphate. A total of 186 assays were performed. Significant differences among groups were found for TPP activation (t test, $p<0.01$). Differences were most prominent for the undifferentiated schizophrenic patients. Results from our study indicate that undifferentiated schizophrenic patients have an abnormality of thiamine metabolism which derives from its processing enzyme and is not dependent on nutritional status. Thiamine deficiency may therefore modulate the expression of clinical symptoms in a subset of patients with schizophrenia or may altogether bear witness to a different disease process.

242.5

THE USE OF EBV-TRANSFORMED LYMPHOID CELLS IN THE STUDY OF SCHIZOPHRENIA. T. Ranson, A. Mezu, J. Lancaster, P. Sweetnam and H. Kulaga*#. NovaScreen®, Baltimore MD 21224; #NIMH, St. Elizabeth's Hospital, Washington, D.C. 20032

Two continuous lymphoid cell lines are now being used to further the studies of schizophrenia at the cellular level. The lines are unique having been established from subjects in the E. Fuller Torrez monozygotic twin studies at the National Institute of Mental Health. Cell lines SLM, schizophrenic twin, and LG, psychiatrically normal twin, were started from EBV-transformed PBMC's, and have been maintained in culture for over one year. Twenty distinct receptor systems have been examined by radioligand binding and minimal or no binding was detected to the $GABA_A$ receptor complex, 5HT-1A, 5HT-2, Dopamine-1, Dopamine-2, delta opioid, mu opioid, kappa opioid. Both cell lines express the dopamine-4 receptor and dopamine uptake site as determined by ^{3}H-clozapine and ^{3}H-WIN binding, respectively. No apparent alteration in either binding affinities and receptor density were apparent for either receptor. This maybe of interest given the SLM cell line was established while the subject was undergoing Haldol treatment. Chronic drug studies are now underway *in vitro* to further examine the potential for biochemical alterations as a resulting from drug interactions.

242.6

THE SPECIFIC ACTIVITY OF THE SYNAPSIN PROTEINS IS MARKEDLY REDUCED IN THE BRAINS OF SOME SCHIZOPHRENICS. E. M. Dudek, S. Leonard*, R. Freedman, and M. D. Browning. Dept. of Pharmacology, University of Colorado Health Sciences Center, Denver, CO.

The synapsin proteins are a family of synaptic vesicle-associated phosphoproteins that play a role in regulation of transmitter release and participate in synapse formation. We have recently shown that the synapsins may play a role in forms of synaptic plasticity thought to underlie normal cognitive function. We hypothesized that schizophrenia, which is often characterized by morphological deficits and cognitive impairment, might be associated with alterations in the synapsin proteins and examined the levels of synapsin proteins in postmortem hippocampus obtained from 6 schizophrenics and 7 controls. Utilizing a quantitative western blot technique we found the specific activity of synapsins I and IIb in the seven control brains to be 98.4 ± 11.2 and 79.7 ± 9.4 u/mg protein, respectively. Two brains from schizophrenic subjects exhibited synapsin levels essentially identical to the control brains. However, in 4 of the schizophrenic samples the concentrations of synapsins I and IIb were markedly decreased to 31 ± 7.4 and 40.8 ± 5.6, respectively. No significant differences in PMI, age, cause of death, or storage time of postmortem brain could be correlated with the reduction of specific activity in the schizophrenic samples. It is also unlikely that the differences are due to tissue loss in the brains of schizophrenics as our findings relate to the absolute concentration of synapsins in the hippocampus. The data are thus consistent with a deficit in synapsin regulation of synaptic function in the brains of some schizophrenics.

242.7

ANTICHOLINERGICS MODIFY POSITIVE AND NEGATIVE SYMPTOMS IN SCHIZOPHRENIA R. Tandon,* J.R. DeQuardo, JF Greden. University of Michigan Schizophrenia Program, Ann Arbor, MI 48109-0120

Anticholinergic drugs have been assumed to have no effects on schizophrenic symptomatology. Studies indicate, however, that anticholinergics may antagonize the beneficial effects of neuroleptics on positive symptoms, and increase positive and decrease negative symptoms in drug-free schizophrenic patients. To further study this issue, we conducted a double-blind placebo-crossover study, comparing the effects of biperiden (a centrally-active agent) and glycopyrrolate (an anticholinergic agent with no central activity) on positive and negative symptoms in twenty otherwise medication-free (minimum two-weeks) schizophrenic (DSM-III-R and SADS/RDC) inpatients. After a three-day period when they received no drugs, they received either biperiden (4 mg po bid) or glycopyrrolate (1 mg po bid) for 3 days and they were then crossed over to the other medication for the next 3 days. The order of drug was randomized. Patients were rated on the BPRS by blind raters at four timepoints : baseline 1, baseline 2-day 4, agent 1-day 7, agent 2-day 10. Post-biperiden ratings were compared to post-glycopyrrolate and baseline ratings. Glycopyrrolate had little effect on any symptom cluster. Biperiden was observed to significantly increase positive symptoms and decrease negative symptoms. Following biperiden withdrawal (in the biperiden-first group), positive symptoms decreased. These data indicate that cholinergic modulation significantly affects positive and negative schizophrenic symptoms and suggest the need for systematic trials of cholinergic and anticholinergic agents in the treatment of positive and negative symptoms of schizophrenia, respectively.

242.8

ISOLATION OF A TRANSFORMING AGENT FROM HUMAN NEUROBLASTOMA CELLS EXPOSED TO SCHIZOPHRENIC CSF S. Shirabe[1,2], J.R. Stevens[1]* & J.P. Schwartz[2]. 1) NIMH at St. Elizabeths Hosp., Washington DC 20032 & 2) CNB, NINDS, NIH, Bethesda MD 20892.

Schizophrenic (Sch) but not control (Con) CSF contains an agent which can transform the human neuroblastoma cell line SK-N-SH (EP). After transformation, the agent can be detected in culture medium (CM). Centrifugation of CM on a Percoll density gradient indicated that the agent banded with a density of 1.08-1.11 g/ml, suggesting that it is particulate. The agent is sensitive to $CHCl_3$ and proteinase K (PK) but resistant to DNase or RNase. Since these results suggested that the agent might be viral in nature, we labeled Sch- and Con-cells with ^{32}P-phosphorus for 16 hr in order to determine whether the particle contained nucleic acid. The CM was treated successively with DNase and RNase; SDS and PK; phenol and $CHCl_3$ extraction; followed by centrifugation at 100000xg. Agarose gel analysis showed an appox. 25kb band in Sch- but not Con-cell medium, which was DNase-sensitive, RNase-resistant, and could be digested with EcoRI, indicating that it is a double-stranded DNA. These results suggest that the growth-promoting agent derived from Sch CSF may be a DNA virus.

242.9

EEG MAPPING OF PSYCHOTIC SYMPTOMS IN ACUTE SCHIZOPHRENIA. D. Lacroix*, P. Rappelsberger, K. Steinberger, K. Thau, H. Petsche. Academy of Sciences, Institut of Neurophysiology, Dept. of Psychiatry, Univ. of Vienna, Währingerstraße 17, A-1090 Vienna, Austria. *Dépt. de Psychiatrie, Hôpital Notre-Dame, Univ. de Montréal, 1560 Sherbrooke E., Montréal, Canada H2L 4M1.

EEG were obtained from 18 acutely psychotic patients with eyes closed and opened using 19 scalp electrodes (10/20 system). Signals were recorded against averaged signals from both ear lobes (TC 0.3s, Filter 35 Hz). Spectral parameters such as amplitude and coherence (local and interhemispheric) were computed for 6 frequency bands. For data analysis, patients were subgrouped according to their symptoms using a Brief Psychiatric Rating Scale: group #1 (n=6) differed from group #2 (n=7) with regard only to the presence of hallucinations and group #2 from group #3 (n=4) with regard only to the presence of thought disorders. Patients were drug-free for at least three days. Patients prone to hallucinations (comparison of group #1 and 2) showed higher local coherences (LC) between posterior temporal, parietal and occipital electrode sites, lower LC in frontal regions and higher delta amplitude. Thought disorders (comparison of group #2 and 3) were mostly related to higher LC between frontal, central and anterio-medial temporal sites and to lower LC between left posterior temporal and left parietal sites. These results suggest dysfunctions in brain areas related to cognitive systems (mental imagery, language and executive functions) which may be involved in the expression of symptoms.

242.10

NEGATIVE SYMPTOMS IN OLDER SCHIZOPHRENIC PATIENTS M.J. HARRIS , D.V. JESTE*, J. KUCK, L.A. MCADAMS

Objective: We studied the relationship of negative symptoms of schizophrenia to variables such as psychopathology, cognitive impairment and neuroleptic-induced extrapyramidal symptoms (EPS) in older patients.

Methods: We evaluated 53 outpatients with DSM-III-R schizophrenia and 30 normal controls over the age of 45. The mean (with SD) age of the schizophrenic patients was 59.4 (8.3) years, duration of illness 19.2 (14.1) years, and neuroleptic dose at the time of evaluation 414.8 (666.9) mg chlorpromazine equivalent. The evaluations included the Brief Psychiatric Rating Scale (BPRS), Scales for the Assessment of Positive and Negative Symptoms (SAPS and SANS), Hamilton Rating Scale for Depression, the Mini Mental State Examination (MMSE), the Abnormal Involuntary Movement Scale (AIMS) and Simpson-Angus EPS Scale.

Results: Schizophrenic patients had significantly more negative symptoms than normal controls ($p<0.001$). In schizophrenic patients, negative symptoms (total SANS score) showed the strongest association with overall psychopathology (total BPRS score, Pearson's $r=.37$ $p<0.02$) and symptoms of depression (total Hamilton's score, $r=.37$ $p<0.03$). Total SANS score also correlated with the bradykinesia score ($r=.36$ $p<0.05$) of the EPS Scale. The severity of negative symptoms tended to correlate inversely with age, and positively with total SAPS Score. There was no association between total SANS score and chronicity of illness, global cognitive impairment (total MMSE score), neuroleptic dose and tardive dyskinesia.

Conclusions: Negative symptoms in older schizophrenic patients correlated with overall psychopathology, depressive symptoms, and bradykinesia, but not with chronicity, cognitive impairment or neuroleptic dose.

242.11

CLOSED AND OPEN LOOP SMOOTH PURSUIT EYE MOVEMENTS IN SCHIZOPRENIA. A.D. Radant and D.W. Hommer*, VA Medical Center & U. of WA. Seattle, WA 98108

Two processes, a failure to suppress inappropriate saccades and an inability to match eye velocity to target velocity, impair overall smooth pursuit performance . The relative contribution of each of these processes to the pursuit abnormalities of schizophrenia is not known. To address this issue we used infra-red oculography to measure the eye movements of 20 neuroleptic-treated schizophrenic patients and 31 normal controls during a constant velocity, predictable tracking task (closed loop pursuit) as well as during the initiation of smooth pursuit (open loop pursuit) elicited by two step ramp tasks. The frequency of intrusive saccades was increased ($p < .05$) and smooth pursuit gain was decreased ($p< .001$) among the schizophrenic patients while pursuing a predictable target. The frequency of catch-up saccades did not differ between the two groups. One of two measures of open loop gain was significantly lower in the schizophrenic patients, but neither the latency of pursuit nor the accuracy of the initial saccade during the step ramp tasks differed between schizophrenics and normals. Schizophrenics made slightly more frequent saccades during fixation than controls ($p <$ .06). Intrusive saccades during fixation predicted closed loop smooth pursuit gain more robustly than did measures of open loop gain. These results suggest that a failure to suppress intrusive saccades may play a more important role in causing schizophrenic smooth pursuit abnormalities than any deficit in the smooth pursuit system itself. Such a tendency is consistent with dysfunction of prefrontal cortex and/or associated sub cortical structures.

242.12

CHANGES IN DISTRIBUTION OF NADPH-DIAPHORASE (NADPH-d) NEURONS IN THE FRONTAL CORTEX OF SCHIZOPHRENICS. S. Akbarian, W.E. Bunney, S.B. Wigal, S.G. Potkin, E.J. Hagman, W.G. Tourtellotte, and E.G. Jones, Dept. of Anatomy and Neurobiology, and Dept. of Psychiatry and Human Behavior, University of California, Irvine, and Dept. of Neurology, University of California, Los Angeles.

Neurons containing the enzyme NADPH-d are selectively spared in chronic neurodegenerative illness and in neonatal hypoxia/ischemia. We analyzed the topographical distribution and the density of NADPH-d neurons in the gray and white matter of the prefrontal cortex of 6 schizophrenics and 6 controls (matched for age, gender and autolysis time) by using an enzyme-histochemical method.

Four of the six schizophrenics had a significant decline in the density of NADPH-d neurons in the external and internal cortical layers. By contrast, the density of NADPH-d neurons in deeper regions of the white matter of the same 4 cases was strikingly elevated. These findings fit well into theories about a disturbance of prenatal brain development, affecting cell migration, in schizophrenia, but do not support theories postulating a postnatal brain lesion or a neurodegenerative process as the underlying basis of schizophrenia.

Supported by NIMH grant MH 44188-03 and a DFG fellowship.

242.13

REGIONAL CEREBRAL BLOOD FLOW DURING SINUSOIDAL PURSUIT, FIXATION AND SACCADIC EYE MOVEMENTS IN PATIENTS WITH SCHIZOPHRENIA AND NORMAL CONTROLS. R.E. Litman, K.F. Berman, T.R. Zeffiro, B. Martin*, D. Pickar. Lab. of Experimental Therapeutics, National Institute of Mental Health, Bethesda, MD 20892.

Saccadic eye movement abnormalities have been reported during performance of fixation and smooth pursuit eye movement tasks in patients with schizophrenia. To investigate the pathoplysiologic mechanisms underlying saccadic dysfunction in schizophrenia, we used oxygen-15 water method for measuring regional cerebral blood flow (rCBF) with positron emission tomography in 5 male patients with schizophrenia and 5 age matched male normal controls. rCBF wasmeasured during performance of sinusoidal smooth pursuit, fixation, and visually-guided saccadic eye movement tasks. Data were collected on the Scanditronix PC2048-15B brain tomograph which produces 15 slices with reconstructed resolution of 6-6.5 mm in three planes. rCBF data were normalized (i.e. each pixel of rCBF expressed as a percentage of the whole mean brain metabolism) for data analysis. Group differences in mean rCBF for the frontal eye fields as well as structures which control of saccadic eye movements will be presented for all eye movement tasks. Implications of these findings for the pathophysiology of eye movement dysfunction in schizophrenia will be discussed.

242.14

SUBSTANCE ABUSE AND THE NEUROBIOLOGICAL ASPECTS OF SCHIZOPHRENIA. W.B.LAWSON*, and J. CLOTHIER, Dept. of Psychiatry, U. of Arkansas Sch.of Med. and John C. McClellan VAMC, N.Little Rock, AR 72114.

Substance abuse is common among schizophrenic patients. Over half may abuse alcohol and nearly 17% may be cocaine abusers. We initiated an 8 week Schizophrenia/Addiction Treatment (SCAT) program with a thorough neuropsychiatric evaluation which included a SCID and clinical DSM IIIR diagnostic assessment, dementia workup, neuropsychological assessment, single photon emission tomography (SPECT) scan, and EEG. All patients abused cocaine, alcohol, or both. They continued their medication but were free from drugs of abuse for at least 7 days. Thus far 28 patients have been studied. Ten of 13 patients that required antiparkinsons medication and 4/4 intolerant to haloperidol due to dystonia were cocaine abusers. Tc-90 Single Photon Emission Tomography(SPECT) was done on 17 patients. All, including cocaine and noncocaine abusers, showed hypoperfusion defects with diverse abnormalities and most showed abnormal neuropsychological performance. Left sided and frontal-temporal deficits were most common.

242.15

PUPILLARY CONSTRICTION FOLLOWING EYEBLINKS IN NORMAL SUBJECTS AND SCHIZOPHRENIC PATIENTS. I.C. Bruce*, P.W.F. Poon, S.L. Lai, F.H.Y. Chan, P.W.H. Lee and F. Lieh-Mak. Depts of Physiology, Electrical & Electronic Engineering, and Psychiatry, University of Hong Kong, Hong Kong.

Abnormal pupillary responses have long been noted as one of the 'soft' neurological signs associated with schizophrenia. This report describes results obtained from a portable device for screening populations for such signs.

Subjects (50 controls and 49 schizophrenic outpatients receiving medication) were asked to fixate on a point while images of the left eye were captured on videotape. Subsequently, the video images were digitized (two 40 sec epochs per subject) and pupil area calculated by counting pixels above a threshold level. Eyeblinks were detected as rapid excursions of pupil area to zero, followed by a rapid return, and were used to create average responses of the pupil to eye opening at the end of the blink.

Compared to controls, the schizophrenics showed increased blink rates, decreased exposed pupil areas (due to the eyelid partially covering the pupil) and sluggish or absent pupillary responses to eye opening following the eyeblink. It is suggested that these signs, in addition to the tracking defect reported by previous workers and confirmed by our observations, may serve as biological 'markers' for the disease, and that this non-invasive method may be useful in screening populations at risk for developing schizophrenia. [Supported by CRCG, HKU]

242.16

SCHIZOPHRENIA AS A FAILURE OF REGULATION OF COMPUTATIONAL TEMPERATURE. D. K. McFarlane*, SmartTech Systems Engineering, 1293 Orlando Dr., Haslett, MI 48840.

Advances in computational neuroscience allow exploration into precise mechanisms that may underlie schizophrenia. Of particular interest are simulated annealing models (e.g. harmony theory, Boltzman machines, Alopex) in which the probability distribution of system states is governed by a global parameter T, called computational temperature in view of its similar role in thermodynamic systems: raising T reduces the differences between the probabilities of different system states. An appropriate annealing schedule of raising and lowering T allows these models to simulate normal cognitive processes. The current study aims to display how abnormal annealing schedules may result in behavior common to schizophrenia. In particular, chronically elevated T keeps the system shifting between otherwise improbable states (loose association), early lowering of T will quench the system toward non-optimal states (bizarre ideation, inappropriate affect), and chronically lowered T will result in poverty of thought, flat affect and possibly catatonia. The physiological substrate of T may be dopaminergic projections from the ventral tegmental area to prefrontal and anterior temporal cortex. Thus, anti-dopaminergic neuroleptics act by stabilizing T, alleviating uncontrolled flights of thought but not restoring the fine temporal control required for optimal annealing.

242.17

EFFECTS OF CLOZAPINE (CLOZ), LOXAPINE (LOX) AND HALOPERIDOL (HAL) ON SYNAPTIC TRANSMISSION IN THE CA_1 AREA OF THE HIPPOCAMPUS. A. Baskys, G. Remington, E. Palmer*, and J. M. Wojtowicz. Clarke Inst. of Psychiatry and Depts. of Physiology and Psychiatry, University of Toronto, Toronto M5T 1R8, Canada.

Among the neuroleptics, Cloz is believed to be least prone to cause movement disorders in schizophrenic patients and is often referred to as "atypical" neuroleptic. To better understand neuronal mechanisms of neuroleptic action, we compared effects of Cloz (50-100 μM) with those of Lox (5-20 μM) and Hal (1-5 μM) on synaptic transmission in the area CA_1 of the hippocampus. Experiments were done in *in vitro* slices prepared from 15-35 day old Wistar rats. Extracellular field potential recordings from CA_1 neurons were used to monitor the slope of the dendritic synaptic potential evoked by 0.1 Hz stimulation of Schaffer collaterals. Cloz (50 μM) reduced synaptic transmission by 17% (SE=3%, n=6). The response returned to the control level after 10-15 min exposure. Both Lox and Hal increased synaptic transmission by 30-50% (n=5). All effects were concentration-dependent and could be reversed upon wash-out of the drug. These results demonstrate a different action of Cloz on synaptic function. Supported by Sandoz Canada.

242.18

NON-CHOLINERGIC DEMENTIA IN ELDERLY SCHIZOPHRENICS. M. Davidson, V. Haroutunian, P. Knott*, P.D. Kanof, P. Powchik, L.M. Bierer, D.P. Perl, D.P. Purohit, and K.L. Davis. The Mount Sinai School of Medicine. New York.

Cortical cholinergic deficits have been associated with most dementias of organic origin, such as Alzheimer's disease (AD), and Parkinson's disease. Recent evidence suggests that a large proportion of elderly schizophrenics suffer from cognitive impairments resembling dementia. To determine the neurochemical basis of this dementia, we have quantified cholinergic markers and biogenic amines in six different cortical regions (Brodmann 8, 32, 44, 22, 36, and 17). Schizophrenic cases (SCHIZ, n=24) with no neuropathological evidence of AD were compared to a cohort of non-schizophrenic chronically hospitalized psychiatric cases devoid of AD-like neuropathology (NON-SCHIZ, n=19), neuropathologically verified AD cases (n=10), and a group of elderly controls (NL, n=9). As expected, markers of cholinergic, dopaminergic and serotonergic activity were significantly (ps< 0.01) diminished in most cortical regions of the AD group. Average regional cholinergic marker levels were not affected in the SCHIZ and NON-SCHIZ groups. Choline acetyltransferase activity in Brodmann areas 32 and 36 was negatively and significantly (ps<0.03) correlated with clinical dementia rating (CDR) scores in the NON-SCHIZ cohort, but not in the SCHIZ group. These results suggest that unlike other dementing diseases, the dementia observed in elderly schizophrenics is unrelated to cortical cholinergic marker activity.

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SYMPOSIUM. NEW WAVES IN CELL CALCIUM. M.R.Hanley, Univ.of Calif., Davis (Chairperson); K.Campbell, HHMI, Univ. of Iowa Sch.Med.; R.Y.Tsien, HHMI, Univ. of Calif, San Diego; D.Clapham, Mayo Foundation.

The regulation of cytosolic free calcium is a convergent control point in excitable cells for a remarkable variety of processes from secretion to gene expression. Most of the major gene products involved in calcium regulation in excitable cells have been cloned and expressed, permitting evaluation of explicit signalling functions for identified gene products. This Symposium will focus on intracellular calcium homeostasis and its modulation by receptor activity. Repeating themes will be how genetic or pharmacological probes and single-cell imaging have created a new appreciation of the sophistication of calcium control.

The functional and structural definition of intracellular "calcium pools", and their perturbation by drugs or genetic manipulation, will introduce the session (M.Hanley). The release of calcium from stores is mediated by InsP3 and ryanodine-gated calcium channels in brain and skeletal muscle, which will be considered in the context of their complementary functions. (K.Campbell). Insights into the molecular mechanisms of calcium signalling based on the use of novel reagents for detecting or altering intracellular messengers in single living cells will be described (R.Tsien). Lastly, the use of confocal laser microscopy has revealed complex spatial and temporal calcium patterns in *Xenopus laevis* oocytes expressing ectopic receptors and transduction genes (D.Clapham).

244

SYMPOSIUM. THE ROLE OF SENSORY INFORMATION IN THE GUIDANCE OF VOLUNTARY MOVEMENT. D.I. McCloskey, Univ. New South Wales, and A. Prochazka, Univ. Alberta (Chairpersons); C.J. Vierck, Univ. Florida Col. Med.; J.D. Brooke, Univ. Guelph; P.J. Cordo, Good Samaritan Hosp. & Med. Ctr.; P.R. Burgess, Univ. Utah

The purpose of this symposium is to encourage the development of a psychophysics of voluntary movement and emphasize the concept that the motor system controls what it senses. To this end, four speakers will present data they believe indicates sensory information is of great importance in the control of voluntary movement. **Dr. Vierck** will describe the permanent impairment that occurs in monkey hand control after lesions that interrupt spinal sensory pathways. **Dr. Brooke** will discuss the importance of sensory information in adjusting to different loads and how ambiguous instructions influence performance. **Dr. Cordo** will present evidence that kinesthetic sensory information is used to accurately trigger the opening of the hand in a throwing movement. **Dr. Burgess** will present data jointly collected with Mark Latash and Gerald Gottlieb at Rush Medical Center and show how from a common database it is possible to arrive at either an equilibrium point model of voluntary movement or a quite different sensory guidance model depending on whether one applies the concept that one can control only what one senses. **Dr. McCloskey** and **Dr. Prochazka**, the two chairpersons, who will not be speakers, will then lead a thirty-minute general discussion on the subject and prepare a written summary of what in their view has been settled and what needs further attention.

247.1

EXPRESSION OF FOS-LACZ CORRELATES WITH CELL DEATH. R.J. Smeyne*, M. Vendrell, T. Curran, and J.I. Morgan. Roche Institute of Molecular Biology. Roche Research Center, Nutley, NJ

Cell death has been shown to be an important component of development in both vertebrates and invertebrates. Current hypotheses regarding the mechanism that both lead to, and underlie cell death include both genetic as well as epigenetic processes. Our laboratory has generated a c-fos-lacZ transgenic mouse that recapitulates expression of the proto-oncogene c-fos. During examination of embryonic and early postnatal mice we observed Fos-lacZ expression in several structures known to have components that undergo cell death; including the medial edge epithelium of the palate, the enamel knot of the developing incisor, the thymus, ossifying chondrocytes and a small population of ventral horn motor neurons. In order to determine if Fos-lacZ was expressed during periods of cell death, we examined two experimental models: neuronal death in the *weaver* mutant mouse, and neuronal death induced by kainic acid administration. In the early postnatal *weaver* cerebellum, expression of Fos-lacZ is observed in the post-mitotic premigratory cell population of the EGL, a site of expression not seen in wild-type mice. Following IP injection of neurotoxic doses of kainic acid, expression of Fos-lacZ was observed in degenerating cells of the hippocampus and amygdala. The kinetics of Fos-lacZ expression first appear several days following injection and remain detectable for approximately 2 weeks. These studies suggest that expression of the proto-oncogene, c-fos, may be involved in, or responsive to, the process of cell death.

247.2

THE RELATIONSHIP BETWEEN TIME OF BIRTH AND TIME OF DEATH OF CELLS IN THE DEVELOPING RAT RETINAL GANGLION CELL LAYER L. Galli-Resta*, M. Ensini and B. Margheritti Istituto di Neurofisiologia CNR 56127 Pisa Italy.

In most neural centres, neurons are generated in a limited time window peculiar to the animal species. Many of these cells soon undergo cell death in a predetermined time interval. We have investigated whether a relation exists between the time of birth and the time of death for the cells of the rat retinal ganglion cell layer. On fixed gestational days, BrU, which is incorporated by cells synthesising DNA, was administered to pregnant rats. On each postnatal day between P1 and P20, one or more pups were sacrificed. Retinae were whole-mounted and reacted with an antibody against BrU. In this way we could follow the time course of the total number of cells born on specific gestational ages and find when cells among them die. We have found that, among the cells undergoing naturally occurring cell death in the ganglion cell layer, the earlier the cells are born, the earlier they die.

247.3

AN INCREASE IN NEURONAL CYTOPLASMIC CALCIUM RESULTS IN INTERNUCLEOSOMAL DNA FRAGMENTATION. Rajiv Joseph*, Wei Li and Enji Han. Dept. of Neurology, Lab. of Experimental Hematology and Stroke, Henry Ford Hospital, Detroit, MI 48202.

Internucleosomal DNA fragmentation, secondary to endonuclease activation, is characteristic of programmed cell death. First noted in thymocytes following treatment with glucocorticoids, DNA fragmentation has also been noted in developing neurons, and in primary neuronal cultures treated with glutamate. As calcium is essential for endonuclease activation, we questioned whether activation of cytoplasmic calcium may directly or indirectly cause DNA fragmentation in neurons. For this purpose, we used PC12 and NCB-20 (Chinese hamster cortical neurons x mouse neuroblastoma, gift from M. Nirenberg, Bethesda, MD) cell lines. The calcium ionophore, A23187, caused a dose-dependent (as low as 10 nM) activation of cytoplasmic calcium and loss of cell viability, in association with DNA fragmentation, in both cell lines. DNA fragments, typical of internucleosomal digestion, were consistently detected in the incubating medium. Release of DNA fragments into the medium was time-dependent and observed within an hour of treatment, preceding loss of cell viability. Furthermore, calcium added directly to PC12 nuclei also produced DNA fragmentation. The mechanism by which an increase in cytoplasmic calcium causes activation of a nuclear enzyme, such as endonuclease, is unknown. Our observation that incubation of PC12 nuclei with calcium also results in DNA fragmentation, indicates that calcium may activate endonuclease directly. However, this effect of calcium was less impressive compared to when DNA fragmentation was caused in intact cells by activation of cytoplasmic calcium, suggesting that calcium may also act indirectly for instance through a regulatory protein. In either event a critical change in the concentration of cytoplasmic calcium, either directly or indirectly activates endonuclease, resulting in the fragmentation of genomic DNA, and neuronal death.

247.4

WALLERIAN DEGENERATION IN THE MAMMALIAN CNS: EARLY DETECTION WITH MAGNETIC RESONANCE IMAGING. F.J. Lexa, R.I. Grossman and A.C. Rosenquist*. Univ. of Pennsylvania Sch. of Med., Philadelphia, PA 19104.

Wallerian degeneration is a fundamental response of the nervous system to injury. Although manifestations of this process on magnetic resonance (MR) imaging have been reported in animal models and humans, it remains incompletely characterized. We undertook this study in order to examine the utility of measuring magnetization transfer rates (MTR) for delineating dynamic changes which occur after controlled injury in a feline model in which anatomic pathways are well understood. Using standard neurosurgical techniques all known visual cortical areas in the cat were ablated. Gradient imaging was performed serially at 1.5 Tesla, with and without a saturation pulse to create a magnetization transfer effect. At varying intervals, the animals were sacrificed for histologic analysis with light and electron microscopy.

Within the first two weeks there is a statistically significant increase in MTR relative to the control hemisphere within the white matter connections between the lateral geniculate nucleus (LGN) and the visual cortex ($p < .02$) at a time when no effects are visually detectable on conventional spin-echo images or light microscopy. Electron microscopy at 8 and 11 days confirmed early changes of Wallerian degeneration. Between 16 and 28 days, this reverses to a decrease in MTR in both thalamo-cortical white matter ($p < 0.01$) and the LGN ($p < 0.05$).

This application of magnetic resonance can reliably detect changes at a time when degeneration is not detectable by other non-invasive imaging techniques or even light microscopy. Furthermore, temporal changes in MTR appear to correspond well with known histologic phases of Wallerian degeneration.

This work was supported by the American Society of Neuroradiology through a Basic Science Fellowship Award to F.J.L. and by N.I.H. grants NS 29029 (R.I.G.) and EY 02654 (A.C.R.).

247.5

CALCIUM-MEDIATED NEURONAL DEGENERATION FOLLOWING SELECTIVE LASER ACTIVATION OF PHOTOACTIVE NANOSPHERES. V.L. Sheen,*E.B. Dreyer, J.D. Macklis. Dept. of Neurology, Prog. in Neuroscience, Harvard Medical School, Children's Hospital, Boston, MA, 02115.

A model of pathologic neuronal degeneration for studies of development and transplantation is provided by noninvasive, selective, photolytic cell population lesions to targeted neuronal subtypes following intracellular incorporation of cytotoxic chromophores. The histologic time-course of neuronal injury with this approach is prolonged, occurring over several days to weeks, without significant inflammatory response, suggesting features unique to this model and distinct from injury due to chemical toxins such as excitotoxic amino acids. In order to better define the mechanisms involved, resting cytosolic calcium levels were measured in vitro from cortical neurons cultured from C57B/6J mice following laser exposure. Neurons were targeted by latex nanospheres conjugated with chlorin e_6 as a singlet oxygen producer. Decline of neuronal membrane integrity was studied in parallel by observation of propridium iodide (PI) incorporation, serving as an indicator for progressive cell injury, electrophysiologic decline, and death.

Singlet oxygen production within lysosomal granules caused an irreversible rise in cytosolic calcium, predominantly from influx of extracellular calcium. Time to peak calcium levels depended on the dosage of laser exposure. The increase in cytosolic calcium was observable within minutes following laser illumination. Incorporation of PI followed a slower time course (hours to days). Either the calcium channel blocker, nimodipine (0.1 uM), or calcium-free medium, reduced neuronal death by approximately 50% as assessed by PI incorporation. These results suggest that neuronal degeneration following singlet oxygen production by selective laser activation of photolytic chromophores occurs by both calcium dependent and independent mechanisms. This may be due in part to release of cytotoxic lysosomal contents. HD28478, HD18655, Alzheimer's Assoc, Rita Allen Fdn, and Lion's Fdn.

247.6

The Ability of Diphenylpiperazines to Prevent Neuronal Death in Dorsal Root Ganglion Neurons In Vitro after NGF Deprivation and In Vivo After Axotomy Keith M. Rich,* Janet M. Dubinsky, and Marc E. Eichler Washington University School of Medicine, St. Louis, MO 63110

Flunarizine, a class-IV Ca^{2+} entry blocker, is an effective cerebral protecting agent in models of brain hypoxia, ischemia, and in metabolic intoxication. Recently, DRG neuronal protection *in vivo* after sciatic nerve injury and *in vitro* after NGF withdrawal has been demonstrated with the diphenylpiperazine, flunarizine. We investigated the ability of the diphenylpiperazine, dihydropyridine, benzothiazipine, and phenylalkylamine groups of calcium channel antagonists to prevent neuronal death in primary dissociated rat dorsal root ganglion (DRG) neurons *in vitro* after nerve growth factor (NGF) deprivation and *in vivo* after sciatic axotomy. Only cinnarizine, a diphenylpiperazine calcium channel antagonist similar to flunarizine, protected DRG from death *in vitro* and *in vivo*. *In vivo*, cinnarizine given subcutaneously (50 mg/kg) protected DRG neurons in rat pups after unilateral sciatic nerve crush on PND-1. Counts of L_4/L_5 DRG neurons in these rats revealed 88% cell survival in cinnarizine-treated animals (compared to uninjured side) with only 69% survival in control animals (the difference was significant, Students *t*-test, $p<0.05$). Using an ^{35}S-methionine protein synthesis assay, we found that the efficacious dose of cinnarizine *in vitro* (10 μM) was significantly lower than flunarizine (40 μM); this lower dose may have less toxicity and greater therapeutic value. The role of $[Ca^{2+}]_i$ in trophic-factor-induced death remains unclear. DRG neurons in cell culture show an initial fall in $[Ca^{2+}]_i$ between 24 and 48 hours after NGF deprivation; this fall occurs prior to morphologic changes consistent with death. This decrease in $[Ca^{2+}]_i$ can be prevented with either flunarizine or cinnarizine. Other types of voltage-dependent calcium channel antagonists did not protect neurons from trophic-factor-induced death and did not prevent this fall in $[Ca^{2+}]_i$ in neurons "dying" after NGF deprivation. DRG neurons become NGF-independent after membrane depolarization in high extracellular potassium; these depolarized neurons show a sustained rise in $[Ca^{2+}]_i$. E-15 DRG neurons maintained in cell culture for 10 days and then deprived of NGF for 24 hours were unable to be rescued from death ("committed to die") by replacing the NGF-containing medium. This "commitment point" to die paralleled the loss in the ability of these neurons to show a normal influx of calcium after depolarization in high potassium. Thus, the loss of membrane integrity for calcium regulation is an important factor associated with irreversible trophic-factor-dependent death.

247.7

EFFECTS OF DIFFERENT NEUROMUSCULAR BLOCKING AGENTS ON NATURALLY OCCURRING MOTONEURON DEATH DURING DEVELOPMENT IN VIVO. L. J. Houenou*, D. M. Prevette and R. W. Oppenheim. Department of Neurobiology & Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27157

The formation of normal nerve-muscle connections during vertebrate embryonic development is followed by the degeneration and death of approximately 50% of postmitotic motoneurons (MNs). Previously, it has been shown that the chronic blockade of embryonic neuromuscular activity (motility) with postsynaptic membrane potential stabilizing agents, including d-tubocurarine (dTC), resultes in the survival of virtually all postmitotic MNs (e.g., Pittman & Oppenheim, 1978). In the present study, we have compared the effects of depolarizing, i.e., decamethonium (dMET), and stabilizing, i.e., dTC, neuromuscular blockers on the survival of chick lumbar spinal cord MNs on embryonic day (E)10 following treatment from E6 to E9. The results show that both dMET and dTC significantly decreased embryonic motility to 25% of control. However, dMET did not affect the survival of MNs, while dTC enhanced MN numbers by 80%, compared to controls. Because both dMET and dTC bind to postsynaptic acetylcholine receptors, but at different sites, a group of embryos were simultaneously treated with both agents. In this case, MN survival was comparable to that obtained with dTC alone. Together, our results indicate that depolarizing and stabilizing neuromuscular blockers have different effects on avian MN survival in vivo and suggest that postsynaptic membrane depolarization may be a major factor that controls the fate of MNs during embryonic development. Additional experiments are underway to determine whether dMET treatment affects muscle innervation patterns and whether other depolarizing agents act similar to dMET.

247.8

DEATH OF CEREBRAL CORTICAL NEURONS AFTER CLOSE AXOTOMY. P. S. Fishman* and D. A. Parks. VA Research Lab., Dept. of Neurology, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Although the neuronal response to axotomy is highly variable, adult corticospinal neurons survive long after their axons are severed in spinal cord or medullary pyramids. To determine if similar cortical neurons that project across the corpus callosum (transcallosal neurons) survive lesions that sever axons close to the cell body (close axotomy), neurons were first pre-labeled with the fluorescent dyes True Blue or Fluorogold by retrograde transport in adult mice. A uniform field of labeled transcallosal cells was formed by applying a dye-soaked gelatin sponge to a large area of contralateral cortex. Seven days later a stab wound was made through cortex contralateral to the label site dividing the field of pre-labeled transcallosal neurons into 2 groups: 1) neurons medial to the wound with intact axons projecting to the contralateral cortex, 2) neurons lateral to the wound that had undergone axotomy. Animals (20) were sacrificed between 1 and 5 wks after injury, while labeled but uninjured control animals (22) were sacrificed 1 to 7 weeks after labeling. Although control animals showed a variable number of labeled cells, density of labeled cells was uniform over a large area of cortex (4 mm^2). In contrast, injured mice had progressive loss of neurons lateral to the stab wound. Loss of labeled neurons was particularly evident when comparing densities of labeled neurons in cortex immediately adjacent to wound sites. There was little direct damage to cortical neurons by the stab wounds, with labeled cell densities medial to the wound within the range of uninjured controls (500-2000 cells/mm^2). Directly lateral to the wound cell densities were markedly reduced, with less than 10% of the expected cell density over a region within 1 mm lateral to the wound by 4 wks after injury. There appeared to be a gradual increase in labeled cell density with distances beyond 1 mm from the wound. In transcallosal neurons axotomy within 1 mm of the cell body results in extensive cell death. A minimum amount of axoplasm or remaining axon collaterals may be needed for cortical projection neurons to survive after axotomy.

247.9

NEURONAL APOPTOSIS : DEVELOPMENT OF A STUDY MODEL FOR THE IDENTIFICATION OF DEATH-RELATED PROTEINS. B. Pettmann* and P. Villa Laboratoire de Neurobiologie Ontogénique, Centre de Neurochimie du CNRS, 5, rue Blaise Pascal, 67084 STRASBOURG Cedex, FRANCE.

Programmed cell death which leads to the selective death of some neurons during development is a well known phenomenon. The idea that cell death is an active process in which the cells actually commit suicide through the biosynthesis of specific proteins is based on the observation that neurons cultured in the absence of their neurotrophic factor and which would therefore normally die, can be rescued by addition of translation inhibitors like cycloheximide. The proteins involved named "killer" proteins remain to be identified.

We have developed a study model consisting in cultures of neurons isolated from chicken ciliary ganglions at embryonic day 8 (CG8). Programmed neuronal death occurs in this ganglion and half of the neurons will die between E7 and E12. In vitro, the CG8 neurons survive only in the presence of a neurotrophic factor, either basic fibroblast growth factor (bFGF) or ciliary neurotrophic factor (CNTF). In their absence, the cells will die within 24-48 hours, but will be saved if cycloheximide or actinomycine D is added. In this system we have studied the biosynthesis of proteins. Fifty microcuries of ^{35}S methionine were added to the neurons immediately after seeding, for 18 hours, in the absence or in the presence of CNTF or bFGF. After 18 h, cells were scrapped, proteins were extracted and submitted to 2D-electrophoresis. We compared the pattern of synthesized proteins on autoradiograms of 4 gels of cells cultured in the absence of trophic factors with 3 gels of cells treated with CNTF and 2 with bFGF. In all the autoradiograms of the untreated cells, we observed a strong stimulation of the synthesis of 4 proteins with molecular weights between 33 and 45 kD and isoelectric points between 5.5 and 6.2. These proteins are hardly visible in the autoradiograms of the treated cells.

We are currently trying to 1) investigate the presence of these proteins in another system of apoptosis (dorsal root ganglionic neurons and NGF) ; 2) get enough materiel to allow microsequencing or antibody production.

247.10

A MODEL SYSTEM FOR CHARACTERIZING CELLULAR AND MOLECULAR EVENTS IN PROGRAMMED NEURONAL CELL DEATH. R. N. Pittman,* A. J. DiBenedetto, and S. Wang. Dept. of Pharmcology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104

Selected sublines of PC12 cells can be grown under culture conditions in which 90-95% of the cells become dependent on NGF for survival. Following removal of NGF in the absence or presence of serum, approximately 85% of the cell death can be blocked with the RNA synthesis inhibitor actinomycin D. This is consistent with the activation of cell death genes following removal of NGF. Properties of our PC12 model system are similar to those of primary cultures of sympathetic neurons (Martin et al., 1988; J. Cell Biol. 106:829). Committment to cell death occurs about 14 hrs after the removal of NGF, and can be blocked by KCl, bFGF, and dbcAMP, but not by protease inhibitors, EGF, or the endonuclease inhibitor ATA (DNA laddering, however, does occur following removal of NGF). In agreement with published studies (Rukenstein et al., 1991; J. Neurosci. 11:2552), PC12 cells also undergo transcription-independent cell death when placed in serum-free medium; however, a large number of differences exist (eg. sensitivity to actinomycin D, KCl, and ATA) between this transcription-independent cell death and the transcription-dependent cell death described above. Studies are in progress to identify molecular steps involved in the irreversibe committment of PC12 cells to a dependence on NGF for survival, identifying cell death genes, and characterizing early transcriptional events in cell death. Supported by Univ. of PA Research Foundation.

PRESYNAPTIC MECHANISMS II

248.1

CORRELATION OF SINGLE CALCIUM CHANNEL OPENINGS AND ACETYLCHOLINE RELEASE FROM THE RELEASE FACE OF A CHOLINERGIC NERVE TERMINAL. E.F. Stanley*. NINDS Biophysics Lab. NIH Bethesda MD 20892.

We have previously reported the direct recording of single Ca channels from the release face of a cholinergic presynaptic nerve terminal (Neuron 7:585). We now report an attempt to simultaneously detect ACh release.

Calyx-type presynaptic nerve terminals were acutely dissociated and patch electrodes were applied to the release face. The patch pipette included an assay solution that emits photons on exposure to ACh. Patch current and single-photon output were recorded simultaneously. Quantal transmitter release was identified as short bursts (SBs) of photons (≥2 photons/0.3 ms). SBs were very infrequent at a -70 mV holding potential. Ramp depolarization increased SBs ($p<<0.01$), particularly over the voltage range of Ca channel activation. SBs were preceded by calcium channel opening events with a minimal and modal latency of 0.2 and 0.5 ms.

248.2

CALCIUM OSCILLATIONS AND EXOCYTOSIS IN PITUITARY GONADOTROPES. A.Tse*, F.W.Tse, W.Almers and B.Hille. Dept. Physiol. and Biophysics, U. of Washington, Seattle, WA 98195.

In adult male rat gonadotropes (identified by reverse hemolytic plaque assay), GnRH receptor stimulation induces an oscillatory release of intracellular Ca (Shangold et.al., PNAS 85:6566) from an IP_3-sensitive store (Tse & Hille, Science 255:462). We examine whether this Ca release stimulates secretion from gonadotropes by simultaneously measuring $[Ca]_i$ (indo fluorometry) and exocytosis (capacitance measurement). Gonadotropes are whole-cell voltage clamped at -70 or -90 mV (where voltage-gated Ca channels are closed), and a sinusoidal wave of 30 mV (p-p) is applied at 800 Hz. GnRH ejected from a puffer pipet results in cyclic elevations of $[Ca]_i$. At 20-25 ^{o}C, the resting $[Ca]_i$ in gonadotropes is 86 + 36 nM (mean + S.D.; n = 12) and the peak elevated $[Ca]_i$ ranges from 0.49 μM to 3.8 μM. Each cycle of Ca elevation is accompanied by a burst of exocytosis (up to 365 fF/cycle), sometimes followed by endocytosis. These phenomena persist in the absence of extracellular Ca, can be triggered by flash photolysis of caged IP_3 (10 μM) but are largely suppressed by buffering $[Ca]_i$ with intracellular EGTA or BAPTA. The rate of exocytosis increases initially with the rise of $[Ca]_i$ but typically reaches a maximum (up to 538 fF/s, corresponding to ~400 vesicles/s) before $[Ca]_i$ peaks. Voltage-gated Ca entry induced by depolarizing voltage steps rarely supplies enough Ca for exocytosis. Therefore, oscillatory release of Ca from the IP_3-sensitive store provides the major control of stimulus-secretion coupling in gonadotropes. (Supported by AM-17803, GM-39520, HD-12629, Mellon Foundation and McKnight Foundation.)

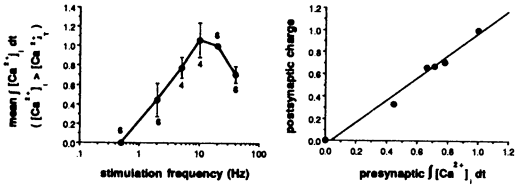
248.3

RELEASE OF LHRH IS LINEARLY RELATED TO THE TIME INTEGRAL OF PRESYNAPTIC CA^{2+} ELEVATION ABOVE A THRESHOLD LEVEL IN BULLFROG SYMPATHETIC GANGLIA.

Y.-y. Peng* & R. S. Zucker. Dept. of Molecular & Cell Biology, Univ. of California, Berkeley CA 94720

In the bullfrog sympathetic ganglia, the amount and rate of LHRH release from the preganglionic C terminals evoked by a train of electrical stimulation increased as the stimulation frequency increased from 2 to 20 Hz. At 40 Hz the amount of release decreased to less than that at 20 Hz (Peng & Horn, 1991). Here, the $[Ca^{2+}]i$ at presynaptic terminal boutons caused by electrical stimulation was directly monitored by fura-2.

We found that as stimulation frequency increased from 0.5 to 40 Hz 1) the peak $[Ca^{2+}]$ ($[Ca^{2+}]_p$) and its rate of rise increased; 2) the decay of $[Ca^{2+}]_i$ transients followed up to 3 exponentials; 3) release of LHRH was linearly related to $\int[Ca^{2+}]_i dt$ where $[Ca^{2+}]_i > [Ca^{2+}]_T$. For a given set of boutons on a C cell, $[Ca^{2+}]_T$, the threshold level of $[Ca^{2+}]_i$ for LHRH release was estimated from the $[Ca^{2+}]_p$ evoked by 0.5 Hz stimulation which did not induce release of LHRH. The mean $[Ca^{2+}]_T$ was 185 nM (n=6). Supported by NIH grant NS15114 to RSZ.



248.4

CALCIUM MICRODOMAINS IN SQUID GIANT PRESYNAPTIC TERMINALS STUDIED WITH N-AEQUORIN-J. R. Llinás*, M. Sugimori and R. B. Silver, Dept. of Physiology & Biophysics, NYU Medical Center, 550 First Avenue, NY, NY 10016, and Section and Dept. Physiology, Cornell University, Ithaca, NY 14853-6401.

The presence of specifically located calcium channels at the active zone of presynaptic terminals indicates that the calcium-dependent release of transmitter requires high calcium concentration profiles to trigger synaptic vesicle fusion (Llinás, Soc. for Neurosci. Symposia 2:139 1977). Calculations based on presynaptic current measurements indicate that $[Ca^{2+}]_i$ against the interior plasmalemma surface could rise to 10^{-4} M (Simon & Llinás, Biophys. J. 48:485, 1985). To test this hypothesis a hybrid synthetic n-aequorin-J having a sensitivity to $[Ca^{2+}]_i$ in the order of 10^{-4} M was injected into the preterminal of the giant synapse of the squid *Loligo pealii*.

The photon emission by n-aequorin-J following increased $[Ca^{2+}]_i$ was visualized using a VIM camera operated in the photon counting mode. The results indicated $[Ca^{2+}]_i$ microdomains on the order of 10^{-4} M occurs at particular sites, or quantum emission domains (QEDs), that correspond in size, number and distribution to the active zones in the squid giant synapse. In addition, QEDs tend to occur as short lasting events that are never continuous in time, suggesting calcium-dependent calcium inactivation of individual active zones. This mechanism may be responsible for the "one vesicle per active zone" rule.

We extend our deep gratitude to Dr. Osamu Shimomura, who kindly provided the n-aequorin-J used in this study. (AFOSR89-0270)

248.5

POSSIBLE ROLE DURING EXOCYTOSIS OF SYNAPTOPHYSIN-LIKE Ca^{2+}-ACTIVATED CHANNEL IN NEUROSECRETORY GRANULES OF NEUROHYPOPHYSIS. J.R. Lemos*, C.J. Lee, G. Dayanithi, and J.J. Nordmann. Worcester Found. Exp. Biol., Shrewsbury, MA 01545 & Centre Neurochimie, Strasbourg, France.

A large amplitude channel, from purified bovine neurosecretory granules (NSG), was incorporated into artificial lipid bilayers. This channel opens only in the presence of free Ca^{2+}, but is inhibited by relatively high Ca^{2+} concentrations. Release of vasopressin from permeabilized neurohypophysial terminals also shows a similar biphasic dependence on Ca^{2+}. Release is selectively inhibited by low concentrations of the long-chain alcohol octanol, but not by high concentrations of ethanol, as is the NSG Ca^{2+}-activated channel. Furthermore, Ca^{2+}-evoked release and channel activity are both inhibited by 10 carbon-long chain TEA-analogs. Most importantly, both the Ca^{2+}-activated NSG channel and Ca^{2+}-dependent release are inhibited by SY-38: a monoclonal antibody directed against the putative Ca^{2+}-binding site of synaptophysin, an integral NSG membrane protein. The close correlation between channel and release properties leads us to conclude that this synaptophysin-like NSG channel may be involved in peptide secretion. (Supported by grants from IREB, NIH, and NSF).

248.6

ROLE OF L-TYPE CALCIUM CHANNELS IN SLOW DEVELOPING POTENTIATION IN *APLYSIA*. S.M. Fredman*Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

Slow developing potentiation (SDP) is a form of enhanced synaptic transmission exhibited at the A-B neuron synapse in the cerebral ganglion of *Aplysia*. Brief intracellular tetanic stimulation (4x 500 msec @20 Hz) of single A neurons causes a long-lasting (~25 min) increase in EPSP amplitudes in B neurons (Fredman, 1991). Previous results (Fredman,1990) suggested that SDP was due in part to the presynaptic activation of PKC. Evidence is now presented suggesting that PKC may be acting on L-type Ca^{2+} channels. SDP required more Ca^{2+} than did synaptic transmission alone. Intracellular injection of nonspecific Ca^{2+} blockers (Ni^{2+}), and Ca^{2+} chelators (EGTA), blocked SDP prior to blocking synaptic transmission. Bathing the cerebral ganglion in 50 μM ryanodine to increase Ca^{2+} sequestration, reduced SDP without effecting non-potentiated EPSPs. SDP was also reduced by L-type Ca^{2+} channel blockers. Verapamil (150 μM) and several dihydropyridines (10-100 μM nifedipine, 10-50 μM isradipine,10 μM darodipine) all significantly reduced SDP. The dihydropyridines, particularly at high concentrations, had multiple effects.The falling phase of the presynaptic action potentials were broadened resulting in an increase in baseline EPSP amplitudes. Despite this increase, the relative amplitude of EPSPs following tetanizing trains were reduced compared to controls. Taken together these results suggest that SDP is mediated by increased presynaptic Ca^{2+} due, in part, to the activation of L-type Ca^{2+} channels. In light of previous findings, they suggest that L-type channels may be phosphorylated by PKC, increasing their opening and intracellular Ca^{2+}, thus enhancing synaptic transmission.

This work was supported by NINDS grant NS28199 and NIGMS (MBRS) grant GM08037. Isradipine and darodipine were gifts from the Sandoz Research Inst.

248.7

THE ROLE OF VESICULAR MOBILIZATION IN TONIC TRANSMITTER RELEASE AND POTENTIATION IN THE CRAB T-FIBER GIANT SYNAPSE. J.-W. Lin* and R. Llinas. Dept. Physiology and Biophysics. NYU Medical Center. NY NY 10016.

The crab T-fiber giant synapse is formed between the stretch receptor afferent, T-fiber, and promotor neurons. The presynaptic element, T-fiber, is a non-spiking neuron which releases transmitter tonically. The large size of the presynaptic terminal allowed double eletrode penetration and the active control of membrane potential at the release sites. Following a prolonged (300 ms) presynaptic depolarization, transmitter release activated by test pulses was enhanced. This potentiated transmitter release exhibited kinetic properties that are best described by a model requiring the control of synaptic vesicular mobilization. A mathematical model was constructed that divided the synaptic vesicle population into two groups. The first group is stationary and can be converted into a second, mobile and releasable population. The conversion is dependent upon the activity of the presynaptic terminal, i.e. a prolonged presynaptic depolarization would activate it, by way of calcium influx, in order to maintain tonic release. In addition, this mechanism can account for the potentiation described above. Specifically, as the conditioning pulse is terminated, the tonic transmitter release stops immediately while the vesicle mobilization continues for a brief period. This process leads to an accumulation of releasable vesicles at the release site and potentiated release. At a microscopic level, autocorrelation analysis of the synaptic noise during tonic release revealed an intrinsic periodicity that was not found in the synaptic noise of the squid giant synapse. This periodic behavior presumably reflects a cycling time which determines the rate of repetitive release at individual release sites. (Supported by NIH grant NS13742 and NS07942-02).

248.8

QUANTAL TRANSMITTER SECRETION FROM MYOCYTES LOADED WITH ACETYLCHOLINE. Y. Dan* and M-m. Poo. Dept. of Biol. Sci., Columbia Univ., N.Y., NY 10027.

Acetylcholine (ACh) was loaded into isolated *Xenopus* myocyte through a whole-cell recording pipette, which also served to record the myocyte membrane current. Within minutes after the onset of recording (with 10 mM ACh in the pipette), pulsatile inward currents resembling miniature endplate currents (MEPCs) were observed. These MEPC-like events increased in frequency and amplitude with time, and were abolished by extracellular application of curare and -bungarotoxin. The frequency of the events increased markedly after repetitively depolarizing the myocyte membrane potential or after treatment that elevated cytosolic Ca^{2+}. These events reflect quantal ACh secretion by the myocyte, and the secretion appeared to be a result of exocytosis of ACh-filled cytoplasmic compartments. The latter notion is supported by the finding that vesamicol, a drug that blocks ACh uptake into synaptic vesicles, reduced the frequency and amplitude of these events, and that cytosolic application of cholinesterases after ACh loading resulted in a gradual disappearance of the events without significant change in their mean amplitude. Finally, evoked ACh secretion was also observed occasionally after applying depolarizing voltage steps to the myocyte, and the efficacy of secretion was improved by elevating external Ca^{2+}. Taken together, these results indicate that transmitter secretion does not require secretion pathways unique to neurons, and the essence of presynaptic differentiation may reside in the provision of transmitter supply and synapse-specific proteins whose functions are facilitatory in nature.

248.9

QUANTAL ANALYSIS OF EXCITATORY SYNAPTIC TRANSMISSION: THEORY AND EXPERIMENTAL APPROACH FOR DETECTION OF SMALL QUANTA EVENTS. Guosong Liu* & I.L. Feldman. System Neurobiology Lab., Dept. Physiological Science, UCLA, Los Angeles, CA 90024-1527.

Quantal analysis is an important tool for analysis of pre- and post-synaptic mechanisms underlying synaptic transmission. However, the experimental conditions for quantal detection have not been well defined. Our results from analysis of simulated synaptic currents suggest that direct resolution of quantal peaks requires the ratio of quantal peak (q) to quantal standard deviation (σ) > 2.2. We studied the factors that influence this ratio and developed experimental techniques that enhance it.

For measurement of synaptic current, q/σ ratio is reduced by: slow frequency response of voltage-clamping system (caused by series resistance); distal origin of synaptic current; and high background noise. To distinguish the current amplitude distribution from noise, digital filtering techniques were compared, based on enhancement of q/σ ratio. Among three filters (Wiener, Gaussian, and Match filter) tested, the Wiener filter provided the best improvement. Other experimental conditions for optimal detection of quantal peak were also determined.

Application of these techniques led directly to observation of quantal excitatory synaptic transmission involving spinal cord neurons. The quantal size is 55-100 pS, substantially smaller than previously reported. Further evidence of quantal transmission is the close relationship between quantal size before and after the application of tetrodotoxin (TTX). After TTX, although the frequency and amplitude of EPSCs were reduced, individual quantal peaks with intervals close to preTTX values remained. Therefore, the apparent skewness of the miniature EPSC distribution results from the summation of multiple events with different peak amplitudes, evenly spaced. Since the range and shape of the miniature EPSC distributions were similar to those observed when single boutons are stimulated, our results suggest the presence of more than one release site in boutons spontaneously releasing transmitter. Support: NIH Grant NS24742.

248.10

RELEASE PROPERTIES, FACILITATION AND DEPRESSION OF EXCITATORY SYNAPSES ON DENTATE GRANULE CELLS, STUDIED BY SINGLE AXON ACTIVATION.
Morten Raastad *, Institute of Neurophysiology, University of Oslo, Norway.

The excitatory response to a second stimulus given shortly after an identical first one, can be larger (facilitation), or smaller (depression) than the initial response. In hippocampal slice preparations with blocked inhibition, the synapses between Schaffer-collaterals and CA1 cells demonstrate facilitation, while depression is more commonly seen in the synapses between perforant path fibres and the dentate granule (d.g.) cells.

Double stimulation of single axons (Soc.Neurosci.Abstr 1991, 593.9) was used to study depression and release properties of excitatory synapses on d.g. cells. Reliable activation of one synaptically connected axon was possible by weak electrical stimulation with a small glass electrode, using a range of stimuli around the threshold for the appearance of EPSCs.

Intracellularly recorded single fibre EPSCs from d.g. cells showed considerable variability in amplitude, like the synapses in the CA1 area. Unlike the latter synapses, however, the release probability was near 100% with 2 mM Ca^{++} and 2 mM Mg^{++} in the extracellular solution. In this situation, there was a negative correlation between the individual amplitudes of the first and second response, and with a smaller average amplitude of the second response, corresponding well with the observed depression. By reducing the Ca^{++} concentration to 1 mM and increasing the Mg^{++} concentration to 4.5 mM a clear population of failures appeared in the amplitude histograms. Under these conditions, the average second response was facilitated, corresponding to a reduced number of failures in response to the second stimulus. In conclusion, facilitation required a situation in which the release probability could increase, and thereby overcome the simultaneously present depression. Supported by NAVF/RMF 326.91-031.

248.11

PEPTIDES HOMOLOGOUS TO THE RAB3 EFFECTOR DOMAIN STIMULATE NEUROTRANSMITTER RELEASE IN HELISOMA NEURONS. J.E. Richmond* and P.G. Haydon. Dept of Zoology and Genetics, Iowa State University, Ames, IA 50011.

Small GTP-binding proteins of the rab gene family are involved in vesicular transport through the secretory pathway. Of the rab GTPases, rab3A is selectively expressed in neurons and is associated with synaptic vesicles. In the present study we asked whether perturbation of rab3 affects neurotransmitter release at cultured *Helisoma* synapses. Using chemical synapses that form between contacting somata we first injected the non-hydrolyzable GTP analogue, GTPγS. Under conditions where action potentials were evoked presynaptically, the frequency of spontaneous miniature currents (MIPSC) increased in response to GTPγS, whereas GTP had no effect. To more specifically perturb rab3 we microinjected synthetic peptides with sequences spanning the putative effector domain of rab3A. Injection of rab3AL caused a dose-dependent increase in MIPSC frequency with a half-maximal effect at about 30μM, and a three-fold increase in MIPSC frequency at a concentration of 40-60μM. No significant increase in evoked release was observed. The increased MIPSC frequency was sustained in high Mg^{2+}/zero Ca^{2+} external saline indicating that the enhanced release was not due to an increase in Ca^{2+} influx. Injection of the peptide rab3(29-38) caused a similar increase in MIPSC, while injection of a peptide bearing little homology to the rab3 effector domain (ral), had no effect on MIPSC frequency. Together these results demonstrate that manipulations aimed at perturbing rab3-target interactions, modify synaptic transmission and provide evidence for a role of rab proteins in regulating neurotransmitter release.

248.12

IMAGES OF SYNEXIN IN RAT BRAIN: HETEROGENEOUS DISTRIBUTION WITHIN LIMBIC STRUCTURES AS DETERMINED BY COMPUTER-ENHANCED NEURO- IMMUNOCYTOCHEMISTRY. **H.B. Pollard* R. Etcheberrigaray, H. Caohuy, D. L.Alkon, and J. L. Olds**, Laboratory of Cell Biology and Genetics, NIDDK; and Neural Systems Section, NINDS, NIH. Bethesda, MD. 20892.

Synexin (Annexin VII) is a calcium-binding protein with membrane fusion and calcium channel properties, which occurs in brain with an additional specific, cassette-exon defined domain of 22 amino acids edited into the unique N-terminal region. The exact function is not known, but we report here that an affinity purified polyclonal antibody directed against the 22 residue peptide from human brain synexin is heterogeneously distributed in the rat brain. The specific localization includes (1) hippocampal pyramidal cells in CA1 and CA3, granule cells of the dentate gyrus, and Schaffer collaterals which are axonal projections of CA3; (2) cingulate cortex, layers 2-6; (3) somatosensory cortex, layers 2-6, including heterogeneous column-like structures; (4) Thalamus, including ventromedial lateral (VML) and ventral posterior medial (VPM) nuclei, which are motor projections to the motor cortex; (5) axons in the corpus callosum; (6) the lateral habenula, which shares with inferior colliculus the highest intensity of deoxyglucose metabolism in rat brain; (7) the ventral aspects of the pars reticularis of the substantia nigra, which contains GABA-ergic terminals from neurons in the caudate-putamen; and (8) axons in the corpus callosum. A second polyclonal antibody against a peptide from the C-terminal domain of synexin prepared in a different animal gave nearly identical distribution in rat brain. High power views demonstrate unambiguously that synexin is enriched in neuronal somata and axons. We conclude that there seems no evidence for molecular heterogeneity in synexin distribution in rate brain, and that furthermore, the distribution seems limited to specific regions of the limbic system and axons.

AXON GUIDANCE MECHANISMS AND PATHWAYS III

249.1

HEPARIN-BINDING GROWTH-ASSOCIATED MOLECULE (HB-GAM) IN AXON GROWTH AND GUIDANCE. H. Rauvala, J. Merenmies, R. Pihlaskari, E. Raulo, E. Castrén and P. Panula. Institute of Biotechnology and Department of Anatomy, Univ. of Helsinki, Helsinki, Finland, SF-00380.

HB-GAM (p18) was isolated from perinatal rat brain as a developmentally regulated, secretory protein that enhances neurite outgrowth from brain neurons in vitro. Molecular cloning of HB-GAM has revealed a conserved lysine-rich sequence. To characterize the role HB-GAM in brain development, we studied its distribution in developing rat forebrain by in situ hybridization and immunohistochemistry. A strong expression of the HB-GAM mRNA was found in cortical neuroepithelium and in developing layers of the cerebral cortex. The most prominent expression of the HB-GAM protein was found in tracts that follow developing axonal processes in several areas of the brain, for example in cerebral cortex, corpus callosum and fimbriae. To study, whether such tracts of HB-GAM could influence neural patterning, pathways of brain-derived and baculovirus-derived recombinant HB-GAM were created on culture wells. The HB-GAM pathways were found to strongly enhance patterned growth of axons from rat forebrain neurons. We suggest that HB-GAM plays a role in axonal pathfinding in perinatal rat brain.

249.2

EXPRESSION OF THE BARRIER-ASSOCIATED PROTEINS EAP-300 AND CLAUSTRIN IN THE DEVELOPING CENTRAL NERVOUS SYSTEM.
Craig F. McCabe, Robert M. Beckstead*, and Gregory J. Cole, Department of Anatomy and Cell Biology, Medical University of South Carolina, Charleston, SC 29425.

Immunohistochemistry of embryonic chick central nervous system (CNS) and immunocytochemistry of retinal cells were performed to compare and map the expression of two barrier-associated molecules. EAP-300 (Embryonic Avian Polypeptide of 300 kDa) and claustrin (a 320 kDa extracellular matrix keratan sulfate proteoglycan) were both transiently expressed in CNS regions that are considered nonpermissive to either neuron migration or axon growth. In the developing spinal cord, EAP-300 and claustrin were both expressed by the marginal zone early in development, and by the roof plate later in embryogenesis. In the developing rhombencephalon, immunoreactivity for both molecules was also observed first in the marginal zone, and later expression was restricted mostly to the midline. In the mesencephalon, EAP-300 and claustrin were also localized to the midline, and this expression represented a continuation of the expression observed in the spinal cord roof plate and hindbrain ventral midline. In the developing retina and cerebellum, EAP-300 and claustrin were differentially expressed. In retina, EAP-300 and claustrin were expressed by Müller cells and the optic fiber layer, respectively. In cerebellum at embryonic day 12 (E12), EAP-300 was expressed by Bergmann glia, but claustrin was not expressed until E15. Immunocytochemical staining of retinal and cerebellar cultures indicated that EAP-300 was expressed by a subset of radial astrocytes, as confirmed by double labeling experiments with a specific marker for radial astrocytes. These data indicate that in the absence of claustrin expression, EAP-300 was expressed specifically by radial astrocytes during developmental periods of neuron migration. Also, the coexpression of EAP-300 and claustrin in CNS regions considered to be nonpermissive to neurite extension suggests that these two developmentally regulated proteins may be associated with barrier function in the developing CNS. **Supported by EY07130.**

249.3

A HEPARAN SULFATE PROTEOGLYCAN IN DEVELOPING AXONAL PATHWAYS W. Halfter*, Dept. of Neurobiology, University of Pittsburgh

Two monoclonal antibodies against heparan sulfate proteoglycan (HSPG) were generated by immunizing mice with embryonic chick retina basal laminae (clone 3A12) and embryonic chick optic nerve (clone 6D2). Both antibodies recognized a new type of HSPG that appears transiently in developing chick axonal tracts. In early stages (E3), the antibodies labeled basal lamina only. During further development, in addition to basal laminae a strong staining of the optic nerve and other fiber tracts in the brain and spinal cord was found. The axonal staining peaked between E8 and E10 and decreased afterwards. By E14, the axonal staining had disappeared, while the labeling of basal laminae persisted. Western blot analysis showed that the antigen had a molecular weight of 600kD that dropped to 250kD upon treatment of the sample with heparitinase or nitrous acid, showing that the antibodies recognized the core protein of a HSPG. Antibodies against HSPG from non-neuronal tissue, such as the EHS mouse tumor, or embryonic chick muscle, did not label axonal tracts, suggesting a unique HSPG in developing nervous tissue. The function of the protein might be to localize adhesion proteins and growth factors to the growing axons before trophic support from the target becomes available.

249.4

LASER INACTIVATION OF HNK-1 PERTURBS LATERAL LONGITUDINAL FASCICLE FORMATION IN THE ZEBRAFISH HINDBRAIN.

E.M. Shah and D.G. Jay*. Department of Cell. and Developmental Biology, Harvard University, Cambridge, MA 02138

During zebrafish development, the trigeminal axons project to the Rohon-Beard axons to form the lateral longitudinal fascicle. The coincident specific expression of the glycosyl moiety, HNK-1, on both of these neuronal subtypes (Metcalfe et al. Devel. 110, 1990) suggests that HNK-1 may be involved in target recognition when these neurons interact. By applying the technique of chromophore assisted laser inactivation (CALI) (Jay and Keshishian., Nature, 348:548, 1990), we have endeavored to characterize the *in vivo* function of the molecule(s) recognized by the HNK-1 antibody during zebrafish neural development.

We have developed an efficient method for vital labeling of the zebrafish neural cell surfaces with HNK-1 antibody. Yolk injections of HNK-1 antibody between 10 and 15 hours post-fertilization result in 89% of the injected embryos exhibiting HNK-1 staining of the trigeminal and Rohon-Beard neuron surfaces at 17 hours post-fertilization. Embryos injected with malachite-green labeled HNK-1 antibody at the 4 somite stage, irradiated at the 14 somite stage and fixed at the 20 somite stage exhibited a perturbation in the normal morphology of the lateral longitudinal fascicle in 55% (10/18) of the cases. We observed either disordered fascicles, differential growth rates of contralateral fascicles and/or failure of the Rohon-Beard and trigeminal axons to meet. Embryos injected with malachite green-labeled HNK-1 antibody, but not irradiated, exhibited perturbations in 14% (5/34) of the cases, while those injected with control reagents and irradiated had perturbations in 20% (2/10) of the cases. These preliminary results using CALI suggest a role for an HNK-1 immunoreactive cell surface molecule in the formation of the lateral longitudinal fascicle in the developing zebrafish.

249.5

GROWTH CONES FOLLOW WIDELY DISTRIBUTED DIRECTIONALITY CUES IN THE ZEBRAFISH BRAIN. J.P.Kanki, S. Akhtar, and J.Y.Kuwada*. Dept. of Biology, University of Michigan, Ann Arbor, MI. 48109-1048.

Growth cones follow precise, cell specific pathways to reach their targets in the zebrafish CNS. To do this growth cones must extend in the correct direction along their appropriate pathways. Directionality cues may be limited to a pathway or be more widely distributed. To determine the distribution of directionality cues, the epiphysial neurons, located laterally at the base of the epiphysis, were transplanted to ectopic sites in the brain of isochronic host embryos and allowed to develop further. Donor cells but not host cells were labeled with FITC-dextran by the prior injection of dye at the 1-2 cell stage. This allowed the visualization of the transplanted cells. Epiphysial growth cones normally extend ventrally from the epiphysis. The growth cones of orthotopically transplanted neurons also extend ventrally, indicating that the transplant method does not unduly damage the donor cells or the host environment. Similarly,the growth cones of donor neurons in ectopic sites overwhelmingly extended ventrally although in principle they could have extended in any direction from their ectopic sites. This was true even at a site where host growth cones were known to extend dorsally. These data suggest that directionality cues that epiphysial growth cones can read are widely distributed in the embryonic brain.

249.6

BEN, A NOVEL ADHESION MOLECULE TRANSIENTLY EXPRESSED DURING AXONAL MIGRATION OF MOTONEURONS AND CLIMBING FIBERS. O. Pourquié, M.E.R. Halloncl, and N.M. Le Douarin*. Institut d'Embryologie du CNRS et du Collège de France, 49 bis Avenue de la Belle Gabrielle, 94130 Nogent-sur-Marne FRANCE.

We have previously reported the identification by means of a Mab, of a 95-110 kd membrane glycoprotein that we call BEN. This protein is expressed in the chick embryo on various cell types among which hematopoietic and neural cells. We have shown that BEN expression is selectively associated with axonal migration on the peripherally projecting neurons, including the motor and sensory neurons. Its distribution during avian cerebellum ontogenesis was investigated by immunocytochemistry and was suggestive of a selective expression on the climbing fibers. We have used a quail-chick chimera grafting system which enabled us to obtain chimeras with a cerebellum exclusively composed of quail cells whereas the inferior olivary nucleus was composed of chick neurons. These animals where studied in double staining with a Mab which recognizes chick neuronal fibers at the exclusion of quail ones together with antibodies directed against the BEN protein. We could show that the BEN positive fibers were of chick origin implicating that their source was extrinsic to the cerebellum. We conclude that BEN is transiently expressed during cerebellum development on the climbing fibers. Using polyclonal antibodies we have shown that BEN is implicated in neuronal aggregation. Biochemical analysis of the purified protein has revealed that it is heavily glycosylated in a tissue specific manner and bears the HNK-1 epitope. We have cloned a full length cDNA encoding this protein. Analysis of its sequence shows that it is a new member of the Ig superfamily with an original structure. Two neural adhesion molecules SC1 and DM-GRASP that share an identical sequence have been simultaneously identified. These proteins are similar in structure to the human melanoma marker MUC18 and are likely to define a new subgroup of adhesion molecules of the Ig superfamily. We conclude that BEN/SC1/DM-GRASP might represent an avian homologue of insects fasciclins.

249.7

F3 NEURONAL CELL ADHESION MOLECULE IS POLARIZED ON AXONS AND ITS SOLUBLE FORM PROMOTES NEURITE OUTGROWTH. G.N.Rougon[1], P. Durbec[1], C. Faivre-Sarrailh[1], J.P. Ternaux[2]* and G. Gennarini[3]. [1]CNRS 179, Université de Marseille-Luminy; [2] CNRS 418 Marseille ; France, [3] Università di Bari, Italy.

The establishment of the complex network of specific neuronal connections is critically dependent on the correct outgrowth of axons to their cellular targets. The F3 molecule is a member of the immunoglobulin superfamily anchored to membranes by a glycane-phosphatidylinositol, and is predominently expressed on a subset of axons. We investigated its distribution by light and electron microscopy in cerebellum and found that the granule cell axons strongly express F3 as soon as they begin to grow, consistent with a role in promoting directional outgrowth of axonal processes. In adult cerebellum, F3 is present at three types of synaptic sites, suggesting it might also play a role in the formation and maintenance of synapses. In each type of synapse F3 is present at only one synaptic site, never at both, indicating that F3 mediates heterophilic interactions. (Faivre-Sarrailh et al. J. Neurosc. 1992. 12; 257-267).

The question as to whether soluble forms of F3, that are detected in developing nervous system, would be functionally active was addressed in vitro on culture of mouse sensory neurons. Preparations enriched in soluble F3 have no effect on neuron attachment and survival but enhance neurite initiation and neurite outgrowth in a dose-dependent manner (Durbec et al. J. Cell Biol. in press).These results suggest that the soluble forms of adhesive proteins with neurite outgrowth promoting properties act at distance of their site of release in a way reminescent of growth and trophic factors.

249.8

RESTRICTED EXPRESSION OF N- AND R-CADHERIN ON NEURITES OF THE DEVELOPING CHICKEN CNS. **C. Redies[1,2,*] and M. Takeichi[1]. [1]Dept. Biophysics, Fac. Science, Kyoto University, Kyoto 606, Japan, and [2]Dept. Biochem., Max Planck-Institute Dev. Biology, Tübingen, Germany**

The expression of two cadherins, N- and R-cadherin, was mapped in the CNS of chicken embryos of 6-11 days incubation. Throughout the neural axis, both molecules are expressed in a developmentally and topographically restricted manner. *In the spinal cord,* the laterally located fibers of the dorsal funiculus express N-cadherin while the medially located fibers do not. These two fiber systems have a different course within the CNS. *In the hindbrain,* N-cadherin is expressed by the descending trigeminal (general somatic sensory) tract and R-cadherin by the visceral motor fibers of the vagus and glossopharyngeal nerves. Other tracts and nuclei expressing either N- or R-cadherin are found *in the mid- and forebrain.*

The possibility that N-cadherin provides a guidance cue for axon migration within the CNS by a homophilic adhesion mechanism was investigated *in vitro*. Axon outgrowth from E6 sensory ganglia was studied on N-cadherin-transfected neuroblastoma cells. The N-cadherin-positive sensory axons defasciculate and follow the cell-cell borders between transfected cells where high levels of N-cadherin are expressed. These results suggest that the topographically restricted expression of N-cadherin during chick brain development may play a role in guiding N-cadherin-positive neurites along CNS paths which express the same molecule.

249.9

SUBSETS OF OLFACTORY AXONS THAT EXPRESS LACTOSERIES GLYCOCONJUGATES ARE ASSOCIATED WITH EXTRACELLULAR LACTOSE-BINDING LECTIN, L-14, AND LAMININ. G. A. Schwarting* and N. K. Mahanthappa, E.K.Shriver Ctr., Waltham, MA 02254; and and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

A homogeneously distributed subset of neurons in the mature olfactory epithelium of rats express terminally lactosaminylated glycolipids that react with the monoclonal antibody, 1B2. In adult rats, 1B2+ axons can be detected in the outer portion of the nerve layer of the olfactory bulb (OB), and can be seen branching through the inner portion of the nerve layer into individual 1B2+ glomeruli; about 10% of glomeruli are 1B2+. L-14, a lactose-binding lectin capable of binding both 1B2+ glycolipids and laminin-associated polylactosamine, is also expressed in the olfactory system. Immunocytochemistry using antiserum to L-14 (gift of D. Cooper, UCSF) has demonstrated that this lectin is in the outer portion of the OB nerve layer and on structures branching toward the the glomerular layer, overlapping with 1B2+ regions. We show that 1B2+ axons and L-14 co-localize with a subset of laminin+ tracks leading to 1B2+ glomeruli. These data suggest that L-14 may promote non-integrin-mediated, laminin interactions with elongating, 1B2+ olfactory axons.

249.10

MAbs #4 & 199 SPECIFICALLY INHIBIT ATTACHMENT OF NG108-15 CELLS TO LOW, BUT NOT HIGH AMOUNTS OF LAMININ. N. R. Smalheiser* and B. J. Collins. Dept. of Pediatrics, Univ. of Chicago, Chicago, IL 60637.

E14 chick brain membrane proteins were purified by DEAE, con A and SDS-PAGE; a band containing cranin was injected into mice to raise monoclonal antibodies. MAbs #4 and 199 recognize a non-integrin ~125 kDa band (N.S., Trans. Am. Soc. Neurochem. 22: 203 '91) which binds to laminin affinity columns in a calcium- and conformation-dependent manner. MAb #4 Ag is co-expressed with laminin in chick CNS and regenerating goldfish optic nerve (Collins et al. Eur. J. Neurosci. suppl. 4: 295 '91).

To learn if MAb #4/199 antigens are involved in cell responses to laminin, MAbs were purified from hybridoma spent medium (cells grown either in serum-free medium, or containing 10% FCS) by hydroxyapatite and ammonium sulfate precipitation. MAbs (100 µg/ml) were added with NG108-15 cells and plated in serum-free medium on plastic Petri dishes treated with laminin (1-5 ng/mm^2) and blocked with BSA (0.3%). Short-term (1.5-7 hr) and long-term (16-24 hr) assays gave similar results: Both MAb #4 and 199 inhibited cell attachment to laminin (1 ng/mm^2) by 60-70% relative to controls (BSA or mixed mouse IgG, 100 µg/ml), but did not inhibit attachment at all to high laminin (5 ng/mm^2), bovine plasma fibronectin (0.5-1 ng/mm^2), or polylysine. A MAb against ACAM (GC-4) did not inhibit attachment to any surface tested. These data suggest that MAb #4/199 antigens play specific roles in laminin-mediated adhesion. Supported by NS 26055, HD 09402, and the March of Dimes Birth Defects Foundation.

FORMATION AND SPECIFICITY OF SYNAPSES IV

250.1

KINETICS AND ULTRASTRUCTURAL 3D RECONSTRUCTION OF DEVELOPING TUBEROUS ELECTRORECEPTORS IN THE ELECTRIC FISH EIGENMANNIA. H.A. Vischer.* Neurobiology Unit, S.I.O., UCSD, La Jolla, CA 92093-0202.

Epidermal tissue of embryonal and juvenile developmental stages of Eigenmannia, a South American, weakly electric fish, was processed for electron microscopy. Serial thick sections (0.5 µm) through developing electroreceptors were photographed with a JEM-1000 High-Voltage Electron Microscope at 1000 kV. Images were subsequently digitized and processed with a reconstruction program on a PC to render 3D pictures. Additional tissue was prepared for histochemistry with Safranin O, a marker for mitotic figures in dividing cells. After the afferent fiber penetrates the basal lamina and invades the receptor primordium, it birfurcates into two classes of branches: 1) Branches that form synaptic contacts with primordial receptor cells and 2) collateral branches that sprout to the periphery of the primordium. These latter collaterals do not form synaptic contacts and degenerate during further development. Synaptic contacts are smaller at early developmental stages than at older stages. On the other hand, presynaptic ribbons are longer and interconnected at their apical ends in early stages and shorter and solitary after synaptic maturation. Supporting cells derive from receptor-primordium cells and divide within the supporting cell layer. At the periphery of this layer, they differentiate into new primordial receptor cells which subsequently shift upwards to the receptor cell layer and mature.

250.2

MOLECULAR ANALYSIS OF THE CA1 SUB-REGION OF THE HIPPOCAMPUS S. M. Nair , S. A. Mackler, Y. Cao and J. H. Eberwine*. Univ of PA Phila PA 19104.

We characterized and compared the relative levels of mRNA expression in CA1 tissue sections to that in single pyramidal neurons from the rat hippocampus by using the antisense RNA (aRNA) amplification procedure. The aRNA generated by this method has previously been shown to represent the poly (A+) RNA population.

Analysis of CA1 sections enables characterization of mRNA populations from discrete numbers of hippocampal cells, but may not detect those that are unique to subtypes of cells since these sections contain many neuronal and glial cell types. In contrast, we performed a similar analysis in both acutely dispersed single live pyramidal cells and in the live slice preparation, where some synaptic connectivity and glial interactions remain.

In-situ transcription (IST) was used to generate cDNAs corresponding to the poly (A+) RNA population from CA1 sections. In separate studies, a patch pipette electrode was used to simultaneously record from cell bodies of single neurons as well as to initiate IST. In each case, the cDNA was made double-stranded and amplified incorporating ^{32}P-CTP into the aRNA product. The aRNA was used as a probe to screen slot blots containing selected cDNA clones - called 'Expression Profiling'.This permits characterization of the relative mRNA abundances from each preparation.

Among 24 different mRNA species examined in the aRNA population, CA1 tissue sections revealed high relative levels of mRNA expression for a Ca^{++} channel, as well as for nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Differences between expression profiles of acutely dispersed single CA1 neurons relative to those in fixed tissue sections are likely due to the heterogeneity of cell types. Also, observed differences in patterns of gene expression between dispersed CA1 neurons and those in the live slice preparation may be partly due to the influence of synaptic and glial connectivity on the physiological state of the neuron.

Further studies along these lines may help characterize the influence of cellular interactions on physiological responsiveness at the level of single neurons.

250.3

SURFACE AND CYTOSKELETAL MARKERS OF POSITION IN THE MAMMALIAN NERVOUS SYSTEM. Z. Kaprielian*, and P.H. Patterson. Division of Biology, 216-76, California Institute of Technology, Pasadena, CA 91125

Spinal cord axons display a rostrocaudal, positional bias in their innervation of sympathetic ganglia and intercostal muscles (Wigston and Sanes, J. Neurosci. 5:1208, 1985). To examine the molecular basis of this positional specificity, mAbs that bind preferentially to rostral sympathetic ganglia were produced. Staining of one of these mAbs, ROCA1 (ROstroCAudal), is highest in rostral sympathetic ganglia and intercostal nerves and declines in a graded manner in the caudal segments. The staining of another mAb, ROCA2, does not display a rostrocaudal difference. Immunoblot analysis shows that ROCA1 recognizes two antigens in membrane preparations of peripheral nerves and ganglia: (1) a Triton X-100-insoluble 60 kD protein and (2) a Triton X-100-soluble 26 kD protein. The 60 kD antigen is preferentially expressed in rostral intercostal nerves, and is identical to the intermediate filament protein, peripherin. The 26 kD protein, on the other hand, is found on neuronal and glial cell surfaces. N-terminal amino acid sequence data obtained from the affinity purified 26 kD protein indicates significant homology with human CD9, a protein expressed on the surfaces of platelets and pre-B lymphocytes. CD9 mAbs induce platelet aggregation and homotypic adhesion of pre-B cells (Horejsi and Vlcek, FEBS. 288:1, 1991).

ROCA2 also binds this 26 kD protein in peripheral nerves and ganglia. Unlike ROCA1, ROCA2 binding does not display a rostrocaudal gradient, suggesting that it is the ROCA1 epitope, and not the 26 kD protein itself, which is distributed in a rostrocaudal gradient. (Supported by the McKnight foundation, a Markey internal grant in Developmental Biology, and an individual NRSA.)

250.4

CLONING OF THE *DROSOPHILA* NEURAL GENE, PASSOVER. **S.N. Krishnan, E. Frei, G. P. Swain* and R. J. Wyman.**
Dep't. of Biology, Yale University, New Haven, CT 06511

Passover flies fail to jump in response to a light-off stimulus. The mutation disrupts the synapses of the giant fibers, command neurons for this response. *njP181*, a non-jumping mutant was isolated from a P-element mutagenesis and found to be allelic to *Pas*. Revertants of *njP181* were obtained; in all revertants the P-element had been excised. Using genomic sequences adjacent to the P- insertion site, a single 2.7 kb cDNA was obtained from 2 million phages. The cDNA spans 60Kb of genomic sequence and straddles a breakpoint that defines the gene. The cDNA has a 5′ untranslated sequence of 335 bp, an open reading frame of 1083 bp, and a 3′ untranslated region of 1330 bp. The P-element of *njP181* is inserted in the 5′ leader sequence 28 bp from the start of the cDNA. The cDNA codes for a putative protein of 361 amino acids with a calculated molecular weight of 42.9 kd. This putative protein has the features of an integral membrane protein with a single transmembrane domain. The putative extracellular domain is composed of 256 amino acids, followed by a transmembrane domain of 22 amino acids, as judged by hydropathy analysis. *Pas* may start a new gene family: the only highly similar gene is *l(1) optic ganglion reduced* (proteins 46% identical). Both genes are involved in the development of postembryonic neurons. The head capsule, (with eyes and lamina attached) was dissected away from the brain of newly emerged adult flies; in situ hybridization showed strong expression of *Pas* in two large cells in the same location as the giant fiber somata.

250.5

ECTOPIC EXPRESSION OF THE UNC-5 GUIDANCE RECEPTOR IN THE TOUCH NEURONS OF C. ELEGANS STEERS THEIR AXONS DORSALLY ON THE EPIDERMIS. J.G. Culotti*, M. Hamelin, Y. Zhou, and I.M. Scott. Samuel Lunenfeld Res. Inst. of Mt. Sinai Hospital, Toronto, Canada M5G 1X5.

The unc-5 gene of C. elegans encodes a novel cell adhesion receptor of the immunoglobulin superfamily (UNC-5) which is required to guide dorsal growth cone movements on the epidermis (Leung-Hagesteijn et al., submitted). unc-5 has been shown genetically to require unc-6, which encodes a putative epidermal guidance cue, for dorsal guidance (Hedgecock et al., Neuron 4:61-85,1990; Ishii et al., submitted). We wanted to test whether unc-5 expression in neurons that do not grow dorsally on the epidermis would suffice to steer them in a dorsal direction. When ectopically expressed in the set of touch receptor neurons, which normally extend axons longitudinally or ventrally on the epidermis, unc-5 induces dorsal axon trajectories. These abnormal trajectories depend on unc-6, suggesting that UNC-5 acts to redirect the touch cell axons by utilizing its normal guidance functions.

250.6

TERM1: A NEURON-SPECIFIC GLYCOPROTEIN LABELS GROWTH CONES AND DEVELOPING SYNAPSES OF AN IDENTIFIED INTERNEURON. T. Meier, S.Therianos, D. Zacharias and H. Reichert*. Dept. of Zoology, University of Basel, CH-4051 Basel, Switzerland.

TERM1 is a molecular label that is expressed by a pair of individually identifiable developing interneurons in the embryonic nervous system of the grasshopper. During embryogenesis, TERM1 is not expressed by any of the other interneurons in the CNS. The extremely restricted spatiotemporal expression pattern of TERM1 during nervous system development suggests that it may play a role in neuron-specific cell-cell recognition during pathfinding and synaptogenesis.

We have characterized TERM1 at the cellular and biochemical level with the aid of a TERM1-specific monoclonal antibody. Cellular studies involving immuno-electron microscopy show that the TERM1 label is localized on the outer cell surface of the growth cones of the two interneurons during axogenesis. Subsequently, during the phase of synaptogenesis, TERM1 is found at the synaptic terminals of the labeled cells. It is retained at these specific synapses throughout postembryonic life. Immunopreciptation of TERM1 followed by a biochemical analysis shows that the molecular label is a membrane associated glycoprotein with an apparent molecular weight of 50kD. TERM1 is now being isolated for microsequencing, antibody generation and expression cloning. (Supported by the Swiss NSF).

250.7

***DROSOPHILA* EMBRYOS MUTANT FOR ZYGOTIC *TOLL* HAVE ALTERED RP MOTONEURON NUMBERS AND ECTOPIC MOTOR ENDINGS.** M. S. Halfon,[1] C. Hashimoto[2] and H. Keshishian[1]*. [1]Dept. of Biology & [2]Dept. of Cell Biology, Yale Univ., New Haven, CT 06511.

Toll (Tl) encodes a transmembrane protein important in dorso-ventral patterning. A *Tl* enhancer-trap line has previously shown *Tl* transcription in several ventral muscle fibers (Nose & Goodman, 1991. Soc. Neuro. Abs., 17:742), spurring our interest in a possible role of *Tl* in synaptogenesis. We have used anti-Tl antibody to characterize zygotic Tl protein expression. At embryonic stage 14, Tl is expressed on the medial and lateral edges of muscle fiber 7, and is located on membrane contacts between muscle fibers 17 & 16, 16 & 15, and 15 & 28. At stage 15, Tl is no longer expressed on muscle fiber 7 and between fibers 17 & 16, and in stages 16 and 17, the only Tl expression in the musculature is between fibers 16 & 15. The spatial pattern is strikingly similar to that of fasciclin III (fasIII), a molecule believed to be involved in axon pathfinding and synaptogenesis. Due to the similarities in fasIII and Tl expression, we looked at Tl localization in a *fasIII*$^-$ background and fasIII localization in a *Tl*$^-$ background. Both fasIII and Tl are properly expressed in the musculature in the respective backgrounds. In wildtype embryos, there are 3 fasIII positive RP motoneurons. In *Tl* null embryos, ~74% of hemisegments have a wildtype RP number, while 3% have +1 RP, 17% have -1 RP, 1% have -2 RP and 4% have -3 RPs (n=94 hemisegments, 7 embryos). Antibodies against eve and Ubx, which label additional motoneurons, likewise show aberrancies in neuron number. A further Tl$^-$ phenotype is ectopic nerve endings on muscle fibers 6 & 7, which are contacted by the RP3 axon. A similar result is seen when the muscle fibers are denervated by RP ablation (T.N. Chang & H.K., this mtg.). We are investigating whether these aberrant synapses are due to pre- or post-synaptic mechanisms.

250.8

LASER ABLATION OF EMBRYONIC MOTONEURONS IN *DROSOPHILA* RESULTS IN ABERRANT LARVAL TARGET MUSCLE INNERVATION. T.N. Chang*[1] & H. Keshishian, Interdepartmental Neuroscience Program[1] and Dept. of Biology, Yale University, New Haven, CT 06511.

Previous mismatch experiments duplicating or ablating muscle targets implicated cellular recognition of synaptic partners by motoneurons (Chiba *et al.*, Soc Neurosci Abstr 17:213). Here we ablated motoneurons to show that partial or complete denervation induces foreign inputs on muscle targets. We directed the laser through the cuticle at the identified motoneuron RP3 on the left side of every abdominal segment in intact stage 15-16 embryos. Left RP3s were ablated just before or shortly after they made their initial contacts on their targets, contralateral muscle fibers 6 and 7. Unlased right RP3s served as an internal control. We then allowed the embryo to mature to third instar and examined the innervation of muscle fibers 6 and 7. In unlased animals, 6 and 7 innervation includes type I (large varicosity) endings at the middle of the 6-7 cleft; type II (small varicosity) endings are co-localized with the type I's in anterior abdominal segments. Type I endings were missing on the right side of most lased animals, while type II's were either missing or were present but more extensive, sometimes crossing normally restrictive segment borders. Foreign endings were observed at ectopic sites on both muscles 6 and 7; such endings were extremely rare on the control side or in unlased animals. For example, in most right hemisegments of left RP3-lased animals, both type I and II ectopic endings were observed from the transverse nerve. In addition, ectopics were observed branching off from endings on 13, as well as from 15-16 endings, which originate from a separate nerve branch. In all cases, the control side (right RP3s, left musculature) had normal innervation of 6 and 7. Our results show that partial or complete denervation results in novel synaptic inputs from diverse sources.

250.9

THE ROLE OF NEURAL ACTIVITY IN ESTABLISHING NEUROMUSCULAR ENDING MORPHOLOGY DURING *DROSOPHILA* EMBRYOGENESIS. J.E. Jarecki,[1] M.D.S. Anderson,[2] Y-A. Sun*[3], H. Keshishian[3]. Genetics[1] and Biol.[3] Depts., Yale Univ., New Haven, CT 06511 and Div. of Genetics[2], Harvard Medical School, Boston, MA 02115.

The motoneuronal ending anatomy of embryos and larvae is highly stereotyped. Mutations that increase neural activity result in more elaborate larval endings (Budnik *et al.*, J Neurosci, 10:3754). Some of these changes probably occur postembryonically. Both muscle area and motoneuron arbors grow dramatically during this period, with bouton number increasing 10 fold and distinct type I and II (large vs small bouton) processes arising by the 2nd larval instar. In addition, activity may also affect embryonic development. In hatchlings motor endings are relatively simple, yet prefigure the mature larval anatomy. However, they also differ in their size and maturity. For example, synapses on muscle fibers 6&7 have more differentiated boutons than those on 12 &13. We have examined the affect of altered activity in embryos, and found that it influences synaptic development. During late embryogenesis coordinated bodywall peristalsis begins. This movement is neurally evoked and mediated by glutamate (M.A., Soc Neurosci Abstr. 16:973). We used both genetic and pharmacological tools to cause paralysis. Argiotoxin 636, an open channel glutamate blocker that acts postsynaptically, had no effect on ending morphology when injected during stage 17. The role of presynaptic activity was assessed using either TTX or the sodium channel mutant *paralytic (para). para* does not block bodywall peristalsis, and had no affect on ending morphology. However, microinjection of TTX during stage 17 did block peristalsis, suggesting that at least one other sodium channel gene is expressed at this stage. Also TTX retarded maturation on fibers 12 and 13, so that endings resembled growth cones. The affected arbors are 3- to 4-fold more branched than controls and lack boutons. This effect did not occur on muscle fibers 6&7, suggesting fiber-specific differences. These results imply a role for embryonic presynaptic activity in regulating synaptic maturation.

250.10

SYNAPTIC COMPETITION AT DEVELOPING *APLYSIA* SENSORIMOTOR SYNAPSES IN CELL CULTURE: REGULATION BY ACTIVITY AND ELECTRICAL COUPLING. D.L. Glanzman* Dept. of Physiological Science, UCLA, Los Angeles, CA 90024.

Recent morphological data indicate that when two *Aplysia* sensory neurons are grown together in dissociated cell culture with a single, postsynaptic motor neuron, the outgrowth of the two sensory neurons tends to occupy separate postsynaptic regions (Glanzman et al., *Neuron* 7: 903, 1991). This morphological segregation of the outgrowth from different presynaptic inputs may reflect competition between the developing sensory neurons for innervation of the target motor neuron. To determine whether activity can affect this apparent competition, electrophysiological experiments were performed on sensorimotor cocultures, each of which consisted of a motor neuron innervated by two sensory neurons. All sensorimotor cocultures were 2-3 days old. Each sensory neuron was activated once every 5 min, and the size of the excitatory postsynaptic potential (EPSP) evoked in the motor neuron by the test stimulus was recorded intracellularly. After 3-4 test stimuli, one of the sensory neurons was given a brief tetanus (24-48 pulses at 20-25 Hz). This selective tetanic stimulation tended to suppress the synaptic connection made by the nontetanized sensory neuron with the motor neuron, as indicated by a reduction in the size of the EPSP, when there was little or no electrical coupling between the two sensory neurons. The reduction in the size of the nontetanized sensory neuron's EPSP was prolonged, lasting for up to 30 minutes. By contrast, when the sensory neurons were strongly coupled electrically, selective tetanization of one of the sensory neurons did not result in a prolonged suppression of the nontetanized input. These results indicate that the effect of neuronal activity upon the synaptic competition between sensory neurons in developing *Aplysia* sensorimotor cocultures can be influenced by the extent to which the presynaptic neurons are electrically coupled.

250.11

RETROGRADE CONTROL OF FACILITATION AT AN ECTOPIC CENTRAL SYNAPSE. K.A. Killian* and R.K. Murphey. Program in Neuroscience and Behavior, Univ. of Massachusetts, Amherst, MA 01003.

By transplanting sensory neurons (SNs) of the cricket to ectopic sites, we can induce them to synapse with unusual postsynaptic neurons. We have used these mismatched synapses to try to understand the relative roles of pre- and post-synaptic neurons in determining the temporal aspects of transmitter release at a synapse.

Interneuron MGI is located in the terminal abdominal ganglion (TAG) where it synapses with SNs of cercal filiform hairs. These synapses facilitate when activated at 100 Hz (G. Davis, Soc. Neurosci. Abstr. 17:1288, 1992). Campaniform sensilla (CS) located on the tibia of the meso-thoracic leg of locust have been shown to form synapses with thoracic interneurons which only exhibit depression during repetitive stimulation (N. Emptage, unpub.). When homologous CS SNs of the cricket are transplanted to the abdomen, they regenerate and synapse with MGI. We have found that when these ectopic synapses are activated at 100 HZ, facilitation is sometimes observed. Thus, we hypothesize that MGI can induce facilitation at these synapses. Supported by NRSA NS 08847-01 (KAK) and NSF BNS90-96180 (RKM).

UPTAKE, STORAGE, SECRETION AND METABOLISM II

251.1

MOLECULAR CLONING OF THE HUMAN DOPAMINE AND NOREPINEPHRINE TRANSPORTER GENES. K.A. Neve*, C. Li, R.A. Henningsen, S. Smiley, and A. Janowsky VA Medical Center and Oregon Health Sciences University, Portland, OR 97213

The molecular cloning of cDNA for neurotransmitter transporters has revealed that they are highly homologous proteins. They are unusual in that each is expressed in a set of neurons that is often quite small and does not overlap with the sets of neurons expressing other neurotransmitter transporters. As a first step towards identifying determinants of cell-specific expression, we are cloning the genes and flanking regions for the dopamine (DAT) and norepinephrine (NET) transporters. DAT and NET cDNAs were cloned by reverse transcription-PCR, and used as hybridization probes to screen a human genomic library. For each cDNA, 19 positive recombinants were selected and plaque-purified, and genomic DNA from the phage clones is being mapped with restriction endonucleases and sequenced. One phage that hybridized with the DAT probe contains a 15-kb DNA insert that has 0.5 kb of intronic sequence at its 3' end. Upstream from the intron, coding sequence begins within amino acid 96 and continues through the translational start codon. In these 95 amino acids, the human DAT has 91% identity with the rat DAT but only 84% identity with a bovine DAT. A 0.4 kb *SacI-EcoRI* fragment ending 24 bp upstream from the start codon has been subcloned for determination of the transcriptional start site by S1 nuclease protection. Based on a published length of 3.4 kb for a DAT cDNA and 3.6 kb for the message, the promoter should lie within this fragment if there is no intron in the 5'-UTR. (NIDA Contract No. 271-90-7405, VA Merit Review Program)

251.2

CLONING, PHARMACOLOGICAL CHARACTERIZATION AND DELETION MUTATIONS OF THE HUMAN DOPAMINE TRANSPORTER. B. Giros*, S. El Mestikawy, Ch. Pifl, N. Godinot and M. G. Caron. Dpt. of Cell Biol. Howard Hughes Med. Inst., Duke University Durham, NC 27710, USA

We have screened a human substantia nigra cDNA library with probes from the rat dopamine transporter. A 3.5 kb cDNA clone was isolated and found to code for a 620 amino acid protein. The hydropathicity analysis suggested the presence of 12 putative transmembrane spanning domains, a characteristic feature of sodium-dependent neurotransmitter carriers. The rat and the human dopamine transporters were found to be 92% homologous. When permanently expressed in mouse fibroblast Ltk^- cells, the human clone was able to induce a saturable time- and sodium-dependent dopamine uptake. This transport was blocked by psychostimulant drugs (amphetamine, cocaine, phencyclidine), neurotoxins (6-OH-DA, MPP^+), neurotransmitters, antidepressants (amitriptyline, bupropion, desipramine, mazindol, nomifensine, nortriptyline), and various uptake inhibitors (mazindol, GBR 12783, GBR 12909, amfonelic acid). The rank order potencies of the K_i of these substances on the human and the rat dopamine transporter displays a high correlation (r=0.998), when compared under the same conditions, i.e, same expression vector, same stable cell lines, and experiments run in parrallel.

In order to elucidate the role and function of the different domains of this large transmembrane protein, we have constructed a serie of mutants in which we deleted respectively, the C-terminus tail, the N-terminus tail, or the large second extracellular loop. Interestingly, some of these deletions produce an impairment of the dopamine uptake, without any marked modifications of the ^{3}H-CFT binding. These findings should allowed the determination of whether binding and transport are dissociable functions of these transporters.

251.3

UPTAKE OF THE NEUROTOXIN MPP^+ BY THE CLONED HUMAN AND RAT DOPAMINE TRANSPORTERS. Ch. Pifl, B. Giros, N. Godinot and M.G. Caron*. Dept. of Cell Biology, Howard Hughes Medical Institute, Duke University Medical Center, NC 27710.

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces parkinsonism in humans and subhuman primates but not in rats. A crucial step in the neurotoxicity seems to be the uptake of its toxic metabolite proper MPP^+ by dopamine (DA) neurons as revealed by the protective effect of DA uptake blockers. Since primates but not rodents show a preferential retention of MPTP metabolites in the caudate, their different susceptibility to MPTP neurotoxicity may be due to species differences in the DA transporter systems versus MPP^+. To test this hypothesis we stably expressed the cloned human and rat DA transporters in Ltk^- cells; we measured uptake of [^{3}H]DA and [^{3}H]MPP^+ in these cells and, in parallel, in striatal synaptosomes of rats. The DA uptake in clonal cell lines and in striatal synaptosomes showed the rank order of potency of uptake blockers characteristic of a DA transporter. However the cloned transporters had a much lower affinity for MPP^+ than the transport system in striatal synaptosomes (K_m = 20-30 μM vs 200-300 nM). No significant differences between human and rat transporters were observed. We conclude that (1) species differences in susceptibility of humans versus rats to MPTP neurotoxicity cannot be due to differences in the properties of DA transporters and (2) the molecular basis of the high affinity uptake of MPP^+ in striatal synaptosomes remains to be clarified.

251.4

CLONING AND EXPRESSION OF TWO NOVEL GABA TRANSPORTERS FROM RAT BRAIN. L.A. Borden, K.E. Smith, P.R. Hartig*, T.A. Branchek, and R.L. Weinshank. Synaptic Pharmaceutical Corporation, 215 College Road, Paramus, NJ 07652

Neuronal transmission at GABAergic synapses is terminated by the uptake of GABA into the presynaptic terminal and surrounding astrocytes. The previous cloning of the neuronal GABA transporter (GAT-1; Guastella et al., 1990) revealed that this protein is a member of the 12 transmembrane domain superfamily of transporters. We have now isolated clones from rat brain cDNA which encode two novel GABA transporters. The clones, designated GAT-2 and GAT-3, exhibit 68% amino acid sequence identity to one another, but only 53% identity to GAT-1. When expressed in COS-7 cells both transporters display high-affinity for GABA (K_M≈10μM), and transport activity is dependent on external sodium and chloride. GAT-2 and GAT-3 display greater sensitivity to β-alanine than does GAT-1, suggesting similarity to the transporter characterized in glial cultures. However, the lack of sensitivity to guvacine, nipecotic acid, and hydroxynipecotic acid indicate that GAT-2 and GAT-3 are distinct from the previously characterized glial transporter. The identification of these novel clones reveals unexpected heterogeneity in GABA transporters.

251.5

NEUROTRANSMITTER TRANSPORTER & ORPHAN cDNAs IN RAT MIDBRAIN LIBRARIES: STRUCTURAL VARIANTS IN THIS GENE FAMILY. G. Uhl*, S. Shimada, A. Persico, P. Gregor, E. Nanthakumar & S. Kitayama. *Labs. Mol. Neurobiol., ARC/NIDA & Depts. Neurol. Nsci., JHUSM, Box 5180, Baltimore, MD. 21224.*

A sodium dependent neurotransmitter transporter gene family has been defined by GABA, norepinephrine, dopamine (DAT), and serotonin (SERT) transporter cDNAs. While cloning the DAT cDNA, we developed data concerning 20 members of this family identified in screening 5 x 10^5 plaques from a size-selected midbrain cDNA library. 8 of the cDNAs encoded GABAT, 3 DAT, and 1 SERT while 8, including v7-3, were novel. v7-3 and 2 related clones encoded amino acids 60% homologous to other transporters and 12 hydrophobic putative transmembrane domains. The 4th extracellular loop was strikingly long, and displayed N-linked glycosylation sites. v7-3 mRNA was highly expressed in thalamus, hippocampus, olfactory bulb, and cerebellum but not peripheral tissues. *Xenopus* oocytes injected with mRNAs synthesized from the 8 orphan clones failed to display specific uptake of any of 9 candidate substrates. These data suggest diversity in the size and structures of members of this sodium dependent transporter gene family.

251.6

IDENTIFICATION OF A cDNA CLONE WITH HOMOLOGY TO THE ADRENAL CHROMAFFIN GRANULE AMINE TRANSPORTER.
D.L. Peter, Y. Liu, C. Weigmann, N. Brecha, and R.H. Edwards*. Department of Neurology, University of California, Los Angeles, 90024.

We have previously isolated a cDNA clone for the chromaffin granule amine transporter using the genetic strategy of selection in MPP^+. To identify related vesicular amine transporters in the brain, a rat midbrain cDNA library was screened with this cDNA. Sequence analysis of the positive midbrain clones revealed one with significant homology to the chromaffin granule amine transporter. Northern blot analysis of $polyA^+$ RNA indicate expression in the midbrain, pons, medulla, and in a midbrain cell line. In situ hybridization analysis of rat brain demonstrate its expression in aminergic populations.

251.7

OBSERVATION OF TWO VESICULAR COMPARTMENTS IN A SINGLE NERVE CELL.
Andrew G. Ewing*, Helle Kristensen and Yau Yi Lau. Department of Chemistry, Penn State University, University Park, PA 16802.

A critical hypothesis in single-cell neurophysiology is the existence of two vesicular compartments for neurotransmitters. Neurotransmitters in the functional compartment are readily available for stimulated release, whereas those in the nonfunctional compartment are thought to act as long-term stores or perhaps for potentiation of response following extremely heavy neuronal stimulation. The bulk of the analytical data suggesting the presence of these two compartments has been obtained by study of tissue levels of neurotransmitters following inhibition of synthesis. We have used capillary electrophoresis in narrow-bore capillaries to examine whole cell levels of dopamine in single large invertebrate neurons. Variation of the sampling conditions apparently leads to selective lysing of vesicles in the functional vs nonfunctional compartments and, in fact, leads to two different peaks for dopamine in a single electropherogram. The first peak is modelled to contain dopamine (6.1 ± 3.0 fmol (n=3)) from the cytosol and in vesicles poised for exocytosis. The second peak is abolished by long lyse times or the application of reserpine and, hence, appears to contain dopamine (19 ± 9.1 fmol (n=3)) from storage vesicles that are not readily releasable.

251.8

AMPHETAMINE ACTION EXAMINED IN REAL-TIME IN THE GIANT DOPAMINE NEURON OF *PLANORBIS*.
[1]D. Sulzer*, [2]Y.Y. Lau, [2]A. G. Ewing and [1]S. Rayport. [1]Dept. Psychiatry, Ctr. Neurobiology & Behavior, Columbia Univ.; Dept. Neuropathology, NYS Psychiatric Inst., NY 10032. [2]Dept. Chemistry, Penn State, University Park, PA 16802.

In the weak base model of psychostimulant action (Sulzer & Rayport, *Neuron* 5;759, 1990), amphetamine (AMPH) disrupts the synaptic vesicle pH gradient, redistributing catecholamines from vesicles to the cytosol; the elevated cytosolic catecholamine in turn promotes release through reverse action of the uptake transporter (Sulzer *et al., Soc. Neurosci. Abstr.*, 1991). To test this mechanism further, we applied ultra-small volume electrochemical and capillary electrophoresis techniques developed by Ewing *et al.* (this meeting) to the giant dopamine (DA) neuron of the snail *Planorbis corneus*. By injecting DA or AMPH into the neuron and measuring DA efflux in real time with an extracellular carbon-ring electrode, we confirmed that increased cytosolic DA promotes reverse transport. Injection of 20 fmol of DA increased extracellular DA by 40 µM; DA release was rapidly and reversibly blocked by nomifensine (10 µM). Injection of 0.8 pmol of AMPH increased extracellular DA by 1.5 µM. These results are the first direct demonstration of reverse transport of neurotransmitter and suggest that AMPH acts intracellularly to release DA. To examine AMPH effects on vesicular DA, we performed capillary electrophoresis on single neurons. 100 µM AMPH for 15 min increased DA in the cytosol and in vesicles poised for exocytosis (these are not resolved with this technique) from 6.1 ± 3.0 fmol (n=3) to 107 ± 23 fmol (n=6), whereas DA in reserpine-sensitive vesicles decreased from 19 ± 9.1 fmol (n=3) to below detectable levels (< 10 amol; n=3). These results are inconsistent with an exchange diffusion model, in which AMPH should lower cytosolic DA; rather, the increase in cytosolic DA with a decrease in vesicular DA supports the weak base model.

251.9

MECHANISMS OF CALCIUM RESPONSIVE SECRETION IN MEDULLARY THYROID CARCINOMA CELLS.
S.A. DeRiemer,* H.S. Hsiung, & H. Tamir. Depts. of Anatomy and Biological Sciences, Columbia Univ., NY NY 10032.

The parafollicular C-cells of the thyroid respond to elevations of serum calcium by releasing the calcium-lowering hormone, calcitonin, as well as serotonin (5-HT). Both 5-HT and calcitonin are found in the same secretory granules, and they have been shown to be co-released. Medullary thyroid carcinoma (MTC) cells derived from a human C-cell tumor have been used to analyze the coupling between extracellular calcium and 5-HT secretion. Intracellular calcium levels were determined using fura-2 spectroscopy, and 5-HT secretion was monitored by HPLC with electrochemical detection. Changes in extracellular calcium from 0.01 - 7 mM produced proportional increases in both intracellular calcium and 5-HT secretion. This suggests that intracellular calcium acts as the mediator for the signals produced by altered extracellular calcium levels. The intracellular calcium levels correlated with 5-HT secretion ranged from 10 µM to 35 µM suggesting that the secretory apparatus in MTC cells responds to intracellular calcium levels similar to those stimulating secretion in other nerve and endocrine cells. Electrophysiological analysis using whole cell patch clamping on MTC cells revealed the existence of a non-inactivating calcium current resembling, but not identical to a dihydropyridine -sensitive 'L-type' calcium channel. Two of the distinctive properties noted were a slower time couse of inactivation (tails) and less of a difference in the conductance of barium vs calcium than expected from a prototypical 'L-type' channel. Application of the dihydropyridine antagonist nimodipine (10^{-5} M) inhibited stimulated 5-HT release by 80%. This effect was reversed by the dihydropyridine agonist, BAYk8644 (10^{-7} M).

Support provided by grant MH37575 to HT and a Klingenstein Fellowship to SD.

251.10

EXPRESSION OF THE SMALL GTP-BINDING PROTEIN RAB3A IN THE ADULT RAT BRAIN.
O. Stettler*, A. Zahraoui, K.L. Moya and B. Tavitian. INSERM U334 and CNRS URA 1285, C.E.A., S.F.H.J., Orsay, and INSERM U248, Paris, France.

The *ras*-related *rab* genes code for small GTP-binding proteins that are thought to control intracellular membrane trafficking. Some of the rab proteins are localized to the membrane of specific subcellular organelles, and rab3A is associated with small synaptic vesicles. Previous immunohistochemical studies have revealed terminal regions and several specific neural circuits which contain rab3A, but since the protein is not present in cell bodies, those studies were unable to identify the neurons expressing this small G protein. In this study, we have used in situ hybridization with a specific probe to localize the rab3A mRNA in the adult rat brain and we have found an unique pattern of neurons that express this protein. In the forebrain, rab3A mRNAs were mostly detected in neocortical and limbic areas such as hippocampus, enthorinal cortex and rhinencephalic nuclei, while no significant labelling was observed in the striatum, in the hypothalamus or in several thalamic nuclei. Rab3A expression does not directly correspond to any known neurotransmitter expression pattern nor is it completely superimposable to the pattern of expression of other synaptic vesicle proteins. Our results show that the expression of rab3A is spatially restricted and thus, that the protein exerts its function in a subset of neurons in the brain.

In combination with previous studies of other synaptic vesicle proteins, the present results suggest that subsets of presynaptic terminals can be characterized at a molecular level by their vesicular proteins, which may impart unique functional properties. The complex combinations of such synaptic components would provide multiple molecular mechanisms for the precise regulation of exocytosis in the brain.

251.11

GLUCOSE DEPRIVATION INHIBITS NEUROTRANSMITTER RELEASE AND DECREASES UDP-GLUCOSE INDEPENDENT OF ATP. N.A. Veyna*, and R.B. Marchase, Department of Cell Biology, The University of Alabama at Birmingham, Birmingham, AL 35294

It has been previously established that glucose deprivation diminishes electrical responses prior to a reduction in ATP or other high energy phosphates in both retina (Winkler, J. Gen. Physiol. 77:667-692, 1981) and hippocampal slices (Cox and Bachelard, J. Physiol. 352:91-102, 1984). We suggest that this loss of activity is due to a decrease in vesicular neurotransmitter release. Utilizing a synaptosomal model system we find a 41% decrease in release of [^{3}H]norepinephrine in response to 40 mM KCl after a 20 minute incubation in glucose-free buffer (n=10). Luminometric assays were performed on identically treated synaptosomes to determine ATP and UDP-Glc content. A 78% decrease was seen in UDP-Glc pools (n=6), whereas ATP content was decreased by a statistically insignificant 0.8% (n=6).

When synaptosomes undergo a freeze-thaw permeabilization to allow the introduction of radiolabeled UDP-Glc a single protein of 63 kDa is labeled. This protein, PGP 63, contains glucose in a phosphodiester linkage, and its analogue has previously been implicated in vesicular release mechanisms in *Paramecia* (Satir et al., J. Cell Biol. 111:901-907, 1990). Interestingly, PGP 63 is more robustly labeled when synaptosomes containing radiolabeled UDP-Glc are depolarized with 40 mM KCl.

Supported by NIH EY 06714 (RBM) and a Patricia Roberts Harris Predoctoral Fellowship (NAV).

251.12

ACETYLCHOLINESTERASE REGULATION IN *APLYSIA*: EFFECT OF AGE AND CHRONIC SENSORY STIMULATION. B. Peretz*, M. Srivatsan and B. Hallahan, Dept. of Physiology Univ. of Kentucky Med. Cntr., Lexington, KY 40536.

Acetylcholinesterase (AChE) is a major protein in the hemolymph of *Aplysia*. It apparently insures a ready supply of choline for the nervous system and may be involved in trophic function. Both age and chronic sensory stimulation (CSS) affect hemolymph AChE activity (Srivatsan et al., 1992). We wanted to find the source of hemolymph AChE and the effect of age and CSS on its source. The central nervous system (comprised of 4 paired ganglia and the abdominal ganglion) and tissues such as the gill, foot muscle, buccal muscle, liver, heart, and gonad were examined in young (ca 85 days old), mature (ca 170 days old) and old (over 250 days old) animals. Hemolymph had the highest AChE activity of any tissue in the animal, followed by the activity in the gill and foot. With increased age the activity in the two tissues was decreased, and the activity in the nervous system increased. CSS (siphon stimulation for 4 weeks) resulted in decreased AChE activity in the ganglia and other tissues tested in young and mature *Aplysia*, and increased activity in the ganglia and tissues from old *Aplysia*. We next tested for release: Pooled ganglia and sections of the gill and foot musculature were subjected to depolarization with high K^+ (ca 100mM) in seawater of (1050 mOsm) for up to 1 hr; little or none was measured in control tissues in seawater (10 mM KCl). Little release was measured from the ganglia. The gill and foot did release AChE in the three age groups. From the gill, released activity/g. tissue for the young was 68.22 ± 39.27 units (n=6); for the mature was 33.41 ± 18.12 units (n=6); and for the old was 41.77 ± 18.65 units (n=4); 1 unit = AChE that hydrolyzes 1μmole of substrate/min. The foot showed an age-related pattern of release similar to the gill. These results indicate that with age and to CSS hemolymph AChE activity reflects the activity in the tissues. And, the gill and foot may be sources of hemolymph AChE. The mechanisms of AChE release into the hemolymph need to be determined. (NIA)

251.13

DETECTION OF PROTEIN TYROSINE KINASES (TKs) AND THEIR SUBSTRATES IN THE POSTSYNAPTIC DENSITY (PSD) OF ADULT RAT BRAIN. S.Y. Lin[1], K. Wu[1], J.E. Adler[2]*, J. Boelen[3], R.C. Penhallow[3], T.W. Kim[1], Y. Huang[4], J.L. Xu[1] & I.B. Black[1], [1]Dept. Neurosci. and Cell Biol., UMDNJ/RWJ Med. Sch., Piscataway, N.J. 08854; [2]Dept. Neurol., Wayne State Univ. Med. Sch., Detroit, M.I. 48201; [3]Bristol-Myers Squibb, Pharma. Res. Inst., Princeton, N.J. 08543 and [4]Div. Neurosci., NYSPI, New York, N. Y. 10032.

Recent studies showing high level of TK activity in adult rat brain suggest that the enzyme(s) may mediate signal transduction in the nervous system. Since abundant evidence indicates that the PSD is crucial for synaptic function, we examined this structure for TKs and substrates. Our results revealed that the specific PTK activator, Mn^{2+}/Vanadate, enhanced phosphorylation of several proteins (M.W.=20 to 250 kDa) in the PSD and synaptic membrane (SM). In addition, the presumptive TKs were greatly enriched in the PSD, compared with those in SM or total homogenate (H). Moreover, Western blot analysis using rabbit anti-phosphotyrosine antibody indicated that the PSD isolated from cerebral cortex, cerebellum and olfactory bulb contained several tyrosine-phosphorylated proteins (M.W.=20 to 250 kDa), which appeared to be PTKs and their substrate proteins. These phosphorylated proteins were differentially expressed in these brain regions. We conclude that PTKs and their substrates are present in the PSD and may play roles in synaptic function. (NS10259 and HD23315)

HUMAN COGNITION: ELECTROPHYSIOLOGY II

252.1

IMAGING OF STIMULATION AND FUNCTIONALLY EVOKED INTRINSIC OPTICAL CHANGES IN MONKEY AND HUMAN CORTEX. D.W. Hochman*, G.A. Ojemann, M.M. Haglund, Dept. Neurological Surgery, Univ. of Washington, Seattle, WA 98195

In a variety of preparations, it has been demonstrated that changes in the intrinsic optical properties of tissue are associated with neural activity. Using video imaging, we studied stimulation and functionally evoked activity in monkey and human cortex. Pairs of images of macaque visual cortex were acquired during left eye and right eye visual stimulation. Each image consisted of at most 96 frames averaged at 30 Hz. Subtraction of these pairs yielded difference maps of ocular dominance columns (signal/noise: 0.1-0.5). Comparing control images (no visual stimulus) to stimulation images, temporal information regarding the onset and recovery of these optical changes was obtained. Large changes (>15%) were observed in the magnitude of optical absorption of the cortex between control images and stimulation images. After surface electrodes were placed on the cortex of human patients, electrical stimulation evoked large optical changes (>5.0%) in a graded manner near the stimulating electrodes. Negative optical changes were observed surrounding these areas of intense activity. In some cases, epileptiform activity was evoked by the cortical stimulation. The optical changes observed during these afterdischarge episodes corresponded to the electrical activity recorded with surface electrodes. Optical changes were evoked in human cortex during sensory (tongue movement) and language tasks. Both the positive and negative optical changes were larger than those evoked by direct electrical stimulation (up to 30%). Computer analysis of our images enabled us to follow the temporal characteristics of the opticalchanges in the vasculature and many cortical areas simultaneously. (Research supported by the Klingenstein Foundation Fellowship to MMH)

252.2

OPTICAL IMAGING OF EPILEPTIFORM AND COGNITIVE ACTIVITY IN HUMAN CORTEX. M. M. Haglund*, G. A. Ojemann, and D. W. Hochman. Dept. Neurological Surgery, Univ. Washington, Seattle, WA 98195.

Optical imaging in animal somatosensory, olfactory, and visual cortices has revealed high resolution maps of functional activity (Orbach et al., 1985; Blasdel and Salama, 1986; Kauer, 1988; Frostig et al., 1990; Ts'o et al., 1991). After obtaining informed consent, similar video imaging techniques were used in patients undergoing awake craniotomies for intractable epilepsy (n = 15). We obtained maps of epileptiform and functional activity from human cortex. Epileptiform activity evoked by bipolar stimulation for 4 sec (60 Hz, 1 msec biphasic pulses, 2-8 mA) was recorded by surface EEG electrodes both within the optical imaging field and outside the field-of-view. The optical changes correlated with the electrical activity recorded from nearby surface EEG electrodes with the intrinsic reflection signals increasing as the intensity and duration of the afterdischarge activity increased.

After cortical stimulation mapping identified sensory/motor cortex and essential language sites (Broca's and Wernicke's areas), large optical changes were found in somatosensory cortex during tongue movement. In the anterior language areas, naming evoked optical changes that were localized in the anatomical Broca's area and not in areas of speech arrest identified by surface stimulation. In the posterior language areas, naming evoked optical changes in not only essential language areas for naming, but also secondary language areas. These secondary language areas, not identified by surface stimulation, were verified when the subject's language deteriorated as the surgical removal of cortex neared these areas. The extension of high resolution optical imaging to human cortex will open new areas of investigation into the cortical organization of sensory/motor cortex, language, and cognitive processes. (MMH supported by Klingenstein Foundation and American Association of Neurological Surgeons Research Foundation Fellowships; GAO supported by NIH NS21724,17111, and 20482.

252.3

An Electrophysiological Study of Infants' Sensitivity to the sound structure of English Function Words. V.L. Shafer[1,2], D.W. Shucard,[*1,3] J.L. Shucard[1], L.A. Gerken[3], [1]Departments of Neurology, [2]Linguistics and [3]Psychology, SUNY @ Buffalo, 100 High Street, (D-6), Buffalo, NY 14203.

A major issue in the study of language acquisition is the age at which infants attend to specific aspects of language structure. Our work explores the possibility that children are sensitive to the phonological properties of function morphemes even before they have begun to produce their first words. Auditory evoked potentials (AEP) to task-irrelevant pairs of tone probes were obtained to investigate the sensitivity to English function morphemes of 10- to 12-month-old infants. Infants received three conditions; a baseline condition of only tones; and two experimental conditions consisting of task-irrelevant tone probe pairs superimposed on an unmodified version of a story, and a modified version of the same story in which the function morphemes had been replaced by nonsense forms (e.g. ku, po). The pattern of AEP fast habituation and amplitude asymmetry to the tone pairs were examined across conditions. The following results were obtained: 1) There was a general decrease in amplitude from the baseline to the experimental conditions, and 2) the pattern of fast habituation within each cerebral hemisphere differed across conditions. For example, there was less habituation in the right hemisphere relative to the left hemisphere during the unmodified version of the story. The opposite pattern occurred for the modified version. Furthermore, for the second of the tone pairs, there was a reversal of amplitude asymmetry between the experimental conditions. These findings support the notion that 10- to 12-month old infants are sensitive to the phonological and/or prosodic information carried by function morphemes, and 2) that attentional resources in each cerebral hemisphere are allocated differentially among the three condition.

252.4

Fast Habituation of Scalp-Recorded Auditory Evoked Potentials to Paired Tone Stimuli in Infants. J.L. Shucard*, D.W. Shucard, V.L. Shafer, L. E. Ferretti, S. A. Kubow Department of Neurology, SUNY @ Buffalo, 100 High Street, (D-6), Buffalo, NY 14203.

Fast habituation of the averaged evoked potential was described by Callaway (1973) as a general reduction in evoked potential amplitude that occurs to stimuli presented less than 10 seconds apart. When auditory stimuli are presented in pairs, separated by 2 seconds, with an interpair interval of approximately 10 seconds, averaged evoked potentials to the second stimulus may have as little as half the amplitude as that to the first stimulus of the pair. It has been shown that fast habituation depends somewhat on the subject's anticipation of the stimulus, attention to the stimulus, or knowledge of the interstimulus interval. In our laboratory, we have exploited this phenomenon of auditory evoked potential (AEP) fast habituation to study the allocation of cerebral resources under a variety of experimental conditions in infants, children and adults. In this investigation we present data describing the scalp distribution of AEPs associated with paired tone stimuli in infants less than one year of age. AEPs were recorded in awake infants to pairs of auditory tone bursts (100 msec, 600 Hz, approximately 70db) separated by two seconds with an interpair of greater than 6 seconds. Findings showed that 1) there was a marked amplitude decrement from tone 1 to tone 2 in frontal and temporal but not parietal scalp locations; and 2) right hemisphere recordings from frontal and temporal sites but not parietal sites showed higher amplitude responses than left. These findings occurred for AEP components between 100-500 msec. These results indicate that "fast habituation" to paired tone stimuli is present in infants and that it varies with both left to right and rostral to caudal scalp locations. This technique may prove useful for studying the development of orientation and attention in infancy.

252.5

ELECTROPHYSIOLOGICAL MEASURES OF SEMANTIC PROCESSING IN HUMANS. A.C. Nobre*, G. McCarthy. Neuropsychology Laboratory, VAMC, West Haven, CT 06516 and Departments of Psychology and Surgery, Yale University.

Event-related potentials (ERPs) were recorded from cortical surface and depth electrodes from patients performing language tasks. The tasks were designed to manipulate semantic processing and involved categorization of linguistic stimuli viewed successively. One task included intermixed pairs of semantically related or unrelated words, while the other included orthographically illegal nonwords (e.g., 'xmwtf'), pseudowords (e.g., 'flepple'), grammatical connectives (e.g., 'therefore'), and concrete nouns. Scalp recordings showed that these manipulations of semantic processing modulated an ERP which occurred near 400 msec (N400) following word onset. Intracranial studies using an anomalous-sentence task have shown that the anterior-medial temporal lobe is one neural generator for N400 (McCarthy & Wood 1984, Soc. Neur. Abs.). Recordings in the anterior-medial temporal lobe during the two categorization tasks also exhibited an N400 that was elicited by concrete nouns and was modulated by semantic priming. Illegal nonwords and grammatical connectives elicited little N400 activity, however, pseudowords did elicit N400. In addition, neocortical stimulation and recordings were performed in patients with electrode grids over the left hemisphere. Electrode sites which caused disruption of naming and comprehension during cortical stimulation, exhibited focal electrophysiological potentials that were modulated by the task manipulations.

252.6

PARALLEL DISSOCIATION OF HUMAN RECOGNITION/RECALL AND TWO DISTINCT ERP COMPONENTS THROUGH THE MANIPULATION OF ITEM MEANINGFULNESS. Daniel M. Rice* & Thomas F. Locke, Andrus Gerontology Center, University of Southern California, Los Angeles, CA. 90089.

In a first experiment, seventeen young adults had 32 channel topographical ERPs while they were engaged in a continuous verbal recognition memory task in which item meaningfulness (moderate frequency words vs. pronounceable non-words) and item repetition (novel vs. repeat) were manipulated. Items were presented every three seconds on a CRT. Item repetition within 6-18 seconds was associated with 1) an increased positive component over the entire head during the 400-600 msec poststimulus period for both words ($p<.008$) and non-words ($p<.004$), and 2) an increased negative component over the entire head during the 800-1200 msec poststimulus period for words ($p<.003$), but not for non-words. All of these effects were largest in the posterior leads in this nose reference recording. Because there were no significant differences between recognition performance accuracy or response time for words vs. non-words and because the late negative ERP enhancement to repeated words happened after the average response time, this late negative enhancement would not appear to reflect a process which is necessary for recognition memory. In a second experiment, the identical timing and word/non-word list were employed as in the first experiment, but the items were not repeated verbatim. Instead, the first few letters of each item were repeated. Ten young adult subjects were now asked to recall the item recently seen which uniquely corresponded to the letter cue presented. Words were recalled better than non-words in all ten subjects in this letter cued recall task ($p<.0001$). These experiments suggest that 1) moderate frequency words are recalled, but not recognized, better than non-words, and 2) a pattern of ERP effects parallels this dissociation.

252.7

SUPPRESSION OF UNATTENDED-CHANNEL MISMATCH-RELATED ACTIVITY IN HUMAN AUDITORY CORTEX DURING AUDITORY SELECTIVE ATTENTION. M.G.Woldorff*(1), C.C.Gallen(2), S.R.Hampson(2), S.A.Hillyard(1), C.Pantev(2), and F.E.Bloom(2). Dept. of Neurosci., UCSD, La Jolla, CA 92093-0608 (1), Dept. of Neuropharm., Research Inst. of Scripps Clinic, La Jolla, CA, 92037 (2).

The mismatch negativity (MMN) and the corresponding mismatch magnetic field are elicited by infrequent, physically deviant sounds in a sequence of repetitive auditory stimuli. Previously, Naatanen and colleagues had asserted that this wave was unaffected by attention and thus reflected a strongly automatic feature-analysis and mismatch-detection process. These proposals were called into question by two recent fast-rate dichotic listening experiments designed to optimize the selective focusing of attention (Woldorff, Hackley, and Hillyard, 1991). In these experiments, the deviant tones (intensity decrements) in the attended ear elicited deviance-related negative (DRN) waves consistent in latency (peaking at 200 msec), waveshape, and distribution with previously described MMNs, but the MMN elicited by the unattended-channel deviants appeared to be highly suppressed.

We have now recorded the tone-evoked neuromagnetic fields in an experiment with very similar parameters to one of our previously reported MMN experiments. Like its electrical counterpart, the deviance-related field (DRF) in the unattended channel was extremely small. In the attended channel, however, the DRF peaking at 200 msec (as visualized in the grand average across subjects) was of substantial amplitude, and, like the auditory-evoked N100, could be well-modeled as originating from a focal dipolar source in the auditory cortex on the supratemporal plane (STP). Furthermore, the difference wave derived by subtracting the extremely small unattended-channel DRF from the attended-channel DRF also showed this pattern of localization.

These neuromagnetic results indicate that the additional deviance-related activity in the attended channel at 200 msec does not primarily consist of a non-specific N2b component as proposed by Naatanen (1991), but rather arises predominantly from activity in the specific auditory cortical areas on the STP. Moreover, it appears that this specific auditory analysis/mismatch activity can be highly suppressed in one channel when attention is strongly focused on another.

252.8

AMPLITUDE OF EVENT-RELATED POTENTIALS GENERATED IN THE CONTEXT OF MNEMONIC DEMAND IS DIRECTLY RELATED TO MRI DERIVED HIPPOCAMPAL VOLUME. L. deToledo-Morrell*, M. Sullivan, D. Charletta, F. Morrell & C. Spanovic. Depts. of Neurol.Sci., Psychol. & Diag. Radiol., Rush Med. Coll., Chgo, IL 60612.

There is some uncertainty about the neural generators of the P300 component of event-related potentials (ERPs). To examine the proposition that the amplitude of the P300 potential elicited under conditions of mnemonic demand reflects the integrity of the hippocampal formation, we studied 21 subjects electrophysiologically and with a high resolution MRI protocol to assess hippocampal volume. Subjects consisted of patients with various dementias, with structural neocortical lesions, aged and young controls. ERPs were recorded referentially during a modified Sternberg memory scanning task with letters as probes as well as during a visual discrimination (oddball) task that did not contain a memory component. Hippocampal volume was computed from MRI scans of gapless coronal slices taken perpendicular to the long axis of the hippocampus. With memory loads of 3-5 letters, P300 amplitude was significantly correlated with hippocampal volume irrespective of diagnosis or age ($r=0.812$, $p<.001$). In the same subjects, the amplitude of the P300 potential elicited in the spatial oddball task did not relate to hippocampal volume ($r=0.339$, $p>.05$). These results clearly indicate that the neuroanatomical substrate (i.e., the generator site) of the P300 potential may vary depending on the nature of the eliciting task.

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252.9

RETROACTIVE ENHANCEMENT OF A SKIN SENSATION BY A DELAYED CORTICAL STIMULUS, IN MAN.

B. Libet, E.W. Wright, B. Feinstein and D.K. Pearl. Dept. of Physiology, UCSF, San Francisco, CA 94143-0444, and Dept. of Statistics, Ohio State Univ., Columbus, OH

Retroactive or backward masking had been shown earlier with either a second peripheral stimulus (by others) or a delayed cortical stimulus (by us) as the masking agent. We now report that retroactive enhancement can also be produced when a train of stimulus pulses to somatosensory cortex (C) follows a skin stimulus (S) by up to 400 msec or more. Two identical stimulus pulses (S_1 and S_2) were applied to the same skin electrode, S_2 following S_1 by 5 sec. Subjects reported whether S_2 felt stronger than, same as, or weaker than S_1 in the control trials, and with the C stimulus added some time after S_2 in experimental trials. S_2-C intervals ranged from 25 msec to 500 msec or more. In control series S_2 was reported same as S_1 in about 2/3 of trials, and stronger than S_1 in 1/3 of trials. With C added, S_2 was reported stronger in about 2/3 of trials and same as S_1 in 1/3, for all S_2-C intervals to 400 msec; difference from controls was significant at p=<.0001. The long S_2-C intervals effective for retro-enhancement provide strong support for our thesis that the development of a conscious sensory experience, and perhaps awareness of any event, requires a substantial duration of up to 0.5 sec of appropriate cortical activity.

252.10

COGNITIVE PROCESSING IN A THREE CHOICE REACTION TIME PARADIGM AND ITS MODULATION BY TRANSCRANIAL MAGNETIC STIMULATION (TMS). E. Gomez-Tortosa, A. Pascual-Leone, D.W. Alway, P. Nichelli, M. Hallett and J. Grafman *. Cognitive Neuroscience and Human Motor Control Sections, NINDS, NIH, Bethesda, MD 20892

Neural processes involved in a choice reaction time (RT) task may vary according to the number of choices. In a 3-choice RT paradigm we studied 8 right-handed normal volunteers (4 men and 4 women, aged 22 to 50 years). A monitor displayed an asterisk randomly either on the right, on the left, or on both sides of a fixation point. Subjects were asked to respond as fast as possible by pressing either a single key on the same side of the stimulus or both keys at the same time in case of bilateral stimuli. Unimanual RT was equal with right and left hand and significantly shorter than RT for the faster hand in bimanual responses in all subjects. With bilateral stimuli, 6 subjects responded in >60% of the trials with the sequence "right-left", while two preferred the sequence "left-right". Intermanual interval was about 5 ms for the preferred and 25 ms for the non-preferred sequence, but RT for the first key press in responses with the preferred sequence was approximately 200 ms longer than in those with the non-preferred sequence. There was no significant difference in RT between trials with unilateral stimuli and trials with bilateral stimuli responded with the non-preferred sequence. Subthreshold TMS of the sensorimotor area concurrently with the stimuli induced faster RTs in unimanual contralateral responses and biased all subjects to lead bimanual responses with the contralateral hand despite long intermanual interval. Although the task encompassed 3 choices, subjects completed it in only 2 processing steps, one for unimanual responses (short RT) and the other for bimanual responses with the preferred sequence (long RT). Bimanual responses with the non-preferred sequence may represent processing errors in which a unimanual response is initiated but corrected in time; this results in short RTs for the first hand but long intermanual intervals. Lateralized TMS speeds up the pre-movement excitability build-up for contralateral hand movements, thus shortening unimanual RT and inducing processing errors for bimanual responses.

252.11

IMPROVED EEG/MEG SOURCE LOCALIZATION BY COMBINING A CORTICAL SURFACE MODEL WITH SPATIO-TEMPORAL CONSTRAINTS. A.M. Dale* and M.I. Sereno, Cognitive Science Department, University of California San Diego, La Jolla, CA 92093.

We recently described a linear approach to the inverse problem of localizing cortical current sources using EEG and MEG data and anatomical constraints (Dale and Sereno, *NS Abs.*, 1991). This technique works well with shallow sources and is computationally tractable, even for several thousand source dipoles (required for a minimal tiling of the cortical sheet). Optimal linear inverse operators, however, typically mis-localize deeper sources. Even with constraints on dipole location and orientation, and noise and source covariance, there are numerous shallower, spread-out solutions in the case of a deep focal source that are also consistent with the data for a single time step. Recently, Mosher et al. (*NS Abs.*, 1991) described an application of the MUSIC algorithm to the source localization problem using constraints arising from the structure of the sensor data over time. We have integrated a MUSIC-like constraint into our approach, and show with model studies that we can obtain high spatial resolution with moderate numbers of sensors.

The cortical sheet is first found by orthogonal slice combination, flood-filling, and deformable template refinement (Sereno and Dale, this volume). The sensor spatio-temporal covariance matrix is then estimated using a finite series of timesteps from an averaged evoked response. An eigenvalue decomposition of the sensor covariance matrix and the forward solution are used to obtain estimates of the variance of each source dipole. These are inserted into the equation for the optimal linear inverse operator as *a priori* source variance estimates. The inverse operator is then used to calculate a solution for each time step. Our formulation does not require setting an explicit eigenvalue threshold and reduces smoothly to a minimum-norm like approach when the eigenvalues are almost equal (as with white noise).

Model studies using a real cortical sheet (~150,000 vertices subsampled to ~10,000 dipoles) and 61 EEG and 61 MEG sensors distributed around the head show that the combination of the anatomical and spatio-temporal constraints allows us to resolve multiple, relatively deep sources even when they overlap substantially in time. By restricting source dipoles to be locally perpendicular to the cortical sheet, "ghost" sources (e.g., between two temporally correlated sources) that would appear using an unconstrained MUSIC approach are almost always ruled out.

Supported by UCSD McDonnell-Pew Cognitive Neuroscience Center.

252.12

A TECHNIQUE FOR RECONSTRUCTING AND FLATTENING THE CORTICAL SURFACE USING MRI IMAGES. M.I. Sereno* and A.M. Dale, Cognitive Science Dept., University of California San Diego, La Jolla, CA 92093.

In order to more strongly constrain the solution to the inverse problem of localizing cortical current sources using MEG and EEG (Dale and Sereno, this volume), we needed to reconstruct the shape of the entire cortical sheet for individual subjects from MRI images, since this varies substantially among different people. Stacked-section images displayed with interslice interpolation, transparency, and lighting are realistic-appearing, but they lack explicit information about the orientation of a local surface patch. Since manual methods for making a cortical surface model are labor intensive and error-prone, we devised a more automatic, three-stage method for generating a high-quality wireframe cortical surface model.

2-D MRI images have good in-plane resolution (<1mm), but sections are usually thick (3-6 mm), smearing the image of the cortical surface when it is oriented nearly parallel to the slice plane. Therefore three orthogonal series of inversion-recovery sections (needle-shaped voxels) optimized for contrast between the gray and white matter are combined into a single volumetric data set with the same high (sub-slice) resolution in all directions (cubic voxels) using a linear estimation technique.

After automatic skull removal, an initial estimate of the boundary between the cortical gray and white matter is found by recursive flood-filling. White matter (classified by voxel intensity) is initially flood-filled in 3-D from a single seed location. Internal holes and external islands are eliminated by successive "outside in" and "inside out" fills. A single, closed tessellation of the white-matter surface can then be constructed from the faces of filled voxels bordering unfilled voxels.

The surface is refined using a deformable template algorithm. The location of each vertex is updated according to elastic "forces" between neighboring vertices, and repulsive and attractive forces along the local surface normal that depend on the MRI data at the vertex. An adaptation of this technique is used to computationally flatten the cortical sheet to view EEG/MEG (or PET) foci. By freeing the surface from the MRI data, the algorithm causes it to relax toward minimal surface tension, producing a representation of the cortical sheet with no folds, but one that preserves the topology of the folded cortex. The partially flattened representation retains its global shape, appearing as if it has been gently inflated, effectively exposing hidden sulcal cortex. The partially flattened images are reminiscent of a macaque brain, and much easier to interpret than a complete flattening, which requires cuts.

ION CHANNEL MODULATION I

253.1

PROTEIN KINASE A MODULATES AN ENDOGENOUS VOLTAGE-DEPENDENT CALCIUM CHANNEL IN *XENOPUS* OOCYTES. Y. Chen, J. D. Pollock*, Y. Wang, A. DePaoli-Roach, L. Yu, Dept. of Med. & Mol. Genetics, Indiana Univ. Sch. of Med., Indianapolis,IN 46202.

Xenopus oocyte possesses a calcium channel that is activated by membrane depolarization. The calcium influx through this channel activates a calcium-dependent chloride channel and results in a transient outward current (T_{out}). T_{out} is increased by cAMP-dependent phosphorylation of the endogenous voltage sensitive calcium channel in *Xenopus* oocyte. Treatment of oocytes with forskolin, IBMX, or cAMP injection increases the magnitude of T_{out} in a dose- and time-dependent fashion without a change in voltage dependence. The effect of cAMP on T_{out} is mediated through the activity of protein kinase A (PKA). The effect of cAMP and PKA on the increase of T_{out} is blocked by a PKA inhibitor PKI as well as a mutant RII, which inhibits PKA in the presence of cAMP. Furthermore, the increase of T_{out} by cAMP injection can be inhibited by both type I and type II phosphatases. A barium current is augmented by cAMP injection, and the magnitude of the increase is directly proportional to that of T_{out}. These results suggest that the increase of T_{out} produced by cAMP-mediated phosphorylation results from a modulation of the endogenous calcium channel in the oocyte.

253.2

ACTIVATION OF CHLORIDE CHANNELS IN HUMAN NEUTROPHILS BY TUMOR NECROSIS FACTOR α VIA CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE. M. A. Schumann*, P. Gardner, and T. A. Raffin. Division of Pulmonary and Critical Care Medicine, Stanford University School of Medicine, Stanford, CA 94305.

The mechanism underlying tumor necrosis factor α (TNFα)-induced intracellular Ca^{2+} changes and activation of Cl^- channels in polymorphonuclear neutrophil leukocytes is unknown. By means of the whole-cell patch clamp methodology, we studied the mechanism of activation of Cl^- channels by TNFα in human neutrophils. The current was carried by Cl^- ions and was activated by Ca^{2+} ions. Bath application of ionomycin (1μM) activated the control current significantly. Bath application of TNFα (1000 U) resulted in comparable activation of the current in 80% of tested cells. Both amplitude and activation rate of the current were augmented. The activated current was blocked by 10 μM 5-nitro-2(3-phenylpropyl-aminobenzoic acid, a potent Cl^- channel blocker. We found that with the intracellular application of a peptide inhibitor (10 μM) having the same sequence as the inhibitory domain of the multifunctional calcium/calmodulin-dependent protein kinase (273-302), the current ceased to activate, from the level of the control, by the exogenous application of either ionomycin or TNF α. Including 10 μM of the truncated form (nonactive control; 284-302) of the inhibitory peptide in the pipette did not block the TNFα- (or ionomycin-) induced Cl^- current. These results show that Cl^- channel activation by Ca^{2+} in human neutrophils is mediated by Ca^{2+}/calmodulin-dependent protein kinase and this mechanism underlies, in part, the activation of Cl^- channels by TNFα-induced Ca^{2+} changes in these cells.

253.3

PKC ACTIVATION PRODUCES SPIKE BROADENING IN SENSORY NEURONS OF JUVENILE *APLYSIA* PRIOR TO THE DEVELOPMENT OF 5HT-INDUCED BROADENING. E.A. Marcus* and T.J. Carew. Depts. of Biol. and Psych., Yale Univ., New Haven CT 06520

In tail sensory neurons (SNs) of adult *Aplysia*, serotonin (5HT) produces both increased excitability and spike broadening. An important component of 5HT-induced spike broadening is mediated by protein kinase C (PKC)-dependent modulation of the delayed rectifier potassium conductance, Ikv (Baxter and Byrne, 1990). In development, 5HT-induced spike broadening emerges after 5HT-induced increased excitability (Marcus and Carew, 1990). Here we examined whether the lack of 5HT-induced spike broadening in juvenile *Aplysia* reflected an inability of 5HT to activate PKC or alternatively an inability of PKC to modulate Ikv.

Confirming Sugita et al. (1991), we found that activation of PKC via application of phorbol esters (PDAc, 3μM) produced significant spike broadening in adult SNs (x =17.4%, p<0.001, N=13) with no appreciable effect on excitability. We then examined the effects of PDAc on SNs of juvenile *Aplysia* (>1g and ≤ 1.5g) and found that it produced significant spike broadening (x =18.1%, p<0.02, N=8). Moreover, there was no difference between the magnitude of PDAc-induced spike broadening in these juveniles and adults suggesting that the ability of PKC to modulate Ikv is fully mature at this stage. In younger juveniles (>0.5g and ≤1g) PDAc did not produce significant spike broadening (x = 3%, ns, N=8) and the effect of PDAc on spike duration was significantly different from that observed in adults (p<0.001). These data suggest that the ability of PKC to modulate Ikv is not expressed in these juveniles although Ikv appears to be functional at this stage.

We propose a model for the sequential development of neuromodulatory pathways which includes 3 steps: initial expression of an ionic conductance, followed by development of the ability to modulate that conductance with specific second messengers, followed still later by development of the ability to activate the second messenger with a specific modulatory neurotransmitter.

253.4

MODULATION OF AN *APLYSIA* DIVALENT-PERMEABLE CATION CHANNEL BY ENDOGENOUS KINASE AND THE CATALYTIC SUBUNIT OF PROTEIN KINASE A. G.F. Wilson*, and L.K. Kaczmarek. Dept.of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Nonspecific cation channels provide the depolarizing drive underlying bursts of action potentials and also an entryway for cations that mediate secretion. Using bag cell neurons of *Aplysia*, we have studied the modulation of a channel permeable to both monovalent and divalent cations. This channel was present in 50/182 excised patches, but appears only rarely in cell-attached patches. In physiological extracellular solutions (containing in mM: 460 NaCl, 10.4 KCl, 11 $CaCl_2$, 55 $MgCl_2$, 10 Hepes, pH 7.8), the channel exhibits a slope conductance of 30-35 pS, an extrapolated reversal potential close to 0 mV, and is voltage-dependent, the open probability decreasing with hyperpolarization below -60 mV. We have found that ATP (5 mM, the concentration normally present in intracellular solutions) bath applied to inside/out patches results in an increase in channel activity (n= 4/4), presumably via a kinase closely associated with the channel protein. Further, addition of the catalytic subunit of PKA (1-5 μM) decreases the open probability when the starting open probability of the channel is high (n= 6/6), but increases the open probability when the starting open probability is low (n= 3/4). Both effects of the catalytic subunit require ATP and appear to be due to a difference in the behavior of one channel type, since occasionally transitions in activity are observed for single untreated channels. Thus, these effects are best explained by a two phosphorylation site model, where the effect of phosphorylation of the second site depends on whether the first site is phosphorylated. This model is being tested by treating patches with protein phosphatases.

253.5

THAPSIGARGIN ACTIVATES AN INWARD CURRENT AND SPECIFICALLY INCREASES INTRACELLULAR CALCIUM AT THE SOMATA OF BAG CELL NEURONS IN CULTURE. L.S. Kao, R. J. Knox,* E. A. Jonas, J.A. Connor, and L.K. Kaczmarek. Dept. of Pharmacology, Yale University School of Medicine.

A rise in intracellular calcium occurs during a long-lasting afterdischarge in *Aplysia* bag cell neurons. We used thapsigargin, a specific inhibitor of the endoplasmic reticulum Ca^{2+} pump to analyse the effects of calcium released from intracellular stores on the electrophysiological responses of bag cell neurons. We used isolated cells that at the time of the experiments had produced considerable new neuritic outgrowth. Using digital imaging of cells loaded with the calcium indicator dye, fura-2, we found that thapsigargin (0.5μM) produced a rapid and sustained elevation of the basal calcium concentration at the soma. Such changes as occurred in neurites appeared to be the result of passive spread from the soma, suggesting that intracellular calcium stores are specifically localized at the soma. Thapsigargin also produced a decrease in the latency for the generation of action potentials and, in media containing the potassium channel blocker TEA, a sustained depolarization of ~ 10mv. The depolarization was observed when Na^+ was replaced by TEA or Tris but not by N-methylglucamine and was insensitive to 10mM Co^{2+}, 100μM TTX or to removal of extracellular Ca^{2+}. The depolarization was paralleled by the development of a non-rectifying inward current with a reversal potential of around -20mv. Our data suggest that an increase in intracellular calcium at the soma may activate a non-specific cation channel that may contribute to changes in the electrical properties of bag cell neurons at the onset of an afterdischarge.

253.6

ENHANCEMENT OF Ca AND K CURRENTS BY MODULATORY PEPTIDE COTRANSMITTERS CORRELATED WITH POTENTIATION AND DEPRESSION OF CONTRACTIONS OF THE ARC MUSCLE OF *APLYSIA*. V. Březina*[1], E.C. Cropper[1], C.G. Evans[1], I. Kupfermann[2] and K.R. Weiss[1]. [1]Dept. Physiol. & Biophys., Mt. Sinai Sch. Med. and [2]Ctr. Neurobiol. & Behav., Columbia Univ., New York, NY.

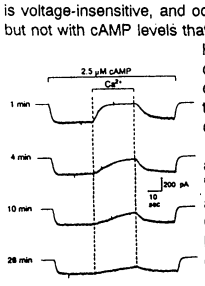
The accessory radula closer (ARC) muscle of *Aplysia* has been studied as representative of the many buccal-mass muscles whose coordinated contractions underlie biting and swallowing. Preservation of the functional integrity of these behaviors even as their frequency and amplitude change, for instance during food-induced arousal, requires coordinated changes in the parameters of these contractions. In the case of the ARC muscle, such modulation is thought to be brought about in large part by appropriate release of peptide cotransmitters of several different families (the small cardioactive peptides (SCPs), myomodulins (MMs), FMRFamide-related peptides (FRPs) and buccalins) from the same two motorneurons whose primary transmitter, ACh, mediates the contractions. At least some of the peptides feed back to presynaptically modulate ACh and cotransmitter release. Another important site of modulation, however, is clearly the postsynaptic ARC muscle itself: contractions elicited by exogenous ACh are potentiated by low concentrations of SCPs and MMs, weakly depressed by high concentrations of SCPs and MM_B but more strongly by MM_A, and strongly depressed by FRPs. We have studied the electrophysiological properties of dissociated ARC muscle fibers to determine whether modulation of ion currents might underlie these effects. Indeed, the pattern of the effects of the peptides on contraction is clearly reproduced in their pattern of effects on two ion currents: SCPs and MMs enhance an 'L'-type Ca current, and SCPs and MM_B slightly activate a characteristic K current whereas MM_A and FRPs activate it strongly. The Ca current most likely supplies much of the Ca^{2+} necessary for contraction, while the K current appears well suited to counteract ACh-induced depolarization of the muscle and thus limit activation of the Ca current. It is thus an attractive hypothesis, which we are currently testing more directly, that the enhancement of the Ca current by the SCPs and MMs is in fact the mechanism by which they potentiate ARC muscle contractions, whereas activation of the K current is the mechanism by which the SCPs, MMs and FRPs, to different degrees, depress those contractions.

253.7

INDIRECT INHIBITION OF OLFACTORY CYCLIC NUCLEOTIDE-GATED (CNG) CATION CHANNELS BY INTERNAL CALCIUM: PROBABLE ROLE IN SENSORY ADAPTATION. Richard H. Kramer and Steven A. Siegelbaum Ctr. for Neuro. and Behav., HHMI, Columbia Univ. P&S., New York, NY 10032

Olfactory signal transduction involves an odorant-elicited increase in cAMP, which directly activates CNG channels. Adaptation is thought to involve an increase in intracellular Ca^{2+}. Here we demonstrate that internal Ca^{2+} profoundly inhibits CNG channels in inside-out "macro" patches containing the dendrite and cilia from catfish olfactory neurons.

Ca^{2+}-dependent inhibition of the cAMP-elicited current is distinct from the open-channel block produced by Mg^{2+}. The Mg^{2+} block is voltage-dependent, occurs only at $[Mg^{2+}]$ > 100 μM and is equally effective at all cAMP concentrations. In contrast, the inhibition by internal Ca^{2+} has a $K_{1/2}$ of 3 μM, is voltage-insensitive, and occurs at low cAMP concentrations (e.g. 2.5 μM), but not with cAMP levels that saturate channel gating (e.g. 50 μM).



Hence Mg^{2+} reduces the maximal cAMP-elicited current, while Ca^{2+} shifts the activation of CNG channels to higher cAMP levels without altering the maximal response. This shift in sensitivity is characteristic of adaptation in sensory systems.

The inhibition of CNG channels by Ca^{2+} is apparently not direct because 1) the effect "washes out" with time after excision (see Figure), and 2) Ca^{2+} has no effect on the cloned catfish CNG channel expressed in Xenopus oocytes. Hence we propose that Ca^{2+} inhibits CNG channels by interacting with a loosely associated protein distinct from the CNG channel protein.

253.8

MECHANISM OF VOLTAGE-DEPENDENT CALCIUM CHANNEL MODULATION BY NEUROTRANSMITTERS IN CHICK SYMPATHETIC NEURONS. A.G. Golard* and S.A. Siegelbaum. Ctr. Neurobiol. and Behavior, HHMI, Columbia University, New York, NY 10032.

Neurotransmitter inhibition of calcium currents (I_{Ca}) can be relieved by large depolarizing prepulses. This effect has been postulated to be due to the voltage-dependent unbinding of a blocking particle (e.g. G protein) to the channel or, alternatively, to reflect a slow voltage-dependent gating step intrinsic to the modulated channel. According to the first hypothesis, the rate of reblock following a depolarizing prepulse should increase with the extent of inhibition (i.e. concentration of blocking particles). We used whole cell patch clamp to study calcium channel inhibition produced by somatostatin (SS) and norepinephrine (NE). At maximal concentrations, both SS (30 nM) and NE (30 μM) inhibit I_{Ca} by 50% at a test potential of 0 mV. This inhibition is transiently relieved by a 15 msec prepulse to +100 mV. Upon repolarization to -80 mV, I_{Ca} reblocks with a τ of 58.4±19 msec (n=7) for SS and of 52.0±0.2 (n=2) for NE. With repeated applications of NE or SS, the extent of inhibition desensitizes and re-block kinetics are significantly slowed (τ increases to >100 msec for both NE and SS). These results favor a model in which a blocking particle is freed by depolarization.

253.9

Ca^{2+}-DEPENDENT REGULATION OF K CHANNELS IN INTACT HUMAN T LYMPHOCYTES. L.C. SCHLICHTER*, I. CHUNG, P.A. PAHAPILL AND P.A. SCHUMACHER. Playfair Neuroscience Unit, Toronto Hospital, Toronto, Ontario, CANADA, M5T 2S8.

T cell activation is dependent on a rise in intracellular Ca^{2+} which is comprised of an IP_3-mediated release from internal stores and a sustained influx across the plasma membrane. The driving force for Ca^{2+} influx is maintained by K^+ efflux and two classes of K channels have been identified in T cells; voltage dependent and Ca^{2+} dependent. However, we have recently determined that the K channels are also regulated by second messenger (kinase) pathways. Since mitogenic stimuli activate both IP_3 and protein kinase C pathways, it is not clear which K channels will be active during mitogenic stimulation. Using patch-clamp recordings in the cell-attached and nystatin-permeabilized configurations to preserve cytoplasmic integrity, we have characterized three K channels; an inwardly rectifying 10-25 pS voltage-dependent channel, a similar conductance Ca^{2+}-dependent channel and a smaller (~9 pS) Ca^{2+}-dependent channel. The voltage-dependent channel is inhibited by a physiologically relevant rise in Ca_i over the entire voltage range (-100 to +50 mV). Steady-state activity of this channel is increased at the resting potential by Ca^{2+} depletion. Conversely, two types of non-voltage dependent K channels are activated by a rise in Ca_i (ionomycin, Ca rebound treatment) or by mitogenic lectins which stimulate both the IP_3 and PKC pathways. The larger of the two channels was sensitive to patch excision, hence it may be under-represented in whole-cell or excised patch recordings. Our results are consistent with a major role for Ca-dependent rather than the voltage-dependent K channels in maintaining the Ca^{2+} influx.

253.10

MODULATION OF Ca CURRENTS BY L-GLUTAMATE IN CULTURED HYPOTHALAMIC NEURONS. T.H. Müller*, H.U. Zeilhofer & D. Swandulla. Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany & Institut für Pharmakologie und Toxikologie, Universität Erlangen-Nürnberg, Erlangen, Germany.

Dissociated hypothalamic neurons taken from foetal rats (E14) and kept in culture for more than 21 days exhibit both low- (LVA) and high-voltage-activated (HVA) Ca currents and different types of glutamate receptors (Swandulla & Misgeld, J. Neurophysiol. 64:715, 1990; Müller et al., J.Physiol. 450:341, 1992). In the present study we investigated the effect of glutamate on Ca currents recorded in whole-cell configuration. External application of glutamate (2 - 100 µM) reduced HVA Ca currents in a dose-dependent manner (~ 50% at 100 µM) whereas LVA Ca currents were not affected. Quisqualate reduced HVA Ca currents with a tenfold higher potency compared to glutamate. Kainate (5 µM), NMDA (20 µM), AP4 (100 µM) and trans-ACPD (100 µM) were ineffective. The action of glutamate on Ca currents was attenuated by including either the GDP-analogue GDB-ß-S, which blocks G-protein activation, or the Ca chelator BAPTA in the pipette solution. The results suggest that glutamate modulates HVA Ca currents by a Ca-dependent mechanism involving G-protein activation.

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253.11

ARACHIDONIC ACID INHIBITS SODIUM CURRENTS IN STRIATAL NEURONS. B.A. MacVicar*, D.D. Fraser, K. Hoehn, and S. Weiss. Neuroscience Research Group, University of Calgary, Calgary, AB, Canada T2N 4N1.

We investigated the effects of arachidonic acid (AA) on neurotransmitter release and on Na currents (I_{Na}) in striatal neurons. In cultured striatal neurons, the TTX-sensitive release of [^{3}H] GABA induced by 5 µg/ml veratrine was depressed by AA (1-10 µM) in a concentration dependent manner (up to 65% at 10 µM). AA did not, however, inhibit the release of [^{3}H] GABA induced by 56 mM K^+ depolarization. This suggested that AA inhibited veratrine-induced release by selectively acting upon the Na channel. We then investigated the action of AA on TTX-sensitive I_{Na} studied under whole-cell voltage clamp in either cultured or acutely isolated striatal neurons. AA (10 µM) reversibly depressed the peak amplitude I_{Na} (45% in culture; 30% in acutely isolated cells) and shifted steady state inactivation by -15 mV in the majority of neurons studied. Na entry was studied in cultured striatal neurons by imaging veratrine-induced changes in SBFI fluorescence, a Na-dependent fluorescent dye. AA (10 µM) reversibly inhibited (81%) the veratrine-induced increases in $[Na]_i$. We conclude that AA inhibits synaptic transmitter release and Na entry in striatal neurons by depressing I_{Na} and shifting steady state inactivation in the hyperpolarizing direction. Supported by Medical Research Council (Canada).

253.12

ACTIVATION OF CA^{2+}-DEPENDENT CURRENTS IN CULTURED RAT SENSORY NEURONES BY INTRACELLULAR APPLICATION OF A CYTOSOLIC SPERM FACTOR OR CYCLIC ADP-RIBOSE (cADPR). K.P.M. Currie, R.H. Scott*, K. Swann+ & A. Galione=. Dept. of Physiology and +MRC Exp. Emb. and Terat. Unit, St. George's Hosp. Med. Sch., London SW17 0RE, U.K. and =Dept. of Pharmacol., Oxford Univ., Oxford OX1 3QT, U.K.

Microinjection of a high molecular weight cytosolic extract from sperm (SF) into unfertilized mammalian eggs activates sustained Ca^{2+} oscillations due to a sensitization of calcium-induced calcium release (CICR). cADPR is a potent Ca^{2+} releasing agent in sea urchin eggs and may also act on a CICR mechanism. We have found that intracellular application of both these agents induces Ca^{2+}-dependent inward currents in cultured rat dorsal root ganglion (DRG) neurones. Cells were voltage clamped at -90mV using the whole cell variant of the patch clamp technique and SF (0.2-2.0 mg/ml total protein conc.) or cADPR (10^{-8} - 10^{-6} M) applied by inclusion in the patch pipette. Extracellular application of SF had no action nor did intracellular application of cytosolic brain extract or serum albumin fraction V. Activity of both agents was abolished by denaturing (heat or freeze/thaw). SF induced activity was attenuated by intracellular ruthenium red and could be triggered or potentiated by activating a series of voltage gated Ca^{2+} currents to load the cell with Ca^{2+}. However, preincubation with caffeine (1mM) did not abolish SF induced activity.

GENE STRUCTURE AND FUNCTION IV

254.1

EXPRESSION OF THE SMALL NUCLEAR RNA-ASSOCIATED PROTEIN SMN IN CELLS IS NOT SUFFICIENT TO DIRECT CGRP SPECIFIC MRNA SPLICING. S.E. Leff*, M.L. Reed, and M.E. Sanjines. Dept. of Pharmacology, Stanford Univ. School of Medicine, Stanford, CA 94305.

The mammalian calcitonin/CGRP gene is alternatively spliced in a tissue-specific fashion. Currently, there are no tissue-specific trans-acting splicing factors identified that control or participate in alternative splicing of mammalian mRNAs. However, one small nuclear ribonucleoprotein associated polypeptide, SmN, has been described that is expressed selectively in brain, heart and pituitary, tissues which favor the production of CGRP mRNA from a transgene. We examined whether the ectopic expression of SmN in cells alters the pattern of alternative splicing of a transfected rat calcitonin/CGRP in: 1) HeLa cells, which express no endogenous SmN and; 2) COS cells, which express a relatively moderate level of SmN. In both instances, expression of SmN was not sufficient to significantly increase the production of CGRP mRNA from the rat calcitonin/CGRP gene. However, expression of SmN protein in HeLa cells was associated with a post-transcriptional suppression of Sm B/B' levels that might arise from either an effect on synthesis or degradation of Sm B/B' proteins. Thus the cell specific expression of SmN protein does not appear to be sufficient to direct on CGRP mRNA specific splicing. Current studies are attempting to determine whether SmN expression is necessary for a cell to produce CGRP mRNA efficiently.

254.2

CHARACTERIZATION OF CIS-ACTING ELEMENTS IN THE GLUTAMINE SYNTHETASE PROMOTER. J. F. MILL* and H. J. PUROHIT. Lab. of Molecular Biology, NINDS, NIH, Bethesda, MD 20892.

Glutamine synthetase (GS) gene expression is confined to astrocytes in the CNS. Previously, a series of deletion and site-directed mutants of the promoter region were generated by PCR, cloned into pSV_0CAT, and expressed in various cell lines.

We identified three regions of importance for the regulation of GS expression, a site homologous to AP2 located at -222, an enhancer region at -315, and a silencer region at -797. We have shown that these sites are functionally important for GS expression in astrocytes.

We now demonstrate that these regions bind trans-acting factors using a gel retardation assay with synthetic oligonucleotide probes. Interestingly, the site we identified by homology to the AP2 consensus sequence did not bind any proteins. By extending the site to include an upstream G/C rich region we were able to demonstrate binding with nuclear extracts from astrocytes, however, purified AP2 protein was unable to bind. Thus the site must bind an unidentifed, non-AP2, protein.

We have also been able to show that the two other sites are capable of binding proteins from astrocyte nuclear extracts. The silencer binds a protein which is not present in HeLa nuclear extracts, while the enhancer region binds proteins from both HeLa and astrocyte nuclear extracts.

254.3

NICOTINE AND MUSCARINE STIMULATE EXPRESSION OF PNMT PROMOTER CONSTRUCTS TRANSFECTED INTO PRIMARY CHROMAFFIN CELLS. M.J. Evinger~, L.M. Hemmick~, S. Regunathan#, D.J. Reis#, and M.E. Ross+, ~Dept. Neurobiol. & Behav., SUNY Stony Brook, #Div. Neurobiol., Cornell Univ. Med. Coll., +Dept. Neurology, Univ. of Minn.

Exposure of bovine adrenal medullary cells to nicotine or muscarine stimulates accumulation of phenylethanolamine N-methyltransferase (PNMT) mRNA with each of these cholinergic agonists acting through separate receptor and second messenger systems. We sought to establish whether the effects of these agents are mediated through defined regions in the 5' upstream sequences of the PNMT promoter. Primary cultures of bovine chromaffin cells were transfected with PNMT-CAT or -luciferase promoter-reporter constructs, then exposed to either nicotine (50 uM) or muscarine (100 uM). Both nicotine and muscarine stimulate by 2-5 fold expression of the 3 kb and 0.9 kb constructs but not the 0.3 kb construct. The relative effects of these agonists are comparable for both CAT and luciferase constructs when transfected either by calcium phosphate or electroporation. The muscarinic response appears specific for the m4 receptor subtype: a) only m4 subtype mRNA is expressed in bovine chromaffin cells (Fernando et al., 1991), b) the m4 selective antagonist 4-DAMP blocks muscarine-stimulated expression of PNMT constructs at concentrations that inhibit accumulation of endogenous PNMT mRNA, and c) 4-DAMP relieves muscarinic inhibition of forskolin-stimulated cAMP accumulation in these cultures. This indicates that muscarinic stimulation of the transfected PNMT gene is mediated through a cyclic nucleotide coupled m4 receptor. We therefore conclude that 5' upstream sequences (>-300 bp) in the PNMT gene convey responsiveness to neural stimuli transduced individually through nicotinic and muscarinic receptors on chromaffin cells.

254.4

TRANSCRIPTIONAL REGULATION OF SOMATOSTATIN (SRIF) GENE BY A CYCLIC GUANOSINE 3'5' MONOPHOSPHATE (cGMP) MECHANISM IN THE PERIVENTRICULAR NUCLEUS OF THE RAT. M.C. Aguila*. Department of Physiology, Neuropeptide Division, UT Southwestern Medical Center, Dallas, TX 75235-9040.

We had previously reported that growth hormone-releasing factor stimulates cGMP and SRIF release without altering cAMP accumulation from median eminences (ME's) incubated in vitro. Therefore, the possible role of cGMP on SRIF release and on the regulation of SRIF mRNA was examined in periventricular nucleus (PeN) of male rats incubated in vitro. SRIF mRNA levels were determined in PeN explants subjected to 2 and 6 hrs culture in Waymouth's medium in the presence of various concentrations (10^{-9} to 10^{-6}M) of dibutyryl cGMP (dbcGMP); sodium butyrate served as control. Sodium nitroprusside (SNP) was used to activate the guanylate cyclase pathway. Levels of SRIF mRNA were determined by S_1 nuclease protection assay using a [^{32}P]labelled rat SRIF riboprobe. SRIF release was measured at 30 min and 6 hrs by RIA. After 6 hrs of incubation SRIF mRNA levels and SRIF release were significantly ($p<0.025$) increased by 10^{-7}M dbcGMP, whereas sodium butyrate did not modify them. Likewise, SNP (10^{-6}M) augmented ($p<0.001$) SRIF mRNA levels at 6 hrs. These results indicate that cGMP regulates the expression of SRIF gene in the PeN and they suggest that cGMP may play an important role in the transcriptional regulation of the SRIF gene. (Supported by NIH grant NS26821).

254.5

IN VIVO ANALYSIS OF THE RAT PREPROENKEPHALIN PROMOTER USING AN HSV DEFECTIVE VIRAL VECTOR.
M.G.Kaplitt[1,*], L.Lipworth[1], S.D.Rabkin[2], and D.W. Pfaff[1]. [1]Laboratory of Neurobiology and Behavior, Rockefeller Univ., New York, NY 10021; [2]Program in Molecular Biology, Memorial-Sloan Kettering Cancer Center, New York, NY 10021.

We have employed a defective herpes simplex viral vector as a gene transfer vehicle, in order to characterize tissue or brain region-specific regulatory sequences in the rat preproenkephalin promoter. Previously, we have demonstrated that a defective HSV vector can transfer and express a foreign gene in the adult rat brain (Mol. Cell. Neurosci. 2:320-330). In this study, the bacterial lacZ gene was placed under the control of a 2.7kb fragment of the rat preproenkephalin promoter. The transcription unit was inserted into our HSV amplicon and the viral vector dvHENK was then created. Following stereotaxic microinjection of the viral vector, blue cells were noted in the ventromedial hypothalamus and the amygdala, two regions known to express endogenous enkephalin. This indicates that the promoter was functional in the vector. The caudate nucleus usually contains the highest level of endogenous preproenkephalin expression in the brain, however no positive cells have been noted in this region. Other regions are currently being examined. We have also produced defective viral vectors with different fragments of the promoter region, and these are also being tested *in vivo*. This study demonstrates that promoter analyses, once restricted to tissue culture, can now be performed *in vivo* using a plasmid-based HSV defective viral vector.

254.6

A NOVEL MADS-FAMILY TRANSCRIPTION FACTOR IN HUMAN BRAIN AND MUSCLE TRANS-ACTIVATES VIA THE MEF-2 ELEMENT. D. Leifer*, D. Krainc, J. McDermott, Y.-T. Yu, R. E. Breitbart, J. Heng, R. L. Neve, B. Nadal-Ginard, and S. A. Lipton. Depts. of Neurology, Cardiology, and Psychiatry, Harvard Medical School, Boston, MA.

We have identified cDNA clones from human fetal brain and muscle libraries for a novel transcription factor in the MADS family. This family includes the yeast transcription factor MCM1, plant homeotic genes such as AGAMOUS and DEF A, which have roles in flower morphogenesis, and human serum response factor, which may regulate muscle-specific genes and immediate-early genes. Our clones occur in several variant forms that appear to result from alternative splicing. Northern blots indicate that our clones are expressed at abundant levels in human fetal cerebral cortex and skeletal muscle, and at lower levels in cerebellum, but not in other regions of the brain or in other tissues. Our cloned proteins bind specifically to the myocyte-specific enhancer binding factor-2 (MEF-2) regulatory element, which has so far been found to be functionally important in a variety of muscle-specific genes. Our proteins also bind a variant MEF-2 element that is present in the promoter of the brain creatine kinase gene. Moreover, our proteins specifically activate transcription of reporter genes containing the MEF-2 element. Gel-shift experiments have confirmed that an MEF-2 activity similar or identical to our cloned proteins is present in human cortex (Krainc et al., this meeting). Our results suggest that these proteins have a role in development not only of muscle but also of human cerebral cortex and cerebellum, perhaps similar to the role of MADS proteins in plant morphogenesis.

254.7

PROMOTER REGIONS INVOLVED IN BASAL AND HORMONE-STIMULATED ACTIVITY OF TYROSINE HYDROXYLASE (TH) GENE. M.K.Stachowiak*, A.Goc. Barrow Neurological Institute, 350 W.Thomas Rd, Phoenix, AZ 85013.

Cardiovascular effects of angiotensin II (AII) have been attributed to the stimulation of catecholamine (CA) secretion from sympathoadrenal and central CA neurons. We have shown that stimulation of AII receptors in adrenal medullary cells (AMC) increases mRNA level and transcriptional activity of the TH gene, which encodes the rate-limiting enzyme in CA synthesis. AII upregulated also c-Fos- and c-Jun and related antigens. To examine mechanisms of AII induction of TH gene, and possible role of AP1 factor, we constructed plasmid bearing luciferase reporter gene under control of -428/+21 bp fragment of TH promoter. Deletion mutants were prepared by removing either -194/-54 or -269/-54 promoter regions. Luciferase activity in transiently transfected cells was normalized to the amount of intracellular plasmid DNA. Each construct expressed luciferase in bovine AMC as well as in glia-derived SF763 cells. -194/-54 region of TH promoter may be involved in tissue-specific basal regulation since its deletion enhanced TH promoter activity 5-fold in AMC and 2-fold in SF763. Deletion of further upstream region containing putative AP1 site (deletion -269/-54) reduced expression of luciferase. Incubation of AMC with AII induced a 3-fold increase in luciferase expression from the wild type TH promoter. Promoter deletions diminished this stimulation to 2.1-fold (-194/-54 deletion) and 1.8-fold (-269/-54 deletion). We conclude that AP1-containing -269/-195 region is important for basal TH promoter activity, and that multiple regions including -269/-54 fragment participate in AII regulation. Pretreatment of AMC with AII enhanced in vitro binding of nuclear proteins to TH promoter. Binding of Fos-related antigens was observed in extracts from nonstimulated AMC and was enhanced in cells treated with AII supporting the roles of AP1 in basal and AII-induced expression of the TH gene.

254.8

THE CYCLIC AMP RESPONSE ELEMENT IS ESSENTIAL FOR BASAL AND INDUCIBLE TRANSCRIPTION OF THE TYROSINE HYDROXYLASE GENE M.K. Lee, J. Carroll*, H. Ishiguro, T.H. Joh and K.S. Kim. Cornell Univ. Med. Coll. at the Burke Med. Res. Inst., White Plains, NY 10605.

Adenosine 3', 5'-cyclic phosphate (cAMP) exerts its effects on a variety of responsive genes via a conserved octamer motif 5'TGACGTCA3', cAMP response element (CRE). The tyrosine hydroxylase (TH) gene, encoding the first and rate-limiting enzyme in the biosynthesis of catecholamine neurotransmitters, contains a single copy of the consensus CRE in the proximal 5' region at -45 to -38 bp upstream of the transcription initiation site. Functional analysis of the upstream 2400 bp region using a transient transfection assay in catecholaminergic cell lines identified two upstream domains, -365 to -151 bp and -60 to -39 bp, containing important cis-acting elements for TH transcription. Strikingly, site-directed mutagenesis demonstrated that the TH CRE was essential not only for cAMP-inducible transcription but also for basal transcription of the TH gene. The CRE of the TH gene resides in a position nearest to the TATA (8 base pairs apart) among the known genes containing the CRE. DNA binding assays revealed the presence of two specific DNA/protein complexes using either TH CRE or somatostatin CRE oligonucleotides suggesting that the same CRE binding protein activates both genes. These data suggest that the CRE exerts an important dual role as a basal promoter element and an inducible enhancer for TH transcription. Supported by MH24285.

254.9

cAMP-DEPENDENT PROTEIN KINASE (PKA) IS REQUIRED FOR BOTH BASAL EXPRESSION AND INDUCTION OF THE TH GENE. K.S. Kim*, D.H. Park, T. Wessel, J.A. Wagner[1] and T.H. Joh Cornell Univ. Med. Coll. at the Burke Med. Res. Inst., White Plains, NY 10605 and [1]New York, NY 10021.

The role of PKA in the expression of TH and the regulation of catecholamine biosynthesis remains controversial. PKA could exert its regulatory function on TH expression at the transcriptional level via the CRE of the TH gene as well as at the post-translational level via the direct phosphorylation of TH molecule. We have found that the CRE located at -45 to -38 bp is crucial both for basal and inducible transcription of the TH gene, suggesting that PKA may have an important role in TH expression. This CRE might also respond to other signal transduction pathways, such as the Ca++/calmodulin-dependent protein kinase. To address this issue, we utilized several PKA-deficient PC12 cell lines (Ginty et al., JBC 23, 15325, 1991). Specifically, the AB11 and A123.7 cell lines that were rendered PKA-deficient by stable expression of the mutant regulatory subunits of PKAI provided a useful model system to assess the functional importance of PKA in catecholamine biosynthesis. We also used the A126-1B2 cell line, a PC12 mutant deficient only in PKAII, which was isolated after chemical mutagenesis (Van Buskirk et al., MCB 5, 1984, 1985). The AB11 and A123.7 cell lines contained about 15% and 30% activity compared to the wild type PC12, whereas the A126 1B2 line did not show any detectable TH activity. Northern blot analysis indicated that PKA plays an essential role in the expression of TH primarily at the transcriptional level. These observatons were supported by *in situ* hybridization and immunocytochemical analysis. We suggest that PKA is an essential component of the signal transduction pathway for both the basal and inducible expression of TH. Supported by MH24285.

254.10

CHARACTERIZATION OF A PROTEIN THAT SPECIFICALLY BINDS PHOSPHORYLATED CREB. J.C. Chrivia, R. Kwok* and R.H. Goodman. Vollum Institute, Oregon Health Sciences University, Portland, Oregon 97201-3098.

Transcriptional regulation by cAMP depends in many cases on a DNA element designated the "cAMP-responsive enhancer," or CRE. CREB, a leucine zipper transcription factor, has been shown to bind the CRE sequence. In response to increased levels of cAMP, CREB is phosphorylated at its consensus site for cAMP-dependent protein kinase (PKA). Mutation of this site prevents activation of CREB. How activated CREB induces transcription is unclear but presumably it acts either by directly contacting the core transcriptional machinery or indirectly by binding to an adapter protein.

To identify proteins that interact with the phosphorylated form of CREB, CREB was labeled with γ^{32}P ATP and PKA and used as a probe to screen λgt11 expression libraries. This approach led to the isolation of a cDNA clone for a CREB-binding protein (CBP) of 2440 amino acids (268 KD). Analysis by Western blot confirmed the presence of the 268 KD CBP protein in a number of cell lines, and in addition demonstrated the presence of a shorter 220 KD isoform. Northern blot analysis of rat brain mRNA revealed CBP mRNA of 9 and 10 kb in length. Using immunohistochemical techniques CBP was found to be localized exclusively to the nucleus.

To determine whether CBP interacted with CREB in solution, recombinant CBP was added to a mixture of CREB and radiolabled CRE, and complexes were analyzed by gel shift assay. If non-phosphorylated CREB was used, CBP had no effect on the mobility of the CREB-CRE complex. If CREB was phosphorylated by PKA, addition of CBP supershifted the complex.

We propose that CBP interacts with the activator domain of the transcription factor CREB, that this interaction requires phosphorylation of CREB by PKA, and this association may alter or mediate CREB activity.

254.11

ANALYSIS OF THE GENE PROMOTER OF THE 67,000 Da FORM OF RAT GLUTAMIC ACID DECARBOXYLASE. Michael J. Morales* & David I. Gottlieb. Anat & Neurobiol, Washington U Sch Med, St. Louis MO 63110.

Glutamic Acid Decarboxylase (GAD) is the key enzyme responsible for GABA biosynthesis. There are two forms of this enzyme, 67,000 Da and 65,000 Da, that are encoded by different genes. To study the genetic regulatory apparatus that governs development of GABAergic neurons, we are examining the transcription of the 67,000 Da form of GAD from rat.

We previously reported the cDNA sequence of GAD_{67} (Wyborski, *et al.*, Mol. Brain Res. 8:193-198 (1990)). Further analysis suggested that GAD_{67} mRNA from adult rat brain had multiple 5' ends, ranging from 218 to 250 nucleotides upstream from the translational start site. We obtained a genomic clone from bacteriophage λ that contained the first two exons of the GAD_{67} gene plus 12 kBp of upstream sequence. The translational start site resided on Exon 2, while Exon 1 consisted of 5' untranslated sequence. The sequence of the region upstream of the transcriptional start site revealed four consensus "GC Boxes" within 30 nucleotides of the start site and a "TATA Box" another 125 bp upstream. This distance suggests that the TATA box is not widely used in adult rat brain.

To asses the activity of this putative promoter, we transiently transfected reporter constructs into P19 embryonal carcinoma cells. This cell line, in its undifferentiated state, expresses GAD_{67} mRNA. A 280 bp region that contained the GC Boxes and TATA Box supported expression of the reporter gene. A 2.2 kBp region upstream of the transcriptional start site (including the promoter) increased expression of the reporter 10-fold.

An important aspect of P19 cells is that they can differentiate into cells resembling neurons. GAD_{67} transcription is induced approximately 50-fold in differentiated P19 cells (See Abstract by G. Bain, *et al.*). Stable transfection of reporter constructs containing portions of the GAD_{67} gene into P19 cells, followed by differentiation, should allow us to identify *cis*-acting elements required for GAD_{67} induction.

CATECHOLAMINE RECEPTORS: ADRENERGIC II

255.1

CHARACTERIZATION OF THE SIGNAL TRANSDUCTION PATHWAYS OF THE THREE α_1-ADRENERGIC RECEPTORS J.W. Lomasney*, A. Hirakata, L.F. Allen, D. Capel, A.D. Proia, M.G. Caron and R.J. Lefkowitz. Depts. Pathology, Ophthalmology, Medicine and HHMI, Duke U. Med. Ctr., Durham, NC 27710.

Many hormones, growth factors and neurotransmitters exert their effects by causing profound changes in cellular lipid metabolism. Epinephrine (EPI) and norepinephrine have been shown to activate various phospholipases (PL) including PLC, PLA_2 and PLD via α_1-adrenergic receptors (AR). Our laboratory has identified by molecular cloning three distinct α_1-ARs (α_{1A}, α_{1B}, and α_{1C}). The purpose of this study was to determine the interaction of α_1-AR subtypes with PLC , PLA_2 and/or PLD.

Rat-1 fibroblast cell lines which permanently express each of the α_1-AR subtypes were incubated with either [^{14}C] arachidonate (AA) or [^{3}H] inositol. PLC activation was assessed by isolating inositol phosphates (IP) using anion exchange chromatography. PLD catalyses a transphosphatidylation reaction in which the phospholipid phosphatidyl group is transferred to ethanol (Et) to produce phosphatidylethanol (PEt); the formation of PEt was determined by thin layer chromatography (TLC) and used to assess PLD activity. PLA_2 activity was assessed in the presence of the diacylglycerol lipase inhibitor RHC-80267 by determining the release of AA using TLC. In α_{1C}-Rat-1 cells, 100 μM EPI stimulates the formation of IPs 15 fold, and PEt 27 fold. RHC-80267 markedly reduced monoacylglycerol (MAG) by >90% whereas the EPI-stimulated increase (~2 fold) in AA was not significantly changed, suggesting that the AA is derived from PLA_2 catalyzed hydrolysis of phospholipids. All effects were blocked by the α_1 antagonist prazosin. In α_{1B}-Rat-1 cells, IPs increased 8 fold, PEt 5 fold and AA ~1.5 fold. In α_{1A}-Rat-1 cells IPs increased 2 fold, however, PEt and AA were minimally increased. These findings suggest differences in the transmembrane signalling pathways of the α_1-AR subtypes. The α_{1C}-AR potently stimulates PLC, PLD and PLA_2, the α_{1B}-AR less potently stimulates PLC, PLD and PLA_2, and the α_{1A}-AR weakly stimulates PLC and minimally stimulates PLD and PLA_2.

255.2

RELATIONS BETWEEN α_{1a}- AND α_{1b}-ADRENOCEPTOR SUBTYPES: AN ELECTROPHYSIOLOGICAL ASSESSMENT IN HYPOTHALAMIC NEURONS. L.-M. Kow* and D.W. Pfaff. The Rockefeller University, New York, NY. 10021

We previously found that excitatory neuronal actions and lordosis-facilitating effects of α_1-agonists, methoxamine and phenylephrine, in ventromedial hypothalamus (VMH) of rats were both blocked by the α_{1b}-antagonist, chloroethylclonidine (CEC), indicating that the α_{1b}-subtype is involved in mediating these functions (Brain Res. in press). To investigate whether α_{1a}-subtype is also involved, effects of α_{1a}-antagonists, WB-4101 (1-10 μM) and 5-methyl urapidil (5-MU, 1-10 μM), on the excitatory action of α_1-agonists on VMH neurons were examined *in vitro*. Like CEC, both WB-4101 (on 19 cells) and 5-MU (on 16 cells) completely blocked or severely attenuated the α_1-action on all the neurons tested, but did not block the excitatory actions of carbachol or oxytocin. These together with our previous findings with CEC suggest that all the VMH neurons excitable by α_1-agonists possess both α_{1a}- and α_{1b}-subtypes. This suggestion was tested in 20 VMH units excited by α_1-agonists. The excitatory response of each of these units was subjected first to CEC (50-100 μM), whose effect is reversible, and then to 5-MU or WB-4101. In each case, the α_1-action was completely or nearly abolished by either α_{1a}- or α_{1b}-antagonist alone. These findings not only confirmed that every VMH neuron excitable by α_1-agonists possess both α_{1a}- and α_{1b}-subtypes, but also indicate that both subtypes are necessary for mediating the neuronal action of α_1-agonists. Thus, in these hypothalamic neurons the α_{1a}- and α_{1b}-subtypes appear to be functionally linked.

255.3

DIFFERENTIAL EXPRESSION OF THREE α_2-ADRENERGIC RECEPTOR mRNAs IN RAT BRAIN. M. Scheinin*, J.W. Lomasney, D. Hayden-Hixson, U.M. Schambra, M.G. Caron, R.J. Lefkowitz & R.T. Fremeau Jr. HHMI, Duke U. Med. Ctr., Durham, NC 27710, and BDRC, UNC-CH, Chapel Hill, NC 27599.

Molecular cloning has revealed the existence of at least three distinct genes which encode related α_2-adrenoceptor (α_2-AR) subtypes. To begin to relate the receptor subtypes to functional catecholaminergic subsystems, we have determined the distribution of the three corresponding mRNAs in rat brain with in situ hybridization using ^{35}S-UTP- labeled antisense cRNA probes. The distributions of the three mRNAs were strikingly different, but not entirely exclusive. α_{2A}-AR mRNA (RG20 [1]) was most abundant in the locus coeruleus (LC), and was also present in several other regions of the brain stem, hypothalamus, and cerebral cortex (most notably layers 2-3 and 6). Expression of α_{2C}-AR mRNA (RG10 [1]) was strongest in the striatum. Labeling was also seen throughout the cerebral cortex, in the dentate gyrus and CA1 region of the hippocampus, and in the olfactory tubercle. Some regions of the hypothalamus and amygdaloid complex, inferior colliculi, pontine nuclei, and scattered, large cells in the granule cell layer of the cerebellum appeared to express both α_{2A}-AR and α_{2C}-AR mRNA. Labeling with the α_{2B}-AR probe (RNGα_2 [2]) was largely restricted to several thalamic nuclei. The exclusive presence of α_{2A}-AR mRNA in the LC, where a large part of the noradrenergic innervation of the brain originates, suggests that this receptor subtype functions as a presynaptic autoreceptor. Since cells expressing α_{2A} mRNA were also observed in noradrenergic projection areas, including the cerebral cortex, the α_{2A}-AR appears to mediate postsynaptic signaling, as well. The differential expression patterns of the receptor subtypes suggest different physiological functions for the three α_2-AR subtypes, warranting more detailed identification and characterization of the involved cell types and neuronal pathways. Such information may provide important clues for targeted drug development.

References: [1]Lanier et al., JBC 266:10470,1991; [2]Zeng et al., PNAS 87:3102,1990

255.4

[^{11}C]MK-912 AND [^{11}C]WY26703 AS POSITRON EMITTING RADIOLIGANDS TO LABEL ALPHA-2 ADRENERGIC RECEPTORS *IN VIVO*: ASSESSING THEIR USE IN RHESUS MONKEY BRAIN. R.C. Pleus*, C.Y. Shiue, G. Shiue, J. Rysavy, H. Huang, J. Sunderland, K. Cornish, L. Bai, M. Frick and D.B. Bylund. Depts. of Pharmacology and Physiology, Univ. of Nebraska Med. Ctr., and Creighton University Ctr. for Metabolic Imaging, Omaha, NE 68198-6260.

In humans, alpha-2 adrenergic receptor density is associated with depression and borderline personality disorder. Also, agonists for these receptors can lower blood pressure, reduce symptoms of opiate withdrawal, induce anesthesia, and improve cognitive deficits.

Positron emitting ligands for dopaminergic, cholinergic, benzodiazepinergic, serotonergic, and opioid receptor systems have been developed for positron emission tomography (PET). However, there are no suitable alpha-2 adrenergic receptor ligands currently available for PET studies. We have methylated the desmethyl precursor of two alpha-2 adrenergic receptor antagonists using [^{11}C]H_3I to obtain [^{11}C]WY26703 and [^{11}C]MK-912. *In vitro* analyses of the unlabeled compounds demonstrate that both compounds exhibit subnanomolar affinity for subtypes of alpha-2 adrenergic receptors (i.e., alpha-2A, -2B, and -2C). *In vivo* studies in rodents demonstrate that both compounds pass the blood-brain barrier and both exhibit specific binding in brain when rats coinjected with atipamezole (alpha-2 antagonist) were compared to those injected with the [^{11}C] radioligand alone. To test these radioligands further, [^{11}C]WY26703 and [^{11}C]MK-912 will be injected into Rhesus monkeys. Brains will be imaged by PET and blood samples will be obtained to assess plasma radioactivity clearance and unchanged [^{11}C]WY26703 and [^{11}C]MK-912. These data will indicate that these two radioligands may have potential use in human PET studies. (Supported by NIH grant GM40784).

255.5

G PROTEINS COUPLED TO ALPHA-2 ADRENERGIC RECEPTORS Y. Okuma and T. Reisine*. Dept. Pharmacology, Univ. PA, Philadelphia, PA 19104

Alpha2-adrenergic receptors mediate many of the biological effects of epinephrine and norepinephrine. G proteins couple these receptors to cellular effector systems. To identify which G proteins are coupled to alpha2a-receptors (AR), cloned AR stably expressed in LLCPK1 cells were solubilized and AR/G protein complexes were immunoprecipitated with antisera directed against different alpha subunits of G proteins. Immunoprecipitated receptors were detected using a binding assay. Antisera directed against the C-terminal region of Gia and Goa immunoprecipitated AR. Antisera against internal regions of the three subtypes of Gia also immunoprecipitated the receptor. Antisera against beta-36 also immunoprecipitated the receptor but not antisera against beta-35. The binding of agonists to the receptor alters the association of the receptor with G proteins. Following clonidine binding to the receptor, antisera against the C-terminal region of Gi and Go were less effective in immunoprecipitat- in the AR whereas antisera directed against internal regions of Gi, Go or beta-36 were equally effective in immunoprecipitating the agonist-bound and agonist free AR. These findings indicate that Ga and beta-36 remain coupled to the agonist bound AR but a conformational change occurs in the receptor/G protein complex such that the epitope for C-terminal directed Gia and Goa antisera is made inaccessible. Since these conformational changes in the AR/G protein complex occur when agonist bind to the receptor, they may be important for the activation of the AR signal transduction pathway. Supported by MH45533 GGM34781

255.6

SYNTHESIS AND CHARACTERIZATION OF A HIGH AFFINITY RADIOIODINATED PROBE FOR THE IMIDAZOLINE/GUANIDINIUM RECEPTIVE SITE (IGRS). S. Lanier, B. Ivkovic, J. Neumeyer*, V. Bakthavachalam, Dept. Pharmacol., Medical Univ. of South Carolina, Charleston, SC 29425, Research Biochemicals Inc., Natick, MA 01760.

Imidazoline and guanidinium compounds recognized by α-adrenergic receptors (AR) elicit diverse effects in the central nervous system and in peripheral tissues. Many of these responses appear unrelated to interaction with α-AR or other known receptor systems suggesting the presence of a specific imidazoline/guanidinium receptive site (IGRS). Radioligand binding studies indicate the presence of such a site in brain, kidney and liver. To facilitate the structural analysis of IGRS, we prepared functionalized analogs of cirazoline, a ligand that exhibits high affinity (Ki=0.8nM) for IGRS. Radioiodination of one analog, 2-(3-aminophenoxy)methyl imidazoline (AMPI) generates a high affinity ligand ^{125}I-AMIPI (Kd=2nM) selective for IGRS as determined by binding assays in rabbit kidney membranes. ^{125}I-AMIPI was diazotized and converted to the azide AzIPI, a potential photoaffinity adduct for IGRS. Incubation of rabbit kidney membranes with ^{125}I-AzIPI followed by photolysis results in covalent labeling of a major Mr=55000 and a minor Mr=61000 species as visualized by SDS/PAGE-autoradiography. The labeling of both species is blocked by the imidazoline idazoxan or the guanidinium analog guanabenz but is not effected by coincubation with the α-AR selective compounds rauwolscine, prazosin or epinephrine. A similar labeling pattern was observed with rat brain membrane preparations. The pharmacological specificity of labeling implicates the Mr=55000/61000 species as the ligand-binding subunit of IGRS. The availability of these probes should facilitate efforts to define the functionality of IGRS and a potential endogenous ligand. (Supported by NIH GM 46605-01, NS24821 and CTR2235.)

255.7

EXPRESSION OF FUNCTIONAL IMIDAZOLINE RECEPTORS IN RAT ASTROCYTES. S. Regunathan*, D.L. Feinstein and D.J. Reis. Div. of Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021.

In many tissues clonidine and structurally related imidazolines bind not only to α_2-adrenergic receptors (AARs) but also with high affinity to a non-adrenergic imidazoline receptor (IR) which is expressed in several isoforms. In brain, IRs have been identified by membrane ligand binding (Wikberg et al., *J. Neurochem.* 55:192, 1990) or by autoradiography (Hudson et al., *Br. J. Pharmacol.*, 102:4P, 1991) with regional variability. However, the cellular localization of the IR is not known. We have characterized the binding of ^{3}H-idazoxan to membranes prepared from primary cultures of rat cerebral cortical astrocytes and neurons obtained from neonatal rat. ^{3}H-idazoxan binds specifically (85% of total binding) to astrocytic membranes. The binding is saturable to a single high affinity site with an apparent K_D of 4 nM, and a B_{max} of 220 fmol/mg protein. Over 80% of binding is to non-adrenergic receptors (not blocked by 10 μM epinephrine). ^{3}H-idazoxan is displaced by cirazoline > idazoxan > clonidine > amiloride >>> rauwolscine = epinephrine consistent with binding to an idazoxan-preferring (I-2) subclass of IRs. In contrast, the binding of ^{3}H-idazoxan to neuronal membranes was only 25% of astrocytes and completely displaceable by epinephrine. Thus, neuronal binding was exclusively to AARs. Incubation of astrocytes with 100 μM idazoxan, but not vehicle, increased, by 15-fold, the amount of GFAP mRNA as measured by quantitative PCR. We conclude: (a) astrocytes derived from neonatal rat express IRs of the I-2 subclass; (b) the receptors are functional and can stimulate expression of the GFAP gene; (c) the majority of binding of ^{3}H-idazoxan observed in cortex is probably astrocytic; (d) since ^{3}H-idazoxan binding in brain varies topographically these results suggest regional variability in astrocytic phenotypes. *In vivo*, astrocytes may be a target cell for the actions of imidazolines in brain.

255.8

ISOLATION OF, AND PRODUCTION OF ANTIBODIES TO, THE IMIDAZOLINE RECEPTOR PROTEIN. H. Wang*, S. Regunathan, M.P. Meeley and D.J. Reis. Division of Neurobiology, Department of Neurology & Neuroscience, Cornell University Medical College, New York, NY 10021.

The imidazolines clonidine and idazoxan bind in brain to α_2-adrenergic (AAR) and to imidazoline receptors (IR). The structure of the IR is not known. We sought to isolate the IR of bovine adrenal chromaffin cells which express IRs but not AARs (Regunathan et al., *Mol. Pharmacol.* 40:884, 1991). Chromaffin cell membranes were solubilized and receptor proteins purified on para-aminoclonidine- or idazoxan-affinity matrices by elution with idazoxan. A major receptor protein was isolated with an affinity for ^{3}H-idazoxan (K_D: 3.7 nM) comparable to that for the receptor in the intact membrane (K_D: 4 nM). The number of binding sites of the isolated receptor protein (B_{max}: 465 pmol/mg protein) was enriched 1000-fold over membrane receptor (350 fmol/mg protein). The rank order of potency for inhibition of ^{3}H-idazoxan binding (K_I) to the isolated receptor protein was: cirazoline $\geq$ idazoxan > clonidine and amiloride >> rauwolscine $\approx$ epinephrine which paralleled that of membrane. Immunization of rabbits with the IR protein yielded specific polyclonal antibodies which reacted with IRs, as demonstrated on Western Blot, by immunoinhibition and immunoprecipitation of ligand binding. The anti-IR protein antibodies specifically stained cultured bovine adrenal chromaffin cells as well as subpopulations of rat adrenal medullary cells and neurons and glia in specific areas of rat brain (Ruggiero et al., *Soc. Neurosci. Abstr.*, 1992). We conclude that IRs differ biochemically from AARs and that the binding site is contained in a protein of 70 K_D which may or may not represent the complete receptor. Antibodies to the IR protein will permit mapping the distribution of IRs in brain and periphery to provide clues to function and provide a probe for isolation and cloning of the IR.

255.9

IMIDAZOLINE RECEPTOR-ASSOCIATED BINDING PROTEIN IN CENTRAL NERVOUS SYSTEM. D.A. Ruggiero*, T.A. Milner, H. Wang, S. Regunathan, M. Anwar and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

We sought to localize in rat brain a specific polyclonal antibody raised in rabbits to an imidazoline receptor-associated binding protein (IRBP) purified from bovine adrenal gland (Wang et al., *Soc. Neurosci. Abstr.*, 1992). IRBP-like immunoreactivity (IRBP-LI) was detected in neurons, neuronal processes and non-neuronal cells by light and electron microscopy (EM) and verified by control studies. Perikarya containing IRBP-LI were uncommon. A prominent cell group was observed in the med. habenular n. Immunostained neuronal processes were anatomically restricted. Labeled punctata were localized: in medulla to dorsal motor vagal n. and n. ambiguus, n. tractus solitarii (NTS; particularly medial n. centralis), superficial lamina of spinal trigeminal n. (and contiguous loci in spinal dorsal horn), dorsal cap of Kooy in inferior olivary n.; in pons to dorsal and external divisions of lat. parabrachial n. and surrounding the locus ceruleus; in midbrain to the lateral interpeduncular nucleus (i.e. component of fasciculus retroflexus); in forebrain to ventrobasal thalamic complex, midline thalamic n., and an arc extending between the central amygdaloid n. and bed nucleus of stria terminalis. Non-neuronal cells containing IRBP-LI included: astrocytes in white and gray matter sometimes aligned with penetrating vessels, and radial, subependymal and subpial glia. By EM, IRBP-LI in the NTS was localized to axons and terminals and astrocytes containing dense bundles of filaments. The results are consistent with a view that, in the CNS, imidazoline receptors are regionally distributed and are expressed both in neurons, particularly presynaptically, and glial cells.

255.10

DISTINCT SUB-CELLULAR LOCALIZATION OF IMIDAZOLINE AND α_2-ADRENERGIC RECEPTORS IN BOVINE CEREBRAL CORTEX. M.P. Meeley*, S. Regunathan, S. Roberts, S. Bramwell and D.J. Reis, Div. of Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021.

The imidazolines clonidine (CLON) and idazoxan (IDA) bind to α_2-adrenergic (AAR) and imidazoline receptors (IR) in brain. In several organs, IRs of the IDA-preferring (I-2) subclass are expressed in mitochondria (Parini et al., *J. Biol. Chem.*, 260:155, 1991). To determine whether IRs and AARs are differentially distributed between subcellar compartments in brain, synaptosomes and mitochondria were isolated from fresh bovine cerebral cortex by Percoll density gradients and membranes prepared. Binding of ^{3}H-IDA to synaptosomal and mitochondrial membranes was specific (K_D = 8 and 5 nM, respectively). Epinephrine (EPI) (10 µM) displaced 80% of binding of ^{3}H-IDA to synaptosomal but not mitochondrial membranes. ^{3}H-IDA was displaced from mitochondria by: cirazoline > IDA >> CLON >> amiloride > EPI = rauwolscine. The order in synaptosomes was: IDA > CLON > rauwolscine > cirazoline > EPI. Displacement of ^{3}H-IDA to synaptosomes not mitochondria by CLON was inhibited by Gpp(NH)p. ^{3}H-IDA binding to mitochondria but not synaptosomes was inhibited by K^+. Clonidine-displacing substance (CDS), a purported endogenous ligand for IRs, displaced binding of ^{3}H-IDA to mitochondrial membranes with high affinity. We conclude: (a) in bovine brain, AARs are localized primarily to synaptosomal membranes and regulated by GTP; (b) IRs, concentrated in mitochondrial membranes, are not G-protein linked and are regulated by K^+; (c) CDS binds to IRs of brain with high affinity; (d) Since IRs of the I-2 subclass are preponderantly glial (Regunathan et al., *Soc. Neurosci. Abstr.*, 1992) agents interacting with these IRs bind to receptors differentially localized with respect to cellular and subcellular compartments in brain.

255.11

^{125}I-CYANOPINDOLOL (ICYP) BINDING SITES IN THE TREE SHREW BRAIN. G., Flügge*, O. Ahrens and E. Fuchs. German Primate Center, Göttingen, FRG.

The ß-adrenoceptor (ß-AR) system in the brain is of special interest because it is affected by antidepressant treatments. When studying ß-AR it has to be considered that there are considerable species differences with respect to distribution and density of ß-AR subtypes. We are interested in central nervous catecholaminergic receptors of tree shrews (*Tupaia belangeri*), a species which provides a useful model for investigating effects of psychosocial stress. We therefore localized and quantified ß-AR in the tree shrew brain by *in vitro* autoradiography with ICYP which labels $ß_1$ and $ß_2$ subtypes.

Strongest ICYP binding was detected in all layers of the neocortex (mostly $ß_1$), the superior colliculus (mostly $ß_2$), and the molecular layer of the cerebellar cortex (mostly $ß_2$). Moderate binding was found in the inferior colliculus, the molecular layer of the hippocampus, the pulvinar nucleus (equal portions of $ß_1$ and $ß_2$) and the granular layer of the cerebellar cortex (mostly $ß_2$). In contrast to the rat, other regions like the dorsomedial medulla oblongata and the substantia nigra showed only very low binding. In summary, (1) the number of ICYP binding sites in the tree shrew brain is lower than in the rat and (2) the ratio of ß-AR subtypes differs between the brain regions.

VISUAL CORTEX: NEURONAL RESPONSE PROPERTIES

256.1

SPATIAL FREQUENCY CHARACTERISTICS OF LGNd AND 17-18 NEURONS IN ADULT SPLIT-CHIASM CATS. L. Richer*, M. Ptito, F. Leporé, J.-P. Guillemot Dépt. de Kinanthropologie and Psychologie, Univ. du Québec and Univ. de Montréal, Montréal, Québec, H3C 3P8.

Behavioral and physiological studies have demonstrated that high spatial frequencies and contrast sensitivity are strongly attenuated during transfer across the callosum. We have evaluated the spatial frequency characteristics of neurons in adult split-chiasm cats at two levels: in the LGNd and in the callosal recipient zone of areas 17-18. Recordings were carried out at least one month after splitting the optic-chiasm. Extracellular activity was displayed and analysed in conventional manner. Preparatory surgery to recordings was carried out using Halothane-N_2O anaesthesia and single cell activity was recorded under paralysis, local anaesthesia and N_2O. Stimulation consisted of sinusoidal gratings, swept at optimal orientation, direction and temporal frequency. Results indicated that LGNd cells in the split-chiasm cats were limited to the A1 layer and were X- and Y-like. The X-like cells had a higher spatial frequency cut-off than the Y-like cells, as in the normal cat. The visual acuity and contrast threshold of these cells were also similar in the normal and the split-chiasm cats. Cortical recordings show that about one third of the cells are still binocular in the callosal recipient zone. Binocular input originating either from the direct geniculo-cortical or callosal pathways show similar sensitivity to spatial frequency (ranging in different cells from low to high) and contrast thresholds (<10% to <20%). The spatial frequency ranges of both narrow band and low pass cells were similar in the normal and the split-chiasm cats. The high spatial frequency cut-off was lower than in the LGNd both for the direct geniculate and the callosal input. Moreover high spatial frequency cut-off seemed to be related to ocular dominance and not to the source of the visual input.

256.2

COLOR RECEPTIVE FIELDS IN MACAQUE V1: THE SPATIAL AND TEMPORAL STRUCTURE OF ISOLATED CONE INPUTS. *R. Clay Reid and Daniel Y. Ts'o**. Laboratory of Neurobiology, The Rockefeller University, NY.

We have studied the spatial structure of color selective receptive fields in the striate cortex of *Macaca fascicularis*. Optical imaging of the intrinsic signal provided maps of color-selective and highly monocular regions so that cytochrome oxidase blobs could be tentatively identified *in vivo*. White-noise checkerboard stimuli were used to map the spatiotemporal structure of receptive fields. For some circularly symmetric receptive fields, the spatial and temporal interactions between center and surround were also studied with concentric annuli modulated by white noise. The separate contributions of each of the cone types (L, M, and S: long, medium, and short wavelength-sensitive) were studied with the method of silent substitution.

Red/green parvocellular neurons in the lateral geniculate nucleus have been studied previously with similar stimuli. The antagonistic on and off inputs are segregated by cone type: rarely does one cone type provide spatially opponent linear on and off inputs to a receptive field (Reid and Shapley, Nature, 1992). In the cortex, modified type II cells with strong broad-band suppressive surrounds have been reported (Ts'o and Gilbert, J. Neurosci., 1988). Nonlinear suppression made such neurons difficult to study with two-dimensional white noise stimuli. Concentric annuli modulated by white noise were more effective in driving these cells and helped characterized the suppressive nonlinearity.

S cone input to blob cells was not always opposed by the sum of L and M cones signals. Often, the S cone input was opposed by one cone type, usually L, while the other cone type had the same sign as the S cone. (Supported by grants EY06393, EY08240, ONR N00014-91-J-1865 and the Whitaker Foundation).

256.3

COLOR PROCESSING IN THE CYTOCHROME OXIDASE-RICH BLOBS AND BRIDGES OF MACAQUE STRIATE CORTEX. C.E. Landisman* and D.Y. Ts'o. Laboratory of Neurobiology, The Rockefeller University, NY, NY 10021.

To better understand the organization of color processing in striate cortex, we have used a combination of optical imaging and electrical recording. Previous work has shown that the cytochrome blobs contain a predominance of monocular color selective cells whereas interblobs contain broadband cells (Livingstone and Hubel, 1984). Subsequently, Ts'o and Gilbert (1988) found clustering of red-green and blue-yellow opponency such that individual blobs seemed dedicated to one or the other. They also observed color selective cells that were in the bridges connecting blobs both within a single ocular dominance columns and across ocular dominance columns.

We have shown that color selective regions, as revealed by optical imaging, overlap the monocular blobs as well as span ocular dominance columns. We postulated that such color regions may correspond to two blobs connected by a bridge. Guided by optical maps of color regions and ocular dominance, we targeted sites of color regions spanning ocular dominance columns with electrode penetrations. A series of penetrations across one color region revealed a shift from monocular cells of one eye, to binocular cells, to monocular cells of the other eye. Interestingly, at times a bridge was found to connect a red-green blob to a yellow-blue blob. Histological reconstruction confirmed that the color selective regions correspond to areas of dense cytochrome oxidase staining, which includes both the bridges and the blobs.

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256.4

POSITION AND PHASE INDEPENDENCE OF NEURAL RESPONSES TO NON-CONVENTIONAL GRATINGS IN MACAQUE AREA V4. J. L. Gallant, J. Braun*, and D. C. Van Essen. Biology Division 216-76, Caltech, Pasadena CA 91125.

We previously reported that a substantial minority (~15%) of neurons in area V4 respond better to non-conventional grating patterns (concentric, hyperbolic, or radial) than to conventional sine-wave gratings (Gallant et al., Invest. Ophth. Vis. Sci. 1992). We have now investigated the position and phase dependence of neural responses in the subset of cells selective for non-conventional gratings in area V4 of anesthetized macaque monkeys. In general, cells that responded best to a non-conventional grating remained selective for that pattern when the stimuli were placed at various positions that overlapped the cell's classical receptive field. Also, the great majority of non-conventional cells showed no significant tuning for the spatial phase of the optimal non-conventional grating. Some cells responded well to only a single class of non-conventional grating (most commonly concentric), whereas other cells responded vigorously to multiple classes of non-conventional grating but not to conventional gratings.

The position and phase independence of many non-conventional cells in V4 suggests highly nonlinear spatial processing. These cells are unlikely to represent an orthogonal set of basis functions derived from differential geometry and previously proposed on theoretical grounds. Rather, they may represent the local energy associated with complex surface patterns. This may constitute an intermediate stage of form processing, between the low-level processes of V1 and the more complex characteristics of IT.

256.5

CLUSTERING OF VISUAL RESPONSE PROPERTIES IN CORTICAL AREA V4 OF MACAQUE MONKEYS. E.A. DeYoe*, S. Glickman, & J. Wieser; Dept. Cellular Biology & Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226.

To identify physiological correlates of segregated projections to V4 from thin- and inter-stripes of V2, we used long tangential penetrations through the crown of the prelunate gyrus to record neuronal responses to drifting sinusoidal gratings modulated in both luminance and hue. Eight luminance-matched colors were presented at each of 8 spatial frequencies. Responses (spike rates) were analyzed by constructing standard tuning curves for hue and spatial frequency, and by computing a measure of information conveyed by each cell (Optican & Richmond, 1987) about hue and spatial frequency separately or about all 64 stimulus conditions together. In each of 5 penetrations (108 recording sites in 4 animals) passing 3.5-4.5 mm A-P across the prelunate gyrus, we observed clustering of cells that conveyed significant information about hue (>0.2 bit) or frequency (>0.1 bit), or conveyed little information about either parameter (<0.1 bit). The latter cells responded poorly to formal test stimuli but sometimes responded well to more complex figures. Few cells (2 of 108) conveyed significant information about both color and spatial frequency simultaneously. Clusters ranged in size from 2-10 successive recording sites spanning 0.25-1.4 mm. These results suggest that V4 contains at least 2, and possibly 3, types of functional subdivisions for the processing of color and form.

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256.6

SENSITIVITY OF MONKEY INFERO-TEMPORAL CELLS TO LUMINANCE, MOTION AND TEXTURE DEFINED PATTERNS. Gy. Sary, R. Vogels* and G.A. Orban. Lab. Neuro- en Psychofysiologie, Medical School, KULeuven, Belgium.

We investigated whether TE units are sensitive to form attributes irrespective of the cue that defines this attribute. In one experiment we recorded from 415 TE cells while the monkey was discriminating the orientation of gratings of which the boundaries were defined by a discontinuity in either luminance, direction of motion or texture. We found that from the 145 cells which were tested for all three grating types one third responded to the grating stimuli irrespective of the defining cue. There was no correlation between the sensitivity for the orientation of the luminance, motion or texture boundaries. For those few cells that were significantly orientation tuned for at least two types of boundaries, the preferred orientations tended to match.

In a second experiment, using the same monkey, we presented 8 different patterns during fixation (e.g. a cross, star, triangle) and recorded from area TE. The patterns were defined either by a difference in luminance, motion or texture. Several cells responded to the patterns irrespective of the defining cue. Surprisingly, pattern selectivity usually differed according to the defining cue: e.g. selectivity for particular luminance defined patterns but no selectivity for the motion defined patterns. Also, the preference for particular patterns did not necessarily match for the different defining cues. The results of both experiments suggest that in TE sensitivity for form attributes depends on the cue which defines the form attribute.

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256.7

EXTRARETINAL SIGNALS IN RELATION TO PARALLEL PATHWAYS IN MACAQUE VISUAL CORTEX. Vincent P. Ferrera and John H. R. Maunsell* Dept. of Physiology and Center for Visual Science, University of Rochester, Rochester NY, 14642.

The visual cortex of macaque monkeys has been divided into two functional streams which have been characterized in terms of sensory processing (color/form vs. motion) and in terms of behavioral goals (object recognition vs. spatial orientation). As a step towards unifying these two views of cortical processing, we have compared the behavioral modulation of sensory signals across the two streams in monkeys trained to do a visual short-term memory task. We recorded from individual neurons in areas MT, MST, 7a and V4 while monkeys performed a delayed match-to-sample task using direction of motion as the matching criterion. This task allows us to determine if sensory responses are modulated by extraretinal signals related to the direction of the remembered sample. We sorted responses as a function of the remembered direction and calculated a modulation index, MI = (max. response - min. response)/(max + min). In the motion pathway, we found virtually no extraretinal signals in MT (avg. MI 0.022±0.007 s.e., 29 cells), but progressively stronger extraretinal signals in later stages, i.e. MST (avg. MI 0.234±0.025 s.e., 30 cells) and 7a (avg. MI 0.323±0.04 s.e., 42 cells). In contrast to MT, strong extraretinal signals for direction matching were found in V4 (avg. MI 0.411±0.031 s.e., 56 cells), a relatively early stage of the color/form pathway, even though this pathway is not generally viewed as playing a significant role in motion processing. Some cells in V4 were also tested while the animal performed a color matching task and showed memory-related modulation of their response when either color or direction was used as the matching criterion. We conclude that extraretinal signals related to the match-to-sample task are found earlier and are stronger in the temporal (color/form) pathway than in the parietal (motion) pathway, regardless of the stimulus dimension involved.

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256.8

RESPONSES OF INFERIOR TEMPORAL (IT) NEURONS DURING VISUAL SEARCH. L. Chelazzi,* E.K. Miller, J. Duncan, A. Lueschow, and R. Desimone. Lab. Neuropsychology, NIMH, Bethesda MD, 20892.

The responses of IT neurons are known to be modulated by mnemonic and attentional processes. We studied IT responses in two rhesus monkeys performing a visual search task that required both processes. Each trial began with the brief presentation of a sample stimulus at fixation. Following a delay interval of 700-3000 ms, during which fixation had to be maintained, a pair of test stimuli was shown 3-5° from the fovea and the animal was required to saccade to the stimulus (target) that matched the sample. Many cells displayed differential sustained firing during the delay interval and for most of them the magnitude of delay activity paralleled the sample preference. When a given sample was predictable from trial to trial, appropriate sample-dependent activity developed well in advance of actual sample presentation. This sustained activity is not an invariant characteristic of IT responses in short-term memory tasks, however, as it was infrequently seen in a standard matching to sample task with individual test stimuli presented at the fovea. In addition to the delay activity, some cells in the search task responded differentially to the test stimuli depending on their spatial configuration, or depending on the particular sample presented on a given trial, or depending on both factors. We conclude that all of the visual information needed for the animal to perform the search task is present in IT, for at least some cells. Supported in part by HFSPO, NATO, AFOSR, ONR, DFG.

256.9

CODING OF EXTRAPERSONAL VISUAL SPACE IN BODY-PART CENTERED COORDINATES. M. S. A. Graziano* and C. G. Gross. Psychology Department, Princeton University, Princeton, N. J. 08544

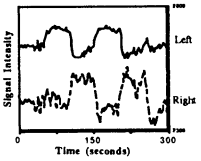
Several areas in the macaque brain contain bimodal neurons, which respond both to visual and tactile stimuli. These areas include 6, 7b, and the putamen. They are monosynaptically interconnected, forming a bimodal system. Each area contains a somatotopic map. In addition, the neurons with tactile RFs on the face or the arms often respond to visual stimuli adjacent to the tactile RF, within about 10 cm of the skin. Thus each area contains a somatotopically organized map of the visual space that immediately surrounds the monkey. This system of bimodal areas could code the location of visual stimuli in extrapersonal space.

We recorded from single neurons in these areas in anesthetized, paralyzed macaques. For a class of neurons that had tactile RFs on the arm and visual RFs adjacent to the tactile RFs, when the arm was moved, the visual RF moved with it. Thus, these neurons appear to code the location of visual stimuli in arm-centered coordinates. More generally, we suggest that the bimodal cells represent extrapersonal space in a body-part centered fashion, rather than in a head-centered or trunk-centered fashion.

256.10

FUNCTIONAL NEUROIMAGING BY MRI: HUMAN VISUAL SYSTEM J.W Belliveau*, K.K Kwong, J.R Baker, C.E Stern, R Benson, I.E Goldberg, M.S Cohen, D.N Kennedy, R.B.H Tootell, P.T Fox, T.J Brady, B.R Rosen, MGH-NMR Center, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114. *PTF at Univ Texas at San Antonio.*

Recent advances in our laboratory have yielded high speed MRI techniques that are sensitive to changes in cerebral blood volume, blood flow, and blood oxygenation (see abstract by Kwong et al.). These non-invasive methods were used to investigate the functional organization and frequency response of human primary visual cortex. Seven normal subjects underwent dynamic NMR imaging using a prototype high-speed echo planar imaging device (1.5 Tesla GE Signa modified by ANMR, Wilmington, MA). Cortical activation was measured using an oxygenation-sensitive gradient echo imaging sequence sensitive to variations in T2* (TR = 2000 ms, TE = 50 ms) and a T1-sensitive (blood flow) spin echo inversion recovery sequence (TI = 1100 ms, TR = 3500 ms, TE = 42 ms). Activated cortical areas are revealed by subtraction of averaged baseline (resting) images from all subsequent images. Frequency response studies were carried out using full-field, bilateral, pattern-flash visual stimulation goggles (Grass Instruments, Quincy MA). The stimulus rate was varied between 2 and 32 Hz. Additional studies were carried out using full-field and hemifield presentation of a counterphased checkerboard stimulus. Functional images exhibit sensitivity to stimulation frequency, and correspond well with previous PET studies. Alternating hemifield experiments show that the cortical response tracks the stimulus presentation (see figure). The ability of this technique to generate high temporal and spatial resolution data should foster greater understanding of the unique structure-function correlates involved in visual processing.



256.11

IDENTIFICATION OF EARLY VISUALLY EVOKED POTENTIAL COMPONENT SOURCES *IN VIVO* USING MAGNETIC RESONANCE IMAGING. V. P. Clark*, E. Courchesne, S. A. Hillyard, and M. Grafe. Dept. of Neurosciences, School of Medicine, U. C. San Diego, La Jolla, CA 92093-0608.

Procedures which are used to characterize and localize the component sources of visually evoked potentials (VEPs) such as the Brain Electrical Source Analysis (BESA) algorithm have been used to make claims regarding functional differences between striate and extrastriate cortical regions in humans. However, considerable inter-subject variability in the topographic position of striate cortex may lead to the erroneous identification of functionally defined regions on the basis of cerebral topographic or cranio-cerebral position alone. Here we present a method using magnetic resonance (MR) imaging to directly identify and differentiate the myeloarchitecture of striate and extrastriate cortex *in vivo*. A high-resolution MR protocol using a 1.5 Tesla magnet (Signa) was employed. Signal contrast between grey and white matter was enhanced by the use of a 5" surface coil, imaging sequences optimal for detecting grey/white contrast in the occipital lobe, and a large number of excitations to reduce noise. Also, partial voluming of adjacent laminar layers was reduced by using an imaging plane orientation orthogonal to the surface of the cortical region in question, and utilizing the highest possible spatial resolution (i.e., thin slices, small field of view, and a dense spatial matrix). After imaging, the MR data matrix was analyzed to identify upper and lower gray matter boundary locations, and adjacent data points falling within equivalent laminar levels were averaged together. Differences between laminar levels and topographic locations were tested for significance. Using this method, the stria of Gennari (which is a defining myeloarchitectonic feature of striate cortex) and extrastriate cortex were identified and differentiated *in vivo*. Correspondence of MR signal intensity, myeloarchitectonic, and cytoarchitectonic data from a post mortem brain confirms this identification. With improved spatial resolution of MR imaging, this method has the potential to distinguish up to 50 myeloarchitectonic regions *in vivo*.

VEP data has been collected from experimental subjects and the results of BESA compared to MR images of subjects' cerebral cortical topography. The approach outlined here allows the identification of the myeloarchitectonic areas that generate the early components of the VEP.

256.12

THE BRAIN STRUCTURES INVOLVED IN VISUAL ORIENTATION DISCRIMINATION IN MAN: A PET STUDY. G.A. Orban*, P. Dupont, R. Vogels, G. Bormans, J. Nuyts, C. Schiepers and L. Mortelmans. Lab. Neuro- en Psychofysiologie, Medical School, KULeuven and PET Center, Dept. of Nuclear Medicine, UZ GHB, Leuven, Belgium.

Using positron emission tomography and changes in regional cerebral blood flow measured with (O-15) water we compared the brain structures involved in two types of orientation discrimination tasks: a temporal same-different task and an identification task. In the first task subjects had to compare the orientation of two successively presented gratings (2-3° orientation difference, median % correct 87). In the second task subjects had to identify the grating presented as vertical or not (1-2° orientation difference, median % correct 83). Control tasks included a detection task and passive fixation task. Grating stimuli were identical in all tasks and were presented foveally. Fixation was controlled by EOG. Data were obtained from 7 right-handed subjects with a CTI-931/8/12 PET scanner. Approval was given by the Ethics committee of the KULeuven Med. School. The analysis of the subtraction images was done with the SPM software (Hammersmith Hospital London) and with technique of Fox and Mintun (J. Nucl. Med. 89).

Comparison of the same different and identification tasks revealed a marked lateralization in the right hemisphere of the significant foci. These included the cuneus, the gyri occipitalis medius, temporalis inferior, fusiformis, parahippocampalis, cinguli anterior, and pulvinar. Activation of the same foci was also observed after subtraction of detection from same-different. Comparison of the identification and detection task revealed in addition to gyrus lingualis and fusiformis several nonvisual structures (e.g. cerebellum, gyrus cinguli). These results suggest that same-different tasks require more visual processing than the simpler identification task, underscoring the task dependency of visual processing.

NEURAL PLASTICITY II

257.1

AUDITORY COMPENSATION OF EARLY VISUAL DEPRIVATION IN THE CAT'S ANTERIOR ECTOSYLVIAN CORTEX. J.P. Rauschecker* and M. Korte. Neuroethology Unit, NIMH, Poolesville, MD 20837, U.S.A. and Max-Planck-Institut für biologische Kybernetik, W-7400 Tübingen, Germany.

Binocular deprivation (BD) from birth leads to improvement of sound localization in cats. The superior colliculus (SC) in the midbrain tectum is one candidate structure in which the neural basis for this behavioral change could be sought. Indeed, a four-fold increase of auditory-responsive units is found in the SC of visually deprived cats (Rauschecker and Harris, 1983). The major auditory cortical input to SC comes from the anterior ectosylvian cortex (AES), and this projection is strengthened in visually deprived cats.

Neuronal responses in AES to visual, auditory, and somatosensory stimuli were studied in normal and visually deprived cats under halothane anesthesia. Just like in the SC, drastically changed proportions of neurons with responses to the different modalities were found. Neurons in the fundus of the anterior ectosylvian sulcus (area AEV; Mucke et al., 1982; Olson & Graybiel, 1987), which normally react exclusively to visual stimuli, responded vigorously to auditory and somatosensory stimuli. The few neurons that could still be driven by visual stimulation were now bimodal, responding also to other sensory modalities. No increase of unresponsive units was found.

Auditory spatial tuning width for the azimuth of a sound source was compared for neurons in the anterior ectosylvian region of normal and blind-reared cats using broad-band stimuli. A spatial tuning (ST) index was calculated for each cell as the min/max ratio between responses in 7 different azimuthal positions (Rajan et al., 1990). The distribution of ST indices for blind cats was significantly shifted towards smaller values indicating sharper tuning ($p<0.001$, median and Smirnov tests). In addition, the proportion of omni-directional units was 44% in normal cats and only 14% in visually deprived cats. The half-widths of spatial tuning curves were also significantly reduced in all BD animals. It seems possible that these changes in the cortical AES region form the neural basis for the improvements in sound localization behavior of visually deprived cats and that the changes in the SC are only secondary.

257.2

VISUAL INPUTS ROUTED TO THE AUDITORY PATHWAY IN FERRETS: BEHAVIORAL RESULTS. L.S. Carman*, S.L. Pallas+ and M. Sur. Department of Brain and Cognitive Sciences, M.I.T. Cambridge, MA 02139 +Division of Medicine, Baylor College of Medicine, Houston, TX 77030

A retinal projection to the auditory pathway of the brain can be induced in neonatal ferrets by ablating or reducing normal retinal ganglion cell targets (the superior colliculus and lateral geniculate nucleus) and deafferenting the auditory thalamic nucleus (medial geniculate nucleus, MGN) (Sur et al., 1988). This 'rewired' retinal projection confers visual responsivity on cells in the MGN and in primary auditory cortex (A1) (Roe et al., 1990, 1991). We sought to determine (i) whether this rewired projection is capable of mediating behavior, and (ii) whether rewired ferrets perceive a light stimulus presented to the rewired A1 as visual or auditory. Unilaterally rewired ferrets were trained to distinguish between auditory stimuli and a light stimulus presented to the non-rewired hemisphere. The perceptual function of the rewired projection to A1 was then examined by presenting test light stimuli to the rewired hemisphere. Prior isolation of remnant visual thalamus in the rewired hemisphere with ibotenic acid ensured that the visual stimuli activated the rewired pathway. Rewired ferrets consistently responded to test stimuli as though they were visual. To confirm that the responses to test light stimuli were mediated by the rewired projection and its representation in A1, the auditory cortex of rewired ferrets was ablated; ferrets responses were reduced to chance level. These results indicate that the functional modality of a sensory cortical region can be respecified by novel sensory innervation. Supported by EY07719

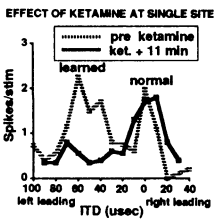
257.3

LEARNED AUDITORY RESPONSES IN THE BARN OWL'S OPTIC TECTUM ARE PREFERENTIALLY SUPPRESSED BY THE NMDA RECEPTOR BLOCKER KETAMINE. M.S. Brainard*, D.E. Feldman and E.I. Knudsen. Dept. Neurobiology, Stanford University, Stanford, CA 94305.

Space-specific neurons in the barn owl's optic tectum (OT; superior colliculus) are normally tuned to the interaural timing differences (ITDs) which are produced by sounds at the locations of their visual receptive fields. Neurons in owls raised with laterally displacing prisms become tuned for abnormal ITDs corresponding to the locations of their optically displaced visual receptive fields. We have found that these learned responses to abnormal ITDs are strongly suppressed by ketamine.

Owls were raised with prismatic glasses that laterally displaced the visual field. Beginning at 60 days of age, extracellular recordings were used to measure unit tuning for the ITD of sounds delivered via earphones. At some recording sites, units responded both to the ITD which was normal for that location in the tectum ("normal ITD"), and to the ITD which corresponded to the prismatically displaced visual receptive field ("learned ITD"). At those sites, responses to repeated presentations of normal and learned ITDs were monitored for a 20 minute control period, and then for up to 100 minutes following injection of ketamine HCL (10-25 mg/kg; IM).

Ketamine suppressed responses to learned ITDs by 40 to 90% during the 20 minute period following injection, with gradual recovery over the next hour. In contrast, ketamine had a consistently smaller effect on responses of units to normal ITDs. The same levels of ketamine blocked responses of OT units to iontophoresed NMDA but not quisqualate. These results suggest that NMDA receptors may participate differentially in the expression of responses to the learned versus normal range of ITDs.



Supported by NIH: RO1 DC00155-12, NSF: RCD 8758111 & HHMI: predoctoral fellowship.

257.4

INDUCTION BY SEIZURES OF F1/GAP-43 GENE EXPRESSION IN HIPPOCAMPAL GRANULE CELLS. A. Routtenberg*[1], P. J. Meberg[1], and C. M. Gall[2]. 1. Cresap Neurosci. Lab., Northwestern Univ. Evanston, IL 60208. 2. Dept. of Anatomy and Neurobiology, Univ. of California, Irvine 92717.

F1/GAP-43 mRNA is highly expressed in pyramidal cells of the adult rat hippocampus, but is nearly absent in the granule cells even at the time of mossy fiber outgrowth in the neonate [Neuroscience 45:721 (1991)]. To determine whether granule cells can be induced to express F1/GAP-43 mRNA, we studied transcript levels after seizures, which are known to increase the expression of other genes [Prog. Brain Res. 83:371 (1990)]. Seizure-inducing electrolytic lesions were made in the hilus with stainless-steel electrodes, and then mRNA levels were measured in contralateral hippocampus by quantitative *in situ* hybridization. F1/GAP-43 expression was induced in granule cells. Grain density was almost 20-fold higher at 24 hr, as compared to 6 or 12 hr post-lesion. Labeling over granule cells had declined by 48 hr post-lesion; but even at 10 days was still higher than controls. This sustained elevation of F1/GAP-43 mRNA expression may be important for the sprouting of mossy fibers shown to occur after seizures in other paradigms. F1/GAP-43 expression also increased 2-3 fold in CA1 pyramidal cells, but the peak expression occurred at 48 hr post-lesion. The changes were attributable to the seizures, as labeling in all cell fields was similar to controls when similar lesions were made with a platinum electrode which does not induce seizures. These data provide evidence that F1/GAP-43 expression can be induced in adult neurons which normally do not express F1/GAP-43. This increased expression by seizures may be a useful paradigm for studying whether regulation of F1/GAP-43 in the mature CNS is by transcription [J. Neurosci. 12:691 (1992)] or mRNA stability [Molec. Cell. Neurosci. 2:402 (1991)]. [Supported by MH25281-18 and AFOSR-90-0240 to A.R. and NS-26748 to C.G.]

257.5

AFFERENT STIMULATION IN THE ENTORHINAL CORTEX INCREASES THE CA1 RELEASE OF GABA, GLUTAMATE AND ASPARTATE, IN VIVO, ASSOCIATED WITH INCREASED EXPRESSION OF NGF AND BDNF mRNA IN THE RAT HIPPOCAMPUS. T. Falkenberg*, W.T. O'Connor, U. Ungerstedt, H. Persson[1], and N. Lindefors. Department of Pharmacology and [1]Department of Medical Chemistry, Laboratory of Molecular Neurobiology, Karolinska Institutet, P.O. Box 60 400, S-104 01 Stockholm, Sweden

The present study was undertaken to understand the relative role of afferent stimulation in the entorhinal cortex on the synaptic release of neuroactive aminoacids and their involvement in regulation of gene expression of neurotrophic factors. Transmittor release was measured, *in vivo*, by means of microdialysis in the dorsal CA1 region of the hippocampus in awake freely moving rats as well as behavioural effects following injection of different doses of quisqualate into the lateral part of the lateral entorhinal cortex. The entorhinal cortex is the major source of cortical input to the hippocampal formation and we have shown using a similar paradigm as in this study that afferent transsynaptic stimulation differentially regulate nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) mRNA expression. Furthermore, pretreatment with the benzodiazepine receptor agonist diazepam completely prevented the increase in NGF and BDNF mRNA expression seen following afferent activation. Preliminary results indicate that quisqalate injection results in dose-dependent release of GABA, glutamate and aspartate and dose-dependent effects on behaviour, such as increased exploratory behaviour, epileptogenic activity and stereotypic movements. Changes in behavioural activity accompanied changes in basal transmittor levels. Similar effects were also seen after injection of quisqualate into the perirhinal cortex, however, epileptogenic activity could not be observed. Experiments are in progress to determine, with *in situ* hybridisation, if there are transmittor release dependent changes in mRNA levels of NGF and BDNF following afferent activation with different concentrations of quisqualate. Thus we are currently investigating the possibility that changes in release of GABA, glutamate and aspartate differently regulate gene expression of BDNF and NGF that may mediate changes in neuronal plasticity in the hippocampus.

257.6

BRAIN LOCALIZATION OF ACTIVIN AND INHIBIN IN RATS WITH NEURAL PLASTICITY RESPONDING TO DEFICIENT NUTRIENT CENTRALLY AND DURING INGESTION UNDER PROTEIN OR L-LYSINE DEFICIENCY K. Torii[1]*, K. Oosawa[2], M. Funaba[3], A. Okiyama[1], T. Murata[2], M. Takahashi[4] and T. Ono[5] [1]Ajinomoto Co. Inc.; [2]ERATO, R&D Corp. of Japan, Yokohama; [3]Azabu Univ., Sagamihara; [4]Univ. of Tokyo; [5]Toyama Med. Pharm. Univ., Toyama, Japan

Each L-amino acid(AA) in plasma and brain remains unchanged all day long while normal diet is available. Once L-lysine(Lys) deficient diet offered to rats, Lys in plasma and brain declined. When solutions of AAs were offered, they selected the Lys solution and their food intake and growth normalized. The recording of single neuron activity in the lateral hypothalamic area (LHA) of these rats suggested that the neural plasticity occurred, specifically responding to deficient nutrient, Lys, centrally and during ingestion of AA. Also the release of possible neurotrophic factors, activin A (β_A-β_A) or inhibin (α-β_A), in plasma of rat with or without deficiency both protein and Lys were determined respectively by *Hydra Japonica* assay. The present study determined the brain localization of activin A and inhibin using rabbit polyclonal antibody against several peptides of partial sequence both α- and β_A-subunit. Sprague-Dawley strain male rats (N=3, each group), 6 weeks of age, fed a diet with or without protein or Lys deficiency for 4 days, then each brain, fixed by gluthalaldehyde, was serially sectioned 30 μm in thick. The localization of immunochemically positive area for antibodies against α-subunit in the brain were seen in the arcuate nucleus (AN), the ventromedial hypothalamus, CA1 layer of the hippocampus and the nucleus of tractus solitarius (NTS). In the case of antibodies against β_A-subunit, the AN, the amygdala and the NTS were also positive. The degrees of positive area were quite comparable to each other nutritional treatment. These results suggest that the neural plasticity is not merely happen in the lateral hypothalamic area induced by Lys deficiency, and that activin A and/or inhibin may be involved in the plasticity in brain to maintain AA homeostasis, coupling with changes of preference and appetite for AA.

257.7

ACTIVITY-DEPENDENT PLASTICITY OF INHIBITORY AND EXCITATORY AMINO ACID TRANSMITTER SYSTEMS IN CULTURED RAT CEREBRAL CORTEX . G.J.A. Ramakers*, H. van Galen, M.G.P. Feenstra, M.A. Corner and G.J. Boer. Netherlands Inst. for Brain Res., Meibergdreef 33, Amsterdam, The Netherlands.

Chronic suppression of spontaneous bioelectric activity in rat cerebral cortex cultures increases neuronal cell death and results in electrophysiological alterations, indicative of changes in the balance between excitatory and inhibitory neurotransmitter systems. To delineate mechanisms that could underlie this disbalance, we investigated the effects of chronic silencing with tetrodotoxin (TTX) on the content and release of glutamate, aspartate and gamma-aminobutyric acid (GABA) in culture. Chronic TTX treatment decreased the content of all amino acids investigated. However, relative to the general neuronal marker NSE (neuron-specific enolase) only GABA was decreased, indicating a disproportionate loss of GABAergic neurons upon chronic silencing. The K+- induced release of GABA, glutamate and aspartate increased about tenfold between 7 and 21 days *in vitro* in control cultures. Chronic TTX treatment significantly increased the depolarization-induced release of glutamate and aspartate at 7 days *in vitro*, and at all ages increased the ratio of the evoked release of the excitatory amino acids to that of GABA more than twofold. These observations support the hypothesis that chronic silencing increases the ratio of excitatory to inhibitory synaptic activity through several mechanisms, all of which may be involved in activity-dependent functional neuroplasticity in vitro as well as in vivo.

257.8

FUNCTIONAL ADAPTATION TO CORTICOSPINAL TRACT DISRUPTION IN THE HUMAN BRAIN. JJM Kew, PN Leigh*, RE Rassingham, RSJ Frackowiak, DJ Brooks. MRC Cyclotron Unit, Hammersmith Hospital, London, UK; Department of Neurology, Institute of Psychiatry, London, UK.

To determine the way the brain adapts to a corticospinal tract lesion, we studied cortical function in 12 patients with amyotrophic lateral sclerosis (ALS), a disease causing neuronal loss in the motor cortex and degeneration of the corticospinal tract. Six normal human volunteers were studied for comparison. Positron emission tomography (PET) was used to image regional cerebral blood flow (rCBF) in subjects at rest and during performance of paced, freely-selected movements of a joystick with the right hand.

Comparison of the increase in rCBF caused by the task between the two groups of subjects showed significantly ($p<0.001$) greater activation in ALS patients in the lower third (face area) of the contralateral sensorimotor cortex, the contralateral anterior insula, and the ipsilateral cingulate cortex (dorso-caudal area 24). Accompanying facial movements were not observed in patients.

The ventral expansion of the upper limb output zone in ALS patients suggests that recruitment of neurons lying in the face output zone but projecting to upper limb target motoneurons may occur in response to a corticospinal tract lesion. Recruitment of insular and cingulate motor areas may represent further functional adaptation. (Supported by the Medical Research Council)

257.9

REGIONAL CORTICAL GYRAL VARIATIONS IN HUMAN MONOZYGOTIC TWINS. D. R. Weinberger*, A. J. Bartley, D. W. Jones, J. R. Zigun. Clinical Brain Disorders Branch, National Institute of Mental Health, Washington, D.C. 20032

Early hypotheses about the role of physical forces in the development of human cortical gyri have yielded to the current view that gyral patterns are determined primarily by genetic factors and reflect underlying connectivity. The advent of high fidelity renderings of cortical surfaces from thin section (1.5 mm) MRI and a quantitative cross correlation method for comparing gyral patterns on such images (Bartley AJ, et al, Neuroscience Abs, 1992) have made it possible to test in living subjects hypotheses about human cortical development. We evaluated cortical gyral patterns on volume renderings of the cortical surface from MRI scans of five pairs of normal monozygotic (MZ) twins. Pattern concordance was greatest within twin pairs for cerebellar folia (mean ± sem cross correlation value = .45 ± .02) and least for dorsolateral prefrontal cortex (.21 ± .02). Concordance in other cortical areas ranged from .25 to .33. No consistent lateralization effects were seen. In random pairings of unrelated twins, concordance values were considerably lower. This study demonstrates that while MZ twin pairs have greater cortical pattern similarity than pairs of unrelated individuals, even within MZ twins, there is considerable variability in cortical gyral patterns. Greatest variance is found in highly evolved, late maturing prefrontal cortex, suggesting that individual experiential factors play a role in the development of human cortical gyral variation.

257.10

AGE RELATED PLASTICITY AND THE HIPPOCAMPAL MEMORY IMPAIRMENT. E.J. Holmes*, Lab. for Neuropsychology, Hampton University, Hampton, VA 23668.

Previous studies from this laboratory have demonstrated that adult rats which received large bilateral lesions of the hippocampus in infancy were less impaired on an 8-choice radial arm maze task than animals which received analogous lesions during early adulthood. These studies also demonstrated that animals which were operated at approximately 3 weeks of age were the least impaired when tested as adults with the level of impairment increasing as the age at the time of surgery increased. Therefore, it appeared that the younger the animal was at the time of surgery, the greater the sparing of impairment should be when tested for spatial memory capacity during adulthood. To test this hypothesis, ten adult animals which were operated at approximately 2 weeks of age (i.e., 10-15 days old) were tested on the same radial arm maze task following a post-operative period equal to that given the other operated groups. Surprisingly, the results showed that the adults operated at at 2 weeks of age were markedly more impaired than the adults operated at 3 weeks (U=0, $p < .05$, Mann-Whitney U-test) and performed most like the subjects operated in early adulthood. These results are quite provoking and serve to help deflate the notion of "the earlier the brain damage, the better the recovery." Clearly, the timing of the damage is critical and suggests significant ongoing changes in both neurochemical and neuroanatomical development during this period.

257.11

SELECTIVE NEUROANATOMICAL PLASTICITY AND DIVISION OF LABOR IN THE HONEY BEE (*APIS MELLIFERA*). G.S. Withers*, S.E. Fahrbach, & G.E. Robinson, Neuroscience Program & Dept. of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

The division of labor in a honey bee colony is organized in part by age of the worker bee. Younger bees typically work within the hive; older bees forage for food. To determine whether neuroanatomical plasticity is associated with this behavioral maturation, unbiased volume estimates of all major brain regions were made using Cavalieri's Direct Estimator. Tissue was sampled from bees at three distinct stages: inexperienced 1-day old bees, nurse bees caring for brood (typically 7-10 days of age) and pollen foragers (21 days and older).

While there was no overall change in the volume of the brain, our survey revealed two significant regional changes. Nurse bees had the greatest volume of olfactory glomeruli relative to both 1-day old bees and foragers. This may reflect the important role of chemosensory cues in regulating behavior within the dark hive. An entirely different pattern was seen in the mushroom bodies (corpora pedunculata). In this region, the volume of neuropil of was significantly increased in foragers, while the volume occupied by the intrinsic cell population was significantly decreased. As the mushroom bodies are important in both multimodal integration and learning, their function is likely to be critical in foraging behavior. This is the first evidence of substantial region-specific plasticity associated with the acquisition of complex behaviors in an invertebrate.

257.12

LONG-TERM INCREASE IN ACTION POTENTIAL AMPLITUDE AND SOMA EXCITABILITY IN *APLYSIA* SENSORY NEURONS FOLLOWING PERIAXONAL IMMUNE REACTION . A.L. Clatworthy*, G.A. Castro, E.T. Walters. Dept. of Physiology & Cell Biology, Univ. Texas Medical School at Houston, TX 77225.

Implantation of cotton string around nerves containing axons of sensory neurons (SN) causes a foreign body reaction ("granuloma") associated with a long-term decrease in spike threshold and accommodation during 1 s depolarizing test pulses delivered to the SN soma (Alizadeh et al. *Soc. Neurosci. Abstr.* 16:597, 1990). Using similar methods we have found that additional properties of the soma are altered 6-9 days after ipsilateral implantation of string around pedal nerves, and that the soma alterations occur in the apparent absence of injury to the axons. For SNs on the implanted side vs. contralateral SNs, spike amplitude was greater (90 vs. 84 mV), spike duration was longer (3.8 vs. 3.3 ms), the AHP was smaller (3.4 vs. 3.8 mV), and input resistance was greater (40 vs. 28 MΩ) ($p<.05$ in each case; n=14 animals with 4-8 SN pairs tested per animal). Replicating our previous findings, both spike threshold and accommodation were lower in these same animals (1.0 vs 1.2 nA, and 3.2 vs 1.2 spikes/1 sec pulse)($p<.02$). Stimulation of nerves distal to the granuloma always evoked a spike in the SN soma in cells that also responded with a spike to proximal nerve stimulation, indicating normal conduction through the implanted region. Furthermore, light microscopic examination of sections through the granuloma revealed no evidence of axonal injury. We are beginning to examine potential contributions from immunocytes, nerve stretch, and injury of the nerve sheath to this long-term SN plasticity.

257.13

LONG-TERM ALTERATION OF SOMA MEMBRANE PROPERTIES IN PEDAL MOTOR NEURONS AND MUCUS MOTOR NEURON R2 OF *APLYSIA* FOLLOWING AXONAL INJURY. M. F. Dulin and E.T. Walters*. Dept. of Physiology & Cell Biology, University of Texas Medical School at Houston, TX 77225.

Crushing the axons of sensory neurons in *Aplysia* causes a delayed hyperexcitability of the soma that lasts for weeks (Walters et al. *Science* 253:797, 1991). We have now examined motor neurons using similar procedures. Relative to paired contralateral controls, pedal motor neurons with crushed axons displayed a significantly greater AHP (14.2 vs. 8.6 mV)($p=.03$) and tendencies for a lower spike threshold (1.2 vs. 1.8 nA)($p=.09$) and greater resting potential (-46.7 vs. -41.9 mV)($p=.2$) 5 days after unilateral pedal nerve crush performed under strong anesthesia (n=6 animals with 4-6 cell pairs per animal, 2-tailed tests). In contrast to sensory neurons, pedal motor neurons did not display noticeable alterations in spike duration or accommodation ($p>.3$). Axons of mucus motor neuron R2 were injured by nearby crush of the right connective and branchial nerve (8 crushed and 10 control cells). Preliminary results indicate a tendency for spike accommodation to decrease (17.5 vs. 12 spikes/1 s pulse, $p=.08$), resting potential to increase (-59.3 vs. -52.3 mV, $p=.18$), and AHP to decrease (5.6 vs. 8.6 mV, $p=.23$). These early results indicate that long-term, injury-induced plasticity of electrophysiological properties occurs in motor neurons as well as sensory neurons in *Aplysia*. Furthermore, the plastic changes differ between sensory and motor neurons, and may differ between different classes of motor neurons.

258.1

OLFACTOMEDIN: A NOVEL EXTRACELLULAR GLYCOPROTEIN UNIQUELY EXPRESSED IN OLFACTORY NEUROEPITHELIUM. R. R. H. Anholt*, H. Yokoe, and D. A. Snyder. Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

Olfactomedin is a 57 kD glycoprotein synthesized by Bowman's glands and sustentacular cells and deposited in the extracellular matrix of the olfactory neuroepithelium where it remains confined to the viscous lower mucus layer. The protein exists as a 120 kD homodimer formed via a disulfide bond. We purified olfactomedin from olfactory tissue of Rana catesbeiana by lectin affinity chromatography and determined its N-terminal sequence. We then used a 16-fold degenerate 47-mer oligonucleotide to screen a cDNA library and identify a clone with a 1.9 kb insert which encodes olfactomedin. Partial sequence analysis of this cDNA reveals a polyadenylation site near its 3'-end and an open reading frame at its 5'-end. It encodes a hydrophobic leader peptide followed by a highly hydrophilic domain which contains, as expected, several potential N-linked glycosylation sites along with cysteines, which may form intramolecular disulfides to stabilize the conformation of the polypeptide. The 250 amino acid sequence, thus far determined, does not display homologies to any known proteins. The abundance of this unique olfactory glycoprotein at the chemosensory surface suggests that it may be essential for the structural integrity, morphogenesis or chemosensory function of the olfactory membrane. Supported by NIH grant DC00394 and ARO grant DAAL03-89-K-0187.

258.2

PATTERNS OF EXPRESSION OF PUTATIVE OLFACTORY RECEPTORS A. M. Cunningham, N. S. Levy, D. K. Ryugo and R. R. Reed.* Depts of Molecular Biology and Genetics, Neuroscience and Otolaryngology-HNS, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Olfactory signal transduction has been shown biochemically to involve a GTP-dependent increase in cAMP levels in olfactory neuronal cilia. Subsequently, novel components of this G-protein coupled cascade have been identified: an olfactory specific G-protein, G_{olf}, an adenylate cyclase and a cyclic nucleotide-gated ion channel. The receptors involved in odorant binding remained unknown until efforts to identify them by sequence similarity to other G-protein coupled receptors succeeded in defining a new large gene family (Buck & Axel, 1991). Work in our laboratory has added new members to this family and we are studying their genomic organization.

We have generated anti-peptide antibodies designed to recognize most members of this family. Immunohistochemical studies have confirmed expression of the receptor proteins exclusively by neurons in the rat olfactory neuroepithelium, both in dendrites and sensory cilia. Current immunoEM studies of olfactory cilia will define the relationship of these receptors to other components of the transduction apparatus. Recently we have produced antibodies to a small subset of the receptors. These antibodies recognize only a subpopulation of the sensory neurons (approx. 10-20%) and the expressing cells are distributed uniformly in the neuroepithelium. Information about receptor distribution within neurons in the epithelium and the connections of these cells in the olfactory bulb will form the basis of our understanding of the mechanism of olfactory sensory coding at a molecular level. To further this, we are performing in situ hybridization studies with probes made to specific receptors.

It has been shown that some odorants can stimulate the phosphatidylinositol (PI) pathway. As we have shown virtually all neurons in the epithelium express this receptor family, it may be that some of these receptors are capable of activating the PI pathway. We have expressed receptors in a mammalian cell line in an attempt to study receptor-ligand relationships and second messenger generation. The combined results of these approaches will provide insight into the mechanism of olfactory receptor stimulation and how odorant information is conveyed to the higher centers.

258.3

EXPRESSION AND ANAYLSIS OF COMPONENTS OF THE OLFACTORY SIGNALING SYSTEM IN XENOPUS OOCYTES. Jonathan Bradley, Yasuhito Uezono, Norman Davidson, Henry Lester*, and Kai Zinn. Division of Biology, California Institute of Technology, Pasadena, CA 91125

In order to identify and characterize olfactory receptors for specific odorant chemicals, we have developed a method for generating a electrophysiological signal in response to cAMP elevation in Xenopus oocytes. To do this, we expressed the cystic fibrosis transmembrane regulator (CFTR), a chloride channel that is controlled *via* phosphorylation by cAMP-dependent protein kinase A (see abstract by Y. Uezono et al.). When rat nasal epithelial polyA+RNA is injected into oocytes together with synthetic CFTR mRNA and a mixture of odorants applied to the oocytes, a large (up to 800 nA)chloride current is observed. We made an olfactory cDNA library, screened it at low stringency with a PCR probe recognizing the ofactory 7-helix receptor gene family recently identified by Buck and Axel (Cell 65, 175), and isolated a large number of hybridizing clones. Pools of synthetic mRNAs from these clones were injected into oocytes together with CFTR mRNA and tested with odorant mixtures. We now have preliminary data indicating that single clones can mediate odorant responses.

In other work, we have cloned cDNAs encoding a new cyclic nucleotide-gatedcation channel expressed in rat nasal epithelium, and are characterizing this channel by heterologous expression. Support: NS28182, GM29836, CFF, Pew Foundation, McKight Foundation, Drown Foundation.

258.4

BASAL CELLS MODULATE RECEPTOR CELL FUNCTION IN *NECTURUS* TASTE BUDS BY A SEROTONERGIC MECHANISM. Douglas A. Ewald* & Stephen D. Roper Dept. of Anatomy and Neurobiology, Colorado State Uuniversity, Fort Collins CO 80523 and the Rocky Mountain Taste and Smell Center, Denver CO 80262

Basal cells, which comprise only 10% of the total cell population of *Necturus* taste buds, are involved in the majority of morphologically identifiable synapses in the taste bud (Delay & Roper, J. Comp. Neurol. **277**: 268, 1988). In slices of lingual epithelium, focal chemical stimulation (140 mM KCl) of the apical tips of taste receptor cells elicits receptor potentials in taste receptor cells and postsynaptic responses in basal cells (Ewald & Roper, J. Neurophysiol. **67**: 1316, 1992). It is now known that Merkel-like basal cells in the *Necturus* taste bud contain serotonin (5-HT; Welton, Taylor, Delay & Roper, AChemS. Abstr. #48 & #194, 1992). We have bath applied 5-HT (100 μ M) to thin slices of lingual epithelium that contain taste buds while recording intracellular responses from taste cells. Under these conditions 5-HT increased the input resistance of receptor cells (mean $\pm$ S.E.: 54 $\pm$ 9%, N= 14) and thereby decreased the electrotonic decay of receptor potentials from the apical to the basal ends of the receptor cells (39 $\pm$ 13% increase in amplitude in the same cells). During 5-HT application we also observed an increase in the postsynaptic potentials recorded in basal cells (45 $\pm$ 17%, N=9), presumably due to the increased amplitude of the presynaptic (receptor) potential. Next, direct electrical depolarization of basal cells (two or more 1-sec pulses to 20-50 mV above resting potential, applied through the intracellular recording electrode) was used in an attempt to stimulate the release of endogenous 5-HT. This procedure increased the amplitude of subsequent postsynaptic responses recorded in the same basal cell (42 $\pm$ 10%, N=8) and thus mimicked the effect of bath-applied 5-HT. These results are consistent with the view that 5-HT, released from basal cells, can modulate receptor cell function and alter synaptic transmission in taste buds. Supported by NIH grant DC01238.

258.5

PATTERNS OF ADRENERGIC AND PEPTIDERGIC INNERVATION OF HUMAN OLFACTORY MUCOSA. **Y. Chen[1], M.L. Getchell*[1,2], N.S. Rama Krishna[3], Z.Y. Su[3] and T.V. Getchell[1,2,3], 1, Div. of Otolaryngol., Dept. of Surgery; 2, Sanders-Brown Center on Aging; 3, Dept. of Physiol. & Biophys., University of Kentucky College of Medicine, Lexington, KY. 40536.**

Tyrosine hydroxylase (TH)- and dopamine-β-hydroxylase (DβH)-immunoreactive (ir) fibers innervate blood vessels and Bowman's glands in the lamina propria of human olfactory mucosa. In this study, colocalization of neuropeptide Y (NPY) and DβH immunoreactivity and the relation of TH- and DβH-ir fibers to olfactory marker protein (OMP)-ir fibers were investigated using double staining immunofluorescence techniques. Sections were stained with antibodies to DβH and NPY, TH and OMP, or DβH and OMP simultaneously. Colocalized DβH- and NPY-ir was observed primarily in fibers around blood vessels in the deep vascular layer of lamina propria; few were found near Bowman's glands. TH- and DβH-ir fibers were present in the epineurium and perineurium of OMP-positive nerve bundles; the adrenergic fibers appear to innervate vasa nervorum, blood vessels in the sheaths that supply enclosed olfactory nerve fibers. No fibers contained colocalized OMP- and TH-ir or OMP- and DβH-ir. Thus vasomotor activity and secretion are regulated by adrenergic and peptidergic input, and sympathetic nerve fibers regulate blood flow to olfactory nerves. Supported by NSF BNS-88-21074 (MLG) and NIH DC-00159 (TVG).

258.6

EFFECTIVENESS OF HOMOLOGOUS SERIES OF NONREACTIVE AIRBORNE CHEMICALS IN EVOKING NASAL IRRITATION AND ODOR. J. E. Cometto-Muñiz and W. S. Cain*. John B. Pierce Lab. and Yale Univ., New Haven, CT 06519.

By testing anosmic and normosmic subjects we assessed the relative sensitivity of the nasal trigeminal and olfactory nerves in the detection of nonreactive airborne chemicals. Anosmics, lacking a functional sense of smell, provided nasal irritation thresholds. Normosmics provided odor thresholds. The stimuli comprised homologous ketones and selected secondary and tertiary alcohols and acetates. Anosmics detected all compounds, albeit at much higher concentrations than normosmics. As seen before with homologous alcohols (Physiol. Behav. **48**(5) 719-725, 1990) and acetates (Pharmacol. Biochem. Behav. **39**(4) 983-989, 1991), both types of thresholds decreased with carbon chain length, odor declining more rapidly. Nasal irritation was evoked at a fairly constant percentage of vapor saturation (≈32%) irrespective of molecular size or chemical functionality. Such a relationship does not hold for odor thresholds. The outcome suggests that nasal irritation from nonreactive substances relies heavily on a physical, rather than chemical, interaction with the nasal mucosa. Supported by NIH Grant DC00284. J.E.C.-M. is a member of the Carrera del Investigador Científico, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), República Argentina.

258.7

NOVEL MICROVILLAR CELL IN THE OLFACTORY EPITHELIUM OF CARTILAGINOUS FISH. S. Takami* and P.P.C. Graziadei, Department of Biological Science, Florida State University, Tallahassee, FL 32306.

It has been reported that the olfactory epithelium (OE) of cartilaginous fishes contains exclusively microvillar receptor cell but no ciliated receptor cells. We will report here that the OE of the clearnose skate (*Raja eglanteria*) contains a novel type of microvillar cells. The OE of juvenile *Raja* of 2-4 months after hatching was observed by using transmission electron microscopy (TEM) and scanning (S) EM. By TEM, we have confirmed the microvillar receptor cells which have been reported in other fishes; these cells contained well developed lamellae of rough endoplasmic reticulum (rER) in the perikaryal region and their dendritic ending had some microvilli (thickness; about 100 nm) on the surface. We also found a second type of receptor cell-like perikarya which contains a well developed Golgi apparatus and many smooth vesicles around it, but rare rER. Apical processes (dendrites) of these cells contained some mitochondria, sparse microtubles and numerous smooth vesicles, and projected a hemispherical ending from the luminal surface. This ending appeared to have a few thick microvilli (thickness; 250-300 nm) which were even thicker than cilia of sustentacular cells. Each microvillus was filled with a bundle of filaments and this bundle could be traced down to the dendrite as far as 7 μm. By SEM, hemispherical endings with a few short and thick processes were confirmed. The above observations strongly suggest the presence of two types of olfactory receptor cells in skates; one with "stiff" microvilli and the other with microvilli of more conventional structure.

(The specimens were kindly provided by Dr. Carl A. Luer, Mote Marine Laboratory. Supported by NIH Grants NS20699 and DC01071-01.)

258.8

HYPERTHYROIDISM STIMULATES CELL GENESIS IN THE OLFACTORY EPITHELIUM OF PREMETAMORPHIC, *XENOPUS LAEVIS* LARVAE. G.D. Burd*. Depts. of Anatomy and Molecular & Cellular Biology, Life Sciences South, University of Arizona, Tucson, AZ 85721.

In *Xenopus* larvae, thyroxine (T_4) pellets (10%) placed adjacent to the nasal capsule stimulate cell genesis in the olfactory epithelium and produce an increase in the number of olfactory axons (Burd. 1990. In: ISOT X, Døving, ed.). Blocking thyroid hormone synthesis with propylthiouracil (0.01%) reduces cell genesis in the olfactory epithelium (Burd and Thomas. 1990. Chem. Sen. 15:558) and the number of olfactory axons (Burd. 1992. J.Comp.Neur. 315:255). In the present study, I examined whether olfactory axon number and olfactory epithelium volume and cell genesis were increased in tadpoles treated for 6 days with 5 nM T_4 in rearing solution (a dose similar to blood levels of larvae entering metamorphic climax). Treatment began when larvae reached stage 48, well before normal synthesis of thyroid hormone begins. Six days after hormone treatment, T_4-treated larvae and sibling controls were injected (I.P.) with 1 μCi ^{3}H-thymidine. Twenty-four hours later, the animals were processed for electron microscopy and autoradiography. I found that this T_4 treatment stimulates a 5-fold increase in the number of ^{3}H-thymidine labelled cells in the olfactory epithelium and a 2-fold increase in the volume of the sensory epithelium. The number of olfactory axons also increased, but not significantly. In summary, thyroid hormone, at levels equivalent to physiological values in larvae entering metamorphic climax, induces cell genesis and expansion of sensory epithelium in premetamorphic larvae. A significant increase in olfactory axon number is expected to occur at later time points. Supported by Whitehall Foundation and Arizona Disease Control Research Commission.

ACETYLCHOLINE: RECEPTORS

259.1

MOLECULAR CHARACTERIZATION OF CURARE BINDING SITE OF THE *TORPEDO* ACETYLCHOLINE RECEPTOR. M. E. O'Leary* and M. M. White, Department of Physiology, Medical College of Pennsylvania, Philadelphia, PA 19129

Curare is a well-characterized antagonist of nicotinic acetylcholine receptors. Despite its designation as a competitive antagonist, it is not clear if curare interacts with the same residues as does ACh, or rather binds to an adjacent, yet overlapping domain; both mechanisms are consistent with the competitive classification. We have recently described two conserved tyrosine residues (αY190, αY198) that are important components of the agonist binding site of the *Torpedo* AChR. We have further characterized these residues by examining the curare inhibition of mutant receptors expressed in *Xenopus* oocytes. In voltage clamp experiments, curare inhibits wild-type receptors by binding to a single class of high affinity sites (K_i=40 nM). Phenylalanine substitution of αY198 (αY198F) causes a 10-fold increase in the curare affinity (K_i=3.1 nM), and measurement of the rate of recovery from inhibition by curare shows that the main effect of the αY198F mutation is to decrease the off-rate for curare by approximately 10-fold. Our data are consistent with the notion that this mutation stabilizes curare binding by increasing the hydrophobic interaction between curare and the binding site. In contrast, the αY190F mutation reduces the curare affinity 10-fold (K_i=400 nM), suggesting that this tyrosine residue is important for high-affinity binding, possibly through a cation-π interaction with the quaternary or tertiary ammonium moieties of curare. Supported by NIH grants NS23885 and NS08880.

259.2

NICOTINIC RECEPTOR δ SUBUNIT D180 and E189 CONTRIBUTE TO THE BINDING OF ACETYLCHOLINE. C. Czajkowski*, C. Kaufmann and A. Karlin, Center for Molecular Recognition, Columbia University, New York, NY 10032.

The negative subsite of the nicotinic receptor interacting with the quaternary ammonium group of ACh is about 1 nm from αC192 and αC193. Recently, we showed that Torpedo δ164-224 contains D or E residues about 1 nm from αC 192/193 (J. Biol. Chem. 266:22603, 1991). We have now mutated three D/E residues in this region of the mouse δ subunit to N/Q and expressed the mutant δ subunits, together with wild-type α and β subunits, in Xenopus oocytes. Voltage-clamp data show that wild-type αβδ receptors are half-maximally activated (Kapp) by 3 μM ACh. The mutations δD180 to N (δD180N), δE186Q, and δE189Q have Kapp of 227 μM, 6 μM, and 36 μM, respectively. The affinity of ACh, measured by its inhibition of α-bungarotoxin binding, changes comparably to Kapp. δD180 and δE189, which are conserved in all species of δ, γ, and ε, but not β, are likely to be part of the negative subsite at both the α-δ and α-γ ACh binding sites. Supported by NIH grants NS07065 and NS07258 and by MDA.

259.3

EXPRESSION OF A SYNTHETIC GENE FOR α-BUNGAROTOXIN IN *E. coli*. E. Hawrot*, N.J. Messier, C.A. Vaslet, S.H. Hsu, L.N. Gentile, Section of Molecular and Biochemical Pharmacology, Division of Biology and Medicine, Brown University, Providence, RI 02912.

The snake venom-derived polypeptide, α-bungarotoxin (BGTX), has played a key role in the biochemical and molecular characterization of nicotinic acetylcholine receptors (nAChR) obtained from fish electric organs and from the neuromuscular junction of skeletal muscle. Although site-directed mutagenesis of the nAChR has been pursued effectively to study structure-function relationships in the nAChR a similar approach has not previously been applied to BGTX.

We have constructed a synthetic gene for BGTX based on the primary amino acid sequence of this 74-amino acid protein and using the codon usage preferences of *E. coli*. The synthetic gene was assembled from six overlapping synthetic oligonucleotides and the final annealed, ligated product was amplified by PCR. The BGTX-gene was then fused to a gene encoding a highly soluble T7 coat protein. A protease site recognized by Factor Xa was engineered at the junction site to allow selective release of the recombinant BGTX. Crude extracts of transfectants containing the recombinant BGTX gene contained BGTX activity as determined by competition binding assays utilizing ^{125}I-labelled authentic BGTX and *Torpedo* nAChR-enriched membranes. The fusion protein, purified by ion-exchange chromatography, is active although its specific activity is reduced by approximately 100-fold compared to authentic BGTX. After Factor Xa cleavage, the major released product co-migrates with authentic BGTX on reverse-phase HPLC. These studies suggest that recombinant BGTX refolds correctly and that site-directed mutagenesis studies currently in progress will shed light on the mechanisms underlying receptor recognition and binding.

(Supported by NSF-IBN-9021227 and NIH-GM32629).

259.4

REGULATION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR GENE EXPRESSION.

R.T. Boyd*, Department of Pharmacology and The Neuroscience Program, The Ohio State University College of Medicine, Columbus, Ohio, 43210.

The neuronal nicotinic acetylcholine receptors (nAChR) are composed of ligand binding subunits (α) and structural subunits (β). Different combinations of α and β subunits produce nAChR subtypes with different pharmacological and ion conducting properties. Transcriptional regulation may be an important determinant of receptor subtype in a neuronal population and thus influence transmission through a ganglia or group of neurons by controlling the nAChR subtype(s) present.

In order to understand the transcriptional regulation and promoter structure of the rat neuronal nicotinic acetylcholine receptor alpha 3 gene, 2781 nucleotides of genomic DNA were sequenced upstream of the start of the coding region. Known transcriptional regulatory elements such as octamer site, AP-1 site, CCAAT box, and two TATA boxes were shown to be present. A series of fragments from this region were cloned upstream of a luciferase gene (Promega, pGL2-Basic) and the resulting series of constructs were transfected into PC12 cells. Sequences required for the expression of this gene were defined in this manner.

PC 12 cells were grown in the presence of agents that have been shown to modulate cholinergic receptors such as TPA, NGF and nicotine. The effects of these agents on the level of several nAChR subunit RNAs (α3, β2, β4, α5, α7) were determined. This work was supported in part by the AHA (Ohio Affiliate) and the Bremer Foundation.

259.5

(+)2-METHYLPIPERIDINE: AN ALLOSTERIC MODULATOR OF CENTRAL NEURONAL NICOTINIC RECEPTORS? S.P. Arneric*, J.P. Sullivan, J. Raszkiewicz, M.L. Hughes, C. Briggs, M.J. Buckley, M.W. Decker, A.M. Hettinger and D.S. Garvey. Neuroscience, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064-3500

Previous work suggested that (+)2-methylpiperidine [(+)2-MP] is a putative allosteric modulator of brain receptors labelled by [^{3}H]nicotine (Sloan et al., *Life Sci.* 37:1367, 1985), since it increased the specific binding of [^{3}H]nicotine. This study sought to determine: 1) Can this effect be replicated using the new nicotinic ligand [^{3}H]cytisine? 2) Are there physiological correlates consistent with enhancement of nicotinic transmission?

Preincubation of rat whole brain synaptic membranes with 10-1000 pM (+)2-MP enhanced specific binding of [^{3}H]cytisine up to 125 % of control; (-)2-MP was without effect. No effect of (+)2-MP was observed on the rate of association or dissociation of [^{3}H]cytisine. (+)2-MP caused a dose-related (3-10 mg/kg, i.p.) enhancement of the habituation process normally seen in mice; an effect which can be dissociated from impairment of locomotor activity. (+)2-MP (0.1 mg/kg, i.p.) also made effective a subthreshold dose of (-)nicotine to diminish the avoidance response elicited by the elevated plus maze, but was without effect alone. Moreover, (+)2-MP , but not (-)2-MP, (0.1-1 mg/kg, i.v.) enhanced (up to 145 % of control) endogenous central nicotinic transmission assessed by increases in rat cortical blood flow elicited by basal forebrain activation. In contrast, (-)nicotine induced hypothermia and lethality were not potentiated in mouse by (+)2-MP (up to 100 mg/kg, i.p.). Nor were there significant effects to potentiate nicotinic synaptic transmission in the rat superior cervical ganglion. CONCLUSIONS: (+)2-MP may be a selective modulator of central neuronal nicotinic receptors. The allosteric nature of this interaction requires further study before drawing any analogy to the glycine/NMDA modulatory site. Its pharmacologic profile appears to potentiate the beneficial, not detrimental, effects of nicotinic receptor activation.

259.6

PHARMACOLOGICAL CHARACTERIZATION OF TRANSIENTLY EXPRESSED NEURONAL NICOTINIC SUBTYPES J.R. Forsayeth* and B.A. Dodson Dept. of Anesthesia, Univ of Calif, San Francisco, San Francisco, CA 94143

Characterization of neuronal nicotinic acetylcholine receptor (neuronal nAChR) subtypes within the CNS has been formidable due to low receptor number and lack of specific subtype ligands. In this study we report on a transient expression system using COS cells that permits the rapid and reproducible expression and pharmacological characterization of neuronal nAChR subtypes. Briefly, cDNA plasmids for each subunit were subcloned into pCDLSRα296 as blunt fragments inserted between the Pst 1 and Eco R1 cloning sites. COS cells (a simian kidney cell line) were transfected by a DEAE-dextran/ DMSO technique (Gu Y, Neuron 5:147-157, 1990). Specific L-^{3}H-nicotine or ^{3}H-ACh binding to expressed nAChR was determined by filtration (Dodson B, Ann NYAS 625:649-652, 1991) with non-specific binding defined as that occurring in the presence of excess (1mM) unlabeled L-nicotine or carbachol, respectively. No specific ligand binding was detected with cells transfected with α4 subunit alone or α4 in combination with β3. The α4β2 combination resulted in low expression but high affinity binding (B_{max}≈6 fmol/mg protein, K_d≈4.5nM) of both ligands, whereas α4β4 resulted in high expression but low affinity binding (B_{max}≈72 fmol/mg protein, K_d≈ 30nM). The addition of β3 to the α4β2β4 mixture (i.e., α4β2β3β4) resulted in >20-fold increase in high affinity binding such that high and low affinity binding to the α4β2β3β4 combination was comparable to that for the α4β2 and the α4β4 combinations, respectively. These results suggest the neuronal nAChR to be pentameric with the high affinity α4β2 and low affinity α4β4 dimers linked together by the β3 subunit, in a fashion similar to that reported for the muscle nAChR (Blount P, Neuron 3:349-357, 1989). [Supported by NIH Grant NS28062 (JRF) and UCTRD Grant RT352 (BAD)]

259.7

THYMOPOIETIN INTERACTS IN A SIMILAR MANNER WITH α-BUNGAROTOXIN RECEPTORS IN DIFFERENT RAT BRAIN REGIONS: QUANTITATIVE AUTORADIOGRAPHY. R. Afar*, P.B.S. Clarke, G. Goldstein and M. Quik. Dept. Pharmacol., McGill U., Montréal, Canada & Immunobiol. Res. Inst., Annandale, NJ, U.S.A.

The α-bungarotoxin (α-BGT) binding site in nervous tissue has long been known to a possess nicotinic pharmacology. Recently, an α-BGT binding subunit (α7) has been cloned and expression studies have demonstrated activation by acetylcholine and inhibition by low concentrations of α-BGT. As well, other subunits (α8, α5) that may represent α-BGT binding proteins have been reported. Thymopoietin (TPO), a thymic polypeptide hormone, has been previously shown to inhibit ^{125}I-α-BGT binding to rat brain membranes and brain sections. The present experiments were done to quantitate the interaction of TPO with the α-BGT receptor, in order to evaluate whether or not TPO might be interacting in a homogeneous manner with all α-BGT binding sites. Quantitative image analysis of inhibition of ^{125}I-α-BGT binding in the presence of increasing concentrations of TPO was performed on different brain areas. Formaldehyde fixed rat brain sections (20 μm) were incubated with 1 nM ^{125}I-α-BGT in the absence and presence of TPO (10^{-11}- 10^{-6} M). TPO (1 μM) almost completely inhibited ^{125}I-α-BGT binding in all brain areas visualized. Quantitation of displacement curves performed on different brain areas revealed that TPO affected these regions with similar potency. IC_{50} values for TPO obtained over six regions ranged from 16.5 ± 9.8 nM to 32.4 ± 9.9 nM. The results suggest that TPO recognizes all high affinity (1 nM) α-BGT binding sites in rat brain. Supported by MRC (Canada) and FRSQ.

259.8

POLYMERASE CHAIN REACTION ANALYSIS OF MUSCARINIC RECEPTOR SUBTYPES IN THE MOUSE COCHLEA. D.G. Drescher[1]*, S. Upadhyay[1], E.R. Wilcox[2] and J. Fex[2]. [1]Lab. of Bio-otology, Wayne State Univ. Sch. of Med., Detroit, MI 48201; [2]Lab. of Molecular Biology, NIDCD, NIH, Bethesda, MD 20892.

Cloning studies have indicated that there exist at least five genes for muscarinic acetylcholine receptors (mAChRs), termed m_1-m_5, which lack introns in their coding sequences (Bonner et al., *Science* 237: 527-532, 1987; *Neuron* 1: 403-410, 1988). Generally, the receptors corresponding to m_1, m_3, and m_5 couple to G proteins and stimulate phosphatidylinositol turnover, while those corresponding to m_2 and m_4 are linked to adenylyl cyclase inhibition via G_i proteins. Biochemical and pharmacological investigations have suggested that mAChRs are present in the mammalian cochlea, consistent with evidence that efferent neurons to the auditory organ use acetylcholine as a neurotransmitter. In the present study, total RNA was extracted with guanidine thiocyanate from the cochleas of 16-day-old CBA_J mice. Messenger RNA was purified from the total RNA using oligo dT cellulose, and the mRNA was treated with DNase to degrade genomic DNA. After reverse transcription, resulting cDNA was amplified by polymerase chain reaction (PCR), using primers specific for nucleotide sequences m_1-m_5. PCR products corresponding to subtypes m_1, m_3, and m_5, but not to m_2 and m_4, were amplified. These results suggest that muscarinic acetylcholine receptors of these odd-numbered subtypes are expressed in the mammalian cochlea, consistent with a coupling to cochlear phosphoinositide metabolism. (Supported by NIH Grant R01 DC 00156, ONR Contract N00014-88-K-0067, and the NIH Intramural Research Program.)

259.9

STUDIES WITH AN AFFINITY LIGAND, 4-BROMOACETAMIDOPROCAINE (BAP), ON AN ADRENAL CELL LINE TRANSFECTED WITH THE M1 MUSCARINIC CHOLINERGIC RECEPTOR (mCR) GENE. D. X. Wang, S-S. Sheu, and L. G. Abood*. Department of Pharmacology, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

A mouse adrenal cortex tumor cell line (Y1) transfected with an M1 mCR gene M1/zem228 (obtained from Neil Nathanson) has been used to probe the site and mechanism of action of BAP, an affinity ligand for mCR recognition site. With ^{3}H-QNB binding, the transfected Y1 cells had Kd values of 10 pM and 100 pM and Bmax value of 400 fmol/mg and 800 fmol/mg respectively. The apparent Ki of BAP for inhibition of ^{3}H-QNB binding was about 0.1 μM. At 1 μM BAP there resulted complete irreversible inhibition of ^{3}H-QNB binding and 50% inhibition of carbachol-stimulated phosphoinositide turnover. At 0.2 μM BAP, the carbachol-stimulated calcium transient, as measured by fura-2 fluorescence with a photomultiplier detector, was irreversibly inhibited almost 50%. The study has extended findings of others that the transfected Y1 cell line is useful for examining the functional characteristics of the mCR and examining the site of interaction of BAP. Supported by DA 00464.

260.1

FREE RADICALS GENERATED BY CALPHOSTIN INHIBIT PROTEIN KINASE C. S.S.-H.Wang* and S.H.Thompson. Neurosciences Program and Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950.

Calphostin A and C are lipophilic, light-sensitive perylenequinones that generate singlet oxygen upon illumination. They also inhibit protein kinase C (PKC) with high potency (IC_{50} = 250, 50 nM) and specificity, but only in the presence of light. We propose that calphostin acts by partitioning into the membrane and producing singlet oxygen, which then irreversibly modifies the diacylglycerol-binding domain of PKC. Our assay is inhibition of muscarinic receptor-mediated calcium release in N1E-115 cells by the PKC activator phorbol 12-myristate 13-acetate (PMA).

Inhibition by PMA is blocked by 100 nM calphostin A. This block is reduced in the presence of 1 mM of the antioxidant 2-mercaptoethanol and does not occur in the absence of light. At higher levels of illumination, calphostin causes progressive failure of calcium regulation, an effect not seen in cells injected with the pseudosubstrate inhibitor PKC(19-36). This leads us to conclude that calphostin can affect free radical-sensitive membrane constituents other than PKC. We speculate that free radicals can act as endogenous second messengers that inhibit PKC. (Supported by BNS 9021217 to S.H.T. and MH10088 to S.S.-H.W.)

260.2

PROTEIN KINASE C INHIBITORS DO NOT COMPLETELY BLOCK THE REDUCTION OF I(AHP) BY PHORBOL ESTERS IN HIPPOCAMPAL CA1 NEURONS. K. L. Engisch and B. E. Alger*, Dept. of Physiol., Univ. MD Sch. of Med., Baltimore, MD 21201.

Activation of the slow afterhyperpolarization in CA1 hippocampal pyramidal neurons produces accommodation (the reduction of action potential firing in the presence of a maintained depolarization). Modulation of the Ca^{2+}-activated K^+ current underlying the slow afterhyperpolarization, I(AHP), can increase or decrease cell excitability. Phorbol esters inhibit I(AHP), suggesting that it can be regulated by PKC activation. To test this hypothesis, we examined whether inhibitors of PKC could prevent the action of phorbol esters on I(AHP). Using whole-cell voltage clamp to record I(AHP) in rat hippocampal CA1 neurons in the slice preparation, we found that inclusion of the peptide pseudosubstrate inhibitor PKCI(19-31) in the intracellular pipette solution at 70 μM had only a small effect on the inhibition of I(AHP) by 1 μM PDA (% inhibition of I(AHP), control, 34.4 $\pm$ 4.3, N=19; PKCI(19-31), 20.1 $\pm$ 3.1, N=9; $p < 0.05$, t test). Cells were perfused with the peptide for at least 45 min prior to PDA application. We also bath-perfused 30 μM sphingosine for 45 min to 1½ hr before applying PDA and found a similar, small effect on PDA inhibition of the I(AHP) (% PDA inhibition in sphingosine, 22.9 $\pm$ 2.8, N=13).

Our results show that PKCs of cells in the slice preparation require doses for inhibition many times the published half-maximal doses of PKC inhibitors measured in cell-free assays. These findings have implications for the interpretation of studies which use PKC inhibitors to support the involvement of PKC. For example, because 10 μM sphingosine prevents LTP induction in the hippocampal slice (Malinow, et al., 1988), it has been suggested that PKC or CAMKII activation are required for LTP. We conclude that either sphingosine acts on LTP via CAMKII, or LTP induction involves PKC isozymes different from those associated with I(AHP).

260.3

DIFFERENT SENSITIVITY OF IP_3-DEPENDENT INTRACELLULAR Ca^{++} STORES BY MULTIPLE PHOSPHOINOSITIDE LINKED RECEPTORS IN HUMAN NEUROBLASTOMA CELLS. **A. Fatatis*, A. Bassi, G.F. Di Renzo and L. Annunziato. Sect. of Pharmacology, Dept. Human Comm. Science, II Sch. of Med, Univ of Naples, Naples ITALY.**

In LAN-1 human neuroblastoma cells, 1 minute pulses of CCh, Bk, ATP and ET were all able to elicit Ca^{++} release from IP_3-dependent intracellular stores, monitored by fura-2 single-cell microfluorimetry. The dose-response curve of the 4 agonists showed that CCh elicited the highest increase of $[Ca^{++}]_i$. When ET, BK and ATP were superfused consecutively at 2 minute intervals in a random sequence and in absence of extracellular Ca^{++}, a condition which prevents the refilling of IP_3-dependent stores from extracellular Ca^{++} sources, the 2nd and the 3th agonist application still evoked a $[Ca^{++}]_i$ increase, although progressively reduced, suggesting a partial refilling from the intracellular space. However, if CCh was added consecutively as a 4th stimulus, a sustained elevation of $[Ca^{++}]_i$ reappeared. On the other hand, when CCh was applied as 1st stimulus, all the other phosphoinositide linked agonists, subsequently applied, were unable to elicit $[Ca^{++}]_i$ elevation. The same phenomenon happened when CCh was applied as 2nd or 3th stimulus. All together these results demonstrated that when ET, BK and ATP have exhausted their Ca^{++} releasing actions, CCh was still able to elicit an additional $[Ca^{++}]_i$ increase from IP_3-dependent internal stores.

260.4

PHOSPHATIDYLINOSITOL HYDROLYSIS IS FACILITATED BY NMDA AND HARMALINE IN ADULT RAT CEREBELLUM: AN ACTION OF NITRIC OXIDE. J. Li* and S.S. Smith. Anat. Dept, Neuro Ins, Hahnemann U., Phila.

Harmaline evokes the synchronized discharge of the inferior olivary nucleus (Llinas and Muhlethaler, 1988), and is thus an effective tool for increasing climbing fiber discharge to the cerebellum. Previous studies from this lab have demonstrated that NMDA increases the hydrolysis of phosphatidylinositol (PI) in neonatal cerebellum in the presence of low level $GABA_B$ receptor stimulation (Smith and Li, 1991), an effect mediated by the gaseous second messenger nitric oxide (NO; Li and Smith, 1991). In the present study, PI turnover was determined using [H^3]- inositol in 160 μM cross-chopped slices of cerebellar tissue from female rats at post- natal day 7, 14 or 20 and in the adult. 12 hs and 90 min prior to sacrifice, animals were injected with harmaline (120 μgs/kg) or vehicle. Following a 20 min incubation of cerebellar slices with NMDA (100 μM), chloroform extraction and anion exchange chromatography isolated total inositol phosphates. NMDA alone did not significantly alter PI turnover, as previously reported. However, harmaline treatment increased NMDA-stimulated PI turnover up to 70% above basal levels in the adult ($P < 0.05$), an effect dependent upon the full maturation of the climbing fiber system. The permissive effect of harmaline on NMDA-stimulated PI turnover was first observed after day 14 in the prepubertal rat (a 20-30% increase). In the adult, this effect was inhibited completely by the application of the NO synthase blocker L-N_G- nitroarginine (100 μM), which suggests that the NO signalling system is involved in this permissive action of climbing fiber stimulation on excitatory amino acid-induced hydrolysis of PI. Climbing fiber activation is thought to release NO directly (Southam and Garthwaite, 1991). Products of PI hydrolysis evoked by this combined mechanism, Ca^{++} release and protein phosphorylation, may underlie changes in synaptic efficacy in the adult cerebellum. (Suported. by NS25809 & the Dept. of Anatomy)

260.5

SUSTAINED EFFECTS OF PILOCARPINE-INDUCED CONVULSIONS ON BRAIN INOSITOL LIPID SIGNALLING AND BRAIN MORPHOLOGY. K.M.Savolainen*, M.-R.Hiryonen and [1]L. Paljärvi. Div. Env. Hlth, Natl. Publ. Hlth. Inst., and [1]Dept. Pathol., Univ. Kuopio, Kuopio, Finland.

Sustained effects of pilocarpine-induced convulsions on brain phosphoinositide (PI) signalling, and histology, were studied in young (10 weeks) and old (24 months) male rats. Pilocarpine doses were 300 and 175 mg/kg for young and old rats, respectively. Diazepam was used to stop convulsions 2 h after their initiation. Rats were followed for 5 days, and then brain inositol, inositol-1 (Ins1P) and 4-monophosphate (Ins4P) were studied by gas chromatography, and histology by light microscopy. Brain inositol decreased, and Ins1P increased, similarly in young and old rats, except in thalamus where Ins1P remained stable in aged rats. Ins4P did not change in young rats, but in old rats it increased in the frontal cortex and caudate. Widespread brain damage, present in all exposed rats, was more prominent in the old ones. These results suggest that old rats are more sensitive than the young ones to pilocarpine-induced cholinergic convulsions and associated brain damage, possibly due to differences in PI metabolism, as suggested by the differences in Ins4P levels. Supported by the Research Council for Environmental Sciences of The Academy of Finland.

260.6

DISTRIBUTION OF G_s AND G_i PROTEINS CORRELATE WITH ADENYLYL CYCLASE ACTIVITY IN POSTMORTEM HUMAN BRAIN. J.J. Warsh*, L.T. Young, P.P. Li, Siu K.P. and S.J. Kish. Clarke Institute of Psychiatry, Toronto, Ontario, Canada M5T 1R8

Abnormalities in receptor coupling to adenylyl cyclase and stimulatory (G_s) and inhibitory (G_i) G-proteins have been demonstrated in neuropsychiatric disorders, however, little is known about either the brain regional distribution of G-proteins or the actual relationship between brain G-protein levels and G-protein-linked functional responses in normal human brain. Postmortem brain was obtained from 7 subjects who were free of neurologic or psychiatric disorder with autopsy intervals to -80°C less than 24 hours. Using specific polyclonal antisera, G_s and G_i α immunoreactivities were determined by Western blotting in various brain regions. On the same tissue samples, basal and stimulated adenylyl cyclase activity was determined by radioimmunoassay. The highest levels of G_s 52 kDa and G_i α subunits occurred in frontal cortex, and highest G_s 45 kDa levels in caudate compared with other brain regions. Although basal adenylyl cyclase was similar in all regions, both GTPγS- and forskolin-stimulated adenylyl cyclase activity were highest in frontal cortex, caudate and cerebellum. Furthermore, forskolin-stimulated cAMP formation correlated significantly with both G_s 45 kDa and G_i α levels whereas only G_i α correlated with GTPγS-stimulated cAMP formation. These findings underscore the important regional relationship between these G-protein α subunits and adenylyl cyclase activity in human brain.

260.7

DIFFERENTIAL LOCALIZATION AND pH DEPENDENCY OF THE INOSITIDE IP_3, IP_4 and IP_6 BINDING SITES IN RAT BRAIN: AN AUTORADIOGRAPHIC ANALYSIS. A.R. Parent* and R. Quirion. Douglas Hospital Res. Ctre and Dept. Psychiatry, McGill University, 6875 LaSalle Blvd., Verdun, Québec. H4H 1R3.

It is well established that the inositol lipids mediated signal transduction in several cellular populations. Many neurotransmitters, hormones and growth factors act at membrane receptors to induce hydrolysis of phosphatidylinositols generating various inositol phosphates (IP). The best known members of this family are the 1,4,5-IP_3 and 1,3,4,5-IP_4, which are associated with Ca^{2+} homeostasis. Recently, it was also proposed that IP_6 could be related to Ca^{2+} uptake processes. As mean to study the possible roles of the IPs in neuronal tissues, we have localized and quantified specific binding sites for 1,4,5-IP_3, 1,3,4,5-IP_4 and IP_6 under acidic and basic conditions and using their respective tritiated ligands. [^{3}H]-1,3,4,5-IP_4 binding densities are concentrated in the hilus and molecular layer of the hippocampal formation while an opposite pattern is seen with [^{3}H]-1,4,5-IP_3; the highest amounts being concentrated in the oriens and radiatum laminae. This contrasting profil of distribution is also observed in others brain areas. Morever, while highest binding of [^{3}H]-IP_3 is seen at pH 8.5, the opposite pattern is found for [^{3}H]-IP_4 with high binding levels at pH 5.0. For [^{3}H]-IP_6, labelling is more specifically localized to neuronal cell bodies (e.g. granular layer of the dentate gyrus > pyramidal cells of the hippocampus and olfactory bulb = piriform cortex = septohippocampal cells > hilus). Morever, [^{3}H]-IP_6 is most abundant at basic pH. The differential localization of specific receptor sites for these three IPs related to Ca^{2+} homeostasis suggests that each are mediators of a specific set of functions in enriched cellular population. Additionally pH's dependency of each IP binding sites can be correlated to intracellular pH events. Supported by MRCC, Alzheimer Society of Canada.

260.8

DIFFERENTIAL REGULATION OF PROTEIN KINASE C ISOZYMES IN AGING RAT BRAIN. F. Battaini*, V. Ladisa^, S. Bergamaschi^, L. Lucchi ^ S. Govoni^ and M. Trabucchi. Chair of Toxicology, IInd Univ. of Roma, ^Inst. Pharmacol. Sci., Univ. Milano, Italy.

Previous studies have demonstrated age-related modifications in protein kinase C (PKC) levels and activity in central and peripheral tissues. Selected PKC isoforms, in particular, seem to be implicated in a number of functions that are modified during aging such as neurotransmitter release and synaptic plasticity. In this study we have analyzed PKC activity and the various isozymes expression in aging Wistar rat brain areas. PKC activity (histone IIIS as substrate) does not show age-dependent changes across young (3 months), adult (8 months) and aged (28 months) animals in soluble and membrane fractions from various brain areas investigated (cortex, cerebellum and hippocampus). However the pharmacologically-induced (PMA 160 nM for 15') enzyme translocation from the soluble to the membrane fraction, assessed as kinase activity, is impaired in aged rats. Isozyme mRNA expression (investigated by *in situ* and Northern hybridization) indicate that, among the calcium-dependent isoforms the α and β type show age-dependent decrease in cortex. The γ isoform is barely detectable in this brain area. Hippocampal subfields and cerebellar α, β and γ isoform expression do not seem to be affected by age in this strain of rats.

These observations indicate that aging, although not modifying PKC activity assessed *in vitro* under optimal conditions, affects PKC translocation and thus its capability to respond to appropriate stimuli. This could be ascribed to age-related modifications in endogenous environment and/or availability of PKC activators. In light of the involvement of the various PKC isoforms in selected neuronal functions, the analysis of all PKC isozyme mRNA coupled to the immunodetection of the gene products will help to better define processes particularly sensitive to aging.

260.9

PROTEIN KINASE C CHARACTERIZATION IN FIBROBLASTS FROM ALZHEIMER'S DISEASE (AD) PATIENTS.
S. Bergamaschi, M. Trabucchi, F. Battaini[1], V. Ladisa, G. Binetti[2], A. Bianchetti[2] and S. Govoni*. Inst.Pharmacol.Sci., Univ. of Milano, [1]Dept. Exptl.Med.Biochem. Sci., IInd Univ. of Roma, [2]Alzheimer's Dept. of the Sacred Heart Hospital of Brescia, Italy.

Alterations at the level of PKC have been reported in brain and in skin fibroblasts from patients with AD. In particular, the amount of PKC, as measured by immunoblotting techniques, is reduced in fibroblasts from patients with sporadic and familial AD. The reduced amount of the enzyme is not correlated with a reduced PKC activity using an exogenous substrate (Cole et al. Arch.Neurol. 46,1195,1989). In the present study biopsies of shoulder skin of patients with sporadic AD and controls were obtained after ethical committee approval at the Alzheimer's Dept. of the Sacred Heart Hospital in Brescia. 10 cell lines from sporadic AD and 6 cell lines from age-matched controls were obtained. PKC distribution between the soluble (S) and particulate (P) fraction was evaluated by means of [^{3}H]-phorbol ester (PdBu) binding. A modified procedure from that described by Wehner (Brain Res. 523,181,1990) was used to prepare S and P fractions. In the S fraction the binding values of [^{3}H]-PdBu were: Bmax: 7.8±1.5 pmol/mg prot.; Kd: 3.7±1 nM. Specific binding represented 70% of total binding. At least 20-30 µg of protein/sample were needed for optimal binding detection. The distribution of the binding between P and S indicated a preferential presence of PKC at cytosolic level. In order to determine the subtypes of PKC present in control and AD fibroblasts the same cell lines were used also for the extraction of mRNA for Northern blot analysis.

260.10

DUAL REGULATION OF ADENYLYL CYCLASE BY MUSCARINIC RECEPTORS IN RAT OLFACTORY BULB. Pierluigi Onali* and Maria C. Olianas, Department of Neurosciences, University of Cagliari, Cagliari, Italy.

We have previously reported that in rat olfactory bulb muscarinic agonists stimulate basal adenylyl cyclase (a.c.) activity in a GTP-dependent and pertussis toxin-sensitive manner. In the present study we show that carbachol (CCh) potentiates the Gs-mediated stimulation of a.c. by either CRH or VIP. The synergistic interaction occurs at free Ca++ below 100 nM, requires micromolar GTP and consists in an increase of the Vmax of the enzyme. On the other hand, the Ca++/calmodulin stimulated (type I) a.c. is inhibited by CCh. Cholinergic agonists also reduce the maximal enzyme stimulation by forskolin with potencies equal to those displayed in increasing basal a.c.. Moreover, the Ki values of pirenzepine, methoctramine and pFHHSiD in antagonizing the muscarinic stimulation and inhibition of a.c. are similar. These results indicate that in rat olfactory bulb a pharmacologically homogeneous muscarinic receptor population can either up- or down-regulate cyclic AMP formation possibly by affecting the activity of different molecular forms (i.e. type I and type II) of a.c..

260.11

GENOMIC CLONING OF THE NEURON-SPECIFIC SPLICING REGION OF THE HUMAN INOSITOL (1,4,5)-TRISPHOSPHATE RECEPTOR (IP_3R).
Danoff, S.K.,* McInnis, M.G., Kearns, W., Snyder, S.H. and Ross, C.A. Departments of Psychiatry and Neuroscience, and Center for Medical Genetics. Johns Hopkins Univ. Sch. of Med., Baltimore, MD. 21205

The phosphatidyl inositol second messenger pathway is an important signalling system in neurons, as well as in other cells. We have previously shown that the IP_3R messenger RNA in rat neuron differs from that in non-neuronal tissues by 120 nucleotides [Danoff, et al. PNAS, 88 2951-55 (1991)]. Using PCR amplification of first strand cDNA, we now demonstrate that these two alternative forms are also expressed in human RNA. We have cloned a portion of the human genomic DNA containing the region of the putative alternative splice site. Both messages are derived from a single gene, produced by an alternative splicing event involving three small exons. A cryptic splice acceptor site in the third alternative exon results in the variable expression of the codon for Gln 1716. We are currently pursuing the chromosomal localization of the human IP_3R.

260.12

MOLECULAR CLONING OF A STRIATAL SPECIFIC ADENYLATE CYCLASE WHICH MAY BE INVOLVED IN DOPAMINE SIGNAL TRANSDUCTION. C.E. GLATT* and S.H. SNYDER. Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

A comparison of forskolin autoradiography and in situ hybridization maps of all of the currently known adenylate cyclase isoforms demonstrated a marked discrepancy between adenylate cyclase protein and mRNA expression in the rat striatum. This suggested that a major member of the adenylate cyclase family had yet to be identified.

Screening of a cDNA library from rat striatum with full length probes from the types I, II, and III adenylate cyclases detected clones which hybridized at low but not high stringency. Sequencing of several clones demonstrated a novel cyclase with high homology to known adenylate cyclases.

Northern blot analysis showed a high level of expression of this form in the striatum but no other brain region or tissue examined. In situ hybridization analysis demonstrated expression in the caudate-putamen, nucleus accumbens and olfactory tubercle.

A full length clone has since been obtained identifying a predicted open reading frame of approximately 3.7 kilobases. A full length expression construct is being prepared to allow a functional assesment of the protein coded for by this gene.

The unique pattern of expression of this gene suggests that it is involved in dopaminergic signal transduction in the striatum.

261.1

CHARACTERISATION OF AvGp50 IN THE AVIAN NERVOUS SYSTEM. P.L. Jeffrey*, A.M. Shepard and K.A. Hancox Children's Medical Research Institute, Camperdown N.S.W. Australia, 2050.

A size fractionated lentil lectin-positive fraction (40-100kDa) of one day old chick forebrain membranes was used to generate a monoclonal antibody, MabSA1.7, which recognises the nervous system specific glycoprotein AvGp50. Expression of AvGp50 is predominant within the molecular and granule cell layers in the cerebellum. AvGp50 is comprised of at least two isoforms and its pattern of expression is developmentally regulated . Removal of the carbohydrate side chains led to a loss of recognition by the MabSA1.7 of the 31kDa isoform and a reduction in recognition of the 34kDa isoform, suggesting that the monoclonal antibody recognises a carbohydrate rather than peptide epitope, confirmed by periodate oxidation /borohydride reduction studies which results in a complete loss of antigenicity AvGp50. We are screening an embryonic day 1 forebrain pUEX-1 expression library with the polyclonal antibody PCA.2 (raised against deglycosylated AvGp50) in order to identify possible cDNA clones of AvGp50. (Supported by the NH&MRC of Australia).

261.2

CELL TYPE AND DEVELOPMENTAL REGULATION OF THE RET PROTO-ONCOGENE DURING PERIPHERAL AND CENTRAL NERVOUS SYSTEM DEVELOPMENT. A.M. Gormley, R.J. Morris, R.V. Stirling*[1] and V. Pachnis[2], Lee Research Centre, Lab of Neurobiology, National Institute for Medical Research, The Ridgeway, London NW7 1AA, UK. [1]Dept Biol, Open University, Walton Hall, Milton Keynes MK7 6AA, UK; [2]Lab of Gene Structure & Expression, NIMR, London NW7 1AA, UK.

Normal development of the nervous system depends on the proper communication between diverse cell types, mediated to a large extent by polypeptide differentiation and growth factors. A number of these factors bind to cell surface receptors that have intrinsic and ligand dependent tyrosine kinase activity. The ret proto-oncogene is a member of the family of tyrosine kinase receptors that was originally identified by its ability to transform NIH3T3 cells. We have previously shown that during the early stages of organogenesis of the mouse embryo, ret mRNA is predominantly localized in the precursors of the peripheral and central nervous system. Here we have used hybridization histochemistry with digoxigenin labelled probes to further define the pattern and cell type specificity of ret expression during later stages of CNS and PNS development. Our findings indicate that ret is expressed in specific subpopulations of neurons of the PNS and CNS, and suggest a role of the ret proto-oncogene in neuronal differentiation.

261.3

POSTNATAL EXPRESSION OF MYOSIN II ISOFORMS IN RAT CORTEX, HIPPOCAMPUS, AND CEREBELLUM. M. E. Miller*, P. D. Chantler, and P. Levitt. Med. Coll. of Penn., Dept. of Anat. & Neurobiol., 3200 Henry Avenue, Phila., PA 19129.

Using an affinity-purified polyclonal antibody generated against murine neuroblastoma (Neuro-2A) myosin II (Miller et al., Neuron (1992) 8:25-44) and a commercially available polyclonal antibody directed against myosin II from human platelets, we are investigating the postnatal expression of myosin II isoforms in rat cortex, hippocampus, and cerebellum. Preliminary results from indirect immunocytochemical studies of fixed, free-floating tissue sections indicate that within these anatomical areas the antibodies discriminate between at least two distinct myosin II isoforms which are expressed in a predominantly non-overlapping fashion.

Within cortex and hippocampus, anti- neuroblastoma myosin II strongly labels cortical and hippocampal neurons (cell bodies, apical/basal dendrites, dendritic spines) from P0 to P10, the labeling intensity decreasing to adult levels after P12. Cortical axons are labeled from P4 to P10, while axons in the hippocampus are labeled from P0 to P12. Anti- platelet myosin II labels cortical and hippocampal axons (P0 to P4), a population of macrophages in the intermediate layers of cortex at the tips of the lateral ventricles (P0 to P4), and many blood vessels and apparent microglial cells (P0 to adult).

In cerebellum, anti- neuroblastoma myosin II labels Purkinje cells (cell bodies/ dendritic arbors) from P0 to adult. Purkinje axons are also labeled from P7 to P12, as are dendritic spines from P7 to adult. Anti- platelet myosin II labels cells in the external (but not internal) granule cell layer (P0 to P12), Golgi epithelial cells and Bergmann glial fibers (P7 to adult), and many blood vessels and apparent microglial cells (P0 to adult). This work demonstrates that neuronal myosin isoforms display a characteristic distribution during postnatal development.

Supported by grant #AR32858 to PDC and #MH45507 to PL

261.4

LOCALIZATION AND PHOSPHORYLATION OF RETINOBLASTOMA PROTEIN IN ADULT AND DEVELOPING BRAIN. G.A. Oyler*, K.A. Harris, and M.L. Billingsley. Dept. Pharmacology, Penn State University College of Medicine, Hershey, PA 17033

Phosphorylation of retinoblastoma protein (Rb) regulates progression through the cell cycle in a phosphorylation-dependent manner. The role of Rb, a growth suppressor protein, in maintenance of terminal neuronal differentiation is not known. Localization and phosphorylation of Rb were investigated in rat and human brain using monoclonal antibodies. Using immunocytochemistry, Rb was localized in neuronal nuclei throughout adult rat brain, and was enriched in hippocampal pyramidal neurons. Developmental studies in rat brain indicated that multiple forms (110-116 kDa) and higher levels of Rb were present from E15-PND7. In adult human and rat brain, Rb was found as a single, 110 kDa unphosphorylated peptide. Phosphorylated forms of Rb were not observed following metrazole-induced seizures in adult rats. Treatment of adult rat hippocampal slices with the phosphatase inhibitor okadaic acid also failed to induce Rb phosphorylation. However, okadaic acid induced Rb phosphorylation in human SMS-KCNR neuroblastoma cells. These results suggest that neurons, unlike neuroblastoma cells, lose the ability to phosphorylate Rb protein during normal development. Thus, Rb expression and lack of phosphorylation in adult brain may be involved in the maintenance of neuronal differentiation.

261.5

CORTICAL REPAIR AND REORGANIZATION FOLLOWING TRAUMATIC MICROINJURY IN THE DEVELOPING RAT NEOCORTEX. J.T. Morgan and M. Marín-Padilla*. Dartmouth Medical School, Hanover, NH USA 03755.

A single superficial microinjury of the developing right somatosensory neocortex was performed in four day-old rats, under hypothermic anesthesia, using a fine surgical needle. The undamaged left hemisphere was used as a control. The pial surface was damaged and secondarily repaired in all animals. At the puncture site, a focal leptomeningeal and/or marginal heterotopia developed. The progressive evolution of the lesion was then studied with H&E, Kluver-Barrera, and rapid Golgi procedures at 1, 8, 11, 21, 44, 56, 71, 173, 191, and 193 days following the trauma. Abnormal vascular patterns, focal cortical dysplasia, abnormal myelin patterns, and pockets of displaced neurons within layer I were found in the damaged area. In addition, the Golgi study showed abnormalities in the neuronal morphologies and in the local circutry in and around the lesioned area. These neuronofibrillary abnormalities are considered to be the result of the focal reoganization that occurs in the damaged area after the injury. The most striking finding, to be presented and discussed, was the number of neurons with abnormal morphologies encountered. These experimentally induced leptomeningeal heterotopias are similar in several aspects to those found in the brains of: a) prematurely born infants who have suffered neonatal asphyxia (Marín-Padilla, *Soc. Neurosci. Abs.,17:* 1991); b) some cases of developmental dyslexia (Galaburda et al.,*Ann. Neurol., 18:* 1985); and c) patients with developmental epilepsy (Farrell et al., *Acta Neuropathol., 83:* 1992). We introduce this experimental model as a possible tool for the study of the development of these kinds of disorders in humans.

(This work was supported by NIH Grant NS-22897.)

261.6

DENDRITIC TOPOLOGY AND PASSIVE ELECTROTONIC PROPERTIES. J. van Pelt, M.A. Corner*. Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.

Dendrites have characteristic geometrical features both in metrical parameters and in their topology, i.e. the pattern in which the segments are connected to each other. A new topological measure, tree asymmetry, is used with values ranging from 0 (completely symmetrical trees) through 1 (maximally asymmetrical trees). The measure is shown to be both efficient and discriminative, with expectations being almost independent of the size of the trees. The population spread in its values for three neuronal types indicates that only a small fraction of the possible topologies actually occurs. Using theoretical models for dendritic growth, the observed variance could be completely explained by assuming random choices for branching segments during growth.

Passive electrotonic properties of dendritic trees in relation to their topology have been studied using a vector implementation of the Laplace-transformed cable equation for branching structures. The structure of the conductance matrix in the current-voltage vector equation is a direct mapping of the topological structure of the dendrite. Topology appears to play a significant role in steady-state and transient spatial attenuation (centrifugal as well as centripetal from the point of activation) and equalization time constants.

261.7

EARLY DEVELOPMENT OF AN IDENTIFIED SEROTONERGIC NEURON IN EMBRYOS OF THE GASTROPOD, *HELISOMA TRIVOLVIS*. J.I. Goldberg* and T.J. Diefenbach. Department of Zoology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9.

One of the earliest events in the development of the *Helisoma* nervous system is the differentiation of a single pair of serotonergic neurons, embryonic neurons C1 (ENC1s). Since ENC1s are identifiable and accessible in live embryos, *in vivo* experimental studies can now be implemented at the cellular level. To provide a foundation for experimental studies, we now describe the sequence of events underlying ENC1 differentiation. Immunofluorescence, 5,7-DHT fluorescence, Nomarski DIC and intracellular recording techniques were used. ENC1 differentiation commenced around stage E15 (15% of embryogenesis) with the weak, granular expression of serotonin immunoreactivity. Neurite outgrowth was initiated soon after (stage E15-16), and was characterized by the rapid ventral extension of primary neurites around the buccal mass to the ciliated target cells in the primordial foot. In the posteroventral region of the foot, the primary neurites from left and right ENC1s turned medially, fasciculated with one another, and terminated in a posteromedial location. Putative guidepost cells were observed at specific points along the route of primary neurite extension. Around stage E18, the ENC1 soma migrated ventromedially beneath the epidermal placode, leaving behind a presumed sensory structure at the surface. Also occurring at this stage was expression of a serotonin uptake system and initiation of neurite branching in the region of the pedal ciliated epithelium. Preliminary electrophysiological experiments indicated that ENC1 is electrically excitable by stage E25, however the exact onset of excitability has yet to be staged. This description of ENC1 development provides a foundation for *in vivo* experiments on the regulatory factors controlling the development of an identified embryonic neuron. (Supported by NSERC of Canada).

261.8

EVIDENCE FOR AUTOREGULATION OF NEURITE BRANCH DEVELOPMENT IN IDENTIFIED SEROTONERGIC NEURONS FROM *HELISOMA* EMBRYOS. T. J. Diefenbach* and J. I. Goldberg, Department of Zoology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9

Embryonic neurons C1 (ENC1s) are cilioexcitatory motor neurons that may influence the development of other embryonic neurons through expression and release of serotonin. From stages E15 (15% of embryogenesis) to E40, ENC1s are the only serotonergic neurons present in the *Helisoma* nervous system. Furthermore, ENC1s elaborate a consistent pattern of neurite branching during this period. Given that neurite outgrowth of adult serotonergic neurons can be regulated by serotonin (McCobb *et al.*, 1988; Murrain *et al.*, 1991), we hypothesized that serotonin released by ENC1 regulates the branching pattern of ENC1 neurites by binding serotonin autoreceptors. To test if a reduction in serotonin synthesis would result in altered neurite outgrowth, stage E15 embryos were treated with the serotonin synthesis inhibitor *p*-chlorophenylalanine (PCPA) for 1 hour, and then allowed to develop in artificial pond water for 48 hours. Resultant changes in outgrowth were then visualized using serotonin immunofluorescence technique. ENC1s of embryos treated with PCPA displayed a marked, transient reduction in serotonin immunoreactivity that was correlated with a 68% reduction in serotonin content per embryo (Control: 488 ± 122 fg/embryo; PCPA: 158 ± 42 fg/embryo). Examination of ENC1 morphology in PCPA-treated embryos revealed a significant increase in the total number of branch points (PCPA: 30.6 ± 2.6; Control: 21.5 ± 1.2). These results suggest that, during ENC1 development, neurite branching is limited by serotonin through an autoreceptor-mediated mechanism. Given preliminary evidence of serotonin uptake by ENC1 target cells, neurite branching may be regulated by the competition between target cell uptake systems and ENC1 autoreceptors for extracellular serotonin.

This research supported by AHFMR (Alberta) and NSERC (Canada).

261.9

INFLUENCES ON POSTEMBRYONIC DENDRITIC CHANGES IN AN IDENTIFIED LEG MOTOR NEURON. K. S. Kent*, Dept. Biol. Structure & Function, Oregon Health Sci. Univ., Portland, OR 97201

During metamorphosis of the moth, Manduca sexta, thoracic leg motor neurons (MNs) persist from larva to adult but undergo dramatic dendritic changes including regression and growth. In order to identify the signals involved in shaping these changes, we have examined the dendritic morphology of an identified leg MN under conditions in which hormonal signals or cell-cell interactions were altered. Ligation / infusion experiments, in which the source of ecdysteroid hormone was removed or removed and replaced, confirmed that ecdysteroid hormone is required for both dendritic regression and growth in thoracic leg MNs. Similarly, manipulation of sensory inputs during the time of dendritic regression or growth confirmed that sensory inputs have little influence over the processes of dendritic regression and growth. Application of a juvenile hormone analogue (methoprene) to the larval legs was used to create mosaics in which larval legs were retained on a pupal insect. Despite retention of larval leg sensory neurons (SNs) and leg muscles, the MN underwent its normal course of dendritic regression. Injections of Mitomycin C into young pupae were used to prevent the generation of adult leg SNs. Despite the lack of adult leg SNs, the MN underwent extensive dendritic growth, in agreement with our previous findings that dendritic growth occurs in the total absence of the adult legs. Together, these results support the hypothesis that dendritic regression and growth in thoracic leg MNs are under hormonal control as has been reported for abdominal proleg MNs (Weeks and Truman, 1985; Weeks and Ernst-Utschneider, 1989; Jacobs and Weeks, 1990). However, alterations of specific aspects of MN dendritic morphology were apparent in the "deafferented" preparations suggesting that sensory inputs shape the growth of MN dendrites in specific regions of the dendritic arbor. (Supported by grants from MRF of Oregon and NSF.)

261.10

INNERVATION AFFECTS THE DEVELOPMENT OF ADULT-SPECIFIC MUSCLES IN *MANDUCA SEXTA*. R.J. Bayline* and R. Booker. Section of Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853.

We are studying the respecification of abdominal muscles during metamorphosis in the moth, *Manduca sexta*, to determine the factors which regulate muscle development. From the onset of pupation (P+0) until four days later (P+4), the larval muscles dedifferentiate, resulting in the loss of myofibril structure as well as a 41% reduction in muscle size. From P+4 through P+12, the nuclei within the dedifferentiated fibers proliferate. The number of muscle fibers also increases dramatically at this time. For example, in the dorsal intersegmental muscle in the second abdominal segment (A2-DISM), fiber number increases from 15-20 at P+4 to several hundred in the adult. As the muscles grow, they alter their attachment points. By P+16, the muscles appear fully differentiated, with Z-bands, myofibrils, and adult attachment points. To determine the role of innervation in muscle respecification, we cut the nerve innervating the muscles during the larval stage and observed the effects in the adult. In the adult A2-DISM, only 5-10 fibers were present on the denervated side. The denervated fibers had the adult attachment points and contained myofibrils, although the fiber diameters were reduced by 33%. To determine the mechanism by which innervation regulates muscle development, we labeled S-phase nuclei with 5-bromo-deoxyuridine three days after cutting the nerve during the pupal stage. Substantially fewer muscle nuclei labeled on the denervated side than on the control side. These results demonstrate that innervation is necessary for the proliferation of muscle nuclei and muscle fibers during respecification. Dedifferentiation, alteration of attachment points, and muscle differentiation proceed normally even in the absence of innervation.

261.11

ABNORMAL SARCOMERES AND LOSS OF ATTACHMENTS TO BODY WALL IN C. ELEGANS MUSCLES DUE TO MUTATION OF β-INTEGRIN GENE pat-3. D.H. Hall* and E.M. Hedgecock. Albert Einstein College of Medicine, Bronx, N.Y. 10461 and Johns Hopkins University, Baltimore, MD 21218.

The gene pat-3 encodes a β1-like integrin subunit in the nematode Caenorhabditis elegans (S. Gettner, pers. comm.). Three mutant alleles have been isolated. The strongest allele, pat-3 (rh54), is embryonic lethal, and affects development of most muscles, plus cell migrations and attachments in non-muscle tissues. pat-3 (rh151) is viable but sterile, and does not appear to disrupt body muscle cells. The mildest allele, pat-3 (rh96), is viable. Using genetic mosaics of rh54, and serial thin section reconstruction of adult mosaics by EM, we compared mutant and non-mutant body muscles to determine the role(s) of β-integrin in muscle development.

Normal body muscles adhere to the body wall cuticle via fibrous linkages to membrane densities along the inner surface of the muscle's plasma membrane. A normal cell contains several sarcomeres lying oblique to the body axis, also anchored to membrane densities. The rh54 mutant phenotype is cell autonomous. Affected cells are not contractile. They detach from the body wall, but remain in close contact with nearby wild type muscle cells, forming both gap junctions and adherens junctions with them and with each other. Mutant cells are longer and much thinner than normal. No sarcomeres are formed, but separate bundles of thick filaments, thin filaments, and dense aggregates are found in the cytoplasm. Some cells form branches which extend dorsoventrally to the opposite muscle quadrant. Interestingly, mutant cells still form "muscle arms" which extend in normal fashion to the nerves and nerve ring in order to receive synaptic inputs. The lethal myospheroid β-integrin mutant in Drosophila causes similar defects in muscle development (Volk et al., Cell 63:525-536, 1990).

262.1

ELECTRICAL ACTIVITY AND GAP-43 EXPRESSION

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GAP-43 has been associated with growth cone motility, synaptic plasticity, and axon outgrowth during development and regeneration. We investigated effects of electrical stimulation on expression of GAP-43 in mouse DRG neurons in culture, using a multi-compartment method of delivering electrical stimulation (Fields, et al., *J. Neurosci.* 10:2950-2964).

Trains of action potentials were delivered to neurons in culture at 10 Hz in 1/2 sec. bursts every 2 seconds for 3 days. No difference in amount of GAP-43 was detected in stimulated cultures compared to unstimulated controls, using ELISA or quantitative video fluorescence immunocytochemistry. Depolarization with 10, 20 or 30 mM K^+ for 3 days in 1,2 or 3 week old cultures failed to alter GAP-43 levels. Consistent with protein measurements, the amount of mRNA encoding for GAP-43 was unchanged following potassium depolarization or electrical stimulation. GAP-43 was regulated by development, however. Levels of GAP-43 mRNA and protein were highest at 3 days *in vitro*, and declined to stable values between 1 and 3 weeks in culture.

The results suggest that GAP-43 expression is not regulated by electrical stimulation, even though growth cone motility, synaptic plasticity and axon sprouting are sensitive to changes in electrical activity. Therefore post-translation modification, such as phosphorylation, may be more important in regulating activity-dependent effects of GAP-43.

262.2

PROTEIN KINASES AND REGULATION OF GROWTH CONE STRUCTURE AND MOTILITY IN GOLDFISH RETINAL GANGLION CELLS.

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Much evidence suggests that cytoplasmic Ca^{++} levels regulate neurite elongation and growth cone motility (Kater et al.1988, TINS 11:315), and Ca^{++} can activate several protein kinases. To test whether protein phosphorylation has effects on growth cone structure and motility, we have locally applied from a micropipette several different kinase inhibitors and activators and a phosphatase inhibitor to the growth cones growing on ependymin while observing with videomicroscopy. General kinase inhibitors sphingosine (50μM in pipette) and H7 (500μM) caused growth cone swelling and collapse. The filopodia, however, remained attached to the substrate as the swelling moved back the retracting neurite. In contrast, neither stimulation of protein kinase A (PKA) by db-cAMP (20mM) or phosphodiesterase inhibitor IBMX (2mM) nor inhibition of PKA by Rp-cAMPS (2mM) caused any change in growth cone shape and behavior. Similarly, neither stimulation of protein kinase C (PKC) by TPA (1μM) or arachidonic acid+diacylglycerol (50μM each) nor inhibition of PKC by calphostin C (1μM) produced any obvious effects. The tyrosine kinase inhibitor Lavendustin A (1μM) was also without effect. ML-7 (5μM), the inhibitor of myosin light chain kinase (MLCK), however, caused growth cone swelling and retraction, as did the calmodulin antagonists calmidazolium (0.1μM) and CGS 9343B (100μM). Finally, the phosphatase inhibitor okadaic acid (50nM) caused growth cone swelling and collapse like that of sphingosine. These results indicate that both increasing and decreasing protein phosphorylation in retinal growth cones can lead to abnormal structural changes. Among the kinases, PKA, PKC and tyrosine kinases may not be critical, while calmodulin dependent kinases, such as MLCK and CamKII, may play an important role in regulating growth cone structure and motility. Supported by NIH grant EY-03736.

262.3

$p59^{fyn}$ Tyrosine Kinase in Growth Cones and Axonal Tracts of the Developing Rat Brain.

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The product of the proto-oncogene c-fyn ($p59^{fyn}$) is a non-receptor tyrosine kinase. This src-related kinase is principally expressed in T-lymphocytes and neurons as two distinct forms, which differ in their kinase domains and are encoded by alternatively spliced mRNA . In the embryonic chick retina, $p59^{fyn}$ is localized in the soma of differentiating neurons and in the outgrowing neuronal processes of the developing plexiform layers, whereas in the mature retina it is found chiefly in cell soma. To investigate whether $p59^{fyn}$ in embryonic neurons is a component of the nerve growth cone, its expression was analyzed in fetal rat brain by immunoblotting and immunocytochemistry. $p59^{fyn}$ was found to be concentrated in the membranes of a growth cone-enriched subcellular fraction isolated from fetal (day 18) rat brain. A synaptic plasma membrane fraction from adult brain exhibited much lower levels of $p59^{fyn}$. Interestingly, in the growth cones of regenerating sciatic nerve, $p59^{fyn}$ was not expressed at elevated levels characteristic of embryonic growth cones. Immunoperoxidase staining of E18 rat brain revealed that $p59^{fyn}$ was localized primarily in axons of developing tracts. Prominent staining was visualized in the lateral olfactory, optic and pyramidal tracts and cerebral peduncles, but not in the striatum and ventricular zone of the cerebral cortex. In the adult brain tracts, no staining was observed, however, immunoreactivity was seen in the olfactory bulb particularly in the glomeruli, mitral cell bodies and their dendrites. These results suggest that the neuronal form of $p59^{fyn}$ may play a principal role in neurite outgrowth of developing CNS neurons.

262.4

TRANSCRIPTIONAL REGULATION OF THE GAP-43 GENE IN CULTURED NEURAL, GLIAL AND NON-NEURAL CELL LINES.

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The GAP-43 gene encodes a 24 kDa phosphoprotein whose expression *in vivo* is restricted to neurons, predominantly those undergoing axonal growth. However, GAP-43 expression has also been demonstrated in astrocytes and in Schwann cells, arguing against strict neuron-specific expression. As an initial step towards understanding the mechanisms that specify the cell-type specific regulation of the gene, we have begun an analysis of the GAP-43 promoter by preparing promoter constructs that are linked to the reporter gene chloramphenicol acetyltransferase (CAT) and then stably transfecting them into neural (PC12), glial (C6) and non-neural cell lines (Rat 2). Analysis of pools of stable transfectants indicate that 230 bp of 5' flanking DNA sequence defines a GAP-43 minimal promoter that is active in both neural and glial cell lines, but not in non-neural fibroblast cell lines. Furthermore, additional sequence regions lying upstream of the first 1000 bp are required for high level expression in both neural and glial cell lines. Further characterization of the sequence regions that define cell type specific transcription is in progress. Supported by USPHS grant HD27226.

262.5

GAP-43 STIMULATION OF G_O IS BLOCKED BY PALMITOYLATION.

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GAP-43 is a neuronal protein bound to the growth cone membrane by a short amino terminal stretch which contains two palmitoylated cysteine residues (J.Cell Biol. 108:613, Nature 341:345). The hydrophobicity of the palmitates is likely to contribute substantially to the membrane localization of GAP-43, as for other fatty acylated proteins. We have studied whether this modification affects GAP-43 function as well as localization. GAP-43 can stimulate G protein activity, and this may alter growth cone motility (Nature 344:836). The GAP-43 domain which stimulates G_O is localized to the first ten amino acids of the protein where palmitolyation occurs. We have compared the ability of palmitoylated and non-palmitoylated GAP-43 N-terminal 1-25 peptides to stimulate G_O. The palmitoylated species have essentially no activity compared to the non-palmitoylated molecules. Palmitoylated GAP-43 protein is also inactive in this assay. Depalmitoylation restores G_O stimulation. The palmitoylated forms are inactive in both detergent solution and phospholipid vesicles. Modification of the GAP-43 cysteines with PCMPS also reversibly blocks G_O stimulation. It is well known that protein palmitoylation is a dynamic modification with a rapid turnover time, so cycles of fatty acid acylation within growth cones may control a cycle of GAP-43 between an acetylated membrane-bound reservoir of inactive protein and a nonpalmitoylated, active pool. This work also demonstrates that palmitoylation can directly modify protein-protein interactions in addition to directing subcellular localization.

262.6

THE IMMUNOSUPPRESSANT FK-506 ENHANCES PHOSPHORYLATION OF GAP43: IMPLICATIONS FOR A ROLE IN MODULATION OF GROWTH CONE FUNCTION AND NEUROTRANSMITTER RELEASE.

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The immunophilins cyclophilin and FKBP are small predominantly soluble proteins that bind with high affinity the immunosuppressant drugs cyclosporin A (CsA) and FK-506, respectively, and which appear to mediate their pharmacologic actions. Interaction of FK-506-FKBP and CsA-cyclophilin with the Ca^{2+}/CaM-dependent protein phosphatase calcineurin inhibits calcineurin activity, suggesting that calcineurin may mediate actions of these drugs. We report extraordinarily high levels of FKBP protein and mRNA in rat brain, with a unique regional localization that is essentially identical to that of calcineurin, indicating a physiologic link between calcineurin and the immunophilins. We also report that FK-506 and CsA, in low nanomolar concentrations, enhance the phosphorylation of endogenous protein substrates in brain tissue and in intact PC12 cells. The phosphorylation of GAP43, a prominent calcineurin substrate, is regulated by FK-506-FKBP and CsA-cyclophilin complexes. Since GAP43 appears to be involved in neurotransmitter release, as antibodies to GAP43 selectively inhibit neurotransmitter release, we have investigated a role for FK-506 and CsA in this process.

262.7

INCREASED EXPRESSION OF FK-506 BINDING PROTEIN DURING PERIPHERAL NERVE REGENERATION

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Ligand binding studies from this laboratory using ^{3}H-FK-506 (see adjacent abstract: Steiner *et al.*) have demonstrated that the immunophilin FKBP is present at high levels in soluble and particulate fractions of rat brain. Previous studies have also shown that the FK-506/FKBP complex acts as an inhibitor of the protein phosphatase calcineurin, which is also enriched in the CNS. These data suggest that through its effects on calcineurin, FKBP may play an important role in brain function by regulating the phosphorylation state of neuronal proteins. One of the most prominent substrates of calcineurin is GAP43, a protein whose expression has been correlated with neurite outgrowth during development and regeneration. We therefore examined the expression of FKBP during regeneration of the facial nerve, a well characterized model of regeneration in which increases in GAP43 levels have been clearly demonstrated. Experimentally the nerve was crushed with forceps 2mm distal to its exit from the stylomastoid foramen, and expression of FKBP and its mRNA was examined in the facial nucleus at various times after the lesion. An increase in FKBP mRNA was observed beginning between 24 and 48 hours after crush using *in situ* hybridization. Elevated levels of mRNA were also observed at 7 days, suggesting that the increased expression may persist during the period of regeneration. Increases in the levels of FKBP were also observed using ^{3}H-FK-506 autoradiography. These data suggest a role for FKBP in nerve regeneration, possibly by regulating the phosphorylation of GAP43.

262.8

WITHDRAWN

262.9

REGULATION OF GAP-43 PHOSPHORYLATION DIRECTLY CORRELATED WITH GROWTH CONE BEHAVIOR IN CULTURE

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Phosphorylation of GAP-43 by kinase C *in vivo* and in tissue culture occurs after axonogenesis has been initiated and is confined to distal axons and growth cones. Within individual growth cones too, phosphorylation of GAP-43 is locally regulated and its distribution is restricted. Here we have used video enhanced microscopy of cultured DRG neurons together with immunocytochemistry with our specific monoclonal antibody to show that changes in the distribution of phosphorylated GAP-43 correlate with two aspects of growth cone behavior: In individual translocating growth cones phosphorylated GAP-43 is confined to the central region where organelles are concentrated, and accompanies the redistribution of the organelles within the body of the growth cone that occurs when the neurite elongates. Phosphorylated GAP-43 is very low in those highly motile distal lamellae where organelles are absent, and in lamellae or filopodia that are actively retracting, but levels of phosphorylated GAP-43 are increased in those areas when they become stabilized. However, whenever one growth cone touches another part of a cell, neuronal or non-neuronal, GAP-43 phosphorylation is rapidly stimulated throughout the whole growth cone overriding its previous restriction. These results suggest that kinase C phosphorylation of GAP-43 may be involved in discriminatory functions of the growth cone, possibly by stabilizing components of the growth cone membrane.

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262.10

Expression of B-50(GAP43) via a defective Herpes Simplex virus vector in cultured non-neuronal cells.

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The expression of the neural phosphoprotein B-50 correlates closely with nerve fiber formation but its causal involvement in nerve fiber growth has not been established. Here we report B-50-gene transfer to cultured cells with a previously described Herpes Simplex virus (HSV) type I defective vector system (Kaplitt et al. Molec. Cell. Neurosci. 2:320-330,1991). Two defective HSV vectors expressing B-50 from the human cytomegalovirus (CMV) promoter were generated. Plasmid pSRa-ori-CMV-B-50 was created by introducing a CMV-B-50 fragment into pSRa-ori, a plasmid containing the HSV-1 cleavage/packaging signal and an HSV-2 origin of replication. A second plasmid, pHCL-CMV-B-50, contains the same sequences with the addition of a CMV-lacZ expression cassette allowing for the identification of infected cells by histochemical staining for ß-galactosidase (ß-gal). Titers of viral stocks were determined by ß-gal histochemistry and immunocytochemical staining for ß-gal and B-50. Similar results were obtained with both methods indicating that viral titers can reliably be determined by immunocytochemical means. Vero cells (African green monkey kidney cells) infected with defective HSV-B-50 viruses exhibited striking morphological changes including filopodial-like structures, ruffled membranes and long thin extensions reminiscent of neuronal processes. Similar processes did not appear in controls treated with vectors not expressing B-50. These results suggest a role for B-50 in the determination of cell shape. Our defective viral vectors will enable the study of B-50's role in regenerative sprouting in vivo.

262.11

DISTRIBUTION OF GAP-43 mRNA IN THE GOLDFISH BRAIN.

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Expression of GAP-43 mRNA has been found in the central nervous system of both immature and mature animals. Within the developing brain, expression of the GAP-43 gene is most abundant and appears to correlate with periods of axonal outgrowth and synaptic organization. In the adult, GAP-43 mRNA is found at lower levels, and is localized to regions of the brain associated with neural plasticity and regeneration. Our study was designed to examine the histological distribution of GAP-43 mRNA in the brain of an adult teleost, *Carassius auratus* (goldfish), which is known for its CNS regeneration capabilities. A 28-base oligonucleotide, corresponding to GAP-43 mRNA was synthesized from the known cDNA sequence of the goldfish GAP-43 gene. The oligonucleotide was radiolabelled and used to probe sections of goldfish brain according to *in situ* hybridization methodology. From these experiments, GAP-43 mRNA was found to be most abundant in the stratum periventriculare and stratum album centrale of the optic tectum, hypothalamic nuclei, the granule cell layer of the valvula, corpus and lateral cerebelli and the inferior olivary complex. Less intense hybridization was found in the stratum griseum centrale of the optic tectum, reticular nucleus, nucleus rotondus, area pretectalis, glomeruli, vestibular and tegmental nuclei, as well as vagal and facial lobes. The goldfish diencephalon appears to be devoid of GAP-43 mRNA. Control studies were performed using a radiolabelled sense oligonucleotide. This probe did not hybridize with GAP-43 mRNA thereby demonstrating specificity of binding of the antisense oligonucleotide to the mRNA. To our knowledge this is the first study to examine the histological localization of GAP-43 mRNA in the goldfish. The presence of GAP-43 mRNA in many brain regions of the adult goldfish suggests that the high degree of plasticity associated with these animals could be related to the expression of GAP-43.

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262.12

MYELIN AND GAP-43 HAVE A PATTERN OF COMPLEMENTARY REGIONAL DISTRIBUTION IN CNS GRAY MATTER OF NORMAL ADULT RATS.

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In the CNS, myelin is present not only in white matter, but also in gray matter areas in varying amounts. Some brain areas are fairly high in myelin, whereas others are only lightly myelinated. Oligodendrocytes and myelin contain proteins inhibitory to fiber growth *in vitro* and for fiber regeneration *in vivo*. In this study we investigate whether these neurite growth inhibitors could also restrict fiber growth and rearrangement of terminals in the normal adult CNS.

We have compared the pattern of myelination in gray matter areas (revealed by myelin staining and MBP immunohistochemistry) with the regional expression of a marker for fiber growth and terminal rearrangement, GAP-43 (revealed by immunohistochemistry), on adjacent CNS sections. In the majority of the CNS regions the myelin and GAP-43 pattern are exactly complementary. Highly myelinated areas like the inferior colliculus, the tegmentum and most brain stem nuclei are low in GAP-43, whereas only lightly myelinated areas as the striatum, the substantia nigra or the substantia gelatinosa of the spinal cord express relatively high amounts of GAP-43. In laminated structures such as the cerebral cortex, the cerebellar cortex or the superior colliculus laminae high in myelin are low in GAP-43 and vice versa.

These results are consistent with a possible role of neurite growth inhibitors from oligodendrocytes in regulating terminal sprouting and rearrangement in the normal adult CNS.

We thank Dr. Karina Meiri for the generous gift of anti-GAP-43 antibodies.

262.13

NEURITE OUTGROWTH IN PC12 CLONES WITH VARIOUS AMOUNTS OF GROWTH-ASSOCIATED PROTEIN B-50 (GAP-43)

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Rat pheochromocytoma (PC12) cells acquire a sympathetic, neuron-like phenotype with neuritic extensions upon NGF induction. This is accompanied by a translocation of the neural-specific phosphoprotein B-50 (= GAP-43, F1 or neuromodulin) to the plasma membrane and an enhanced expression of B-50 mRNA and protein. We selected a number of clonal PC12 cell lines with altered B-50 expression. Protein levels are 2-3 fold enhanced, or 10-20 fold reduced, as determined by ELISA and Western blot.

These clones were further characterized by Northern and Southern blotting. Low expression of B-50 did not markedly reduce the rate of NGF-induced neurite outgrowth, as was reported by Beatge and Hammang (Neuron 6, 1991), but remarkable changes in morphology of neurites and growth cones were observed. These changes could be visualised by immunofluorescence using antibodies against B-50 and cytoskeletal proteins. Transient overexpression of B-50 cDNA under control of the CMV promoter induced the enhanced formation of numerous small extensions, i.e. filopodia, as was found by Zuber et al. (Science 224, 1989) in COS cells. This indicates that B-50 is involved in formation of filopodia and normal growth cone development, but is not under all circumstances essential for neurite outgrowth in PC12 cells. (supported by Prinses Beatrix Fonds).

262.14

ARE B-50 / GAP-43 DECORATED ELECTRON-LUCENT VESICLES INVOLVED IN NEURONAL AXONAL TRANSPORT?

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By immuno electron microscopy we have shown that in developing and adult rat brain B-50 / GAP-43 is predominantly localized at the axonal plasma membrane. Using immuno gold labeling we studied how B-50 is intracellularly targeted to the plasma membrane of developing rat hippocampal neurons in vitro. B-50 was visualized on the cytoplasmic side of the membrane of electron-lucent vesicles (average diameter 100 nm) of the cytoplasm. These B-50 vesicles were present in trans regions of the Golgi apparatus, juxtaposed to microtubules in neurites and growth cones. They accumulated close to the plasma membrane. Interfering with axonal transport by nocodazole (Baas and Black, J. Cell Biol. 111, 495, 1990) altered the distribution of B-50 and these vesicles. Anti-kinesin antibodies were applied to identify axonal motor driven vesicles. We hypothesize that B-50 may be conveyed on membrane vesicles to the plasma membrane as part of the constitutive transport pathway.

262.15

RNA-PROTEIN INTERACTIONS BETWEEN THE 3' UTR OF GAP-43 mRNA AND CYTOSOLIC PROTEINS FROM RAT BRAIN. Implications for the post-transcriptional regulation of the mRNA.

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The growth-associated protein, GAP-43, is a nervous system specific phosphoprotein that has been implicated in the development, regeneration, and remodeling of synaptic connections. Previous work from our laboratory indicated that GAP-43 expression in NGF induced-PC12 cells is controlled by protein kinase C-dependent stabilization of the mRNA (Soc. Neurosci. Abstr.17: 1310). In order to investigate the mechanisms that influence GAP-43 mRNA stability, we performed *in vitro* RNA-protein binding reactions using labeled 3' UTR sequences from GAP-43 mRNA and S100 extracts from adult and neonate rat brains. Complex formation was determined by RNAse T1 protection and gel retardation assays in non-denaturing polyacrylamide gels. The specificity of the interactions was confirmed by competition experiments using an excess of unlabeled homologous or non-homologous RNAs. A 20-100 fold excess of sense RNA fully displaced the labeled 3' UTR sequences from the complex, whereas antisense RNA or unrelated RNAs had no effect on complex formation. Following UV-crosslinking, RNA-protein complexes were analyzed by SDS-PAGE. This demonstrated the formation of three main complexes that migrate with apparent sizes of ~95kDa, ~70kDa, and ~45kDa. Analyses of the complexes by label-transfer experiments revealed that the major GAP-43 mRNA binding proteins have apparent sizes of ~85kDa, ~60kDa, and ~40kDa. Since the stabilization of GAP-43 mRNA was found to require the activation of PKC but was independent of *de novo* protein synthesis, we examined whether RNA-protein complex formation was regulated by specific protein phosphorylation. Treatment of cytosolic extracts with alkaline phosphatase abolishes the formation of the intermediate size complex, suggesting that protein phosphorylation is required for this interaction. The possibility that this RNA-binding protein is the PKC substrate responsible for regulating the stability of GAP-43 mRNA during neuronal differentiation is currently under investigation. Supported by the NIH (NS30255) and the American Paralysis Association (PBI-9006) to NPB.

262.16

MOTONEURON SPROUTING IS NOT ASSOCIATED WITH INCREASES IN GAP-43 MRNA.

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High GAP-43 mRNA levels are associated with axon growth during development or following injury. Another type of axonal growth is collateral sprouting from uninjured neurons in response to partial denervation. We asked whether increased GAP-43 mRNA occurs during collateral sprouting of mouse gluteal motoneurons with axons in the superior gluteal nerve, in response to cutting the inferior nerve. When retrograde tracer (true blue) was applied to the cut inferior nerve and after one week, to allow sprouting to begin, spinal cord was processed for in-situ hybridization with a GAP-43 cDNA probe, only dye-labelled (axotomized) cells had high mRNA levels. When fluorogold was instead applied to the surface of the muscle, and so could be only taken up by the sprouting motoneurons with axons in the superior nerve, fluorescent cells had mRNA levels undistinguishable from those of motoneurons in other parts of the motor column: none containing high GAP-43 mRNA levels were fluorescent. Preliminary observations of sternomastoid motoneurons induced to sprout by marcaine applied to the muscle also reveal no increase in GAP43 mRNA. We conclude that collateral sprouting of motoneuron axons occurs without detectable increases in cell body GAP-43 mRNA expression. (MCB was a Visiting Scientist of the Alberta Heritage Foundation for Medical Research).

262.17

GAP-43 IN DEGENERATING PERIPHERAL NERVE: AXONAL AND SCHWANN CELL ORIGIN.

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We used immunocytochemistry, in-situ hybridization and RT-PCR to investigate the appearance and sources of GAP-43 in rat sciatic nerve distal to axotomy. In normal and short-term transected nerves, GAP-43-IR was restricted to Remak fibres (Schwann cells enveloping unmyelinated axons), but in crushed nerves strong GAP-43-IR was also present both within regenerating axons and around the margins of Schwann cells (bands of Büngner) in the vicinity of the axons, as previously reported (Tetzlaff et al., J. Neurosci. 9:1303-1313). By 21d following resection, all Schwann cells forming bands of Büngner were uniformly GAP-43 immunoreactive, in contrast to the 21d crushed nerves where GAP-43-IR was seen in many regenerating axonal profiles and to a lesser degree with Schwann cells. At 3d and 7d, GAP-43 mRNA was increased in both crushed and resected distal nerve segments compared to the contralateral control nerves. No further mRNA increase was seen at later timepoints after crush, while a massive further increase in GAP-43 mRNA expression was seen in 14d to 50d resected nerves. Thus, GAP-43 mRNA increased in distal stumps after axotomy, and if regeneration was prevented there was a delayed further increase which was sustained for up to 50 days. The latter correlated with a strong IR of all bands of Büngner, indicating that the Schwann cells synthesize significant amounts of GAP-43. The observation of GAP-43-IR around the margins of Büngner bands 3d and 7d after crush, but not 3d and 7d after resection, without differences in GAP-43 mRNA expression, suggest that GAP-43-IR may also be derived from degenerating axonal sprouts during the early stages of regeneration.
Supported by the Canadian Network of Centres of Excellence for Neural Regeneration and Function Recovery.

262.18

EXPRESSION OF GAP-43 IN PC12 CELLS CLONES IS INDEPENDENT OF NEURITE OUTGROWTH.

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The expression of GAP-43, a growth associated protein, has been postulated to function in controlling neuritic outgrowth. We investigated this possibility in a series of PC12 cell clones which express different phenotypes in response to nerve growth factor (NGF) and second messenger stimulating drugs. One PC12 clone, PC12-N21, grew neurites in response to NGF or forskolin (FOR) and increased levels GAP-43 mRNA and protein in response to NGF or phorbol ester (PMA). These cells gave a typical PC12 cell response to NGF, PMA or FOR. A second clone, PC12-N09, did not grow neurites in response to NGF, but did increase GAP-43 levels in response to NGF or PMA. However, in response to FOR the PC12-N09 cells did grow neurites. When PMA and FOR were used together, PC12-N09 cells both initiated neuritic outgrowth and increased GAP-43 expression. These results show that PC12-N09 cells respond to NGF by increasing GAP-43 levels without concomitant neurite outgrowth, but these cells have the ability to express both phenotypes if treated with a combination of drugs that act on different second messenger systems. The behavior of these PC12 clones suggests that NGF stimulation activates at least two different pathways, one which initiates neurite outgrowth and the other which increases the expression GAP-43. **Supported by the NSF (BNS-8909835; RWB), NSF (BNS-9011199; NPB) and American Paralysis Association (PBI-9006; NPB)**

262.19

MEASUREMENT OF RELATIVE AMOUNTS OF PHOSPHO- AND DEPHOSPHO-B-50(GAP-43) PEPTIDES BY FAST ATOM BOMBARDMENT-MASS SPECTROMETRY (FAB-MS) M. Di Luca, P.N.E De Graan, L. De Angelis, W.H. Gispen and F. Cattabeni*, Institute of Pharmacological Sciences, University of Milano, 20133 Milano, Italy and Rudolf Magnus Institute of Pharmacology, University of Utrecht, 3521 GD Utrecht, The Netherland.

The biological role of phosphoproteins depends upon their degree of phosphorylation. Methods currently available to measure the actual *in vivo* phosphorylation state of a protein involve indirect procedures based on ^{32}P incorporation.

B-50(GAP-43) plays a crucial role in neuronal plasticity. The possibility of elucidating its physiological role requires the measurement of its degree of phosphorylation *in vivo*. We have explored this possibility by measuring relative amounts of phospho- and dephospho-forms of the peptide corresponding to amino acid sequence 39-51 of B-50(GAP-43) by FAB-MS. The peptide was phosphorylated by purified Protein Kinase C and the reaction mixture was directly analyzed: the mass spectrum showed two molecular ions at m/z 1583.9 and 1663.9, corresponding respectively to the dephospho- and phospho-forms of the peptide. By changing their relative amounts, linear changes in the ratio of the intensities of the two ions was obtained. The lowest detectable amount of the peptide was 30 pmol, with a signal to noise ratio value of about 3.

This demonstrates the applicability of FAB-MS to quantitate realtive amounts of phospho- and dephospho- peptides derived from B-50(GAP-43) by proteolytic digestion, in the same sample and in the same experimental conditions.

PROCESS OUTGROWTH, GROWTH CONES AND SPROUTING III

263.1

ANTI-β1 INTEGRIN ANTIBODIES INHIBIT REGENERATING RETINAL GANGLION CELL AXON OUTGROWTH AS WELL AS GLIAL CELL ADHESION AND SPREADING. D.S. Sakaguchi* and K. Radke. Dept. Zool. and Genetics, Neurosci Prog., Iowa State Univ., Ames, IA 50011.

The β1 family of integrin receptors have been shown to mediate embryonic axonal outgrowth onto extracellular matrix (ECM) substrates in a number of experimental systems. In the present study we have investigated a role for the β1 integrins during regeneration of retinal ganglion cell axons in *Xenopus laevis*. Late stage tadpole retinas, from eyes that received a conditioning optic nerve crush 2 weeks earlier, were cultured on laminin or ECL (entactin, collagen and laminin ECM preparation) substrates. These explanted retinas were allowed to attach to the substrates and polyclonal antibodies to the β1 integrin subunit (from K. Yamada) added 24 hrs later. IgGs, as well as Fab fragments, inhibited axon outgrowth in a dose-dependent fashion, while control, preimmune rabbit antibodies had no effect.

We also investigated a role for the β1 integrins in mediating glial cell adhesion and spreading. Culturing the *Xenopus* XR1 glial cell line on collagen substrates in the presence of the anti-β1 antibodies resulted in a dose-dependent inhibition of cell attachment. To examine cell spreading XR1 cells were allowed to attach to the substrate and control or anti-β1 antibodies added 12 hours later. This analysis revealed that the anti-β1 antibodies inhibited the spreading of the XR1 cells and in addition, could lead to the retraction of the glial cell lammemapodia. Removal of the antibody containing medium and replacement with fresh medium resulted in the recovery of the XR1 glial cell morphology. β1 family integrins appear to play important functional roles during regeneration and also in glial cell adhesion and spreading *in vitro*, and may play similar roles *in vivo*.

263.2

β1 Integrin-Like Immunoreactivity in the Adult Rat Brain. L.S.Jones*, S.Grooms, L.Terracio, Dev. Bio. & Anat., U. South Carolina, Columbia, SC 29208.

β1 integrin-like immunoreactivity was localized in adult rat brain using a rabbit-raised anti-rat liver β1 integrin antibody (J.Biol.Chem. 264:12686, 1989). Standard immunohistochemical techniques were applied to 10μm paraffin-embedded sections from paraformaldehyde perfusion-fixed brains. Specific staining of integrin-like immunoreactivity was found in vascular structures of the CNS, including microvessels, ventricular ependymal cells, and pia mater. Labelling of medium-sized vessels was particularly striking, as antibody labelled what appeared to be smooth muscle in a "slinky-like" pattern of circumferential organization. The pineal gland was the most densely stained CNS structure. Diffuse staining was present throughout the gray matter of the brain. This staining took on a patterned appearance in certain regions, such as the apical dendritic field of CA1 in hippocampus; in CA1 the labelling appeared to parallel and outline the apically radiating dendrites of the pyramidal neurons, and bore a resemblance to the appearance of GFAP staining in the same region. Faint labelling of astrocytes was noted, and in occasional adjacent sections an astrocyte that appeared labelled both with GFAP and integrin-like immunoreactivity was identified. The localization of integrin-like immunoreactivity in the dendritic fields of hippocampus may indicate that these proteins play a structural role in dendritic morphology. (Work supported by NS27903 and HL40424.)

263.3

EXPRESSION OF INTEGRIN SUBTYPES ON EMBRYONIC CHICKEN SENSORY NEURONS IN CULTURE -- FUNCTIONAL RELATIONSHIPS TO NEURITIC OUTGROWTH. A.W. Lyckman*, C.B. Herbert, R.C. Lewis, L. Suggs, S.P. Massia, G.D. Bittner and J.A. Hubbell. Depts. of Chem. Eng. and Zoology, Univ. of Texas, Austin, Texas 78712.

Neuronal survival and differentiation has a strong dependence on local environmental conditions. With regard to neuritic outgrowth, sensory neurons are differentially responsive to extracellular matrix (ECM) proteins. Many cells, including neurons, carry cell surface receptors for ECM proteins called integrins which may mediate cellular responses to the ECM. Integrins are a superfamily of heterodimeric ($\alpha_x\beta_y$) proteins. Integrins with selective affinities for different ECM proteins derive from specific combinations of α-subunits (at least 11) and β-subunits (at least 7). To better understand mechanisms underlying neuritic outgrowth on different ECM proteins, we used immunofluorescence to determine which integrin subtypes are expressed on embryonic chicken sensory neurons.

Our immunofluorescence studies utilized commercially available monoclonal and polyclonal antibodies to human integrin subtypes (Telios, Chemicon). Cells from dissociated dorsal root ganglia (DRG) of embryonic day 7 chicken embryos were grown in serum-free F-12 medium on different ECM proteins adsorbed to glass. After 12-48h, cells were fixed for 15min with 4% paraformaldehyde and treated overnight at 4°C with antisera diluted with 0.1% Triton X-100 in PBS. Primary antibodies were stained with fluorescently labeled secondary monoclonals (Boehringer-Mannheim). DRG neurons were identified with monoclonal 3A10 from the Developmental Studies Hybridoma Bank. DRG neurons were immunopositive for specific α subunits (α_4, α_5, and α_V); specific β-subunits (β_1 and β_3,); and, specific integrin subtypes ($\alpha_5\beta_1$ and $\alpha_V\beta_3$). DRG neurons were not immunopositive for antisera against α_2. The staining pattern of positive immunofluorescence was usually punctate, and appeared on growth cones, neurites, and the soma. Staining for α_4 also appeared tubulovesicular. Integrin subtype expression was the same whether neurons were grown on collagen IV, fibronectin, laminin or vitronectin. Antisera to $\alpha_5\beta_1$ in living cultures caused retraction of neurites on fibronectin and laminin. This and future studies will be used to predict and assess peptide sequences which promote regeneration of peripheral nerves. Supported by NSF grant ECS - 8915178 to J.A.H and G.D.B.

263.4

GANGLIOSIDE GM1 POTENTIATES LAMININ-INDUCED NEURITE OUTGROWTH IN PC12 CELLS. A. Buriani*, L. Facci, R. Dal Toso and S.D. Skaper. Fidia Research Labs, Abano Terme, Italy.

NGF-differentiated PC12 cells grown on a polylysine surface regenerate few or no neurites in the absence of serum or certain adhesion molecules. Acute addition of laminin to such cultures produces long neurites within several hours. Pretreatment of PC12 cells with the monosialoganglioside GM1 led to significant increases in both the number of neurites and their rate of growth in the copresence of laminin. In effect, GM1 rendered weakly active concentrations of laminin as effective as amounts 2-3 fold greater, thus appearing to shift the laminin dose-response relationship. This effect of GM1 required the presence of an external source of Ca^{2+} and was concentration-dependent, being optimal at 10^{-7}M GM1 in a serum-free environment. GM1 has also been described to potentiate N-CAM and N-cadherin dependent neurite outgrowth in PC12 cells (Doherty et al., J. Cell Biol., in press) in a Ca^{2+}-dependent fashion, suggesting a common signaling pathway in the interaction of GM1 with extracellular matrix and cell adhesion molecules.

263.5

ULTRASTRUCTURAL ANALYSIS OF EMBRYONIC NEURAL CELL ADHESION MOLECULE (N-CAM) EXPRESSION IN RAT DENTATE GYRUS FOLLOWING ENTORHINAL CORTEX LESION. S.D. Styren*, P.D. Miller, C.F. Lagenaur, S.T. DeKosky. Depts. of Psychiatry, Neurobiology, Anatomy & Cell Science, Neurosurgery, and Neurology, Univ. of Pittsburgh Sch. of Med. and Western Psychiatric Inst. & Clinic, Pittsburgh, PA 15213.

Re-expression of developmental proteins following CNS lesions or in degenerative diseases have been demonstrated in some neural systems. We examined changes in embryonic N-CAM expression in adult rat dentate gyrus during axon sprouting and synaptogenesis following ipsilateral entorhinal cortex (ERC) lesion . This lesion denervates the outer 2/3 of the dentate gyrus molecular layer (ML) by disconnecting the perforant pathway afferents, leaving the inner 1/3 unaffected. Light microscopic analysis of dentate gyrus utilizing a monoclonal antibody (recognizing highly polysialyated embryonic N-CAM) demonstrated weak to undetectable staining throughout the ML in normal rats; ultrastructural examination of this zone revealed sporadic weak staining limited to a few dendrites. Ten days post-lesion, intense embryonic N-CAM immunoreactivity was present in the deafferented outer ML. In these animals ultrastructural examination of the outer ML revealed intense immunoreactivity associated with dendrites. Embryonic N-CAM immunoreactivity was most often seen at contact sites between dendrites and axonal profiles, including terminals. Intense staining was localized to the cell surface membrane. In the inner ML embryonic N-CAM was unchanged compared to normal animals. The loss of synaptic contacts in the denervated zone appears to signal rexpression of embryonic N-CAM as a means to re-establish synaptic contacts.

263.6

NEURAL CELL ADHESION MOLECULE (N-CAM) EXPRESSION IN THE RAT DENTATE GYRUS FOLLOWING ENTORHINAL CORTEX LESION. P.D. Miller*, S.D. Styren, C.F. Lagenaur, S.T. DeKosky. Depts. of Neurobiology, Anatomy & Cell Science, Neurosurgery, Psychiatry, and Neurology, University of Pittsburgh School of Medicine and Western Psychiatric Institute & Clinic, Pittsburgh, PA 15213.

We evaluated immunohistological changes in N-CAM expression in adult rat dentate gyrus during the period of axonal sprouting and synaptogenesis following ipsilateral entorhinal cortex (ERC) lesion. This lesion denervates the outer 2/3 of dentate granule cell dendritic fields and induces compensatory sprouting from the subjacent inner 1/3 into the denervated zone, as well as reactive synaptogenesis in the denervated outer molecular layer. Antibodies to total N-CAM and the monoclonal antibody 12F11 (which recognizes N-CAM 140 and 180) intensely stained the inner molecular layer (ML) in controls, and after ERC lesion this intense staining exactly followed the known temporal sequence for the sprouting of axon collaterals into the denervated zone. The area of increased staining expanded to ultimately occupy the inner half of the ML. Monoclonal antibody 12F8 recognizes the highly polysialylated, embryonic form of N-CAM. Controls had very light, uniform ML staining. By 6 days post-lesion, robust staining of the outer 2/3 of the molecular layer developed. This area of intense staining, marking the entire outer ML, receded as axon collaterals from the inner 1/3 entered the denervated zone, so that by 30 days the intense 12F8 staining only occupied the outer half of the ML. This upregulation of embryonic N-CAM persisted at 60 days post-lesion, past the point when synaptic density has returned to normal levels in the denervated zone. Following injury, the hippocampus recapitulates developmental sequences of embryonic N-CAM expresssion. Unlike the developmental sequence, post-injury embryonic N-CAM persists.

263.7

EXPRESSION OF GM2 GANGLIOSIDE BY NEURONS ELABORATING NEURITES IN FETAL RAT CEREBRAL CORTEX CULTURES. K. Dobrenis and S.U. Walkley.* Depts. of Neuroscience and Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

Despite the fundamental importance of dendritic growth, little is known about the regulatory factors involved. Previous studies examining normal dendritogenesis during brain development and ectopic dendrite growth in neuronal storage diseases have suggested a correlation between the appearance of GM2 ganglioside (GM2) and dendritic sprouting (PNAS 88: 11330-34, 1991). To further test the importance of GM2, we are investigating whether a similar relationship is manifested *in vitro*.

Cell cultures were prepared from rat cerebral cortices of embryonic day 15-16, a time at which neurons are commencing neuritic growth. Mechanically dissociated cells were plated on polylysine coated substratum with chemically-defined or fetal bovine serum-containing medium. Cultures were processed by immunofluorescence with monoclonal antibodies to microtubule-associated proteins 2a+2b (MAP2), for unambiguous identification of differentiating neurons, and to GM2. A total of 3907 cells were examined. In serum-fed cultures, cells of neuronal appearance (rounded somas with narrow processes) at 1 day *in vitro* (DIV) primarily bore 1-2 short (<100μm) unbranched neurites. At 14 DIV neurons had lengthy tapering dendritic-like processes and included bipolar-, pyramidal- and stellate-like cells. The percentage of neuron-like cells with MAP2 staining increased over time: 36, 74 and 93% at 1, 2 and 5 DIV, respectively. The percentage of $MAP2^+$ neurons that were $GM2^+$ also increased over these times: 23, 43 and 99%. This value appeared to decline subsequently to 94 and 86% at 8 and 14 DIV. $GM2^+$, $MAP2^-$ neuron-like cells were rare (<4%). In early cultures $GM2^+$ staining was mostly punctate in form and in the somatic domain; over time staining more frequently appeared as patches or rings and extended over dendritic-like profiles. Serum-free cultures show similar results. (NS18804)

263.8

NEURONAL CELLS IN PRIMARY CULTURE EXHIBIT LDL RECEPTOR-MEDIATED ENDOCYTOSIS OF LIPOPROTEINS: A ROLE IN CNS CHOLESTEROL METABOLISM. J. Poirier*, P. Bertrand, R. Alonso, R. Quirion and P. Boksa. Douglas Hospital Research Centre, Department of Psychiatry and Centre for Studies in Aging, McGill University, Montreal, Canada.

Apolipoprotein E, a major plasma protein in humans and rats, is best known in peripheral lipoprotein transport to LDL receptors on liver and adrenal cells. While its role and that of its receptor in neural tissues are less known, the production of apo E during responses of hippocampus to deafferentation and reinnervation implies a role in lipid transport associated with terminal proliferation and synaptic remodelling. To test this postulate, we examined the expression of the LDL receptor in hippocampal neuronal cells in primary culture maintained in serum free conditions to prevent glial proliferation. LDL, which has been labelled with a fluorescent dye, was internalized by the neurites and growth cones at 37°C. The intracellular fluorescence appears to be mostly concentrated at the basis of axons. The extent of the internalization process was significantly reduced (>50%) by incubating cells for 48 hours with 0.1 uM of dexamethasone, a potent glucocorticoid analog. This observation is consistent with the known reduction of LDL receptors prevalence in hepatocytes treated with dexamethasone, and would be expected if the uptake of cholesterol-rich lipoproteins represent the major source of lipids in developing neurons. Supported by the Medical Research Council of Canada and American Alzheimer's disease Association.

263.9

LDL RECEPTOR EXPRESSION DURING SYNAPTIC REMODELLING IN THE HIPPOCAMPUS FOLLOWING ENTORHINAL CORTEX LESIONING. D.Dea*, A. Baccichet, P. Bertrand and J. Poirier. Douglas Hospital Research Centre, Department of Psychiatry and Centre for Studies in Aging, McGill University, Montreal, Quebec, Canada, H4H 1R3.

Apolipoprotein E (apo E) is synthesized and secreted by astrocytes in the hippocampus following lesions of the entorhinal cortex. It was proposed that apolipoprotein E, by analogy to its role in cholesterol transport in circulation, could regulate the salvage and reutilization of cholesterol released during terminal breakdown. This cholesterol could then be transported to neurons by apolipoprotein E-complexes and taken up via the apo E/apo B (LDL) receptor. As a test of this hypothesis, we have examined LDL receptor binding in brain sections of rats undergoing hippocampal reinnervation. The number of neuronal cells labelled by fluorescent DiI-LDL as well as the density of ^{125}I-LDL binding sites in the dentate gyrus were found to increase in parallel with the extent of cholinergic reinnervation occurring in the deafferented hippocampus. Analysis of the mRNA prevalence for the LDL receptor indicates a profile of induction that parallels the receptor binding sites alterations observed in the dentate gyrus area. Previous results showed that the cholesterol released during terminal breakdown is esterified and apparently transported to neurons undergoing reinnervation via the apo E transport system. The present findings indicate that the apo E-complex is taken-up preferentially by neurons though the LDL receptor pathway, where it is presumably used as precursor molecule for the synthesis of new synapses and terminals. Supported by the Medical Research Council of Canada and American Alzheimer's disease Association.

263.10

INOSITOL PHOSPHATE BINDING SITES AND NEURONAL PLASTICITY FOLLOWING ENTORHINAL CORTEX LESIONS. S. Gauthier*, A.R. Parent, D. Dea, R. Quirion and J. Poirier. Douglas Hospital Res. Ctre, Centre for Studies in Aging and Dept. Psychiatry, McGill University, 6875 LaSalle Blvd., Verdun, Québec. H4H 1R3.

Entorhinal cortex lesioning (ECL) produces a loss of more than 80% of the synapses in the outer molecular layer of the hippocampus. However, the loss of synapses is transient. Beginning a few days after denervation, new synapses are formed, virtually replacing the lost inputs within two months. Synaptic remodelling induced by ECL is associated to specific modifications of neurotransmitters, hormones and growth factors. Many of these substances act at membrane receptors to induce hydrolysis of phosphatidylinositols generating various inositol phosphates (IP). The most important members of this family are the 1,4,5-IP_3 and 1,3,4,5-IP_4 which are associated with Ca^{2+} homeostasis. To investigate the potential roles of the IPs in ECL, we characterized and quantified their specific binding sites of 1,4,5-IP_3 and 1,3,4,5-IP_4, using their respective tritiated ligands, after different days post-lesion. [^{3}H]-1,4,5-IP_3 binding sites are maximally increased (30%) between 4 and 8 days post-lesion in hippocampal formation on both sides of the lesion. Interestingly in the cortex, [^{3}H]-IP_3 binding sites increased by up to 25% in the ipsilateral side and by 70% in the contralateral side. [^{3}H]-1,3,4,5-IP_4 binding site changes are delayed and reduced (20%) in magnitude compared to [^{3}H]-IP_3 alterations. The maximal peak is observed between 8 and 14 days after the lesion in the hippocampal formation and the cortex. These results suggest that IPs could be involved in the process of reinnervation following deafferentation observed in the ECL model. The bilateral changes observed in unilaterally lesioned animals suggest a general reorganization of neuronal circuitry that extend beyong the hippocampal formation. Supported by MRCC, Alzheimer Society of Canada and Alcan.

263.11

ABNORMAL NEURONAL OUTGROWTH AND GENE EXPRESSION IN DEVELOPING CEREBELLUM FOLLOWING PERINATAL HYPO- AND HYPERTHYROIDISM. B.C. Figueiredo*[1], G. Almazan, Y. Ma, W. Tetzlaff, F.D. Miller and A.C. Cuello. Dept. of Pharm. and Ther., McGill Univ. Montreal; Dept. of Anat. and Cell Biol. Univ. of Alberta, Edmonton; Dept. of Anatomy, Univ. of Calgary, Calgary, Canada.

We examined the effects of perinatal hypo- and hyperthyroidism on the development of the rat cerebellum by studying the expression of genes that play a role in neuronal outgrowth [GAP-43, tubulin-α1 (Tα1)], as well as those encoding the low-affinity neurotrophin receptor (LNGFR), a constitutive cytoskeletal protein (tubulin 26) and the principal myelin proteins MBP and PLP. One group of pups were made hypothyroid (ho) by feeding dams a 0.4 % propylthiouracil enriched diet (initiated on gestational day 19). The second, a hyperthyroid (Hr) group, were injected subcutaneously with thyroxine (0.3 μg/gm, bwt) daily after birth. Immunostaining for LNGFR using 192-IgG revealed abnormalities in Purkinje cell (PC) axons of ho rats, such as twisting, proximal varicosities and dramatic differences in axonal caliber relative to control rats. The observed increase in LNGFR immunoreactivity (IR), which was greater than the relative increase noted in its mRNA suggests that thyroid hormone may regulate this receptor at a number of cellular levels. The levels of Tα1 and GAP-43 mRNAs were increased in ho cerebellum, thus correlating with the observed abnormal neuronal growth. By contrast, T26 α-tub. mRNA, which is expressed by both neurons and glial cells, was only slightly affected by thyroid hormone imbalance. Interestingly, levels of MBP and PLP mRNAs were altered in ho and Hr rats only during early cerebellar development. This contrast with the permanent changes in these two mRNAs which occur in vitro. Thus, hypothyroidism led to specific increases in Tα1-tub. and GAP-43 mRNAs, and LNGFR (IR, mRNA), all changes that may play a role in the observed abnormal neuronal outgrowth. Supp. by Cent. of Excellence, MRC and MSS (Canada). [1]CNPq and UFC (Brazil).

263.12

ABERRANT DOPAMINE INNERVATIONS OF SUBSTANTIA NIGRA AND INTERPEDUNCULAR NUCLEUS FOLLOWING CEREBRO-VENTRICULAR ADMINISTRATION OF 6-OHDA IN NEWBORN RAT. F. G. Fernandes Xavier, G. Doucet, L. Descarries, T.A. Reader* and M. Geffard. CRSN (Dép. de pathol. et de physiol.), Univ. de Montréal, Qué. CANADA, and Lab. Immunol. Pathol., Univ. Bordeaux II, FRANCE.

In adult rats subjected to bilateral cerebroventricular administration of 6-OHDA neonatally (P3), a relatively dense network of dopamine (DA) axons pervades the substantia nigra (SN), where this transmitter would be normally contained and released by nerve cell bodies and dendrites. Considerable DA axonal sprouting is also observed in the interpeduncular nucleus (IP), which normally receives a very scant DA innervation. To examine the development of these aberrant innervations and determine the critical period for their induction, a first series of rats were lesioned at P3 and perfused with 3.5% glutaraldehyde at P7, P10, P15 or P30; another series were lesioned at P6 or P15, and perfused 30 d later. Brain sections were processed for immuno-cytochemistry with monoclonal antibodies against DA-glutaraldehyde conjugates. All lesions practically eliminated DA cell bodies and dendrites from the SN, but spared many DA neurons in the adjacent ventral tegmental area and rostral midbrain raphe. Following P3 lesioning, some DA immuno-reactive fibers were already observed in SN at P7. The density of this neoinnervation increased sharply until P15, but only slightly further until P30, at which time its intraregional distribution typically resembled that of the DA cell bodies and dendrites in a normal adult. In IP, DA innervation was already much denser than normal at P10 and did not show further increase with longer survival. A similar DA innervation of SN was observed after lesioning at P6 and P15. Thus, the development of both aberrant DA innervations is rapid (2 weeks) and the critical period for their induction extends beyond P15. [Supported by the CNPq (Brazil), the FRSQ and MRC grants MT-10982 and MT-3544).

263.13

THE POSTNATAL DEVELOPMENT OF SEROTONERGIC HYPERINNERVATION IN THE CORTEX OF THE HEMIZYGOUS BRINDLED MOUSE. P.M. Martin*, M. Ohno, R. Mailman, and Kinuko Suzuki, Brain and Development Research Center, and Departments. of Pathology, Psychiatry and Pharmacology, University of North Carolina, Chapel Hill, NC 27599.

Mottled brindled (MO^{br}) is an X-linked mutation in the mouse, maintained in hybrids of the C3H and C57BL strains. Hemizygous males ($MO^{br/y}$) have low copper concentrations in the brain, very low concentrations of norepinephrine, and neuronal degeneration of the cerebrum. They begin to lose weight on approximately postnatal day (P) 10, and die around P15. We have reported that the hemizygous males also have high concentrations of serotonin (5-HT) in the brainstem, and high concentrations of 5-hydroxyindoleacetic acid in the hindbrain and all areas of the forebrain (Satoh et al., 1991). In this experiment, 5-HT immunoreactive fibers in the cerebral cortex and striatum were quantified and compared in hemizygous males and control littermates at P7, P10, P12 and P14 (n=3 pairs at each time point). Forty micrometer Vibratome sections were immunostained with 5-HT antibody. Density of 5-HT immunoreactive fibers was measured using a digitized imaging system in conjunction with darkfield microscopy. Measurements of 5-HT innervation show an age-dependent increase in density of 5-HT immunoreactive fibers in all layers of the cerebral cortex. No differences in innervation were found on P7, but there was a steady increase from P10 to P14, when fiber density in brindled mice was approximately 70% above their matched controls. No significant increase in fiber density was found in the striatum. High performance liquid chromatography showed increasing concentrations of serotonin and 5-hydroxyindoleacetic acid, coupled with a very low concentration of norepinepherine in several brain regions. We have found that brindled mice also show an age-dependent change in 5-HT immunoreactive fibers in the subependymal layer of the lateral ventricles, with fibers becoming increasingly thick and darkly stained, and nodal points appearing very swollen. Preliminary data from mouse pups who were food deprived between P5 and P15 show similar changes in the 5-HT immunoreactive fibers in the subependymal layers. These data suggest that the alterations in 5-HT metabolism and innervation may be initiated, at least in part, to the weight deficits seen in the MO^{br} mice. (Supported by Grants HD03110, NS24453 and ES01104).

263.14

STIMULATION OF THE 5-HT1A RECEPTOR INHIBITS CORTICAL NEURAL GROWTH DURING THE LAST PRENATAL WEEK OF RAT DEVELOPMENT. B. C. Swarzenski* and R. D. Todd. Departments of Psychiatry and Genetics, Washington University, St. Louis, MO 63110.

Serotonin has a variety of effects on pre- and postnatal neural development including neuronal differentiation, neurite outgrowth, and synapse formation. Previous studies from this laboratory demonstrated the presence of 5-HT1A receptors on cultured rat fetal frontal cortical neurons and showed that stimulation of the serotonin 5-HT1A receptor by the specific agonist 8-OH-DPAT inhibits neurite branching during late rat frontal cortical development (E17-E19: gestational age in days). The purpose of the present study was to determine whether the inhibitory effect of 5-HT1A receptor stimulation on frontal cortical neuron outgrowth was present over an extended time during rat embryogenesis. Frontal cortices from Sprague-Dawley rat fetuses ages E14 to E21 were mechanically dissociated to single cell suspensions. The cells were plated in 5% fetal bovine serum at a density of 50 cells/mm2 onto poly (D-lysine) coated culture dishes and after 12 hours in culture at 37° C under 5% CO_2 either 1 μM 8-OH-DPAT (experimental group) or serum medium (control group) was added every 12 hours. After an additional 48 hours in the presence of 8-OH-DPAT or medium 100 consecutive cells in each group were photographed (Nikon Diaphot) and quantitatively analyzed using a Bioquant image analysis system. The data show (i) that the growth inhibitory effects of 5-HT1A receptor stimulation by 8-OH-DPAT are present during a substantial part of rat ontogenesis (at least from E14 until birth), and (ii) that the inhibitory effects include a dramatic decrease in the number of neurite branch points and a less pronounced inhibition in the outgrowth of neurites. Our results suggest that the 5-HT1A receptor has a regulatory role in cortical neural morphogenesis during a substantial period of fetal mammalian development.

263.15

SPROUTING OF SP OR 5HT CONTAINING TERMINALS IN LAMINA II OF RAT SPINAL CORD AFTER DORSAL RHIZOTOMY. **B. Zhang*, and M. Murray. Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, Pa 19129.**

Synaptic plasticity of Substance P (SP) and Serotonin (5HT) containing systems in lamina II of rat spinal cord has been studied using quantitative electron microscopic immunocytochemical techniques (EM-ICC). Dorsal roots were sectioned and dorsal root ganglia were removed from L1 to S2 in 18 rats. After 3, 10 or 60 days rats were perfused, and the L5 segments of the spinal cord removed and prepared for post-embedding immunocytochemistry. Quantitative EM-ICC study demonstrates that the number of SP containing terminals in lamina II decreased by 58% at 3 days postoperatively and recovered to normal numbers in the chronic group. The loss of SP terminals in the acute group is due to loss of SP containing dorsal root afferents, while the recovery in the chronic group suggests replacement of lost terminals by intrinsic SP systems. These results therefore indicate homotypic sprouting in response to dorsal root deafferentation. The number of 5HT containing terminals in lamina II of spinal cord increased by 55% on the deafferented side of the chronic group. The increase in 5HT containing terminals indicates that descending 5HT system shows heterotypic sprouting in response to complete dorsal root deafferentation.

Supported by NIH grant No. NS24707.

263.16

GUANETHIDINE SYMPATHECTOMY INCREASES SUBSTANCE P CONCENTRATION IN THE SYMPATHETIC GANGLIA OF ADULT RATS. P. J. Zollman, E. E. Benarroch, J. D. Schmelzer*, D. K. Nelson and P. A. Low. Neurophysiology Laboratory, Department of Neurology, Mayo Clinic, Rochester, MN 55905.

Chronic sympathectomy produces increased expression of sensory neuropeptides in sympathetically innervated tissue (Aberdeen et al., 1992). We sought to determine whether chronic guanethidine sympathectomy in adult rats results in expression of substance P in the superior cervical ganglion (SCG). Adult rats received intraperitoneal injections of guanethidine or saline for 5 weeks. Six to eight weeks following completion of treatment, concentrations of substance P and neuropeptide Y (NPY) were measured by radioimmunoassay in the superior cervical ganglion (SCG) and thoracic spinal cord. The SCG was also immunostained for NPY and substance P. No differences were observed in thoracic spinal content of either NPY or substance P. We observed depletion of NPY immunoreactive neurons and NPY levels in the SCG (control 9615 ± 2894 vs. guanethidine 236 ± 39, p=0.005). In guanethidine-treated rats, there was a marked increase of substance P levels in the SCG (control 8.7 ± 0.9 vs. guanethidine 39.5 ± 9.19, P=0.005). Substance P was localized in fibers, but not cell bodies of the SCG of guanethidine-treated rats. Thus, sprouting of substance P-containing sensory fibers in the sympathetic ganglia occurs late following postganglionic sympathectomy in adult rats. (Reference: Aberdeen J, Milner P, Lincoln J, Burnstock G. Neuroscience 47:453-461, 1992)

264.1

EARLY DEVELOPMENT OF THE SEGMENTAL INNERVATION OF THE RAT HINDLIMB. K. Mirnics*, R.H. Adolph, H.R. Koerber. Dept. of Neurobiol., Anat. and Cell Sci., University of Pittsburgh, Pittsburgh, PA 15261

Individual dorsal root ganglia (DRGs) and/or ventral horn (VH) segments were isolated in rat embryos fixed at different stages (E13-E16) and filled with one of three carbocyanide dyes DiI, DiA and DiO. Individual experimental preparations included labelling of: 1) single dorsal root ganglia; 2) multiple DRGs (L2-L6) with alternating DiO, DiI and DiA; 3) single ventral horn segments; 4) multiple isolated VH segments (L2-L6) with alternating dyes; 5) single isolated VH segments and the corresponding segmental DRGs with different dyes. After diffusion was determined to be complete, the tissue was sectioned by vibratome and observed under fluorescent microscopy. Results from these preparations reveal that by E13 the plexus is formed and the primordia of the femoral and the sciatic nerve trunks are established. At E14 individual muscle and cutaneous nerves can be identified. At this stage fibers are present in the epidermis of the proximal hindlimb and the nerve trunks extend into the shank. Fibers originating from L3-L5 (DRG and VH) reach the paw by E14.5. At E15 the individual digital nerves are visible and contact the skin of the proximal toes, while the epidermis of the more distal toes is innervated by E16. At all stages sensory and motor fibers are observed to grow in unison. Multiple DRG and VH labelling with different dyes revealed that fibers of different segmental origin are combined in the plexus and diverge in the developing peripheral nerve trunks with little or no mixing. This segmental topographic organization is true of both motor and sensory fibers and can be observed as early as E13. However, afferent and efferent fibers of the same segmental origin intermix extensively in the spinal nerves and form joint fascicles as they enter the plexus and give rise to peripheral nerve trunks. Supported by NS23725 (HRK).

264.2

PATHWAY SELECTION BY REGENERATING PERIPHERAL NERVE AXONS GROWING THROUGH A Y-SHAPED TUBE IS RELATED TO DISTAL STUMP SIZE D.F. Davey*, C.S. Brown and A.D. Ansselin, Microsurgery Research Centre, Sydney and Department of Physiology, University of Sydney, NSW 2006, Australia.

We have confronted regenerating rat sciatic nerve axons with a binary choice at the branch in a Y-shaped tube. The sciatic nerve of young Wistar rats was severed under Halothane anaesthesia proximal and distal to the peroneal-tibial branch. The distal peroneal and tibial stumps were inserted into the ends of the Y branches, and only the peroneal or tibial proximal stump into the base of the Y, leaving a 10 mm gap between the proximal and distal stumps. The tubes were cast from silicone (Sylgard 184; internal diameter 1.2 mm) and allowed for a tight seal when the nerves were sutured in place (ensured by application of a small amount of Vaseline). 8 to 12 weeks later, animals were anaesthetised before perfusion for electron microscopy. The number of myelinated axons in the two branches, and the base of the Y were estimated with a random sampling method. In all cases where regeneration occurred (10 of 17 experiments), axons were found in both branches of the Y. The mean ratio of axons entering the peroneal branch to those entering the tibial branch was 2.3:1 (SD=0.6, n=7) when the peroneal nerve regenerated. For tibial nerve regeneration, the ratio was 2.1:1 (S.D.=1.33, n=3). This result confirms that of Seckel, Ryan, Gagne, Chiu & E. Watkins, (*Plastic Reconstr. Surg.* **78**:793-798, 1986) who reported a mean ratio of 2:1 for peroneal regeneration but is in marked contrast to that of Politis (*Brain Res.* **328**:271-276, 1985) who reported that peroneal axons were almost always 100% correct in selecting the peroneal stump.

264.3

SOMITE AND SPECIFICITY OF SYMPATHETIC PREGANGLIONIC PROJECTIONS. J. W. YIP.* Dept. of Physiology, University of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

The pattern of preganglionic projections in the avian sympathetic system is segmentally specific. Preganglionic axons arising from the T1 spinal cord segment, for example, project predominantly in the rostral direction, whereas T4 preganglionic axons project predominantly in the caudal direction. Individual ganglia, moreover, are innervated in a stereotyped pattern by preganglionic axons arising from several contiguous spinal cord segments. The formation of these patterns is target independent, is not determined by the neurons' segmental origin in the spinal cord, but is influenced by the local environment along which preganglionic axons project.

The somitic mesoderm has been shown to influence the segmentation of peripheral nerves and ganglia. Whether the somitic mesoderm also determines the pattern of preganglionic projections is not clear. In order to address this issue, I have examined: 1) whether the tissue immediately surrounding the sympathetic trunk is derived from the somitic mesoderm, 2) the patterns of preganglionic projections following somite removal, and 3) the direction of preganglionic projections subsequent to rostro-caudal somite transplantations. Quail-chick somite transplantations show that the tissue immediately surrounding the sympathetic trunk is derived entirely from the somite. Anterograde and retrograde HRP studies show that following somite removal, the specificity of preganglionic projections is disrupted. Thus for example, T1 preganglionic neurons, which normally project in the rostral direction, may now project caudally. Consistent with this finding, the segmental pattern of preganglionic neurons projecting to individual ganglia is also altered. Finally, when cervical somites are transplanted to the thoracic region, T1 preganglionic axons project caudally, in addition to the normal rostral direction.

These results indicate that somites are important for the specificity of sympathetic preganglionic projections.

264.4

SOMITE MANIPULATION IN THE CHICK EMBRYO ALTERS SYMPATHETIC PREGANGLIONIC AXONAL TRAJECTORY E.B. Ezerman* and C.J. Forehand. Department of Anatomy and Neurobiology, Univ. of Vermont, Sch. of Med., Burlington, VT 05405.

Sympathetic preganglionic axons exit the spinal cord in the ventral root to project into the spinal nerve at each thoracic segment. After entering the spinal nerve, preganglionic axons make two turns en route to their targets in the paravertebral chain of sympathetic ganglia. First, the axons turn ventromedially to form the ramus communicans, through which they reach the sympathetic ganglion at their segment of origin. Then, the axons turn to project either rostrally or caudally within the chain to innervate ganglion cells at several segmental levels. The cues preganglionic axons use to make these turns are unknown. We have examined whether information derived from an appropriate relationship to somitic tissue during axon outgrowth plays a role in guiding these axons in the chick embryo. At the 22-26 somite stage, midthoracic somites were removed unilaterally and replaced with somites consisting only of either anterior somitic tissue or posterior somitic tissue. Embryos were sacrificed at stages from embryonic day 5-12 (E5-12). Preganglionic projections were mapped in E10-12 embryos by retrogradely labeling preganglionic neurons from the sympathetic chain in vitro. Axon pathways in E5-9 embryos were examined by immunohistochemical detection of GAP-43. In both types of transplants, normal segmentation of the ventral roots, spinal nerves, sensory ganglia and sympathetic ganglia was disrupted. Despite this disruption, normal appearing rami communicantes arose from each of the spinal nerves that did form. However, the pattern of rostrocaudal projections within the sympathetic chain was abnormal.

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264.5

INHIBITION OF NEURITE GROWTH FROM EMBRYONIC RAT DORSAL ROOT GANGLIA (DRG) EXPLANTS BY VENTRAL, BUT NOT DORSAL, SPINAL CORD. GC Kwiat*, J Meredith-Middleton, A Pini, and M Fitzgerald. Dept. of Anat. and Dev. Biol., Univ. Coll., London WC1E 6BT.

We have studied, using *in vitro* organotypic culture methods, central factors which might influence development of the dorsoventral termination pattern of different types of primary afferent neurons in the spinal cord.

Lumbar DRG explants were co-cultured with either dorsal or ventral spinal cord explants in a collagen matrix gel covered with culture medium containing 5% calf serum and 50ng/ml nerve growth factor. DRG neurites were fluorescently labeled with diI by immersion of the DRG explant in a suspension of diI crystals. After 24 hrs in culture the distribution and density of labeled neurites were analyzed using a confocal microscope and image analysis software. Neurite growth from DRG explants from E14-15 embryos typically exhibited a uniformly radial pattern when DRGs were cultured alone, or when co-cultured with dorsal cord explants from the same embryo. In contrast, when DRG explants at this age were co-cultured with ventral cord, DRG neurites were consistently found growing only in directions away from the cord explant. The effect was specific in that ventral cord did not inhibit outgrowth from dorsal cord explants. The inhibition of DRG neurite growth by ventral cord was absent, or greatly diminished, by E18. These results suggest that development of the dorsoventral termination pattern of primary afferent neurons in the spinal cord might involve a transient inhibition of afferent axon growth, and are consistent with *in vivo* results which show that the earliest arriving afferents arborize in intermediate cord regions at a time which overlaps the period of inhibition.

264.6

CELLS OF THE PERIPHERAL NERVE TUBE RELEASE A NEUROTROPHIC FACTOR PROMOTING PROCESS ELONGATION BY CULTURED ADULT FROG DORSAL ROOT GANGLION NEURONS. D.P. Kuffler*, O. Megwinoff, C. Santiago+ and D. Balsolobre+. Inst. of Neurobiology & Dept. of Physiology and Biology Dept.+, Univ. of P.R., Old San Juan, P.R. 00901

Peripheral motor axons regenerate in vivo to precisely re-innervate their denervated targets (Letinsky et al., '76). This regeneration is directed by diffusible neurotropic factors released from pieces of peripheral nerve (the normal pathway of the axons) and denervated muscle fibers (the original targets) (Kuffler, '89). Our present experiments are aimed at isolating and characterizing the responsible long-range targeting neurotropic factors.

Techniques were developed to isolate pure populations of dorsal root ganglion (DRG) neurons from the adult frog, Rana pipiens. These DRG neurons survive in a defined medium without serum or growth factors in tissue culture for more than 4 weeks and extend processes.

To test for the presence of neurotrophic and neurotropic factors released from denervated neuronal targets DRG neurons have been co-cultured with pieces peripheral nerve. The average total process outgrowth was measured for control neurons as well as neurons co-cultured with peripheral nerve. After 10 days in culture the processes of neurons in the co-cultures were ca. 9 times longer than those of control neurons. These results indicate the release a neurotrophic factor from cells of the peripheral nerve tube. Experiments are under way to isolate and characterize this neurotrophic factor and determine whether, in addition to its neurotrophic role, it also functions as a neurotropic factor.

Letinsky, Fishbeck & McMahan ('76) J. Neurocytol. 5:691-718. Kuffler ('89) J. Comp. Neurol. 281:416-425. Supported by an Army Research Office Grant DAAL03-90-G-0189 to DPK and an EPSCoR Grant to CS and DPK.

264.7

MULTIPLE NAVIGATIONAL STRATEGIES IN INDIVIDUAL AXONS OF DEVELOPING MUSCLE NERVE. M.Morgan-Carr and M.Hollyday*. Department of Biology, Bryn Mawr College, Bryn Mawr PA 19010.

Previous studies of developing motor nerves have led to the conclusion that axons grow directly to target muscles, apparently guided by both active and passive cues. We observed the morphology of single axons to learn more about navigational strategies and the location of guidance cues for a compartmentalized and topographically innervated muscle. Individual motor axons of the embryonic chick (stage 26 to 34) were anterogradely labeled by spinal cord injections of HRP. Over 300 axons within the pectoralis nerve were examined with DIC optics in serial 100 µm sagittal sections. Labeled axons could be traced for more than 1mm, often to their terminal endings.

The region examined encompassed the origins of several primary branches to m. pectoralis, and nerves to at least two other muscles. In this region, labeled axons displayed various features suggestive of active exploration and response to local guidance cues. Over 20% of the axons showed abrupt changes in trajectory, including 180° reversals and 360° loops. These loops often gave rise to short, fine axonal buds tipped with growth cones; these were also seen along the main body of the axon and may be interstitial sprouts. Filopodial sprays and lamellopodial "ghosts" were also observed and are thought to be remnants of decision region growth cones, elaborated in search of, or in response to local cues. In addition, 15% of the axons were bifurcated: 60% of the axonal pairs took parallel routes, with the remaining pairs taking disparate routes, usually entering different primary nerve branches, but occasionally even entering different muscle nerves. These navigational features are prevalent in an area of multiple decision regions, where adjacent axons diverge from single points to take completely different routes. These observations suggest that guidance cues are extremely specific and localized in this region, and that axons use multiple navigational strategies to find and respond to them.

Supported by NS-25340 to MH.

264.8

NEUROMUSCULAR SPECIFICITY DEPENDS UPON THE PHYSICAL STRUCTURES ENCOUNTERED BY REGENERATING MOTOR AXONS. P.B. Farel* and M.L. Meeker. Dept. of Physiol. Univ. N. Carolina Sch. Med., Chapel Hill, NC 27599.

Regenerating lumbar motoneuron axons can specifically reinnervate their hindlimb targets during the first third of larval (tadpole) life in the bullfrog, after which time regenerative specificity is lost. Ultrastructural appearance of the Schwann cell-derived basal lamina coincides with the loss of regenerative specificity; however, appearance of immunoreactivity to two major constituents of the basal lamina, laminin and heparan sulfate proteoglycan (HSPG, antibody provided by M.J. Anderson), does not. These data indicate that the molecular components of the basal lamina are present prior to their organization into a discrete structure and that their presence *per se* does not impede regenerative specificity.

We have extended these observations to regenerating axons to show that, following transection, regrowing axons at all developmental stages encounter non-neural elements expressing laminin and HSPG. However, regrowing axons are not found in association with basal lamina tubes at stages when regeneration is specific, whereas axons are always found within basal lamina tubes at stages after regenerative specificity is lost. These data are consistent with the hypothesis that it is development of the the physical constraints imposed by the basal lamina (rather than expression of its molecular constituents) that prevents axons from regenerating to their proper targets.

DEVELOPMENT OF NEUROTRANSMITTER SYSTEMS: AMINO ACIDS

265.1

STRYCHNINE SENSITIVITY OF GLYCINE RESPONSES OF EMBRYONIC RAT SPINAL CORD NEURONS IN CELL CULTURE. M.D. Withers*, R.B. Levine and P.A. St. John. Program in Neuroscience, Div. of Neurobiology and Dept. of Anatomy, University of Arizona, Tucson, AZ 85721.

Cell-cell interactions have been shown to be important in the regulation of neurotransmitter receptors at the mammalian neuromuscular junction, but relatively little is known about regulation of receptors on neurons. We are examining the expression of glycine receptors by rat spinal cord neurons developing in culture. Previous work using binding assays with a radio-labelled form of the glycine antagonist, strychnine, indicated that embryonic rat spinal cord neurons did not express strychnine binding sites before 5-6 days in culture. Presently, we are using electrophysiological techniques to examine responses to glycine at earlier stages. Rat spinal cord cells were dissociated at embryonic day 14 and placed in culture. Using the whole-cell patch clamp configuration we examined the response of the neurons to the application of glycine and the sensitivity of this response to strychnine blockade. Initial results demonstrated the presence of glycine activated currents after 2-3 days *in vitro*; that is 2-3 days before strychnine binding sites were detected. The currents exhibited a reversal potential near the chloride equilibrium potential, suggesting that chloride ions are the charge carriers as with conventional glycine receptors. These glycine-activated currents were not blocked by strychnine at a concentration of 1 µM. The observation of strychnine-insensitive responses to glycine is consistent with recent findings from Becker *et al.* (EMBO J. 7:3717, 1988), which suggest the existence of a strychnine-insensitive neonatal isoform of the glycine receptor. The present results suggest that expression of these neonatal receptors by developing spinal cord neurons in culture precedes expression of the adult isoform by at least 2-3 days. (Supported by NIH NS29657 and NSF 8808506 to PSJ and NS 24895 to RBL).

265.2

GLYCINE- AND GABA-ACTIVATED CURRENTS IN MOTONEURONS OF DEVELOPING RAT SPINAL CORD. B-X. Gao*, and L. Ziskind-Conhaim. Dept. Physiol. and Ctr. Neurosci., Univ. of Wisconsin, Madison, WI 53706.

Motoneuron responses to both glycine and GABA decrease during embryonic development. However, at all ages GABA-induced membrane depolarization is significantly larger than that induced by glycine, although both amino acids generate a similar decrease in membrane resistance (Wu and Ziskind-Conhaim, Soc. Neurosci., 1991, 17:88). To further characterize these changes, and determine the ionic mechanisms underlying the potentials induced by glycine and GABA, whole-cell voltage-clamp recordings were done in thin slices of spinal cords of embryonic rats. In motoneurons of Day 19-21 embryos, both glycine (2 mM) and GABA (1 mM) produced large inward currents (0.6-2 nA), that desensitized in the presence of the amino acids. At the peak current, there was a significant increase in membrane conductance. Our preliminary results show that glycine-activated currents reversed at about -7 mV, close to the estimated equilibrium potential for Cl^- (-5 mV). However, the average reversal potential for GABA-mediated currents was +5 mV. The changes in glycine- and GABA-induced currents during embryonic development will be discussed. Supported by RCDA (NS01314) and NS23808 to LZ-C.

265.3

$GABA_A$ RESPONSES AND ASSOCIATED CALCIUM ELEVATIONS ARE PREFERENTIALLY ELIMINATED IN POSTNATAL RAT SPINAL CORD NEURONS BY SHORT TERM CULTURE. M.K. Walton*, A.E. Schaffner, and J.L. Barker. Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

$GABA_A$ receptor activation has previously been shown to produce depolarizations in spinal cord neurons dissociated from rat embryos, and these depolarizations are associated with an increase in intracellular calcium. We have examined $GABA_A$ responses in spinal cord neurons dissociated from postnatal (PN) rat pups using fluorescent dyes for either membrane potential (MP) (oxonol) or calcium (fura-2). Responses by $GABA_A$ receptors were probed for by bath perfusate addition of muscimol (2µM) and non-NMDA glutamate receptors by kainate (40µM). Tetanus toxin staining was used to identify cells as neurons.

Acutely examined cells revealed the presence of both muscimol and kainate membrane potential responses on the cell bodies of the majority of cells, in similar percentages at embryonic day 17 (E17) and PN days 0, 7, 14. The responses to both muscimol and kainate were depolarizing in acutely examined cells. After 1 day in culture E17 cells continued to show depolarizing responses to both ligands. The PN cells cultured 1 day showed progressive elimination of $GABA_A$ responses in cells from successively older PN ages, declining to none in PN14 cells, while kainate responding percentage showed only a small decrease. Calcium responses to the muscimol depolarization showed a change with developmental age of the cells. PN cells showed a Ca^{2+} rise to a peak and decline towards baseline during the 2-3 min. exposure to muscimol, while embryonic derived neurons showed sustained Ca^{2+} elevation. This was paralleled in the MP recordings by many PN neurons showing a transient depolarization to muscimol, more pronounced when Cl^- was changed to produce stronger depolarization. These results indicate that neurons from PN spinal cord eliminate the $GABA_A$ response as measured by oxonol or fura-2 preferentially over the kainate response when cells are held in culture for 1 day.

265.4

EFFECT OF GABA TREATMENT ON RAT CEREBELLAR GRANULE CELL CULTURE. H.Y. Kim, D.W. Sapp, R.W. Olsen, and N.J.K. Tillakaratne*, A.J. Tobin, Departments of Biology and Pharmacology, UCLA, Los Angeles, California 90024.

To study the molecular basis of the often reported trophic activity of GABA, we have analyzed the effects of GABA and the GABA agonist THIP on primary cultures of rat cerebellar granule cells using quantitative PCR. These immature cerebellar granule cells display only the high affinity GABA binding sites in vitro. Exposure of these cells to GABA or THIP induces an increase in the levels of mRNAs encoding specific $GABA_A$- receptor polypeptides including alpha 1 and beta 2. Our preliminary data suggest that this increase is accompanied by the formation of low affinity GABA binding sites. Furthermore, this induction is abolished by bicuculline treatment in a dose dependent manner. These results suggest that GABA regulates the expression of genes encoding its own receptors and that the effect is mediated by $GABA_A$-receptors. GABA may thus have a regulatory role in the development and cytodifferentiation of the central nervous system. (supported by NS22256).

265.5

GABA$_A$/BENZODIAZEPINE RECEPTOR $\gamma 2$ SUBUNIT GENE EXPRESSION IN DEVELOPING NORMAL AND IN MUTANT MOUSE CEREBELLUM. V. Luntz-Leybman*, A. Frostholm and A. Rotter, Department of Pharmacology, The Ohio State University, Columbus, OH 43210.

The expression GABA$_A$/BZ receptor $\gamma 2$ subunit mRNA was studied during normal development and in adult reeler (rl/rl), weaver (wv/wv), staggerer (sg/sg), lurcher (lc/lc) and Purkinje cell degeneration (pcd/pcd) mutant mouse cerebellum. In situ hybridization with a [^{35}S] cRNA specific probe revealed a diffuse signal, which was present at birth, in the deep cerebellar nuclei and over Purkinje cells in the molecular layer. At postnatal day (P)5-7, labeling became more punctate in appearance as Purkinje cells began to form a monolayer at the interface of the granule cell and molecular layers. Autoradiographic grains were absent in the external germinal layer. Weak labeling was detected over the forming internal granular layer at P5-7, and reached moderate adult levels by P20. Punctate labeling over basket and stellate cells in the molecular layer became visible at the end of postnatal week two. In pcd/pcd and lc/lc mutants, in which Purkinje cells have degenerated, the hybridization signal was absent from the interface of molecular and granule cell layers. In sg/sg mice, where synaptic contacts with granule cells are not formed, no punctate labeling characteristic of Purkinje cells was detected. However, in rl/rl mutants, where all classes of cells are malpositioned, and in wv/wv mutants, in which many granule cells have degenerated, a strong hybridization signal remained over Purkinje cells. Our results suggest that Purkinje cells express the $\gamma 2$ subunit prior to receiving GABAergic inhibitory input, and that $\gamma 2$ expression in mutant animals is not affected by the absence of afferents.

265.6

ONTOGENY OF GABA$_A$/BENZODIAZEPINE RECEPTOR α_6 mRNA EXPRESSION IN MOUSE CEREBELLUM AND COCHLEAR NUCLEI. A. Rotter*, L. Varecka and A. Frostholm, Department of Pharmacology, Ohio State University, Columbus, OH, 43210

Although it has been suggested that granule cells of the cerebellum and cochlear nucleus are derived from a common precursor pool, the GABA$_A$/BZ receptor α_6 subunit mRNA has been reported to be associated only with the granule cells of the adult rodent cerebellum. We have examined the expression of the α_6 subunit mRNA in the cerebellum and cochlear nucleus during postnatal development. A cDNA fragment, encoding a portion of the M3-M4 intracellular loop of the α_6 subunit, was generated by polymerase chain reaction; a [^{35}S]riboprobe transcribed from the cDNA fragment was used for in situ hybridization studies. In the adult mouse brain, a strong hybridization signal was observed over the granule cell layers of the cerebellum and the cochlear nuclei. The hybridization signal in the cochlear granule cells appeared towards the end of the first postnatal week, between postnatal days 6 and 7, coinciding with the appearance of labeling in the cerebellar granule cells. The intensity of the hybridization signal increased gradually, reaching a plateau at approximately postnatal day 25. Our results demonstrate that, in the mouse, α_6 subunit mRNA is not constrained to the cerebellum, and support the suggestion that both cerebellar and cochlear granule cells share a common cellular precursor. These data further suggest that granule cell precursors may be intrinsically programmed to acquire a specific form of the GABA$_A$/BZ receptor, irrespective of their final location in the hindbrain.

265.7

DIFFERENTIAL DEVELOPMENTAL EXPRESSION OF GABA$_A$/BZ RECEPTOR SUBUNIT mRNAs IN THE MURINE INFERIOR OLIVARY NUCLEUS. A. Frostholm*, D. Zdilar, V. Luntz-Leybman, V. Janapati and A. Rotter, Department of Pharmacology, The Ohio State University, Columbus, OH 43210

The developmental expression of the α_1, α_6, β_{1-3}, γ_2 and δ subunits of the GABA$_A$/benzodiazepine (GABA$_A$/BZ) receptor was examined in the murine inferior olivary nucleus by in situ hybridization with antisense cRNA probes. The postnatal appearance and distribution of [^{3}H]flunitrazepam and [^{3}H]muscimol binding sites, α and β subunit-specific ligands respectively, were also studied autoradiographically. The β_3 subunit was transiently expressed in each of the subnuclei of the inferior olive: The signal was strong at birth, increased throughout postnatal week one, and rapidly declined thereafter to low adult levels. A similar pattern of labeling was observed with [^{3}H]muscimol. Low to moderate levels of α_1 subunit mRNA hybridization signal and [^{3}H]flunitrazepam binding sites were also present in inferior olivary neurons at birth, decreasing slowly to adult levels thereafter. Olivary neurons expressed low to moderate levels of β_1, β_2, and γ_2 mRNA throughout postnatal development, while α_6 and δ mRNAs were absent. Our results suggest that the subunit composition of the GABA$_A$/BZ receptor in inferior olivary neurons may change during development, and that this process may be related to the elimination of multiple climbing fiber innervation of cerebellar Purkinje cells, which also occurs during the second postnatal week.

265.8

IMMUNOCYTOCHEMICAL ANALYSIS OF THE EFFECTS OF DIAZEPAM *IN UTERO* ON CORTICOTROPIN RELEASING FACTOR NEURONS AND NORADRENERGIC INPUTS OF PARAVENTRICULAR NUCLEUS IN ADULT RATS. Inglefield, J.R.[1]*, Bitran, D.[2], Olschowka, J.A.[1], and Kellogg, C.K.[2] Depts. of Neurobiology and Anatomy[1] and Psychology[2], University of Rochester, New York, 14642

Earlier work shows that prenatal exposure to diazepam (DZ) modifies the development of hypothalamic noradrenergic (NE) terminals in the rat. Such exposure markedly decreases NE levels, NE turnover rate, and depolarization-induced NE release in the hypothalamus (HYP) of adult offspring. The DZ exposure does not impact NE terminals in hippocampus, cortex, or cerebellum, suggesting selectivity. HYP NE affects stress-induced corticosterone (CS) release by influencing paraventricular nucleus (PVN) corticotropin releasing factor (CRF) neurons. Indeed, there is a reduction in stress-induced CS levels in adults exposed *in utero* to DZ. In the present work, we have studied the effects of DZ exposure on two HYP systems, namely NE and CRF, to determine any associated anatomical changes. Using ICC in conjunction with image analysis, we observed a marked reduction of dopamine-B-hydroxylase (DBH)-immunoreactivity(-ir) (for (nor)adrenergic terminals) within the PVN in adult rats prenatally exposed to DZ. Interestingly, DBH-ir in the subcommisural portion of the bed nucleus stria terminalis was also reduced. However, DBH staining in supraoptic nucleus did not exhibit any change. Preliminary evidence suggests a reduction in PVN CRF-ir as well. Overall, this gives anatomic evidence for the earlier findings in the HYP of adults prenatally exposed to DZ, and confirms a developmental alteration in HYP NE and possibly CRF systems when GABA/benzodiazepine receptors are targeted.

265.9

MANY EMBRYONIC RAT CENTRAL NEURONS EXHIBIT SPONTANEOUS Cl^- CONDUCTANCE WHICH MAY INVOLVE GABA$_A$ RECEPTORS. A.Y.Valeyev*, R.A.Cruciani, G.D.Lange, V.Smallwood, R.Serafini and J.L.Barker. Lab. of Neurophysiology, Instrumentation and Computer Section, NINDS, NIH, Bethesda, MD, 20892, U.S.A.

Whole-cell voltage clamp recordings with Cl-filled pipettes were performed on embryonic central neurons either acutely isolated or cultured for 1-15 days. In the majority of these cells recorded in 1μM TTX and 10 mM $MgCl_2$ the baseline current signal fluctuated at all potentials except 0 mV, which corresponds to the reversal potential for Cl^- ions. Spectral analysis of the fluctuations consistently showed a multi-component Lorentzian fit, suggesting that they were derived from exponentially distributed openings of Cl^- ion channels. Close application of medium consistently and reversibly decreased the holding current and membrane current variance. Spectral analysis of the baseline during application of bathing medium showed a relatively simple 1/f relationship between power and frequency. Presumably, the medium removed diffusible substances accumulating at the cell surface. Both application of antagonists at GABA$_A$ receptors (bicuculline, picrotoxin) and the steroid 5β-Pregnane-3β-OL-20-ONE mimicked the effects of saline on current and varience, strongly implicating GABA$_A$ receptor-coupled Cl^- channels in the baseline.

265.10

GABA$_B$ MEDIATED RESPONSES IN THE *IN VITRO* DENTATE GYRUS OF THE DEVELOPING RAT. D. S. Garant*, S. L. Moshé, and P. K. Stanton. Departments of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

We utilized the paired-pulse paradigm in hippocampal slices *in vitro* to examine the developmental profile of facilitation and suppression of dentate granule cell responses to perforant path stimulation. Reproducing previous results from our laboratory, slices from 15- and 30-day old immature rats demonstrated profound early paired-pulse suppression (interstimulus intervals up to 25 ms) and facilitation (intervals from 50 ms to 250 ms), followed by modest late paired-pulse suppression (intervals from 500 ms to 5 s). Perfusing slices from immature rats in medium containing either of the selective GABA$_B$ receptor antagonists, 2-hydroxy-saclofen (25 μM) or CGP-35348 (100 μM), only slightly facilitated the profile of paired-pulse responses between 100 ms and 5 s. These results suggest that in the dentate gyrus of immature rats, the contribution of GABA$_B$ receptors to the granule cell response to perforant path stimulation is much less than in adults.

(Supported by PHS Grants NS-20253, MH-15788, and the Klingenstein Foundation)

265.11

COMPARATIVE DEVELOPMENT OF GLUTAMATE AND GABA IMMUNOREACTIVE NEURONALPOPULATIONS IN THE CEREBRAL CORTEX OF RAT FERRET AND MONKEY. D.L. Meinecke* and M. L. Schwartz. Section of Neurobiology, Yale Sch. Med New Haven, CT 06510.

Our previous studies in primate show that during development GABA is expressed very early in neurons of the cerebral cortex , prior to the formation of synaptic circuits, suggesting a potential trophic role. In contrast to the information known about GABA, little is known about the expression and distribution of excitatory transmitters in the developing brain. We address this question here using an antibody to glutamate (Arnel) to determine when during embryogenesis cortical neurons become immunopositive for glutamate in the rat, ferret, and rhesus monkey. Material from each of these species was taken through times of neurogenesis, cell migration, and synapse formation.

Double immunolabeling in adults of each of these species revealed that GABA and glutamate are present in separate populations of cortical neurons. In the developing cortex specific GABA labeling can be seen in cells throughout the telencephalic wall at all embryonic ages, including the germinal neuroepithelium, migrating neurons, and the earliest born layers of the cortical plate. In contrast, glutamate immunoreactivity was not present in cells of the germinal zone, or migrating cells. Within the cortical plate glutamate positive cells were not found until far later ages than the first appearing GABA labeled cells at comparable cortical depths. Generally, the first appearance of glutamate positive cortical cells coincided with the ingrowth of major fiber tracts in each of these species. These results support the hypothesis that GABA may have a unique developmental role unrelated to its transmitter role in adult, whereas glutamate seems to increase in cortical neurons after they attain their adult positions and connections, possibly reflecting the emergence of glutamate transmitter pools. Supported by NS 22807-06.

265.12

NEURONS DERIVED FROM P19 CELLS CONTAIN L-GLUTAMATE, GABA AND THEIR RECEPTORS. PA MacPherson [1], DSK Magnuson [2], DJ Morassutti [1], WA Staines [3]*, KC Marshall [2], and MW McBurney [1]. [1]Depts Med & Biol, [2]Phys & [3]Anat/Neurobiol, Univ. Ottawa, Ottawa, Ontario Canada K1H 8M5

P19 cells, a pluripotential embryonic line, can be induced to differentiate with retinoic acid (RA) into a mixed population of both neurons and glia. The neuronal cells obtained are immunoreactive for the NeuN antigen, a characteristic of neurons of the CNS. Immunofluorescence has also been used to identify a variety of neurotransmitters within these cultures, the majority being either L-glu, or GABA. Glutamate immunoreactivity appears within the first 10 days following RA treatment and is found regardless of whether L-glu is contained within the culture medium. When treated with RA, P19 cells both in culture and when grafted into the striata of young adult rats demonstrate an electrophysiological maturation of their membranes. In response to L-glu, intracellular recordings from grafted cells show depolarizations within 10-14 days post RA. These depolarizations are Mg^{2+} sensitive, are reduced by AP5 and blocked by kynurenate, suggesting the presence of both NMDA and non-NMDA excitatory amino acid receptors. In addition, more mature grafts are often observed to develop rhythmical spontaneous synaptic activity with a similar pharmacological sensitivity to that of exogenous glutamate.

265.13

EXPRESSION OF GENES ENCODING GLUTAMIC ACID DECARBOXYLASE IN PLURIPOTENT STEM CELL LINES. G. Bain, T.P Ramkumar, J. Cheng and D. Gottlieb*, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO

The inhibitory neurotransmitter γ-amino butyric acid (GABA) is synthesized by the enzyme glutamic acid decarboxylase (GAD). This enzyme is encoded by two genes in the mammalian genome. Since the expression of GAD in the central nervous system is restricted to GABAergic neurons, it provides an excellent marker with which to analyze the mechanisms which regulate cell-type-specific gene expression in the brain.

The availability of cell lines which express the GAD genes would provide a powerful in vitro system for the analysis of their regulation. The P19 murine embryonal carcinoma cell line is potentially very useful for the analysis of neural gene control. These cells can be induced to differentiate into neurons, glia and fibroblasts by culturing them as aggregates in the presence of retinoic acid. We have therefore examined these cells for GAD gene expression using an RNase protection assay. We have found that both GAD genes are expressed in P19 cells. Undifferentiated cells contain low but detectable levels of GAD mRNA. After neural differentiation is induced, there is a substantial increase in expression for both genes. The presence of normal transcripts from both GAD genes in differentiated P19 cells has been confirmed by RNA blot analysis.

The observation that undifferentiated P19 cells express the GAD genes was somewhat surprising. To determine if this expression is a peculiarity of P19 cells, we analyzed their expression in undifferentiated mouse embryonic stem cells. Both GAD genes are expressed in these cells, although at significantly different levels. This result raises the possibility that GAD may play a role in early embryonic development.

Our data demonstrate that P19 cells inducibly express both GAD genes. They should therefore provide a useful system with which to study the mechanisms which regulate the expression of these two genes.

265.14

SIGNAL TRANSDUCTION MECHANISMS SUBSERVING ACTIVITY-DEPENDENT RELEASE OF GROWTH-PROMOTING PROTEOGLYCANS BY HIPPOCAMPAL NEURONS. M. Sugiura and K.E. Dow*, Queen's University, Dept. of Pediatrics, Kingston, Ontario, Canada.

Excitatory amino acid (EAA) neurotransmitters are involved in activity-dependent neuronal growth during development and in enduring changes in synaptic efficacy in the hippocampus. Neurons produce and release proteoglycans (PGs) which are known to promote neuronal growth and cell adhesion. The present studies were designed to analyze the regulation of PG release by EAA receptor activation of neurons in vitro. Neuron-enriched cultures were established from dissociated hippocampus of 19 day fetal rats. [^{3}H]leucine and [$^{35}SO_4$]-labelled PGs were extracted and isolated using ion-exchange chromatography. Exposure of neurons to the EAA agonist glutamate (100 μM) resulted in an increase in PG release during the 5 minute exposure period. The glutamate effect was partially inhibited by the N-methyl-D-aspartate (NMDA) receptor antagonist D-2-amino-5-phosphonovaleric acid (AP5) and the metabotropic receptor antagonist 2-amino-3-phosphonopropionic acid (AP3). The effect was completely antagonized by the combination of AP5, AP3 and the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Glutamate-induced neuronal PG release was inhibited by sphingosine and the nitric oxide synthase inhibitor, N-nitro-L-arginine. EAA-stimulated activity-dependent responses of neurons which may influence exquisite connectivity in development may be mediated by PGs in the extracellular milieu. By virtue of their binding interactions in the extraneuronal milieu, neuronal PGs with growth modulatory activity may be both necessary and sufficient for enduring changes in synaptic efficacy. The release of these PGs is regulated by both ionotropic (NMDA and non-NMDA) and metabotropic EAA receptor activation. The signal transduction mechanisms subserving EAA-stimulated PG release may involve protein kinase C and/or Ca^{2+}-calmodulin type II kinase. Nitric oxide may be a diffusible messenger responsible for PG release.

265.15

DEVELOPMENTAL INCREASES IN N-METHYL-D-ASPARTATE RECEPTOR-STIMULATED CATECHOLAMINE RELEASE IN RAT BRAIN CORTEX AND HIPPOCAMPUS. L.M. Brown*, Dept. of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

N-methyl-D-Aspartate (NMDA) receptor binding has been shown to exhibit transient increases during postnatal development in rat brain (Tremblay et al., Brain Res. 461 (1988) 393-396). This study was performed to examine postnatal alterations in NMDA receptor-stimulated catecholamine release. The NMDA receptor-stimulated catecholamine release assay has been shown to be a good indicator of NMDA receptor-mediated functional activity (Gonzales and Woodward, JPET. 253 (1990) 1138-1144).

Brain slices (350μm) from the cortex and hippocampus of male and female Sprague-Dawley rat pups of varying ages (1, 7, 14, and 21 days) were used. The slices were washed and incubated in oxygenated Krebs-Ringers bicarbonate buffer and allowed to accumulate [^{3}H]-Norepinephrine ([^{3}H]NE). After uptake of the label, the slices were washed again and aliquots placed into baskets which were transferred through a series of vials containing varying concentrations of NMDA (10, 50, 100, 500 and 1000 μM). Percent [^{3}H]NE overflow was calculated and logistic parameter curve fitting performed using ALLFIT (Delean et al., Am. J. Physiol. 235 (1978) E97-E102).

In the cortex and hippocampus NMDA resulted in a concentration-dependent increase in [^{3}H]NE overflow. The maximal response to NMDA increased with age (1, 7, 14 and 21 days). The cortical responses were (1.55±0.15, 3.67±0.22, 2.94±0.22 and 4.61±0.30) respectively. The hippocampal responses were (1.93±0.19, 2.97±0.12, 3.66±0.15 and 5.31±0.22) respectively. The EC_{50} values also increased with age in both brain regions. These results indicate that significant developmental alterations in NMDA-stimulated [^{3}H]NE overflow are observed postnatally.

265.16

NEUROCHEMICAL ANALYSIS OF DIFFERENTIATION IN THE LA-N-5 HUMAN NEUROBLASTOMA CELL LINE. B. Anton*, C. Evans+, J. Hu, E. Innauer, N.T. Maidment+, J. Talvenheimo# and R.E. Weiss. Depts. of Physiology and Psychiatry+, BRI, UCLA, Los Angeles, CA 90024 and Amgen Inc#., Thousand Oaks, CA 91320.

The effects of the differentiating agents retinoic acid (RA) and nerve growth factor (NGF) on neurotransmitter content and release were studied on the LA-N-5 human neuroblastoma cell line. Cells were cultured in RPMI + 10% FCS medium and plated on different substrates. Both NGF and RA induced extensive neurite formation and a decrease in proliferation when cells were plated on collagen or laminin. Cells plated on plastic showed pronounced morphological changes in response only to RA . Amino acids and catecholamines were assayed by HPLC with fluorometric and electrochemical detection respectively. Cells were exposed sequentially to normal physiological saline (PS) and PS containing 50mM K^+ for 1 h. K^+-induced release of glutamate, glycine and taurine was upregulated after either RA or NGF treatment independent of the plating substrate. Total cell content of these transmitter amino acids was much larger than the apparent releasable pools. These results are compared to our findings on sodium channel density which show similar upregulation (~ 300 %) by both NGF and RA independent of the plating substrate of the cells. These data suggest that LA-N-5 cells may be useful in the study of the induction of neuronal differentiation *in vitro*.

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265.17

CELL VOLUME CHANGES AND TAURINE RELEASE IN BRAIN SLICES INCUBATED IN MEDIA OF DIFFERENT IONIC COMPOSITIONS.

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In the mammalian brain taurine has been claimed to act as osmolyte which is released predominantly from astrocytes upon cell swelling. We endeavored to investigate the relation of cell volume changes to taurine release in mouse cerebral cortical slices superfused with media of different ionic compositions. The release of taurine was enhanced in hypoosmotic media and inhibited in hyperosmotic media. The release was also enhanced, however, by a partial or total omission of Na^+ or Cl^- and by high K^+ concentrations. An enhancement of release was obtained in media in which Cl^- was replaced by a relatively permeant organic anion acetate and in which there occurred massively intracellular swelling in the slices. A marked stimulation was discernible also in media in which an impermeant anion gluconate was used as replacer and in which there occurred no or only negligible swelling of even intracellular shrinking. Most experimental conditions which enhanced the release simultaneously also inhibited the influx of taurine. In view of the low capacity of both low- and high-affinity transport systems of taurine, an inhibition of reuptake may not, however, constitute the sole explanation of the apparent stimulation of release under these conditions. Since a positive correlation between the release of taurine and changes in cell volumes obtains only under certain isolated experimental conditions, we infer that taurine is not released from slices only as a response to cell swelling and may not act as a major osmolyte in the mammalian brain. (Supported by the Emil Aaltonen Foundation, Finland).

NERVE GROWTH FACTOR III

266.1

THE MECHANISM OF NERVE GROWTH FACTOR-INDUCED LONG-TERM HETERODOWN REGULATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR IN PC12 CELLS.

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Differentiation of PC12 cells with nerve growth factor (NGF) produces a sympathetic neuron-like phenotype, which is accompanied by a cessation of cell division. During the differentiation, the epidermal growth factor receptor (EGFR), measured by both binding and EGF-induced phosphorylation, on the cells decreased by 70-90%. Northern blotting indicated that neither of the two EGFR mRNAs (13 and 4.6 kb) were decreased. Western blotting showed that the EGFR protein (170 kD) decreased in proportion to the decreased binding of EGF. Labeling studies showed that NGF had no effect on the rate of degradation of the EGFR. Pulse-chase experiments revealed a series of biosynthetic intermediates leading to the complete EGFR, and, in NGF-treated cells, a failure of the 160 kD EGFR precursor to mature into the 170 kD EGFR protein. The data suggest that the NGF-induced down-regulation of the EGFR is due, at least in part, to an inhibition of some of the final post-translational modifications of the protein, probably at the level of glycosylation.

266.2

THE REGULATION OF NERVE GROWTH FACTOR-STIMULATED CALCIUM UPTAKE BY INTRACELLULAR CALCIUM LEVELS IN PC12 CELLS.

B. Nikodijevic, D. Nikodijevic-Kedeva, A. Kozak, E. Yavin, and G. Guroff*. Section on Growth Factors, NICHD, NIH, Bethesda, MD 20892 and Department of Neurobiology, The Weizmann Institute, Rehovot, Israel.

Nerve growth factor (NGF) stimulates the uptake of calcium and raises intracellular calcium levels in PC12 cells. The stimulation of uptake is maximal after 3-5 minutes of treatment with nerve growth factor and is usually on the order of 50%. The data suggests that the NGF-sensitive calcium channel is different than the L-type calcium channel and the other major calcium channels in PC12 cells. Introduction of phosphatases into permeabilized PC12 cells indicates that the activation of this NGF-sensitive channel is due to a phosphorylation of one of the channel proteins. When calcium levels were lowered by pre-incubation of the cells with nickel, the effect of NGF on calcium uptake was enhanced. Conversely, when intracellular calcium levels were raised by pre-incubation of the cells with high potassium, the effect of NGF on calcium uptake was decreased. Since intracellular calcium levels fluctuate with cell density and change when cells are placed in suspension, these data may indicate why previous reports in this area have been so inconsistent. In the light of recent studies on the enhanced survival of neurons when their intracellular calcium levels have been raised, the actions of NGF on calcium levels may be relevant to the ability of NGF to promote the survival of sympathetic and sensory neurons.

266.3

$p21^{c\text{-}ras}$ Mediates Nerve Growth Factor Signal Transduction in Embryonic Sensory Neurons and PC12 Cells.

N. E. L. Ng and E. M. Shooter*. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

During their development, sensory and sympathetic neurons acquire a dependence for nerve growth factor (NGF) in order to maintain their differentiated state. Little is known, however, about the mechanism by which NGF transduces its signal in these neurons, with much of the knowledge extrapolated from studies using the NGF-responsive pheochromocytoma PC12 cell line. We investigated the role of cellular $p21^{c\text{-}ras}$ in mediating the NGF signal in embryonic sensory neurons and PC12 cells. Changes in the levels of active $p21^{c\text{-}ras}$ in response to NGF were analysed, based on the ratio of the levels of active GTP-bound ras to inactive GDP-bound ras. Following treatment with NGF, ^{32}P-labelled E9 chick dorsal root ganglion and PC12 cells were lysed and immunoprecipitated with anti-ras antibody (Y13-259), guanine nucleotides were eluted, and the GTP and GDP levels were separated and quantitated. In sensory neurons, NGF stimulated a 2-3 fold increase in the levels of ras-GTP within 5 min, with these levels remaining elevated, though at a lower activation state of 1.6-fold of control by 60 min. In PC12 cells, NGF stimulated a 3-4 fold increase in active ras-GTP within 1 min, with the activation remaining elevated at these levels for up to 60 min. In the neurons, a minimum concentration of 20 ng/ml NGF was required to obtain maximal ras activation within 5 min, while in PC12 cells, a minimum of 10 ng/ml NGF was required. At lower concentrations of NGF, ras activation in both sensory neurons and PC12 cells increased slowly with time, with an apparent plateau by 60 min. At this low concentration of NGF, neither sensory neurons nor PC12 cells attained the maximal activation seen with the high concentrations. Treatment of both sensory neurons and PC12 cells with the protein kinase inhibitor K252a inhibited activation of ras in response to NGF. These results demonstrate the involvement of $p21^{c\text{-}ras}$ in mediating NGF signal transduction in embryonic sensory neurons and PC12 cells.

266.4

PTP-P1, A NOVEL PROTEIN TYROSINE PHOSPHATASE DOWN-REGULATED BY NGF.

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Tyrosine phosphorylation of proteins in multicellular organisms is mainly associated with the response of cells to stimuli mediated by receptors for hormone and growth factors and plays an important role in signal transduction and cellular differentiation. Tyrosine phosphorylation is transient and is thought to depend on the activity of both protein tyrosine kinases and protein tyrosine phosphatases. NGF, a neurotrophic factor, can stimulate tyrosine phosphorylation and neurite outgrowth of the rat pheochromocytoma cell line PC-12 trough is receptor trk, a proto- oncogene with tyrosine kinase activity. We repot here the cloning and the characterization of a cDNA from PC-12 cells encoding a novel protein tyrosine phosphatase, PTP-P1. This phosphatase correspond to a 6.2kb transcript and is down-regulated upon NGF-induced differentation of PC-12 cells. The down-regulation of PTP-P1 may be required for the induction of neurite outgrowth by NGF.

266.5

MULTIPLE SIGNAL TRANSDUCTION PATHWAYS LEAD TO INDUCTION OF *C-FOS* EXPRESSION BY NGF IN PC12 CELLS. H. Qi*, R. C. Armstrong and S. Halegoua. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794

In PC12 cells the *c-fos* gene can be transcriptionally stimulated by nerve growth factor (NGF) or by agents which activate distinct second messenger pathways involving A-kinase, C-kinase and Ca^{2+}/calmodulin kinase. Moreover, the A-kinase and C-kinase pathways have been implicated in the mechanims of NGF actions. Recently, the tyrosine kinase Src, the GTP-binding protein Ras and the serine/threonine kinase Raf have also been shown to play critical roles in mediating NGF signals with the order of Src -> Ras -> Raf. To better understand the second messanger systems which regulate the *c-fos* gene in response to NGF in PC12 cells, we have developed a unique approach which uses a pathway-specific reinduction paradigm. Results using this experimental paradigm suggest that two thirds of the *c-fos* induction by NGF involve the C-kinase pathway. The remaining one third of the gene induction is mediated by a novel pathway independent of both A-kinase and C-kinase. The roles of Src and Ras in pathways mediating NGF-induced *c-fos* gene expression are being elucidated using PC12 sublines transfected with *v-src* or activated *ras* cDNA or cDNA encoding a dominant negative *ras* mutant. The relative contributions of Src and Ras to the C-kinase pathway and the novel pathway will be presented. (Supported by NIH grant NS18218).

266.6

RAPID MEASUREMENT OF RESPONSES BY PC12 CELLS TO NERVE GROWTH FACTOR (NGF) USING THE CYTOSENSOR™ MICROPHYSIOMETER. S. Pitchford, B.S. Glaeser*, & K. De Moor, Molecular Devices Corp., Menlo Park, CA 94025.

Study of cellular responses to growth factors typically involves monitoring either signal transduction activation or changes in cell number or morphology in the continued presence of the growth factor of interest. Using the Cytosensor Microphysiometer System to measure alterations in cellular metabolic activity (by monitoring extracellular acidification of a low-buffered RPMI media bathing cells), we have demonstrated early responses of PC12 cells to NGF. The factor stimulated metabolic activity in an apparent dose-dependent fashion between 0.1 and 100ng/ml NGF. The cells were stimulated by only a brief (12 minute) exposure to NGF with extracellular acidification rates reaching a peak between 10 and 20 minutes after the initial introduction of NGF and then declining over the subsequent 2-3 hours. The response to NGF was abolished by pre-incubation of the cells with 25μg/ml genestein, a tyrosine kinase inhibitor, suggesting the involvement of this signal transduction pathway in the metabolic response of PC12 cells to NGF.

266.7

PHARMACOLOGICAL MANIPULATION OF NGF/PROTEIN KINASE C INTERACTIONS IN PC12 CELLS E. J. Martin* and E. M. Meyer, Department of Pharmacology and Therapeutics, College of Medicine, Univ. of Florida, Gainesville, FL 32610

Protein kinase C (PKC) is a phospholipid-dependent, calcium- and diacylglycerol-activated kinase that preferentially phosphorylates serine and threonine residues. In the brain, PKC is involved in gene transcription, neurotransmitter release, synaptic plasticity and receptor transduction. Consequently, this enzyme has been implicated in the second messenger pathways underlying trophic events in the brain, including those triggered by NGF administration. However, to what extent PKC is involved in the events that take place secondary to NGF binding its receptor remains unclear. We therefore designed experiments to study the effects of NGF and phorbol ester applied at different concentrations and for different periods of time. PC12 cells are a commonly accepted model system for studying the complex biological transduction processes initiated by NGF. Cultures of PC12 cells were assayed for PKC activity by permeabilization with low concentrations of digitonin. By permeabilizing the cells, it is possible to introduce a specific substrate peptide and phosphate source [(32P) ATP] into the cells. These components interact with the intracellular environment in such a way that PKC-phosphorylated substrate can be collected on phosphocellulose filters and quantified. We demonstrated in preliminary studies that 10 nM phorbol 12-myristate 13-acetate (PMA) was able to stimulate PKC activity within 15 minutes. This stimulation was attenuated with PKC inhibitor peptide [PKC (19-36)]. NGF at a concentration of 100 ng/ml, but not 50 ng/ml, was also able to stimulate enzyme activity. NGF in combination with PMA produced a thirty percent increase in PKC activity over that of NGF alone. These studies are being extended by measuring the role of PKC activity in other indices associated with cell proliferation and differentiation.

266.8

A PROTO-ONCOGENE SIGNALLING PATHWAY MEDIATES NGF-INDUCED GENE EXPRESSION EVENTS IN PC12 CELLS. G. D'Arcangelo* and S. Halegoua, Dept. of Neurobiology and Behavior, S.U.N.Y. at Stony Brook, Stony Brook, N.Y. 11794.

The rat pheochromocytoma cell line, PC12, is a useful model system for studying the molecular basis of NGF actions. Treatment of these cells with NGF results in the elaboration of a sympathetic neuron-like phenotype, including the extension of neuritic processes and the expression of several genes, some of which encode specific neuronal markers. The signal transduction mechanisms that follow the binding of NGF to its receptor are still largely unknown, however a sequence of proto-oncogene activities including the NGF receptor *trk*, *src* tyrosine kinase, *ras* GTP-binding protein and *raf* serine/threonine kinase have been implicated in the induction of neurite outgrowth.

NGF induces an exquisite temporal pattern of gene expression events which provide a convenient assay for the different signal transduction pathways emanating from the growth factor receptor. In this study, we have used PC12 sublines expressing activated, oncogenic forms of *src*, *ras* or *raf* and sublines expressing dominant inhibitory forms of these proteins to dissect the signal transduction pathways which induce changes in gene expression. Our results describe four classes of gene induction events which identify branchpoints in the NGF signal transduction pathway to gene induction. These results indicate parallel processing from the proto-oncogene components of the NGF signal transduction machinery. (Supported by NIH grant NS18218 to SH and predoctoral fellowship from Hoffman La Roche to GD).

266.9

NGF CORRECTS THE LOSS OF DA UPTAKE ACTIVITY IN EMBRYONIC MESENCEPHALIC CULTURES TREATED WITH MPP^+.

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Nerve growth factor (NGF) promotes the development and survival of sympathetic and sensory neurons in the periphery, however, it is believed that it is neurotrophic for cholinergic but not catecholaminergic neurons of brain. We found that NGF 7S or 2.5S partially restores the biochemistry, morphology and pharmacological responsiveness of striatal dopaminergic neurons in mice treated with MPTP. MPP^+, the neurotoxic metabolite of MPTP, decreases dopamine (DA) uptake activity in embryonic mesencephalic cultures. We have used embryonic (E 15) mesencephalic cultures to investigate the action of NGF on dopaminergic parameters. Cultures were treated with MPP^+, 3μM, for 24 hr, then NGF 2.5S was added to the medium and DA uptake assayed 12 days later. NGF restored DA uptake activity in a dose-dependent manner, with about 500 ng/ml NGF 2.5S returning transport activity to "ear control values. Pretreatment or cotreatment with NGF did not prevent the MPP^+ induced neurotoxicity. Moreover, NGF partially restored the number and the morphology of TH-immunopositive cells in MPP^+ treated cultures. NGF had no effect on the above parameters in control cultures. We postulate that injury induces dopaminergic neuron to become sensitive to NGF.

266.10

KAINATE RECEPTOR MEDIATED DIFFERENTIAL REGULATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR mRNA AND PROTEIN

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BDNF and NGF mRNA have been reported to be increased after seizures induced by electrolytic lesions as well as after injections of the glutamate agonist, kainic acid. However, the receptor subtype specificity of neurotrophin regulation and whether this upregulation results in a concomitant increase in trophic factor protein has yet been determined.

In the present study we report specific regulation of BDNF mRNA and protein in vivo exclusively via the kainate and not the NMDA receptor. Systemic injection of kainic acid (12 mg/kg i.p.) resulted in a dramatic increase in BDNF as well as NGF mRNA throughout several hippocampal and cortical areas. No changes in BDNF mRNA or protein were detected after intracerebral injection of NMDA (1 nmol). The kainate-induced upregulation of BDNF was completely blocked by the kainate receptor antagonist DNQX and not affected by the the NMDA antagonist AP7.

Interestingly, differential modulation of BDNF protein was detected with BDNF peptide antibodies. Little change in BDNF immunoreactivity (BDNF-IR) was noted 6 hr post injection. At 8 hr postlesion, increased BDNF-IR was detected in hippocampal pyraminal and granule cells. At 12 and 24 hours postlesion, increased BDNF-IR was noted in CA1, CA2, and in piriform cortex. Significant cell death was noted in CA3, and reduced BDNF-IR noted in the dentate granule and hilar cells. No significant change was noted in cingulate, temporal and parietal cortices. These data indicate that while there appear to be changes in levels of BDNF protein after kainic acid perturbation, these changes are not as widespread nor do they show the same magnitude and temporal modulation as noted for BDNF mRNA.

266.11

CLONING OF CHICK BDNF: TECTAL FACTOR AND POTENTIAL ALTERNATE PRECURSORS . J. M. Voci and T.H. Large*, Dept. Neuroscience, Case Western Reserve University, Cleveland, Ohio 44106-4975.

Retinal ganglion cell (RGC) survival is dependent on factor(s) from the optic tectum beginning at age E10-11. *In vitro*, BDNF has been shown to support RGC survival and stimulate process outgrowth, suggesting that BDNF may be a tectal-derived factor for developing RGCs. As a first step in examining *in vivo* the BDNF-dependence of RGCs, we have cloned the chick BDNF gene.

A PCR amplified fragment of chick BDNF was used to screen a chicken cosmid library under high stringency conditions. Two BDNF clones were identified, verified by PCR amplification and a cosmid fragment coding for the short BDNF precursor was subcloned and sequenced. Primary sequence analysis reveals that BDNF is extremely well conserved among mammalian, amphibian, and avian species. The mature chicken factor (119 aa) contains only 7 amino acid differences compared to mammalian BDNF. The precursor coding region is almost as well conserved; chick BDNF, like human BDNF, lacks a 2-5 aa insertion at position -72 that is seen in other mammalian species. For mouse NGF, alternate splicing of upstream exons containing start ATGs gives rise to short and long precursor proteins. Consistent with the possibility of long BDNF precursors, the chick gene possesses a splice acceptor site 21 bp upstream from the start ATG and the 7 upstream amino acids are identical to those in mammalian BDNF genes. A potential splice acceptor site 12 bp upstream in mammals is absent in chicken, in agreement with use of the first, but not second, splice site in mammalian BDNF cDNA clones.

Northern analysis reveals relatively low expression of BDNF in the embryonic retina but relatively high expression in the optic tectum, with levels reaching a maximum by E11-13. These results argue that BDNF is unlikely to be an "intrinsic" factor regulating early retinal development, but is expressed within the optic tectum at an appropriate time to regulate the target-dependent survival of RGCs. Expression of chicken BDNF in baculovirus will be used to generate the active factor and antibody reagents necessary for directly testing BDNF actions *in vivo*.

Supported by grants AG00533 to JMV and EY08885 to THL.

266.12

ALPHA-2-MACROGLOBULIN, A CARRIER OF NEUROTRANSMITTERS, FORMS AN INHIBITORY COMPLEX INTERFERING WITH NGF-DEPENDENT NEURITE OUTGROWTH AND SURVIVAL OF CNS NEURONS. D.J. Liebl* and P.H. Koo. Northeastern Ohio Univ. College of Med., Rootstown, OH 44272.

Alpha-2-macroglobulin (α_2M) is a rather ubiquitous protein in extracellular fluids of mammals including CSF. We have recently demonstrated that methylamine-activated α_2M can inhibit neurite outgrowth and damage embryonic sensory neurons (P.H. Koo and D.J. Liebl (1992) J. Neurosci. Res. 31:678-692). The objective of this study is to determine whether α_2M can combine with monoamine-neurotransmitters to also produce neurite-inhibitory and neuron-damaging complexes, and the mechanism of action of serotonin-activated α_2M. Our results demonstrate that α_2M can form stable complexes with serotonin (5HT), histamine, and catechol-amines, and the resultant complexes produced all can inhibit NGF-promoted neurite outgrowth by embryonic chicken dorsal root ganglia. α_2M (1.4 μM) can absorb about 55-65% of the total 5HT (4-8 nM) in solution. At much higher 5HT concentrations , α_2M can combine maximally with 15.2 $\pm$ 1.8 moles of 5HT, in which 4.5 $\pm$ 0.4 moles may be covalently bound. Serotonin-modified α_2M at concentrations greater than about 0.17 μM exerts a dose-dependent inhibition on neurite outgrowth and can seriously damage sensory and CNS neurons. Normal α_2M and free 5HT (up to 188 μM), under identical conditions, produce very little or no effect. The neurite-inhibitory and neuron-damaging activities of 5HT-activated α_2M can be blocked by higher concentrations of NGF. Similar effects can be partially induced in the cerebral cortical culture with anti-NGF IgG antibodies. We conclude that α_2M has natural affinity for all the monoamino-neurotransmitters and the amine-activated α_2M appears to inhibit neurites and neuronal survival by interferring with the NGF action. (NIH NS-30698 and MEFCOM Foundation grant).

266.13

MITOMYCIN C DECREASES EXPRESSION OF NERVE GROWTH FACTOR AND ITS RECEPTOR IN SEAD CELLS. E.L. Imperato*, J.T. Hansen, R. Loy^ and M.F.D. Notter. Department of Neurobiology and Anatomy, and ^Department of Neurology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

SEAD cells were developed by infecting primary rat Schwann cells using a retroviral vector containing the 12S E1A gene. SEAD cells expressed the characteristic Schwann cell markers S-100, nerve growth factor receptor (NGF-rec), and insulin-like growth factor-1 (IGF-1) as detected immunocytochemically. NGF protein also was observed in SEAD cell lysates by ELISA. Mitomycin C (M-C), an antimitotic agent, decreased NGF-rec immunoreactivity and NGF protein expression in SEAD cells; however, there was no effect on IGF-1 immunoreactivity. M-C arrested SEAD cell proliferation but this effect was completely reversible following removal of M-C and a brief recovery period. Decreased mitosis with a decrease in NGF-rec immunoreactivity and NGF protein expression in SEAD cells mimics Schwann cells *in vivo* during later stages of peripheral nerve regeneration. However, Schwann cell proliferation and expression of NGF and its receptor may not be directly related. These are the first observations of a reversible M-C action. Supported by AG09231 (RL), EY06947 (MFDN), and NS25778 (JTH).

266.14

DIFFERENTIAL MODULATION OF NEUROACTIVE SUBSTANCES IN THE CHICK SYMPATHOADRENAL SYSTEM BY NERVE GROWTH FACTOR AND FIBROBLAST GROWTH FACTOR. R. Ramírez-Ordóñez* and J.E. García-Arrarás. Biology Department, University of Puerto Rico, Río Piedras, P.R. 00931.

Environmental factors are known to influence the differentiation of the sympathoadrenal system. Cells in this system synthesize catecholamines (CA) as their main neurotransmitter together with various neuropeptides. Using indirect immunofluorescence and CA histofluorescence we have demonstrated that phenotypes similar to those found in vivo are expressed by cells in vitro. We have studied the effect of nerve growth factor (NGF) and fibroblast growth factor (FGF) on the expression of the CA, epinephrine (E), norepinephrine (NE) and of the neuropeptides, somatostatin (SS), neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), and enkephalins (ENK) in adrenal glands and sympathetic neurons. Cells from 10 day chick embryos were exposed in vitro for 5-6 days to 50ng/ml of NGF or 75ng/ml of FGF. Their neuropeptide content was measured by radioimmunoanalysis and the amount of NE and E analyzed by high performance liquid chromatography. In sympathetic cultures NGF increased the levels of NPY, ENK and VIP increased without changing the level of SS, while FGF decreased slightly the levels of SS and NPY without changing the level of ENK and VIP. NGF had no effect on cultured adrenal cells, but FGF decreased the level of SS without changing the level of the other neuropeptides. In sympathetic ganglion cultures neither NGF nor FGF had any effect on the quantity of NE.

These results demonstrate that, in contrast to the mammalian system, FGF does not mimic NGF effect in chick sympathoadrenal cells . In our system NGF and FGF are modulating differentially the expression of neuroactive substances, suggesting that both neurotrophic factors may have independent roles during the development of the avian sympathoadrenal system. [Supported by grants from NSF (BNS-8801538) and NIH-MBRS Program (RR-8102-18) and partial support of NIH-RCMI (RRO 3641-01).

266.15

RECOMBINANT HUMAN NGF SIGNIFICANTLY ENHANCES THE SURVIVAL OF CHROMAFFIN CELLS *IN VITRO*. M.A. Tokiwa*, L.C. Doering, J.W. Winslow[§] and L.E. Burton[§]. Division of Anatomy, McMaster University, Hamilton, Ontario, CANADA L8N 3Z5 and [§]Genentech Inc., South San Francisco, CA 94080, U.S.A.

Based on the long term survival of transplanted chromaffin cells in peripheral nerve (Doering and Tokiwa, 1991; Brain Res. 551:267) we set out to systematically evaluate the role of the constituent peripheral nerve cells and selected trophic factors on chromaffin cell survival *in vitro*.

Enriched adrenal medulla (AM) cell cultures from 5-7 day old Wistar rat pups were established and grown in 24 well plates by the method of Unsicker et al., 1978; PNAS 75:3498. After 1 week *in vitro*, the AM cells were identified by tyrosine hydroxylase immunohistochemistry and counted.

Co-cultures of Schwann cells/fibroblasts plus chromaffin cells promoted AM cell survival ($P < 0.001$). Conditioned medium from 4 day-old Schwann cell/fibroblast cultures, when added to the AM cultures, also enhanced survival ($P < 0.001$) when compared to the controls.

Recombinant human (rh) factors including neurotrophins (NGF, BDNF) and basic fibroblast growth factor (bFGF) were added alone or in combinations to the enriched AM cell cultures at concentrations of 25, 50, 100 and 1000 ng/ml of medium. Only rhNGF at concentrations $\geq$ 50 ng/ml had a significant ($P < 0.05$) effect on the survival of the AM cells. No other rh trophic factor, alone or in combination exceeded the level of survival obtained with the rhNGF added alone.

At the present time, of the trophic factors tested, rhNGF appears to be the factor that is most critical to chromaffin cell survival *in vitro*.

(Supported by The Parkinson Foundation of Canada)

266.16

A NOVEL FUNCTION FOR THE NGF AND NGF RECEPTORS EXPRESSED ON SCHWANN CELLS: A ROLE IN SCHWANN CELL MIGRATION. E. S. Anton* and W. D. Matthew. Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

Schwann cell migration is an event critical to the normal development and successful regeneration of peripheral nerves. During the development and regeneration of peripheral nerves, migrating Schwann cells are known to express increased levels of NGF and low affinity NGF receptors (NGFr). Compared to a normal nerve, a denervated sciatic nerve contains 50 and 5 fold more NGFr and NGF, respectively (for review see Johnson et. al., 1988) and pre- treatment of a denervated sciatic nerve substrate with NGF results in a 'NGF- laden substratum'. In order to test whether Schwann cells use NGF and its receptors for migration, we studied Schwann cell migration on cryostat sections of normal and denervated sciatic nerves, pre- treated with or without 2.5S NGF. Dorsal root ganglia from 1 day old rats were explanted on to these substrates and the ganglionic Schwann cells that migrated on nerve substrates were visualized after 72 hours in culture with the vital dye, fluorescein di- acetate. Schwann cells migrated significantly farther on a denervated sciatic nerve substrate than on a normal sciatic nerve substrate. Pre- treatment of denervated substrates with NGF significantly enhanced Schwann cell migration on this substrate. The presence of anti- serum to NGF in culture medium abolished the NGF effect. Pre- treatment of normal sciatic nerve substrates with NGF has no detectable effect on Schwann cell migration.

Based on these results we propose that during development and regeneration of peripheral nerves, Schwann cells use their cell surface NGF receptors to migrate in their NGF rich environment in addition to using them to provide tropic and trophic support to NGF responsive neurons.

266.17

NORMAL CNS GLIAL CELLS EXPRESS NEUROTROPHIN RECEPTORS WHOSE LEVELS ARE MODULATED BY NEUROTROPHIN TREATMENT. Shalini Kumar[1], Louis A. Peña[2] and Jean de Vellis[1]* (1) UCLA, Lab. of Biomedical and Environmental Sci., and Neuropsychiatric Institute, Los Angeles, CA 90024, (2) Cold Spring Harbor Lab., Beckman Neuroscience Ctr., Cold Spring Harbor, NY 11724.

We have previously examined the role of a neurotrophin, NGF, and its receptors in the C6 glioma cell line (Kumar et al., *J Neurosci Res* 27:408, 1990). We now extend these findings to primary cultures of pure, normal rat astrocytes and oligodendrocytes. Neurotrophins (NGF, BDNF, NT-3) bind to a common receptor subunit designated LNGFR and to more ligand-specific, tyrosine kinase-containing subunits designated TrkA, TrkB, and TrkC. Northern blot analysis and transcription assays confirmed a basal level expression of the LNGFR subunit in C6 and in oligodendrocytes and but an absence of basal levels in astrocytes. Following neurotrophin treatment, C6 and astrocytes dramatically upregulated LNGFR synthesis. TrkA mRNA was not detectable, however, multiple bands of TrkB transcripts were present at relatively high levels. TrkB was also upregulated by neurotrophin treatment in astrocytes but not in oligodendrocytes. The inductions occurred on a time scale of 4-6 hrs and could be similarly evoked by cycloheximide treatment -- results consistent with observations of receptor autoregulation by its ligand (e.g., EGF and its receptor). In glial cells, NGF upregulates both LNGFR and TrkB but not TrkA, a finding which warrants further investigation. (Supported by DOE, NICHD, and Ford Foundation.)

OTHER FACTORS AND TROPHIC AGENTS: CNTF

267.1

CHARACTERISATION OF THE HIGH AND LOW AFFINITY RECEPTOR FOR CNTF

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Department of Neurochemistry, Max-Planck-Institute for Psychiatry, 8033 Martinsried, FRG

Ciliary neurotrophic factor (CNTF) supports the survival of a variety of embryonic chick neurons in culture at a half maximal concentration of 1-5 pM. We have characterized the binding of iodinated CNTF to E11 chick sympathetic neurons and human neuroblastoma SKN-SH cells. Both cell types express low and high affinity binding sites with K_Ds of 3 nM and 11 pM, respectively. There are about 200 high affinity receptors and 10 000 low affinity binding sites per cell. The specific binding of ^{125}I-CNTF to low and high affinity receptors could be blocked with a 50 times excess of unlabeled CNTF, whereas LIF at 200 fold higher concentrations did not impair binding to either sites. The off-rate of CNTF from high affinity receptors at 4°C is very slow, such that no discernible change in binding could be detected after 3 hours. The off-rate from low affinity binding sites is comparatively fast ($t_{1/2}$= 15 min). In crosslinking experiments with ^{125}I-CNTF under low affinity conditions with high affinity binding blocked by cold CNTF, a single protein could be labelled with a molecular weight of approx. 75 kD. This protein might correspond to a recently cloned low affinity CNTF receptor (S. Davis et al. Science, Vol. 253, 59-63, 1991). Under high affinity binding conditions, a second protein of about 140 kD could be detected. Occasionally, crosslinked products greater than 200 kD could been seen, but the specificity of these labelled proteins is not yet clear. The identity of the cross-linking products is being determined.

267.2

BINDING CHARACTERISTICS OF CILIARY NEUROTROPHIC FACTOR (CNTF) TO SYMPATHETIC NEURONS AND OTHER RESPONSIVE CELL LINES. V. Wong*, D. Pearsall, R. Arriaga, S. Davis, N. Stahl, and R.M. Lindsay. Regeneron Pharmaceuticals Inc., Tarrytown, NY 10591.

A receptor for CNTF which has a molecular mass of 72kD has been cloned (Davis et al., 1991). In this study, we have examined the binding characteristics of ^{125}I-CNTF to its receptor by using a cell line (MG87) stably transfected with the cloned receptor, an Ewing sarcoma cell line (EW1), and cultured primary sympathetic neurons (superior cervical ganglion, SCG). Rat CNTF was iodinated by Bolton-Hunter reagent and the radiolabelled CNTF retained full activity in a ciliary ganglion neuron bioassay. Exposure to CNTF (1ng/ml) for 24 hours resulted in a decrease of ^{125}I-CNTF binding in both SCG and EW1 cells, but not MG87/CNTFR cells. The binding of CNTF to SCG neurons and EW1 cells was saturable and consisted of both high (10^{-12}M) and low (10^{-9}M) affinity, as demonstrated by Scatchard analysis. Binding to MG87/CNTFR cells was only of low affinity (10^{-9}M), suggesting that these responses require a high affinity component. Competition analysis showed a pharmacological rank order of potencies of rCNTF≈ CNTF-myc > hCNTF >> LIF, IL6, NGF, BDNF. Further studies are underway to elucidate the signal transduction pathway that mediates the effect of CNTF.

267.3

LIF- AND CNTF-INDUCED CHANGES IN GENE EXPRESSION IN SYMPATHETIC NEURONS *IN VITRO*. S.E. LEWIS[1]*, M.S. RAO[2], W.T.DAUER[1], A.J. SYMES[1], S.C. LANDIS[2], I.S. FINK[1], AND S.E. HYMAN[1]. [1] Molecular Neurobiology Laboratory, Massachusetts General Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA. 02114 and [2] Dept. of Neuroscience, Case Western University, Cleveland, OH. 44106.

Plasticity of neurotransmitter (NT)/ neuropeptide (NP) phenotype in rat sympathetic neurons has been described in detail and shown to be target tissue dependent. A similar switch from noradrenergic to cholinergic phenotype can be induced in dissociated cultures of superior cervical ganglion (SCG) neurons by treatment with target tissue extracts or with non-neuronal cell conditioned medium. These treatments cause a decrease in catecholamines and neuropeptide Y (NPY) and a concomitant increase in acetylcholine and several peptides, including vasoactive intestinal peptide (VIP), substance P (SP) and somatostatin (SST). Recently, several cytokines have been shown to induce similar changes in NT/ NP expression *in vitro*. We have used the *in vitro* SCG model to investigate changes in mRNA levels for NPs and neurotransmitter synthetic enzymes (NTSEs) induced by two of these cytokines, leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF.) For all of the genes examined, including ChAT, TH, VIP, SP, SST and NPY, the previously observed changes in NT/NP levels are mirrored by changes in the levels of NTSE or NP mRNAs. In addition, the reported inhibition of LIF-induced changes in NT/NP levels by elevated KCl also occurs at the level of mRNA accumulation. We found no significant difference in the time courses for alteration of NP/NTSE mRNAs by LIF vs. CNTF; changes were apparent by northern analysis at 3 to 6 hours of cytokine treatment. To further investigate the mechanism underlying these observed changes in gene expression, we examined the effect of protein synthesis inhibitors on LIF- and CNTF- induced alterations in mRNA accumulation. Both cyclohexamide and anisomycin block LIF- and CNTF- induced changes. Since the required newly synthesized protein may well be a transcription factor, we screened for changes in IEG expression after treatment with LIF or CNTF.

267.4

EFFECTS OF CILIARY NEUROTROPHIC FACTOR ON THE SURVIVAL AND NERVE GROWTH FACTOR-PROMOTED DEVELOPMENT OF CULTURED SYMPATHETIC NEURONS. P. Burnham*, J.-C. Louis, E. Magal and S. Varon, Biology Department, University of California, San Diego, La Jolla, CA 92093.

Ciliary neurotrophic factor (CNTF) has been found to have a wide spectrum of target cells and functions in the nervous system, supporting the emerging view that CNTF acts as a cytokine rather than as a classical neurotrophic factor. In this study we examined the supportive capability of CNTF for sympathetic neurons from the neonatal rat superior cervical ganglion (SCG), traditionally considered to require nerve growth factor (NGF) for long-term survival in culture. If CNTF was provided instead of NGF in low-density cultures depleted of nonneuronal cells, nearly as many neurons survived for 24 hrs as with NGF, and about one fifth the NGF-maintained number of neurons survived for 6 days. The CNTF-supported neurons were smaller than those supported by NGF, had very short processes and showed no immunostaining for the low-affinity NGF receptor (LNGFR), in contrast to the NGF-supported neurons, which developed intense staining for LNGFR over the 6 days in culture. If such cultures were supplemented with Schwann cells, CNTF supported the 6-day survival of nearly as many neurons as did NGF. However, if CNTF was given in combination with NGF in the non-supplemented cultures, the number of neurons surviving for 6 days was decreased by about one third compared to the number supported by NGF alone, and the neurons showed reduced neuritic development and a considerably lower level of LNGFR immunostaining. Supplementation with Schwann cells restored the number of surviving neurons. These results suggest that CNTF can provide trophic support, either directly or in conjunction with nonneuronal cells, to traditional NGF target neurons and can modulate the effects of NGF on its target neurons. Supported by NINDS grant NS-16349.

267.5

CNTF AND LIF REGULATE VIP GENE EXPRESSION BY SIMILAR MECHANISMS. A.J.Symes*,S.E.Lewis, S.E.Hyman and J.S.Fink Molecular Neurobiology Laboratory, Massachusetts General Hospital and Departments of Neurology and Psychiatry, Harvard Medical School, Boston, MA 02114

Neurotrophic molecules have been shown to influence neuronal phenotype, although little is known of the molecular mechanisms through which they act. One such molecule, ciliary neurotrophic factor (CNTF), promotes the differentiation of cultured sympathetic neurons towards the cholinergic phenotype, inducing choline acetyltransferase and neuropeptides including vasoactive intestinal polypeptide (VIP). We have previously identified a human neuroblastoma cell line (NBFL) in which CNTF activates VIP gene expression. In this study we have identified *cis*-acting genomic sequences important to the induction of the VIP gene by CNTF. VIP-luciferase reporter plasmids, containing portions of the 5' flanking sequences of the human VIP gene, were transfected into NBFL cells, treated with CNTF, and the luciferase activity measured. We find that a 50bp region of the VIP gene, distinct from the previously defined cAMP responsive element, subserves transcriptional activation by CNTF. Leukemia inhibitory factor (LIF), another neurotrophic factor that can alter the phenotype of primary sympathetic neurons in a manner similar to that of CNTF, activates VIP gene expression through the same 50bp region in NBFL cells. These studies demonstrate that the neurotrophic molecules CNTF and LIF share common mechanisms of signal transduction.

267.6

REGULATION OF TYROSINE HYDROXYLASE EXPRESSION IN DOPAMINERGIC AND NORADRENERGIC NEURONS BY COOPERATIVE ACTIONS OF CNTF AND CATECHOLAMINES. E. Magal*, P. Burnham, S. Varon and J.C. Louis. Dept. Biology, Univ. of California, San Diego, La Jolla, CA 92093.

While there is growing evidence for a role of ciliary neurotrophic factor (CNTF) on selected sets of CNS neurons, no effects of CNTF have been described so far on the catecholamine neurons. We report here that CNTF promotes the expression of tyrosine hydroxylase (TH) by cultured dopaminergic neurons from E16 rat substantia nigra (SN) and noradrenergic neurons from E18 locus cœruleus (LC). In SN as well as in LC cultures, TH-positive cells decrease progressively to about 30% of their original number determined after 3 hours in vitro. CNTF is able to i) maintain the number of TH-positive neurons in SN and LC cultures for 3 days when given at the time of seeding, and ii) restore the population of TH-positive neurons to its original level when given for 24 hours after a delay of 2 days (SN and LC) or even 5 days (LC). In both cases, however, CNTF is effective *only* when the neurons own neurotransmitter, e.g. dopamine (DA) for SN neurons and norepinephrine (NE) for LC neurons, was applied *simultaneously*. While NE alone does not affect TH expression in LC neurons, DA is able by itself to increase the number of TH-positive cells in SN cultures, albeit to a lesser degree than the CNTF/DA combination. Experiments with selective agonists and antagonists indicate that the effects of DA and NE are mediated through D2-DA receptors and α2-adrenoceptors, respectively. These results define a novel mode of neurotransmitter enzyme regulation in SN and LC neurons by CNTF and the neurons own neurotransmitter, acting in convergence and in an autocrine/paracrine fashion to replace the instructive influences to which the neurons are exposed in vivo. Supported by NINDS grant NS-16349.

267.7

RECOMBINANT HUMAN CILIARY NEUROTROPHIC FACTOR STIMULATES METABOLIC RATE INCREASES IN SH-SY5Y CELLS AS MEASURED BY A CYTOSENSOR MICROPHYSIOMETER. R. M. Johnson*†, P. A. McNeeley†, B. S. Glaeser§ and S. Pitchford§. †Dept. of Neurosciences, Institute of Pharmacology, Syntex Discovery Research, Palo Alto, CA 94304 and §Molecular Devices Corp., Menlo Park, CA 94025.

Information on the transmembrane signalling events and subsequent biochemical processes initiated by ciliary neurotrophic factor (CNTF) receptor activation in neurons is lacking. SH-SY5Y cells, a human neuroblastoma cell line expressing CNTF receptors, were used to study metabolic changes associated with functional ligand-receptor interactions. Real-time measurements of the acidification rate of the external medium bathing the cells after stimulation with recombinant human CNTF (rhCNTF) were continuously monitored by detecting small changes in extracellular pH using a silicon-based microphysiometer. Application of rhCNTF or nerve growth factor (NGF) to resting SH-SY5Y cells increased their metabolic rate in a concentration dependent manner (200 - 1000 ng/ml). Maximum metabolic rate increase was observed with 1 μg/ml rhCNTF and NGF (basal rate in μVolts/sec = 67.6 ± 1.1; rhCNTF, 104 ± 0.5; NGF, 130.9 ±1.0; mean ± S.E.M., N = 6-19). Application of 1 μg/ml basic fibroblast growth factor (bFGF) to the cells did not affect metabolic rate (68.8 ± 2.9 μVolts/sec, N=9). These results are consistent with reports demonstrating SH-SY5Y cells express functional CNTF and NGF but not bFGF receptors. The study provides evidence suggesting that the signal transduction mechanism used by the CNTF receptor in SH-SY5Y cells ultimately stimulates effector systems responsible for increasing a cellular metabolic response.

267.8

CNTF PREVENTS CELL DEATH AND MAINTAINS $p75^{NGFR}$ BUT NOT ChAT IMMUNOREACTIVITY IN DEVELOPING SEPTAL CHOLINERGIC NEURONS FOLLOWING NGF WITHDRAWAL IN VITRO

J.N.C. Kew, C.N. Svendsen*, S.J. Stevens and M.V. Sofroniew. Department of Anatomy, University of Cambridge, U.K

Ciliary neurotrophic factor (CNTF) promotes the survival of various embryonic neurons in vitro and prevents the degeneration of certain neurons after injury in vivo. The effects of CNTF* were examined on septal cholinergic neurons grown in sandwich tissue cultures. Septal cultures (E16 embryos) maintained in the presence of nerve growth factor (NGF) for 14 days from plating contain a population of neurons which stain intensely for $p75^{NGFR}$, ChAT and AChE. Without added NGF, very few cultured septal neurons stain for these markers. At 14 days, removal of NGF and addition of NGF antibodies (NGF-Ab) causes the progressive loss of ChAT, AChE and $p75^{NGFR}$ staining. By four days after addition of NGF-Ab there is widespread degeneration and cell death with fewer than 20% of the cholinergic neurons remaining. Under identical conditions of NGF withdrawal, the addition of CNTF in the presence of NGF-Ab significantly prevents cell death and maintains $p75^{NGFR}$ immunoreactivity in about 90% of the cholinergic cells, while by 4 days ChAT and AchE staining are lost. In addition, CNTF added to septal cultures from plating, leads by 14 days to a similar number of $p75^{NGFR}$, but not ChAT or AChE stained, neurons as seen with addition of NGF. These results suggest that CNTF can act as a survival factor for septal cholinergic neurons in vitro, and can maintain $p75^{NGFR}$ but not ChAT and AChE expression in these neurons. *Recombinant CNTF kindly provided by Regeneron

267.9

AXONAL RETROGRADE TRANSPORT OF CNTF; MODULATION BY PRIOR NERVE INJURY AND SOLUBLE CNTF RECEPTOR. R. Curtis*, Y. Zhu, R.M. Lindsay and P.S. DiStefano. Regeneron Pharmaceuticals, Tarrytown, NY 10591.

Ciliary neurotrophic factor (CNTF) is known to promote the survival and modulate phenotypic differentiation of several neuronal types, including sensory and motor neurons. We show that, like nerve growth factor and the other neurotrophins, ^{125}I-CNTF (rat) can be retrogradely transport and visualized in dorsal root ganglion neurons and spinal cord motor neurons after injection into sciatic nerve. Moreover, the retrograde transport of CNTF is increased by a conditioning crush lesion (1 or 7 days previously) and by coinjection of soluble recombinant CNTF receptor. Depletion of endogenous CNTF from Schwann cells by axotomy did not affect ^{125}I-CNTF transport. Northern blot analysis showed that CNTF receptor mRNA was increased in DRG and ventral spinal cord 24 hr post sciatic nerve crush. This up-regulation of CNTF receptor may be the mechanism by which CNTF transport is enhanced. Retrograde transport of CNTF may represent a critical step in the response of sensory and motor neurons to injury.

267.10

AXONAL RETROGRADE TRANSPORT OF CNTF AND LEUKEMIA INHIBITORY FACTOR (LIF) SHARE A COMMON RECEPTOR MECHANISM. P. S. DiStefano*, R. M. Lindsay and R. Curtis. Regeneron Pharmaceuticals, Tarrytown, NY 10591.

Ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) are ligands for a related family of cytokine receptors and may share common receptor components (N.Y. Ip, et al, Cell, in press). ^{125}I-CNTF and to a greater extent ^{125}I-LIF are retrogradely transported to dorsal root ganglion neurons and spinal cord motor neurons after injection into sciatic nerve. Cross-competition studies showed that LIF blocked ^{125}I-CNTF transport but CNTF could not inhibit ^{125}I-LIF transport. Prior nerve injury and coinjection of soluble CNTF receptor (CNTF-R alpha) enhance both ^{125}I-CNTF and ^{125}I-LIF transport. The interleukin-6 (IL-6) binding-protein, gp130, has been suggested to be component of the CNTF/LIF receptor complex, but IL-6 was without effect on either ^{125}I-CNTF or ^{125}I-LIF transport. These results suggest that sensory and motor neurons respond, via retrograde transport, to both CNTF and LIF through overlapping receptor mechanisms, involving CNTF-R alpha.

267.11

Ciliary Neurotrophic Factor (CNTF) Delays Motor Impairments in the Mnd Mouse, a Genetic Model of Motor Neuron Disease. M. E. Helgren*, B. Friedman, M. Kennedy, K. Mullholland, A. Messer, V. Wong and R. M. Lindsay. Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

CNTF is a trophic factor which supports the survival of motor neurons and certain PNS neurons in vitro. In these studies we have tested the effects of CNTF on preventing or slowing the progression of motor dysfunction in the Mnd mouse, a mutant characterized by adult-onset, progressive motor impairments. We have designed methods to quantify this motor impairment and demonstrated deterioration of locomotor patterns in Mnd mice as evidenced by shift from a reciprocal gait pattern to a synchronous hopping pattern during the progressive stages of the disease. Using this behavioral assay we compared the effects of an 8 week treatment with recombinant human (rh) CNTF (n=42) or a vehicle solution (n=42) in Mnd mice. Animals were tested weekly to evaluate the rate of change in locomotor patterns. Quantitative analysis of foot-fall patterns show that the magnitude and rate of decline in measurements of stride length and intra-step distance are significantly less in CNTF-treated mice as compared to vehicle-treated Mnd mice. Experiments in progress aim to assess anatomical changes that may underlie these behavioral effects. The results indicate that rhCNTF can slow the progression of motor dysfunction in the Mnd mouse and is a useful model in testing therapeutic agents for the treatment of motor neuron diseases.

267.12

INDUCTION OF MOTOR NEURON SPROUTING *IN VIVO* BY CILIARY NEUROTROPHIC FACTOR AND BASIC FIBROBLAST GROWTH FACTOR Mark Gurney*, Hirotaka Yamamoto, and Young Kwon. Department of Cell, Molecular & Structural Biology, Northwestern University, 303 East Chicago Ave. Chicago, IL 60611.

Ciliary neurotrophic factor (CNTF) and basic fibroblast growth factor (bFGF) were tested for effects on sprouting by motor neurons innervating the adult mouse gluteus muscle. Factors were delivered by subcutaneous injection directly over the surface of the superior gluteus muscle once daily for 7 days. Endplates and axons were visualized in a whole mount of the gluteus muscle by combined silver and cholinesterase staining. CNTF (500 ng daily) induced sprouting from both endplates and from the subset of nodes of Ranvier that are closest to the endplate. CNTF increased the occurrence of endplates with sprouts approximately 30 fold to 12.7 $\pm$ 3.5 % (SEM) from 0.4 $\pm$ 0.2% in muscles treated only with BSA. Combined treatment with CNTF and 2 ng bFGF daily potentiated the effect of CNTF approximately 2 fold to 30.3 $\pm$ 2.9%, while bFGF alone had no discernable effect on the occurrence of sprouting from endplates. Sprouting from the node of Ranvier closest to the endplate increased from 0.4% $\pm$ 2% in muscles treated with BSA to 1.9 $\pm$ 0.6% after treatment with CNTF, and to 4.6 $\pm$ 0.6% in muscles treated with CNTF and bFGF, while treatment with bFGF only had no effect. The sprouting stimulus delivered by CNTF showed limited penetrance into the muscle. Beneath the site of injection, 27.9 $\pm$ 6.1% of the endplates on the dorsal surface of the gluteus muscle developed sprouts, while only 7.5 $\pm$ 2.6% of the endplates on the ventral surface of the muscle had sprouts. Thus, CNTF is a candidate for a physiological mediator of sprouting *in vivo*.

267.13

CILIARY NEUROTROPHIC FACTOR (CNTF) PREVENTS MOTONEURON DEATH IN THE DEVELOPING RAT SNB. *N.G. Forger*, S.L. Roberts, V. Wong[1] and S.M. Breedlove, Dept. Psychology, Univ. California, Berkeley, CA 94720 and [1]Regeneron Pharmaceuticals, Tarrytown, New York, 10591.*

Many motoneurons die during the course of vertebrate development, although the molecular mechanisms determining survival remain unknown. CNTF, originally isolated from chick eye as a survival factor for ciliary neurons *in vitro*, also preserves embryonic rat and chick motoneurons *in vitro* and chick motoneurons *in ovo*. We have exploited the perinatal period of motoneuron death in the rat spinal nucleus of the bulbocavernosus (SNB) to test the ability of CNTF to rescue developing rat motoneurons *in vivo*. Most motoneurons in the sexually dimorphic SNB of females normally degenerate between embryonic day 22 (E22) and postnatal day 10 (P10). Female Sprague-Dawley rat pups were, therefore, delivered by cesarean section on E22 and injected with CNTF (1 μg) or vehicle on E22, P1, P2, and P3. Animals were killed on P4 and the number of motoneurons and pyknotic cells determined in the SNB region. CNTF treatment markedly increased SNB motoneuron number (CNTF: 209 $\pm$ 14 vs Vehicle: 122 $\pm$ 10; $p < 0.001$) and decreased the number of degenerating cells to less than 1/3 that seen in controls ($p < 0.001$). These results establish CNTF as the first protein shown to alter naturally occurring motoneuron death in mammals.

Supported by NIH grant #NS28421.

267.14

LOCAL ADMINISTRATION OF CNTF ENHANCES PERIPHERAL NERVE REGENERATION *IN VIVO*. C.F. Da-Silva*[1], F. Langone[2], A. Negro[2] and R.S. Pires[1]. [1]Dept. of Histology, University of Sao Paulo, Brazil and [2]Fidia Research Laboratories, Italy.

The aim of this study was to take advantage of the entubulation repair model to study the *in vivo* role of exogenously administered ciliary neuronotrophic factor (CNTF) during peripheral nerve regeneration.The left sciatic nerve of eight C57BL/6J adult male mice was transected and both the proximal and distal nerve stumps were secured by a single 10-0 suture into a 6-mm length of a polyethylene tube (0.76 mm ID) to give a final gap length of 4 mm. The animals were divided into 2 groups of 4 animals each. One group was implanted with tubes filled with a purified preparation of collagen (Vitrogen, 2.4 mg/ml). The second group was implanted with tubes filled with collagen plus CNTF (1:1, with 100 ng of CNTF in the tube). Recombinant human CNTF was produced and purified from *E. coli* (Negro et al, J. Neurosci. Res., 29:251, 1991). Following a survival time of 6 weeks, the tubes with the regenerating nerve cables were processed for Epon embedding. Myelinated nerve fibers were counted from the mid-portion of the cables with a computer-controlled system (Biographics). The results showed a significant difference in the number of myelinated axons between the collagen + CNTF group (3027$\pm$62, mean$\pm$SEM) and the collagen alone group (1813$\pm$135) ($p<0.05$, Neuman-Keuls). No difference was observed between the CNTF group and a group (n=4) of non-operated mice (3311$\pm$54). The data demonstrate that local application of exogenous CNTF causes a stimulation of axonal regeneration in adult animals. Studies with retrograde neuronal tracers (under way) should reveal a possible selective action of this factor on motor and/or sensory regenerating neurons.

Supported by grants from FAPESP and CNPq.

267.15

CILIARY NEUROTROPHIC FACTOR PROMOTES THE RETENTION OF MULTIPLE INNERVATION OF DEVELOPING SKELETAL MUSCLE FIBERS. Arthur W. English* and Gail Schwartz, Department of Anatomy & Cell Biology, Emory University, Atlanta, GA 30322.

To examine whether developing neuromuscular synapses compete for neurotrophic molecules which are available in limited quantities, the effects of exogenous ciliary neurotrophic factor (CNTF) on postnatal neuromuscular synapse elimination were investigated. CNTF was injected daily for one week into the lateral gastrocnemius muscle beginning on postnatal day 2 (P2). The effect of these injections was evaluated using intracellular recording techniques at ages P6-P16. At doses >200 µg/Kg, CNTF-injected muscles contained more polyneuronally innervated fibers and more synaptic inputs per cell, than either muscles from normal animals or from saline injected contralateral muscles. The amount of polyneuronal innervation remained fairly constant (at the amount expected at P9-P10) even one week after the cessation of injections. Lower doses of CNTF had no significant effect. Thus CNTF promotes the retention of multiple innervation of developing skeletal muscle fibers in a dose-dependent manner. Whether it exerts this effect directly or indirectly on the synapses is not known. Supported by NS20545 and Regeneron Pharmaceuticals Inc.

267.16

SPATIAL AND TEMPORAL EXPRESSION Of CNTF FOLLOWING PERIPHERAL NERVE INJURY. E.D. Rabinovsky*, G.M. Smith, J. McManaman[1] and H.D. Shine. Dept. of Neurosurgery, Baylor College of Medicine, Houston, TX 77030; [1]Synergin, Boulder, CO, 80503.

Ciliary neurotrophic factor (CNTF) is highly expressed in normal peripheral nerves and may be important in the survival of motoneurons and regeneration of injured motoneurons. Both CNTF mRNA and protein decrease in sciatic nerve within 7 days after a crush injury. The changes in CNTF expression are opposite from that of low-affinity NGF receptor ($p75^{NGFR}$) expression, which increases after crush. To examine the regulation of CNTF expression, we characterized the spatial and temporal patterns of CNTF expression after injury. Rat sciatic nerves were crushed, then 1,3,5, and 7 days later removed and cut into 4mm sections (1 segment proximal to crush site and 5 distal). CNTF expression in the segments was measured by western blot and northern blot analysis. Longitudinally sectioned nerves were immunostained for CNTF at 1,3,5 and 7 days after injury. There was no significant change in CNTF mRNA 1 day after crush, but CNTF protein decreased adjacent to the crush site. Both the amounts of mRNA and protein were reduced on day 3 and continued to decrease on days 5 and 7. Immunohistochemical staining localized CNTF to Schwann cell cytoplasm. Injury reduced staining distal to the crush. The decrease in CNTF expression followed the general course of Wallerian degeneration and implies that its regulation is, in part, governed by Schwann cell-axon association. Supported by grants from the Kleberg Foundation and the U.S.Navy (N00014-89-J3003).

268.1

INTERACTIONS OF CHONDROITIN SULFATE PROTEOGLYCANS FROM BRAIN WITH NEURAL CELL ADHESION MOLECULES.
A. Flaccus, M. Grumet, and R.U. Margolis*. Dept. of Pharmacology, New York University Medical Center, New York, NY 10016.

Ng-CAM is a cell adhesion molecule (CAM) that can bind homophilically as demonstrated by the ability of CAM-coated Covaspheres to self-aggregate. In the present study, we found that the aggregation of Covaspheres coated with Ng-CAM was strongly inhibited in the presence of two rat brain chondroitin sulfate proteoglycans (PGs), designated 1D1 and 3F8, at concentrations of <10 μg/ml; in contrast, much higher concentrations of a rat chondrosarcoma chondroitin sulfate PG had no effect on the aggregation. The chondroitin sulfate PGs from brain, but not from chondrosarcoma, also inhibited binding of neurons to Ng-CAM when mixtures of the proteins were adsorbed to polystyrene dishes. Neither of these effects are attributable to the chondroitin sulfate, inasmuch as similar results were observed with the core glycoproteins obtained by chondroitinase treatment of the PGs. To investigate whether cells could bind directly to the PGs, we have used an assay in which cell-substrate contact was initiated by centrifugation. Although no binding to the PGs was detected in gravity assays, neurons bound to the 1D1 and 3F8 PGs but not to chondrosarcoma PG in centrifugation assays. Different forms of the 1D1 PG have been identified in developing and adult brain. The early postnatal form was found to bind neurons more effectively, and neuronal binding to the 1D1 PGs was specifically inhibited by the 1D1 mAb. These results suggest that certain brain PGs can bind to neurons, and that Ng-CAM may be a heterophilic ligand for the 1D1 and 3F8 PGs. The abilities of these brain PGs to inhibit cell adhesion to CAMs may be one mechanism to modulate cell adhesion and migration in the nervous system.

268.2

INCREASED NEURITE OUTGROWTH ON GLIAL SCARS FOLLOWING ENZYMATIC DIGESTION OF CHONDROITIN SULFATE PROTEOGLYCAN
R.J. McKeon* and Jerry Silver. Dept. of Neurosciences, Case Western Reserve University, Cleveland, OH 44106

Recent evidence has demonstrated that an increased expression of chondroitin sulfate proteoglycan (CSPG) is correlated with a limited ability of glial scars to support neurite outgrowth *in vitro*. In order to directly test whether this molecule can actively inhibit neurite outgrowth on glial scars, Millipore filters were, first, implanted into the gray matter of the cerebral cortex in both neonatal or adult rats. Animals were allowed to survive for either 10 days or 1 month. During this time, CSPG is re-expressed in the area of the implant in adult but not neonatal animals and is qualitatively greater after 30 days than at 10 days. Next, the implants were removed, placed in culture, and used as a substrate for dissociated E-6 chick retinal ganglion cells (RGC). RGC's were grown for 48 hrs. in media either with or without 0.5 U/ml chondroitinase ABC, which specifically cleaves chondroitin sulfate GAG sidechains from the core protein. Cultures were then fixed and stained for RGC specific TU-J1. Neurite lengths were measured and digitized "blindly." Statistical analysis revealed a significant enhancement of neurite outgrowth on treated adult glial scars removed one month after implantation ($p<0.001$), as opposed to implants removed from adult animals after 10 days ($p=0.1$). Neurite lengths on implants removed after one month from animals implanted as neonates were virtually identical between the treated and untreated groups. These data are consistent with the hypothesis that CSPG is inhibitory to neurite outgrowth and plays a role in regenerative failure after injury to the adult CNS. Supported by a grant from the Paralysed Veterans of America Spinal Cord Research Foundation and NS 25713.

268.3

THE DEVELOPMENTAL CHARACTERIZATION OF GLIAL HYALURONIC ACID BINDING PROTEIN (GHAP) IN RATS.
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The developmental occurrence and localization of GHAP (glial hyaluronic acid binding protein) was studied in rats utilizing indirect immunofluorescence with the monoclonal antibody 12C5 as the primary antibody. Animals were sacrificed from G12 (gestational day 12) through 90d p.p. (postpartum) with the brain and spinal cord removed, ten micrometer fresh-frozen sections mounted on slides and fixed in -20°C acetone for 10-20 minutes.

GHAP is first detected at G15 in the olfactory tracts and periventricular regions of the brain and in the spinal cord as a diffuse, low level of staining in both the white and gray matter. In the brain, GHAP localizes to the corpus callosum and Purkinje-granule cell layers of the cerebellum by G19 at which time it is also beginning to localize in the ventral white matter tracts of the spinal cord; low levels, however, still remain in the spinal cord gray matter. At 3d p.p., there is focal GHAP staining in all major myelinated tracts in the brain, including the cerebellar fiber tracts. In addition, the olfactory fiber tracts remain GHAP positive and, by this age, the olfactory glomeruli are also GHAP positive. At this age, the spinal cord has its GHAP staining generally restricted to the white matter tracts with decreasing amounts of staining in the gray matter. From this point on, there is gradual refinement of the above patterns to assume the adult staining pattern of white matter tracts in the brain and spinal cord, the olfactory tracts and glomeruli, and with the cerebellum containing moderate staining in the granular cell layer with intense staining in the fiber tracts.

Supported in part by NIH Grant NS 13034 and V.A. Gen. Res. Service Merrit 0002.

268.4

DEVELOPING TASTE PATHWAYS IN SHEEP BRAINSTEM DEMARCATED BY GLIAL AND GLYCOPROTEIN MARKERS.
C.T. King*, L.F. Haus and C.M. Mistretta. School of Dentistry, Univ. of Michigan, Ann Arbor, MI 48109.

During central nervous system development, glia and extracellular matrix molecules demarcate functional pathways and groups of neurons. In the gustatory system, afferent fibers from the VIIth and IXth nerves enter and fasciculate within the brainstem in a stereotyped pattern as demonstrated by Lucifer Yellow labeling. To examine whether glia and/or extracellular matrix boundaries are associated with organization of the solitary tract, we are studying immunocytochemical localization of the glial markers, vimentin (VIM) and glial fibrillary acidic protein (GFAP), and the glycoprotein, tenascin (TN), in the developing sheep brainstem. Horizontal sections of the brainstem from fetuses aged 80-130 days of gestation (term = 147 days) were cut for immunocytochemistry. In midterm sheep fetuses, VIM and GFAP immunoreactive processes are seen within the solitary tract and also provide an outline for the rostral solitary nucleus. Bundles of fibers within the tract are surrounded by a network of intense TN immunoreactivity, suggesting that tenascin promotes fasciculation of the fibers. These patterns are apparent during periods when taste fibers are assembling the solitary tract and neurons in the solitary nucleus are acquiring more complex dendritic arbors and an increasing number of spines. Later in gestation, patterns of GFAP and TN immunoreactivity are essentially unchanged but seem more distinct. Experiments are in progress to determine whether these markers are developmentally regulated and contribute to the initial organization of the solitary tract and nucleus. (Supported by NIH Grant DC00456).

268.5

TENASCIN DISTRIBUTION DURING DEVELOPMENT OF TASTE ORGANS SUGGESTS ROLES IN REGULATING TASTE BUD MULTIPLICATION AND PAPILLA INNERVATION.
C.M. Mistretta* and L.F. Haus. School of Dentistry, Univ. of Michigan, Ann Arbor, MI 48109.

Tenascin is an extracellular matrix molecule with demonstrated roles in segregation, migration and aggregation of cells during tissue differentiation and morphogenesis. We used a rabbit anti-human polyclonal antibody to localize tenascin immunoreactivity in developing fungiform and circumvallate papillae and taste buds, in fetal, perinatal and postnatal sheep. Taste organs were studied from stages of initial papilla morphogenesis through advanced stages of papilla development and periods of taste bud differentiation and proliferation. The spatial pattern of tenascin immunoreactivity (IR) changes during papilla development in fetal sheep: from an initial intense localization in connective tissues beneath the early taste papillae; to diffuse localization within the core of papillae; to a very limited distribution in regions of the papillae core that are directly under epithelium in which taste bud multiplication is occurring. Tenascin IR is absent from the papilla core under nongustatory papilla epithelium. In postnatal animals, tenascin IR is intense and once again is diffuse, throughout the core of taste papillae. At all stages, tenascin IR outlines bundles of nerve fibers within the papillae. In summary, tenascin IR becomes progressively restricted to gustatory portions of taste papillae in fetal and perinatal animals. A role for tenascin in papilla morphogenesis and taste bud multiplication is suggested. Tenascin might modulate adhesion between taste bud and papilla cells and substrate by stimulating de-adhesion at certain areas. In addition, the pattern of IR suggests a role for tenascin in fasciculation of nerve fibers within taste papillae. (Supported by NIH Grant DC00456).

268.6

TENASCIN-LIKE MOLECULES IN A DEVELOPING INSECT OLFACTORY SYSTEM.
C.E. Krull[1]*, D.B. Morton[1], L.P. Tolbert[1], M. Schachner[2], A. Faissner[3]. [1]ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ, [2]Swiss Fed. Inst. of Technology, Zurich, Switz., and [3]Dept. of Neurobiology, Univ. of Heidelberg, Heidelberg, Germany.

Glial cells form boundaries around developing olfactory glomeruli in the antennal lobe (AL) of the brain of the moth *Manduca sexta*, and appear to be necessary for the induction by olfactory axons of glomerular branching patterns in AL neurons. Previously (Krull et al., Soc. Neurosci. Abstr., 1991), we demonstrated that the glial cells in the developing AL are recognized by antibodies against human and mouse tenascin; the binding gradually decreases with maturation. We now have further characterized the proteins labeled by these antibodies at different stages of development using Western blot analysis and have begun tests of the effects of tenascin on neurite outgrowth *in vitro*. The major bands recognized by antibody at all stages of development are two high-molecular weight proteins of approximately 220 and 160 kD. The 220-kD band comigrates with purified mouse tenascin. Following extraction in 2M urea and centrifugation of AL material at 10,000 x g for 5 min, most of the 220- and 160-kD antigens appear in the supernatant, suggesting that they are not integral membrane proteins. Preabsorption of the antibody with mouse tenascin blocks staining of the 220-kD band (as well as the immunocytochemical staining of glia in sections). In tissue culture experiments, AL cells grown on a substrate of con A/laminin plus purified tenascin show decreased adhesion and process outgrowth compared with cells grown on con A/laminin alone. Thus, it appears that molecules of molecular weights and antigenicity similar to vertebrate tenascins are present in the developing AL and that tenascin may inhibit growth of the neurites of AL neurons across glomerular boundaries.

268.7

DO PIONEERING OLFACTORY AXONS INDUCE THE RAT OLFACTORY BULB?

Q. Gong*, M.S. Bailey and M.T. Shipley. Department of Anatomy & Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

We are testing the hypothesis that the earliest olfactory axons induce the formation of the olfactory bulb from the telencephalic vesicle.

GAP43 labels olfactory axons as early as E12 but these axons do not reach the telencephalon. By E13, a few GAP43+ axons enter the rostromedial part of the telencephalic vesicle. These axons penetrate directly to the ependymal zone. At E14, there is no obvious "olfactory bulge", but large numbers of GAP43+ olfactory axons penetrate through the mantle layer and marginal zones to reach the ventricular zone in a highly restricted sector of the telencephalon. By E15, the olfactory bulge is visible as a evagination of the rostromedial part of the telencephalon. At this stage, most GAP43+ olfactory axons terminate in the intermediate zone of this bulge.

Immunostaining of CDA1, an antigen selectively expressed in growth cones, was in excellent agreement with the GAP43 observations. The tract tracer, "fast" DiI, injected into the nasal cavity demonstrated that the GAP43+ axons originate from the olfactory epithelium. DiI labeled axons penetrate to the ventricular zone exactly as do the GAP43+ axons.

These results demonstrate that the olfactory axons reach the mitotic zone of the telencephalon prior to the induction of the olfactory bulb. Pioneering olfactory axons, thus, may modulate the proliferation of the cells in this specific zone of the telencephalon to induce the formation of the olfactory bulb. We have developed a method to double label BrdU and GAP43. This will be used to compare proliferation in the zone innervated by pionerering olfactory axons with other parts of the telencephalon. *(Supported by NIDCD DC00347 and NINDS NS29218)*

268.8

ROLE OF INHIBITORY ECM MOLECULES IN THE FORMATION OF OLFACTORY BULB GLOMERULI.

M. de L. Gonzalez* and J. Silver. Dept. of Neuroscience, Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

We have described the normal anatomy of the olfactory bulb (OB) throughout development, focusing on the role of purported barrier molecules, that are associated with astroglial cells, as potential guidance factors in glomeruli formation. Glomeruli are unusual structures within the OB formed by extremely tight bundles of afferents that fail to grow deeply into the brain. Our data suggests the hypothesis that Cytotactin/Tenascin (CT) and Chondroitin-6-sulfate proteoglycan (C-6s-PG), in the core of the early developing OB, together form a molecular wall that helps position the ingrowing olfactory axons within the olfactory nerve layer (ONL) at the outer edge of the astroglial territory. Using olfactory marker protein antibodies, we found that at E18 olfactory axons are in long strands at the outermost edge of the bulb. By E20-21 axons cluster and form small spheres and by PD1 they gather into distinct glomeruli. Prior to and during the transformation of olfactory afferents into glomeruli that form at the interface between ensheathing cells and astrocytes, there was an enhanced expression of both ECM molecules CT and C-6s-PG associated with VIM/GFAP positive astroglial cell processes, deep to the glomeruli. As glomeruli continue to develop at later stages (PD3-PD9), the GFAP positive astroglial processes beneath them intensify their GFAP, maintain their expression of CT and C-6s-PG, and then migrate peripherally into the ONL to encapsulate and infiltrate the glomeruli. Thus, at later stages, axon/glia interactions occur which result in astroglial rearrangement and interaction with the glomeruli. We suggest that the astroglia and ensheathing cells play a fundamental role in directing the position at which formation of the synaptic glomeruli will occur. Since the olfactory axons continue to elongate during development, the continuous presence of the astroglial barrier may also help the axons condense into their characteristic bundled shape.

268.9

LECTIN BINDING PATTERNS IN THE DEVELOPING RAT TRIGEMINAL SYSTEM.

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The plant lectin peanut agglutinin (PNA) conjugated to HRP was used to demonstrate patterns of extracellular matrix molecules in the whisker pathway of postnatal rats. Infraorbital nerve (IO) lesions at birth were used to disturb the development of the normal whisker related patterns in animals studied from birth (P0) to 20 days old. Details of processing and sectioning have been reported previously (see Steindler & Cooper '86, J. Comp. Neurol. 249:157; Christensen & Woolsey '88, Soc. Neurosci. Abstr. 14:1273). In normal rats, there is no whisker-like pattern in any of the trigeminal centers on P0. Patterns appear in the trigeminal spinal subnuclei - interpolaris and caudalis - on P1. They emerge in the principal nucleus of V and VB thalamus on P2, and may be present in the cortex as early as P4. Patterns persist at all levels until P10 although the intensity of binding is reduced. The whisker patterns are absent at all levels by P20. IO lesions abolish the pattern all levels. In comparison to the timing of PNA binding patterns in postnatal mouse trigeminal system, the rat develops patterns about one postnatal day later but the patterns persist longer. Given other studies which suggest that the rat is farther along in pattern formation of this pathway at birth this is further evidence for a non-sdirective role of these markers in development.

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268.10

NEW AND IMPROVED METHODS FOR ASSESSING DENDRITIC DEVELOPMENT RELATIVE TO SOMATOTOPIC PATCH BORDERS IN THE BARREL NEURAXIS.

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Many developing granule cells in barrel cortex acquire polarized dendritic trees by selective pruning and reorientation relative to thalamocortical patch boundaries. Whether similar rules apply to the developing dendritic trees of trigeminal brainstem neurons is not clear, although we know that most of the cells in trigeminal nucleus principalis (PrV) in adult rats have polarized trees. In attempting to resolve this issue, a number of methods proved inadequate to the task. Golgi staining combined with pattern markers, such as cytochrome oxidase or transported fluorescent dyes, failed to provide sharp whisker patches, silver preservation and/or complete reconstructions. In vivo tracer injections into thalamus always made a lesion that had a confounding effect on PrV dendritic development. Lucifer yellow staining of PrV cells in the fixed slice, while producing complete fills, impeded cytochrome oxidase staining in regions exposed to Lucifer yellow. In our hands, the only successful method to date optimizes conditions for intracellular injection and utilizes postinjection immunohistochemical staining of both Lucifer yellow filled cells and matrix components preferentially present in the inter-patch septa, such as J1-tenascin. Preliminary data suggest that in newborn to 7 day old rats, most PrV dendrites are polarized and they do not cross tenascin-stained, whisker-related, patch borders. Notable exceptions are dendrites from the minority of PrV cells that have large somata and are likely to be responsive to multiple whiskers.

Support: NIH NS29885, DE07662, DE07734.

268.11

EFFECTS OF POSTNATAL BLOCKADE OF CORTICAL ACTIVITY UPON THE DEVELOPMENT AND PLASTICITY OF VIBRISSAE-RELATED PATTERNS IN THE SOMATOSENSORY CORTEX OF RAT AND HAMSTER.

W.R. Bauer*, N.L. Chiaia, S.E. Fish, B.A. Figley, M. Eck, C.A. Bennett-Clarke and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

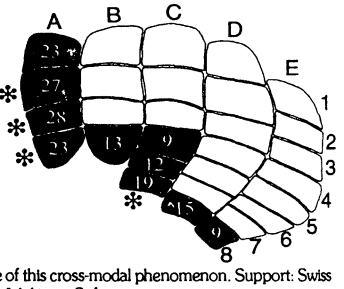
Neuronal activity influences pattern formation in a number of neural systems. In this study, we determined whether or not this was also true in the somatosensory systems of rodents by silencing the primary somatosensory cortex of animals of hamsters and rats with either TTX or a combination of TTX and the NMDA receptor antagonist APV for the first 6-9 days of life. Tetrodotoxin-impregnated implants were also applied to the cortices of rats and hamsters that sustained removal of a single row of mystacial vibrissae follicles or transection of the entire infraorbital nerve (ION, the trigeminal branch which innervates the vibrissa pad) on the day of birth. Neither application of TTX alone nor TTX in combination with APV prevented the formation of the vibrissa-related pattern in the primary somatosensory cortex of either species as visualized with either anterograde tracing with the carbocyanine dye, Di-I, or immunocytochemistry for serotonin (5-HT). Furthermore, TTX implants had no appreciable influence upon the postnatal cortical reorganization observed after either destruction of a row of vibrissae follicles or transection of the ION. These data indicate that normal physiological activity that can be blocked with TTX and APV is not required for either qualitatively normal pattern formation or the postnatal development of lesion-induced abnormal patterns in the somatosensory cortex of either hamsters or rats. NS 28888, DE 08971, DE 07734

268.12

EXPANSION OF THE BARRELFIELD AFTER NEONATAL ENUCLEATION IN MICE.

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

We investigated the effect of neonatal eye removal on the tangential extent of the barrelfield in adult mice. Areas were measured in drawings made from tangentially cut Nissl-stained sections of somatosensory cortex. We compared areas of 29 barrels - shown in figure - corresponding to 29 mystacial vibrissae, between 60 days old mice enucleated at birth (n=13) and their intact littermates (n=13). Multivariate ANOVA showed that the barrelfield was larger in enucleated mice (by 6%; $p<0.0028$). This expansion was almost entirely due to the areal increase of barrels corresponding to the dorsalmost row of vibrissae (row A), and of a set of barrels corresponding to rostral vibrissae near the nose and mouth. These barrels are rendered black in the figure; numbers within barrels indicate the percentage of areal expansion in enucleated versus control mice; asterisks are placed beside barrels that showed significant areal increase ($p<0.0017$); other labels indicate nomenclature for barrels in rows (letters) and arcs (numbers). Assessment of the follicular innervation density in enucleated mice will help to better understand the substrate of this cross-modal phenomenon.



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268.13

RELATIONSHIP BETWEEN SUBCORTICAL AND CORTICAL REORGANIZATION AFTER FETAL CAUTERIZATION OF VIBRISSAE FOLLICLES IN RAT. R.W. Rhoades*, N.L. Chiaia, C.A. Bennett-Clarke, G.J. Janas, M. Eck and C.M. Fisher. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

Cauterization of vibrissae follicles in rats on embryonic day (E-) 15 through E-18 results in an increase in the brainstem areas devoted to the representation of the remaining follicles. Lesions at later ages (E-19 through the day of birth [P-0]) result in smaller and statistically non-significant increases in the brainstem representations of undamaged follicles. This study compared the changes in the areas devoted to the representation of a given follicle in trigeminal subnucleus interpolaris (SpI) and the primary somatosensory cortex (S-I) of rats that sustained vibrissae follicle lesions on E-15 through P-0 with the aim of determining: 1) the degree to which the brainstem changes are magnified or reduced in cortex, and 2) whether the sensitive period for expansion of the representation of intact follicles in SpI and S-I are the same or different. Follicle cauterizations on E-15 resulted in significant increases in the representations of the remaining follicles in both SpI and S-I and there was no significant difference in the magnitude of the changes in these two regions. Similar damage on E-16 through E-18 resulted in significant increases in both SpI and S-I with those in S-I exceeding those in SpI. Damage on E-19 and P-O resulted in non-significant changes in SpI and small, but significant, increases in S-I. These data show that the sensitive period for central reorganization after peripheral damage ends later in S-I than in SpI and that changes in the brainstem are generally magnified in cortex. NS 28888, DE 07734

268.14

ANATOMICAL AND FUNCTIONAL CHANGES IN THE ORGANIZATION OF THE CUNEATE NUCLEUS OF ADULT RATS AFTER FETAL FORELIMB AMPUTATION. N.L. Chiaia*, J.T. Wall, H.P. Killackey, C.A. Bennett-Clarke, and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699

Fetal forelimb removal in rat increases the portion of the somatosensory cortex devoted to the hindlimb representation and it has been suggested that this may result from primary afferent reorganization in the dorsal column nuclei (H.P. Killackey and D.R. Dawson, Eur J. Neurosci., 1:210-221,1989). The present study used both anatomical and electrophysiological techniques to test this proposal. Rats sustained forelimb removals on embryonic day 16 and were used in terminal experiments as adults. The cuneate nucleus ipsilateral to the lesion decreased in volume by an average of 36.7% ($N=7$, $p=<0.001$), but there was no corresponding increase in the volume of the gracile fasciculus and nucleus. Bilateral application of HRP to the sciatic nerves demonstrated that axons which innervate only the gracile nucleus on the intact side of the brainstem were present in the deafferented cuneate nucleus. Injection of HRP into the skin overlying the point of the amputation (the stump) indicated that axons innervating this region filled most of the dorsal one-half of the shrunken cuneate nucleus and overlapped with the sciatic nerve afferents on this side. Receptive field mapping demonstrated a zone in the deafferented cuneate nucleus devoted to the skin overlying the stump and adjacent shoulder. Only 9.1% of the unit clusters ($N=328$) in this region were also excited by hindlimb stimulation. Unit clusters with receptive fields including the skin overlying the stump and the hindlimb were located throughout the rostrocaudal extent of the cuneate nucleus. NS 28888, DE 07734, NS 21105, BNS 90-22168

268.15

EARLY FETAL DEVELOPMENT AND MATURATION OF RAT VAGAL MOTOR NEURONS. L. Rinaman* and P. Levitt. Medical Coll. of Pennsylvania, Dept. Anat. & Neurobiol., Philadelphia, PA 19129.

The embryonic development and maturation of vagal motor neurons was examined, with an emphasis on neurons innervating the proximal gut and their central relationship to gastric sensory afferents. Embryos (E12-E21) were fixed and crystals of dil were placed in the proximal gut or cervical vagus nerve. After 14-30 days, sections were examined to visualize vagal sensory and motor neurons projecting to the dil placement site. At E12, the nodose ganglia contain gastric sensory neurons with central axons that enter superficial layers of the dorsal medulla (tr. solitarius) with little or no arborization. At E13, a few labeled motor neurons (presumably nucleus ambiguus; NA) are seen midway between the dorsal and ventral medullary surface. Some NA neurons appear to be migrating, trailing neurites that reach medially towards the 4th ventricle; others exhibit proximal dendritic arbors. Labeled NA motor axons exit laterally, separate from sensory afferents. At E14, sensory axons penetrate deeper in the dorsal medulla and have a more extensive rostrocaudal distribution, and a new group of labeled motor neurons (presumably dorsal motor nucleus of the vagus; DMV) are seen near the germinal zone of the 4th ventricle. They are completely separate from labeled sensory fibers, do not form a distinct nucleus, and have short trailing processes extending to the outermost cell layer of the ventricular zone. Their axons exit the ventral medulla along with the axons of scattered labeled NA neurons. By E18, the adult nuclear organization of the vagal complex is evident, with distinct NST, DMV and NA; furthermore, some DMV motor neuron dendrites enter the NST sensory terminal field. These data show a remarkable early onset of vagal sensory and motor interactions during embryonic development. Supported by MH45507 and NRSA 1F32NS08900.

268.16

DEVELOPMENT OF AN INTERNEURONAL PATTERN WITHIN THE SPINAL CORD. A. Chen* and R.D. Heathcote. Department of Biological Sciences, University of Wisconsin, Box 413, Milwaukee, WI, 53201.

Although the central nervous system has a high degree of functional organization, it is not clear how much is due to the spatial arrangement of neurons. Recently, a population of interneurons was shown to extend the length of the spinal cord of the larval frog *Xenopus laevis*. The catecholaminergic cells were located in two longitudinal columns in the ventral spinal cord and established a uniformly spaced pattern along each column.

The formation of the unique catecholaminergic pattern was examined during embryonic and larval development. The first tyrosine hydroxylase immunoreactive neurons appeared at 1.4 days (stage 28) of embryonic development. The low numbers and irregular spacing of embryonic cells showed that the pattern emerged gradually during development. The first cells differentiated at random sites along the ventral midline of the spinal cord. Many cells were separated by long distances (>100 μm), and some formed groups of regularly spaced cells within a column. By one week of development (stage 48) the mean distance between cells had decreased to approximately 20 μm, reflecting a sustained increase in the size of uniformly spaced groups of cells. Even though the spinal cord continued to increase in length, the spacing between cells remained constant, indicating that cells were added to new regions of the spinal cord. The domain of catecholaminergic neurons also gradually expanded anteriorly toward the posterior border of the hindbrain during postembryonic development. Thus the mechanisms directing pattern formation during embryogenesis persist into larval life. The establishment of the boundary and spacing of catecholaminergic interneurons could show how at least portions of the central nervous system become partitioned into functional groups of cells.

268.17

DEVELOPMENT AND ORGANIZATION OF THE WHITE MATTER OF THE RAT SPINAL CORD. L. A. Gomez, A. Brusco*, E. M. Lopez and J. Pecci Saavedra. Instituto de Biología Celular, Facultad de Medicina, Paraguay 2155, 1121 Buenos Aires, Argentina.

In a previous study we have demonstrated an organized glial pattern in the brainstem of the rat embryo (IBRO Abstr. 1991, pp 35). In order to investigate the possible participation of radial glia in the assembly of the developing spinal cord, vibratome sections (Embryos aged: E13 to E18) were immunostained for S-100 protein, vimentin or the phosphorylated 160 kDa neurofilament protein.

Vimentin immunostaining revealed radial glial fibers (RGF) extending from the ventricle to the pial surface. In the peripheral zone the RGF became superimposed to form cephalocaudal plates (CP) that ensheathe developing axonal tracts (evidenced by neurofilament immunostaining). This pattern was first evidenced at E13 in a small area of the ventral portion; in correspondence with the increment of axonal tracts the CP became denser and extended ventrolaterally.This glial pattern was only found in the white matter. In the central zone RGF do not form CP; this pattern was found in the gray matter. Immunoreactivity to S-100 protein appeared at E17 in the midline and, at E18, overlapped to vimentin immunostaining. The pattern of glial plates is similar to the organization demonstrated in previous studies in the brainstem. Is is concluded that the glial pattern revealed in this study is the result of interactions between developing axonal tracts with radial glia, and that it could set the structural basis for an organized assembly of the developing spinal cord.

(Work performed with grants from CONICET, Argentina).

268.18

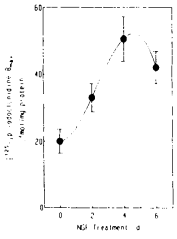
TEMPORAL PATTERNS OF MUSCLE FORMATION IN THE AVIAN LIMB BUD. C. Lance-Jones* and J. Van Swearingen. Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

The muscle cells of the avian limb originate from the adjacent somites and migrate into lateral mesoderm as the limb bud is forming. What factors govern where somitic cells will migrate to within the limb and what fiber types they will become? We have examined the relationship between the time that a muscle cell precursor leaves the somite and that cell's fate with respect to limb position and fiber type. At different stages during the migration period (stages 16-18) chick limb buds were removed to a host embryo's coelomic cavity to isolate the bud from further population by somitic cells. Examinations of stage 32-34 limb bud sections stained with H&E or antibodies to fast and slow isoforms of myosin heavy chain (kindly provided by Crow and Stockdale) suggest that early migrating muscle cell precursors contribute predominantly to muscles that normally contain a large number of slow fibers. Compatible results are obtained in quail-chick limb bud chimeras. We have begun to assess slow muscle fiber numbers in limbs that developed from transplantations of an early chick limb bud onto an older chick host. Preliminary results suggest a depletion of slow fibers, thus supporting the hypothesis that there are distinct slow and fast limb muscle cell lineages prior to migration into the limb. Supported by NIH HD25676.

269.1

I_1-IMIDAZOLINE BINDING SITES IN PC12 CELLS ARE UP-REGULATED BY NERVE GROWTH FACTOR (NGF). P. Ernsberger* Departments of Medicine & Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

Imidazolines bind with high affinity not only to α-adrenergic receptors, but also to I-sites, which can be labeled by [^{125}I]*p*-iodoclonidine ([^{125}I]PIC; proposed I_1) or by [^{3}H]idazoxan (I_2). I_1-Imidazoline sites have been found in adrenal chromaffin cells; in the present study the PC12 cell line was used. Washed P2 membranes were incubated 30 min at 25°C with 0.5 nM [^{125}I]PIC; nonspecific binding was defined by 0.1 mM naphazoline. In PC12 membranes, specific [^{125}I]PIC binding was not inhibited by the adrenergic agents epinephrine (0.1 mM), rauwolscine (10 μM) or SK&F86466 (1 μM) (binding as % of control was 127±6, 110±5, and 93±2, respectively). The imidazol(in)es cimetidine (1 mM) and cirazoline (3 μM) completely inhibited binding (as % of control, 1±5 and 2±3, respectively). Thus, PC12 express I_1 but not α_2 sites. [^{125}I]PIC binding was inhibited by guanine nucleotides (GTPγS = Gpp(NH)p > GTP), implying that I_1 are G-protein coupled. Crude subcellular fractions were prepared and assayed for the density of I_1 and muscarinic sites. Both receptor sites were enriched in plasma membranes relative to mitochondrial, P1 and P3 fractions. Daily treatment with 200 ng/ml NGF increased the density of I_1 sites within 2d (Figure). I_1 density reached a peak of 250±30% of control at 4d, and did not increase further by 6-7 d of treatment. Increased I_1 density coincided with initial neurite outgrowth. The ligand specificity of I_1 binding was not affected by NGF. PC12 cells express I_1-imidazoline sites which are (a) sensitive to guanine nucleotides, (b) neither mitochondrial nor nuclear, and (c) up-regulated by differentiation with NGF. I_1 sites may be developmentally regulated neuronal receptors.



269.2

SPATIOTEMPORAL CHANGES IN EXPRESSION OF TWO FORMS OF A DELAYED RECTIFIER K+ CHANNEL PROTEIN IN NEURONS ARE SIMILAR IN VIVO AND IN VITRO. **N.J. Lenn* & J. Trimmer. Dept. Neurol, Pediatr, Biochem, Cell Biol, SUNY, Stony Brook, NY 11794**

Two major forms of the delayed rectifier K+ channel exist in rat brain neurons, a lower molecular weight polypeptide present from embryonic to adult ages and a higher molecular weight form that increases postnatally. They can be distinguished by their immunoreactivity (IR) to two site-directed antibodies, pGEXdrk1 and KC. IR of these two antibodies with E18 dissociated, cultured rat hippocampal neurons (courtesy of G.Banker) were ranked by random, blinded observations of 20-30 cells per age per antibody on a 0/+/++ scale. pGEXdrk1-IR occurred at all times studied, 6-25 days in vitro (DIV), not changing with increasing DIV (p>0.23 for plasma membrane, cytoplasm and processes). KC-IR, present in the cytoplasm of cell body at all DIV (p>0.9), increased between 10-13 DIV in plasma membranes and between 13-17 DIV in processes (all p<0.0001). Changes in expression of these K+ channel isoforms may be intrinsic to neurons or relate to neuronal interaction, perhaps synaptogenesis.

269.3

Quantification of the Transient Expression of γ-aminobutyric acid (GABA) in the Developing Rat Optic Nerve. J.Y. Lim[1,4], S. Ochi[1,4], M.J. During[3], K. Sakatani[1,4], C.C. Duncan*[2], & J.D. Kocsis[1,4], Dept. of Neurology[1], Dept. of Neurosurgery[2], & Sect. Neuroendocrinology[3], Yale Medical School, New Haven, CT 06510; and VAMC[4], West Haven, CT 06516.

Our electrophysiological studies on developing rat optic nerve indicate the transient presence of $GABA_A$ receptor and endogenous GABA during the first three postnatal weeks. Immuno-electron microscopic studies have localized the GABA in developing rat optic nerve to glia and premyelinated axons. Evidence also indicates endogenous release of GABA which leads to depolarization in the developing rat optic nerve. However, an accurate biochemical quantification of the transient expression of GABA has not yet been established.

To determine GABA content in intact rat optic nerve, high pressure liquid chromotography (HPLC) was employed at 3 postnatal time points: P3(N=3), P19-20(N=2), P>90(N=4). Optic nerves were rapidly removed, frozen in liquid nitrogen, and then assayed for GABA. These measurements, normalized to tissue protein (modified Bradford), displayed a peak of 5.558±0.993 ng GABA/μg protein (mean±standard deviation) at P3. The GABA presence then sharply declined to 1.223±0.072 ng GABA/μg protein at P19-20 and 0.662±0.178 ng GABA/μg protein at P>90. These results are consistent with the time course of GABA activity shown by electrophysiological and immuno-electroscopic studies and serve as an independent, biochemical confirmation of the transient expression of GABA during development of the rat optic nerve.

Supported in part by the NIH and the Dept. of Veterans Affairs.

269.4

EVIDENCE FOR AN UPSTREAM ELEMENT WHICH ENHANCES THE TRANSCRIPTION OF THE MOUSE TYROSINE HYDROXYLASE GENE. W. W. Morgan*, M. Gamez and K. A. Rodriguez. Dept. Cellular and Structural Biology, Univ. Texas Hlth. Sci. Ctr. at San Antonio, TX 78284-7762.

Precise molecular genetic mechanisms limit the expression of the tyrosine hydroxylase (TH) gene to catecholaminergic neurons located within select nuclei of the brain, the sympathetic chain ganglia and paraganglia and adrenal medullary cells. To investigate these mechanisms, chimeric genes were constructed by linking 3.5, 1.1, 0.8 or 0.3 kb of the 5' flanking DNA of the mouse TH gene to a *E. coli* beta galactosidase reporter (Lac Z). Each of these constructs included the +1 initiation site for TH mRNA synthesis. The chimeric genes were introduced separately into PC 12 cells by lipofection. Stably transfected cell lines were established by co-transfection of RSV neo and subsequent G418 selection. Several clones, each originating from a single cell containing one of the above chimeric genes, were expanded for analysis. The enzymatic expression (picomoles/minute/μg protein) of the Lac Z reporter as well as the levels of transcript synthesized from the reporter were more that 50 fold greater in clones stably transfected with chimeric constructs containing 1.1 or 3.5 kb of TH 5' DNA as compared to those containing 0.3 or 0.8 kb of 5' DNA. The size of the transcript produced was the same across the different constructs and was consistent with initiation from the +1 site for TH mRNA synthesis. Analysis of southern blots provided no evidence for marked differences in the number of constructs incorporated into the genome of the PC 12 cells. Collectively, these results provide evidence for the presence of an element between 0.8 and 1.1 kb upstream which markedly enhances the expression of the TH promoter. Supported by DA00755 and GM43763 to WWM.

269.5

DAMAGE TO THE STRIATAL DOPAMINE SYSTEMS IN EARLY DEVELOPMENT SELECTIVELY SPARES THE MATRIX-DIRECTED DOPAMINE SYSTEM. P.A. Frohna*, L. Rioux, B.S. Neal, and J.N. Joyce, Depts. Psychiatry and Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA, U.S.A..

Destruction of the nigrostriatal patch-directed and mesostriatal matrix-directed DA systems in the rat with 6-OHDA have been used as models for exploring regulation of DA receptors, DA-mediated behaviors, and DA biochemistry of the striatum. It is evident that lesions made in early development compared to adult lesions result in very different effects. Recent studies from this laboratory have begun to elucidate the neural mechanisms underlying these age-related differences. We have shown that during development, expression of DA receptors in adults is controlled by the integrity of DA afferents at the time when receptor proliferation occurs. 6-OHDA lesioning of the DA system innervating the patch compartment during early development spares the matrix innervation and causes a loss of D1 receptors from the patch compartment and a behavioral syndrome consisting of a selective D1 supersensitivity

The present study focuses on the consequences of intrastriatal 6-OHDA lesions made at postnatal day 1(P1) on surviving patch- and later developing matrix-oriented DA afferents of the SN-VTA complex. We performed: 1) *In-situ* hybridization histochemistry for tyrosine hydroxylase (TH) and D2-receptor mRNA, 2) TH-immunohistochemistry, and 3) Receptor autoradiography for DA uptake sites (^{3}H-mazindol) and D2 receptors (^{125}I-epidepride), in the SN-VTA complex of P1 6-OHDA lesioned and saline-injected animals at postnatal day 90. There is a significant reduction in the number of neurons expressing TH mRNA and TH immunoreactivity from the ventral tier of the SNpc and SNpr of 6-OHDA treated animals. [^{3}H]mazindol and [^{125}I]epidepride binding are almost absent from the SNpc of this group. The VTA is unchanged between groups in all measures, and may show slightly elevated levels of TH mRNA following 6-OHDA lesion. Thus, the late-developing matrix-directed DA system is spared by P1 6-OHDA lesions but may be biochemically altered in these animals. **Funded by R29 MH 43852.**

269.6

TIMING OF THE LESION IS A CRITICAL DETERMINANT OF THE LONG-TERM EFFECTS OF NEONATAL DOPAMINE (DA) DEPLETION IN THE RAT. B.S. Neal*, S. Daly and J.N. Joyce. Depts. of Psychiatry and Pharmacology, Univ. Penn. Sch. of Med., Phila., PA 19104 and Dept. of Clin. Pharmacology, Royal College of Surgeons, Dublin, Ireland.

The DA system undergoes a great deal of postnatal development, which occurs in three distinct stages (Devl. Br. Res. 60:161,1991). To explore how loss of DA during these stages disrupts the functional expression of DA receptors, neonatal rats received bilateral intrastriatal injections of 6-hydroxydopamine (6-OHDA) on day of birth/postnatal day 1 (P0/P1), P7 or P15. Controls received vehicle injections in lieu of 6-OHDA. On P90 (adulthood), half of the rats were killed and brains processed for neurochemical studies (See also, Frohna *et al.*, this session). The remaining rats began behavioral testing. Rats were challenged with L-DOPA (DA precursor), and various DA agonists (3 doses per drug). P0/P1-lesioned rats exhibited an increased incidence of oral dyskinesias (vacuous chewing, jaw tremor, tongue protrusions) following a low dose of the mixed DA agonist apomorphine (APO; 0.1 mg/kg) and the partial D1 agonists SKF38393 and SKF77434, but not with the full D1 agonist SKF82958 (0.32-3.2 mg/kg). P7-lesioned rats did not exhibit oral dyskinesias; instead, they were hyperactive (*i.e.*, increases in locomotor activity, rearing and jumping). They also engaged in more grooming behavior after APO (0.1-1.0 mg/kg), SKF38393 (1-10 mg/kg), and SKF77434 (0.1-1.0 mg/kg). The D2 agonist quinpirole also induced grooming in the P7 group, suggestive of altered interactions between D1 and D2 receptors. SKF82958 did not induce grooming; rather, the rats were extremely hyperactive, aggressive and exhibited explosive jumping. The P15 group did not respond like the other groups. These findings support our hypothesis that the functional effects are dependent upon the timing of the DA loss. The neurochemical studies are underway to determine what types of receptor changes underlie these behavioral effects. **(Supported by a Tourette Syndrome Association grant and USPHS grant MH43852).**

269.7

THE EFFECT OF *IN UTERO* ADMINISTRATION OF BUTHIONINE SULFOXIMINE ON RAT DEVELOPMENT. E. Reyes*, S. Ott, B. Robinson and R. Contreras. Department of Pharmacology, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131.

Glutathione (GSH) is a tripeptide that is thought to be an essential cell component playing an important role as a cellular antioxidant and scavenger of free radicals. GSH depletion has been shown to render cells more sensitive to various insults, whereas, an increase in GSH has a protective effect. GSH levels can be decreased by inhibition of its synthesis with buthionine sulfoximine (BSO) which inhibits γ–glutamylcysteine synthetase. Several studies have shown that treatment with BSO enhances the toxicity of some drugs and radiation. A previous study indicated that the effects of BSO on the developing embryo were short lived and did not persist to birth. In the above mentioned study the mothers were treated with BSO on days 10 and 11 of gestation. The objective of the present study was to determine the effects of GSH depletion throughout pregnancy on the developing rat. Timed pregnant Sprague–Dawley rats were placed on a liquid BioServ diet containing BSO (2 to 8 mmole/Kg/24h) starting on day one of pregnancy. The mothers were maintained on the diet until gestation day 21 when they were anesthetized with sodium pentobarbital and the pups delivered by Cesarean Section. Various parameters relating to development were determined and GSH levels were measured in brain and liver. A dose response curve showed that a maximum depletion of GSH in the mother's liver was produced by the 8 mmole dose of BSO. An 86% decrease in liver GSH was observed. However, no change was seen in brain GSH levels. GSH levels in brain and liver of the offspring were decreased by 60% and 66%, respectively. No significant effect of treatment with BSO was observed on growth related parameters, such as body weight or brain weight. A significant decrease in neuron specific enolase (NSE) activity in cerebellum and an increase in liver γ–glutamyl transpeptidase activity were observed in pups born to mothers treated with BSO. A decrease in NSE activity and an increase in γ–GTP activity are both consistent with delayed development. Therefore, we conclude that although a decrease in GSH may not produce teratogenic effects it may produce a delay in development and may have a permissive role in teratogenic effects produced by other drugs

(Supported by NIH/MBRS RR 081399 and NIAAA AA 08072.

269.8

RETINOIC ACID RESPONSIVENESS AND DEVELOPMENTAL EXPRESSION OF THYMOSIN ß10 FUSION GENE IN TRANSGENIC MICE. S.-C. Chen, R. J. Smeyne, C. Bocchiaro, J. G. Corbin, R. J. Wurzburger* and J. I. Morgan, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

Thymosin ß10 is an acidic polypeptide that is present in high concentration in the immature nervous system, but that is a minor component in the adult brain. In addition, the expression of this gene is up-regulated by the morphogen, retinoic acid (RA), in a variety of neuroblastoma cell lines eg., B104. The rat thymosin ß10 gene has been isolated and used to identify its RA-responsive element(s). We now report that expression vectors encoding the human retinoic acid receptors can activate chloramphenicol acetyltransferase (CAT) expression from thymosin ß10-CAT chimeric constructs in B104 cells. Through the use of deletion and mutation analyses, the RA-responsive element of the thymosin ß10 gene has been identified on its 5' flanking region.

Using lacZ as a reporter, we have constructed transgenic mice that carry a ß10-lacZ fusion gene and examined its expression during development. This fusion gene contains a 1.3 kb upstream region of the thymosin ß10 gene and its expression was first detected at embryonic day 8.5. High levels of expression of this construct was observed in the embryonic nervous system, especially sensory neurons. The expression of this fusion gene was found to be regulated developmentally. Several other constructs, with different lengths of the thymosin ß10 promoter, have also been used to dissect out the regulatory elements responsible for the developmental expression of the thymosin ß10 gene.

269.9

EXPRESSION OF COMPLEMENT C1qB mRNA DURING BRAIN DEVELOPMENT. SA Johnson*, I Rosovsky, M Etchells and CE Finch. Neurogerontology Division, Andrus Gerontology Center and Dept of Biol. Sci., University of Southern California, Los Angeles, CA 90089-0191.

We recently demonstrated the presence of several complement (C) component mRNAs in neurons and microglia of normal brain, and have found up-regulation of C mRNAs during Alzheimer's disease (AD) (Johnson, et al., submitted). Previous immunocytochemical studies localized C components to plaques and dystrophic neurites in AD brain. Considering the normal cytotoxic role of the C system, a humoral defense arm of the immune system, these data suggest C could play a role in AD pathology. C1qB and C4 mRNAs are also increased in afferent targets after acute deafferenting lesions (Pasinetti, et al., Exp. Neurol., in press), where a lack of neurodegeneration in the afferent target suggests C may be involved in synaptic reorganization. Because C1qB is present in normal human and rat brain, it is important to ascertain the expression of C mRNAs during brain development. Northern blot hybrization showed C1qB mRNA was present in brain from as early as E14. Initial in situ hybridization data at E16 shows cellular localization of C1qB mRNA, predominantly in meningeal zones, with scattered cells in thalamic, hypothalamic, striatal, cortical and hippocampal primordia. Cells of the ventricular zone and cortical plate lamina in the cerebral wall are generally negative, except for a few scattered positive cells. The data are consistant with C expression in blood-borne cells, but probably not neurons, at E16, which is prior to formation of the blood brain barrier. Later developmental stages are being examined. Supported by AG-07909 (CEF) and AG-10673 (SAJ).

269.10

DEVELOPMENTAL REGULATION OF *c-fos* AND ITS RESPONSIVENESS IN SPINAL CORD CULTURES. C. G. Palkovits, D. E. Brenneman, S. C. Fitzgerald, R.W. Summers* and D.V. Agoston, Laboratory of Developmental Neurobiology, NICHD, National Institutes of Health, Bethesda, MD 20892

fos and *fos*- related proteins are transcription factors that couple extracellular stimuli to cellular responses by altering the expression pattern of their target genes. During the "critical period" of neuronal differentiation, the expression of genes is modulated by such extracellular factors. We have choosen *fos*-immunohistochemistry to assess neuronal responsiveness to various environmental stimuli during different stages of neurodifferentiation in the embryonic spinal cord-dorsal root ganglia culture (SC-DRG). The earliest expression of *fos*-immunoreactivity was seen after 7 days in culture (DIC), restricted to neuronal nuclei. This expression was driven by the spontaneous electrical activity and could be blocked by tetrodotoxin (TTX). Changing the medium to fresh serum-rich medium caused up-regulation of *fos*-immunopositivity in the nuclei of neuronal and background cells alike. In neurons but not in background cells, this effect decreased as a function of age and was blocked by TTX. BayK6844 and (+) 202791, agonists of the L-type voltage-sensitive calcium channel (VSCC), dramatically upregulated *fos* expression in neuronal nuclei but not in nuclei of background cells. BayK 6844 was most effective in 15 DIC cells whereas (+) 202791 showed maximum effect in 21 DIC neurons. The antagonists of L-type VSCC Nifedipine and (-) 202791 reduced constitutive *fos* expression an age-dependent way. Pretreatment with BayK 6844 or (+)202791 prevented TTX-induced downregulation of *fos* expression in a similar age-dependent manner. Increasing extracellular calcium levels alone did not increase constitutive expression or prevent TTX-caused downregulation of *fos*-protein. Veratridine depolarization increased *fos* -expression in 4 DIC neurons only, whereas exposure of cells to PMA caused a substantial increase of *fos*- expression in all but the youngest cultures; however mostly in non-neuronal cells. Our results indicate that the expression of *fos* as nuclear signalling molecule, can be used to assess the responsiveness of the developing nervous system to various extracellular stimuli.

269.11

EXPRESSION OF RB, CDC2, NSE, AND FOS BY NEURONAL PRECURSOR CELLS IN THE ADULT BRAIN. H.J. OKANO*, D.W. PFAFF, AND R.B. GIBBS. Lab. of Neurobiol. & Behav., The Rockefeller Univ., NY, NY 10021.

Relatively little is known about cell-cycle regulation during neurogenesis. In the present study we examined the expression of cell-cycle regulatory proteins by neuronal precursor cells located in the hippocampal formation of adults. Male, Sprague-Dawley rats (180-250 g) were killed 2 hours - 4 weeks after receiving a single injection of 3H-thymidine (4μCi/g.b.w. i.p.). Adjacent, 30 μm sections were cut and processed for immunocytochemical detection of mammalian cdc2 (G6 antiserum; Dr. D. Beach), RB (Pharmingen Pharm.), Fos and Fos-related antigens (Dr. M. Iadarola), and neuron-specific enolase (NSE; Chemicon Pharm.). Sections were then dipped in NTB-3 emulsion and radiograms were developed 2-6 weeks later.

3H-Thymidine-labeled (3H-T) cells were detected in the subgranular region of the dentate gyrus (DG). Two hours post-injection (n=3), 79% of these 3H-T cells contained RB-like immunoreactivity (RB-I) and 33.8% contained cdc2-I. After 12 hours the number of 3H-T cells in the DG had doubled. At this time, the percentage of 3H-T cells double-labeled with RB-I decreased to 54.9% while the percentage double-labeled with cdc2-I increased to 42.7%. At later time-points, the percentage of 3H-T cells double-labeled with RB-I decreased to 41.5%, 1.4%, and 0.3% at 24 h, 1 week, and 4 weeks respectively. Similarly, the percentage of 3H-T cells double labeled with cdc2-1 was 43.5% at 24 h, and then decreased to 1.7% and 0% at 1 week and 4 weeks. Very few (0-1%) 3H-T cells in the DG were double-labeled for NSE-I 2-24 h post-injection. This percentage increased significantly to 13.9% and then to 80.6% at 1 week and 4 weeks, suggesting that the majority of these cells had differentiated into neurons. Very few (<1%) 3H-T cells were double-labeled with Fos-I at any time-point. However, seizure activity (induced by pentylene tetrazol) was able to induce Fos-I within 3H-T cells in the DG as early as 1 week post-injection, suggesting that these cells had formed functional connections. The data demonstrate that RB and cdc2 expression are regulated during neurogenesis in the adult hippocampal formation. A similar analysis of neurogenesis in the adult olfactory bulb is in progress and will be discussed. Supported by NIH grant #NS28896.

269.12

EXPRESSION OF THE HLH PROTEINS ND-1, MASH-1, AND ID IN DEVELOPING MOUSE NERVOUS SYSTEM. M. P. Armanini*, G. R. Laramee, J. W. Winslow, and H. S. Phillips, Dept. of Neuroscience, Genentech, Inc., S.S.F., CA 94080.

In situ hybridization was employed to examine the distribution of mRNA for the HLH proteins ND-1, MASH-1, and Id in the developing mouse. A series of embryos from E 7.5 to 17.5 was examined. Hybridization signals for ND-1 and Id were widely expressed in the earlier embryos. With increasing age, Id expression became quite restricted within the nervous system while ND-1 expression was maintained at high levels in several regions of the CNS. MASH-1 signal was transiently expressed in the developing sympathetic ganglia and enteric nervous system as well as in selected regions of the developing CNS. At embryonic days 9.5 to 11.5, mRNA for ND-1, MASH-1, and Id all display prominent expression in the ventricular zone of the neural tube. From E 11.5 to 17.5, Id is progressively eliminated from the ventricular zone and shows very limited expression in CNS. In marked contrast, as development procedes, ND-1 expression remains prominent within the CNS. At all ages investigated, ND-1 expression is maintained at high levels throughout the germinal zone of the lateral ventricle and is prominent in numerous telencephalic structures. MASH-1 expression within the CNS, appeared to be largely restricted to germinal zones. The pattern of ND-1 and MASH-1 mRNA expression suggests some regions of neurogenesis in which ND-1 and MASH-1 might associate with one another, but indicates other regions in which these molecules might be expected to associate with other dimeric cohorts.

269.13

Regulation of c-myc RNA Expression in the Developing Nervous System. G. Weisinger and J.D. DeCristofaro, Dept. of Peds. and Neurobiol., SUNY, Stony Brook, N.Y.

The c-myc protooncogene has been implicated in the regulation of normal cellular growth and differentiation for all but two neuronal cell lines. Recently, Xu et al [MCB 11, 6007 (1991)] demonstrated that c-myc steady state RNA expression is elevated in whole brain total RNA preparations from neonatal mice. This c-myc RNA expression was turned off by 3 weeks of age. We wanted to determine whether c-myc steady state RNA was developmentally regulated in rat nervous system tissues, and if so, whether these tissues are linked to cellular proliferation or not. Total RNA was prepared from numerous nervous system structures from 1 day and 3 week old rat pups and studied by northern analysis. Our data indicates that c-myc RNA is not exclusively associated with cellular proliferation within the developing rat nervous system. In the olfactory bulb and adrenal medulla, there is a tight correlation with cellular proliferation, but in the cerebellum, cerebral cortex and hippocampus, this is clearly not the case. Hence, c-myc may have a nonmitogenic function. Interestingly, c-myc RNA levels in the neonatal cerebral cortex were much greater than that found in the adult rat thymus, a tissue expressing high levels of c-myc RNA. Using this data we want to determine at what transcriptional level these changes occur so that we can develop an understanding of what factors and mechanisms control c-myc, in vivo. Sponsored by NIH RR05736.

269.14

CHARACTERIZATION OF BC1 scRNP, A BRAIN-SPECIFIC CYTOPLASMIC RIBONUCLEOPROTEIN COMPLEX. J.G.Cheng*, H.Tiedge and J. Brosius. Fishberg Center for Neurobiology and Dept. of Molecular Biology, Mt. Sinai Sch. of Med., New York, NY 10029

The brain specific small cytoplasmic RNA (BC1 RNA) in the rat contains many interesting features. It is the first known RNA polymerase III transcript with neuronal specificity. The expression of BC1 RNA in the CNS is regionally and developmentally regulated. Furthermore, it is one of the few RNAs which is actively transported into dendrites. From sequence comparison, BC1 RNA is conserved only in rodents. However, a small RNA that is an analog but not a homolog to BC1 RNA has been characterized in primates.

Four pieces of evidence support the notion that BC1 RNA exists as a ribonucleoprotein complex *in vivo*; first, by running brain extract into CsCl or Cs_2SO_4 gradient the bouyont density of fractions containing BC1 RNA signal is 1.45 gm/ml and 1.25 gm/ml, respectively, which is consistent with the density of a protein-RNA complex; second, the BC1 particle has a larger S value (8.5S) than naked RNA (4.5S) in sucrose gradients; third, the BC1 RNA signal from brain extract migrates with retarded mobility in agarose electrophoresis; finally, specific portions of BC1 RNA are protected by certain proteins and become more RNase resistant. Compared with the signal recognition particle, integrity of BC1 RNP is not Mg^{++} dependent and is more heat resistant. By using conventional purification (chromatography and ultracentrifugation) as well as RNA binding assays performed on protein blots, there are at least one protein which appears to directly interact with BC1 RNA. Necessary amounts of BC1 RNP are being purified for characterization of binding proteins.

GLIA AND OTHER NON-NEURONAL CELLS II

270.1

EXTRACELLULAR MATRIX MOLECULES IN THE RAT AND HUMAN OPTIC NERVE: CHANGES IN PATTERNS OF EXPRESSION AFTER INJURY. W.P.Battisti*, J.Wang, J.Kennerdell[1], and M. Murray, Med.Coll. Pa., Phila., Pa. 19129 and Allegheny Gen'l. Hosp.[1], Pittsburgh, Pa.

The environment of the CNS is important in regulating regrowth following injury. We used immunocytochemistry to compare the distribution of tenascin, chondroitin sulfate proteoglycan (CSPG), HNK-1, and laminin, molecules implicated in facilitating or inhibiting regrowth, in the human and rat optic nerve, species in which regeneration does not normally occur. The human optic nerve is organized in large fasicles containing axons and their associated glial cells, each delineated by basal lamina. Each of the molecules examined shows a unique and constitutive expression in the normal optic nerve. Tenascin staining is ubiquitous and strong labeling is seen over the basal lamina, within axon fascicles, and also defining large interfascicular nonneuronal cells. CSPG and HNK-1 staining are limited to areas of axon fascicles but each with different patterns. Laminin stains external basal lamina. Injured human optic nerve, obtained subsequent to eye trauma or disease, shows a decrease in intensity and a change in the pattern of staining for tenascin, CSPG and HNK-1. Laminin is unchanged. These extracellular matrix molecules have similar staining patterns in the normal rat optic nerve. However, the pattern of expression and intensity is different after crush. In rat optic nerve sacrificed 2 weeks PO, tenascin, CSPG and HNK-1 staining appear normal proximal to the crush but their association with the nonneuronal cells that fill the area at the crush and distally is marked and the pattern of association is distinct. Laminin staining appears within the crush site, but the staining is weak. We are currently examining the population of nonneuronal cells that may contribute to this environment and the colocalization of these cells with the distribution of the ECM molecules. Supported by NS 16556 and ASRI 910161.

270.2

DIFFERENTIAL REGULATION OF CONNEXIN-43 AND -32 mRNA IN THE DEAFFERENTED STRIATUM AFTER A UNILATERAL CORTICAL LESION. H-W. Cheng *and T.H. McNeill. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089-0191.

During development, electrical coupling via gap junctions constitute and important mode of intercellular communication by allowing for direct cell to cell communication between both neurons and glia. However, little information exists regarding the formation of gap junctions between either neurons or glia following experimental brain lesions. To address this issue we examined changes in the prevalence of mRNA for two gap junctional proteins, connexin-43 (Cnx-43) and -32 (Cnx-32), between 1 and 27 days postlesion in the deafferented striatum (ST), following a unilateral cortical lesion. Based on previous immunocytochemical studies, it is suggested that Cnx-43 is associated with the formation of gap junctions between glia; while Cnx-32 is part of the gap junctional complex formed primarily between neurons.

By northern blot and in situ hybridization analysis, we found an increase in the prevalence of mRNA for Cnx-43 but not Cnx-32 in the deafferented ST following the ipsilateral cortical lesion. In addition, the time course for changes in the prevalence of Cnx-43 mRNA was correlated with the temporal sequence of increased glial reactivity as measured by changes in protein and mRNA for GFAP at the lesion site. In the dorsal aspect of the deafferented ST, an increase in Cnx-43 mRNA prevalence was found at 3 days postlesion and reached a maximum at 10 days postlesion. By 27 days postlesion Cnx-43 mRNA was reduced but still elevated over intact controls. These data suggest that the formation of gap junctions between glia are involved in orchestrating the cellular response of reactive astrocytes in the ST following a cortical lesion. However, the precise role of electrically coupled astrocytes during reactive synaptogenesis remains unclear.

270.3

DIFFERENTIAL MODULATION OF GFAP and SGP-2 mRNA FOLLOWING TWO FORMS OF STRIATAL DEAFFERENTATION. P. Elyse Schauwecker*, H-W. Cheng[1], T.J. Collier[2], and T.H. McNeill[1]. [1]Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA 90089, [2]Dept. of Neurobiol. & Anat., Univ. of Rochester, Rochester, NY 14642.

Previous studies have reported that reactive astrocytes play an important role in both removing degenerative debris and the reinnnervation of target neurons following brain injury. However, few studies have correlated the time course of the astrocytic response with the time course of reactive synaptogenesis. In order to address this issue, we examined changes in two astrocytic proteins, GFAP and SGP-2, following a unilateral lesion of either the cortex or substantia nigra (SN), which differ with respect to total synaptic input, transmitter content and reinnervation response. Temporal changes in mRNA prevalence for GFAP and SGP-2 were measured at 3, 10, and 27 days postlesion in the ST following either a nigral lesion with 6-OHDA or unilateral cortical ablation.

Using northern blot and in situ hybridization analysis, we found an increase in GFAP mRNA in the ipsilateral ST following both the nigral and cortical lesions. However, the time course of the GFAP response differed betwen the two lesions. Following the nigral lesion, a transient increase in GFAP mRNA was found at 3 days postlesion which rapidly returned to control levels by 10 days postleison. No changes in SGP-2 mRNA were found. By comparison, both GFAP and SGP-2 mRNA remained significantly increased at all timepoints after a unilateral cortical lesion. These results support the notion that reactive astrocytes perform multiple time dependent functions during reactive synaptogenesis and that the time course of the astrocytic response corresponds to the cellular events that characterize the reorganization of synaptic circuits following a specific deafferentation lesion.

270.4

INCREASES IN GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP)-LIKE IMMUNOREACTIVITY (IR) IN THE RAT SUPERIOR CERVICAL GANGLION (SCG) AFTER DECENTRALIZATION, AXOTOMY AND EXPLANTATION. U. Vaidyanathan, H. Hyatt-Sachs* and R.E. Zigmond. Department of Neurosciences, Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

Studies of the effects of decentralization, axotomy and explantation of the SCG have focused primarily on changes occuring in neurons in the ganglion. Evidence in the literature indicates, however, that some of these manipulations also affect ganglionic non-neuronal cells (e.g., Elfvin et al., Cell Tissue Res. 250:79, 1987). We have examined the effects of all three manipulations on GFAP-IR in the SCG. GFAP staining in control ganglia was seen in satellite cells apposed to some principal neurons. In some instances, immunoreactive cells surrounded a principal neuron. Forty-eight hours after cutting the preganglionic cervical sympathetic trunk or the postganglionic internal and external carotid nerves or after placing ganglia in organ culture, there was a large increase in GFAP-IR in the SCG. Most principal neurons were surrounded by immunostained cells, and the perineuronal area of staining was considerably greater than that seen in control tissue. The magnitude of these changes was greatest after explantation and smallest after decentralization. When only one of the postganglionic trunks of the SCG was cut, the increase in GFAP-IR occurred primarily in that half of the ganglion known to contain the cell bodies of neurons projecting out that trunk.

270.5

POSTGANGLIONIC AXOTOMY AND 6-HYDROXYDOPAMINE (6-HDA) PRODUCE A BURST OF SATELLITE CELL PROLIFERATION IN ADULT RAT SUPERIOR CERVICAL GANGLION (SCG). M. Bachoo, A.K. Hall*, C. Polosa, R. Zigmond, Case Western Reserve Univ. School of Medicine, Cleveland, OH; McGill Univ., Montreal, PQ.

The possibility that increased glial fibrillary acidic protein (GFAP) staining seen in SCG after axotomy (Vaidyanathan et al., this volume) is accompained by glial cell proliferation was tested by immunofluorescent detection of an incorporated thymidine analogue, bromodeoxyuridine (BrDU), using microscopic image analysis. The number of labeled nuclei/unit area was compared between experimental and sham treated rat SCG sections (3-5 animals/ group). A dramatic increase in the number of BrDU labeled nuclei throughout the SCG was seen 48 hrs after internal and external carotid nerve section (16 fold) or 6-HDA administration (75 mg/kg i.v. twice at 12 hr intervals; 11 fold). Double labelling showed BrDU labeled cells to express GFAP but lack fibronectin and laminin. Frequently, BrDU labeled cells were in close proximity and surrounding tyrosine hydroxylase-labeled neurons, suggesting that they were likely to be satellite glial cells.

The time course of cell proliferation following axotomy was determined by administering BrDU for a 24 hr period before sacrifice. Significant proliferation was detected after 1 day (4 fold), dramatically increased at 2 days (14 fold), moderately increased at 3 days (5 fold), and persisting at a low level (3 fold) at 1 and 2 weeks, relative to sham-treated SCG. These results suggest that lack of a retrograde target derived growth factor may produce a cell body response which stimulates satellite cell proliferation.

270.6

REACTIVE ASTROCYTE FORMATION IN THE SPINAL DORSAL HORN IS REDUCED BY ß-ADRENERGIC RECEPTOR BLOCKADE. J. Sutin* and R. Griffith, Dept. of Anatomy and Cell Biology, Emory Univ. School of Medicine, Atlanta, GA 30322.

Astrocytes in primary cultures express ß-adrenergic receptors (ß-ARs) and isoproterenol accelerates the transformation from an ovoid, epithelial-like appearance to a stellate, process bearing morphology (McCarthy et al., J. Neurochem. 10:723 1985). Since astrocytes isolated from several regions of the adult rat brain possess ß-ARs (Shao and Sutin, Glia 6: 1992 in press), we examined the effect of the ß-AR antagonist propranolol on the formation of reactive astrocytes in the rat spinal cord. The lectin *Ricin communis* (1.5 µg) was injected into the sciatic nerve on one side to destroy dorsal root ganglion cells and motor neurons. Seven days later sections from the lumbar spinal cord were processed for GFAP immunocytochemistry and the optical density in Laminae I - III of the intact dorsal horn compared with the corresponding region on the side with primary afferent axon degeneration (N=12). A second group of *Ricin* treated animals received a continuous infusion of propranolol from subcutaneously implanted osmotic pumps (N=12). The pumps delivered 300 µg/hr d,l-propranolol which, taking into account plasma protein binding and clearance half-life, is calculated to produce a steady state free plasma concentration of 4.4 nM l-propranolol. The ß-AR antagonist treatment resulted in a 68% reduction in the increase of GFAP optical density ($p<0.001$) in the dorsal horn after peripheral nerve injury, which we interpret to show a major reduction in glial scar formation. *Supported by a grant from the American Paralysis Association.*

270.7

STIMULATION OF ASTROCYTE PROLIFERATION BY AN MPTP-INDUCED LESION IN THE MOUSE CAUDATOPUTAMEN. W.G. McAuliffe* and R.S. Nowakowski, Dept. of Neuroscience and Cell Biology, Robert Wood Johnson Medical School/UMDNJ, Piscataway, NJ 08854.

MPTP is a dopaminergic neurotoxicant which causes a Parkinsonian syndrome in man, monkeys and mice. Previous work has shown that MPTP causes gliosis, as measured by biochemical detection or immunostaining of glial fibrillary acidic protein (GFAP), in the caudatoputamen (CP). This gliosis appears to be a reaction to the destruction of nigrostriatal dopaminergic nerve terminals. However, it is not clear whether this gliosis is due to the upregulation of GFAP in and hypertrophy of existing astrocytes or if DNA synthesis and cell division of astrocytes also occurs. To investigate this question male C57Bl/6J mice were given a single intraperitoneal dose of MPTP, 50 mg/kg. Forty-eight to 72 hours later these mice were given a single i.p. injection of the thymidine analogue bromodeoxyuridine (BUdR, 50 mg/kg) and sacrificed 30 minutes later. Paraformaldehyde-fixed, paraffin-embedded sections through various levels of the CP were stained with antibodies to GFAP (Dako) or BUdR (Becton-Dickinson) using a Vector ABC kit. The single MPTP injection was sufficient to produce marked increase in astrocytic GFAP staining in the CP by 48 hours. The CP also contained a significant number of BUdR-labeled nuclei reflecting an increased rate of cell proliferation compared to untreated mice. Labeled nuclei were found in various regions of the CP at 48, 54, 60 and 72 hours after MPTP. Mitotic figures were seen occasionally at 48 hours post-MPTP. The size and shape of the BUdR-labeled nuclei indicate that they are astrocytes. We conclude that MPTP causes both hypertrophy and hyperplasia of astrocytes in the caudatoputamen.

270.8

REACTIVE ASTROGLIOSIS IS DELAYED IN THE OPTIC NERVES OF SLOW DEGENERATING C57BL/Ola MICE FOLLOWING RETINA REMOVAL. P. A. Trimmer*. Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, VA 22908.

Following a lesion, there is a loss of propagated electrical activity as well as other degenerative changes in the CNS. In wild type mice (C57BL/6, BL/6), it has not been possible to determine if reactive astrogliosis is triggered by the loss of electrical activity alone or by factors associated with CNS degeneration (ex. macrophage activity and degeneration of myelinated axons). This study capitalizes on the recent discovery of a novel strain of mice, C57BL/Ola (BL/Ola) that express prolonged axon viablity, delayed macrophage recruitment and delayed removal of degenerating debris following a lesion in the PNS (Lunn et al., 1989). BL/6 and BL/Ola mice were subjected to unilateral retina removal and processed for electron microscopy (EM) and immunocytochemistry at 2-28 days post-operation (DPO). At the EM level, degenerating myelinated axons and hyperfilamentous, reactive astrocytes were first detected in BL/6 mice at 7 DPO but similar changes were not detected in lesioned BL/Ola mice until 28 DPO. Increases in glial fibrillary acidic protein immunoreactivity were also delayed in the lesioned BL/Ola mouse optic nerve. The results of this study suggest that the loss of propagated electrical activity that occurs concurrent with retina removal is not a trigger for the formation of reactive astrocytes in the BL/Ola mouse optic nerve. Because reactive astrogliosis is delayed, the most likely source of factors that trigger reactive astrogliosis are the activated macrophages and degenerating myelinated axons whose expression is also delayed in the lesioned optic nerve of BL/Ola mice.

270.9

CHARACTERIZATION OF MACROPHAGES IN NORMAL AND TRANSECTED NERVES. C. Lobato and J.W. Griffin*. Dept. of Neurology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

Resident macrophages, well-characterized in many tissues, have only rarely been described in the peripheral nervous system (PNS). In this study we characterized the numbers, distributions, and immunophenotypes of the resident macrophages of the PNS, and their responses to injury, using as a model system Wallerian degeneration (WD) of the rat sciatic nerve. By immunocytochemistry, the resident macrophages in normal nerve were thin, longitudinally oriented, had long branched processes, and many were Class II-positive, even in germ-free, isolator-reared rats. Over 9% of cell nuclei in normal rat sciatic nerves were associated with resident macrophages.

After nerve transection, the number of macrophages began to increase on day 2, and increased more as time post-transection advanced. Within short-term organ cultures of rat sciatic nerve, there was no increase in macrophage number, indicating that most of the macrophages seen during WD are derived from the circulation.

The prominence of these MHC Class II-positive cells in normal peripheral nerve and their localization near blood vessels makes them candidates for being the antigen-presenting cells of the PNS. During WD of the PNS, circulating macrophages are recruited.

270.10

THE RESPONSE OF MACROPHAGES TO OPTIC NERVE INJURY IN GOLDFISH. J. Wang*, W.P. Battisti, T.C. Eckenrode and M. Murray, Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Macrophages may contribute to the role that the environment plays in successful axonal regeneration. The goldfish optic nerve regenerates readily after crush lesion and this system therefore permits the examination of the role of macrophages in regeneration in the CNS. The monoclonal antibody OX-42 recognizes complement type 3 receptors and its expression is up-regulated in activated macrophages. We examined the expressions of OX-42 antigens on macrophages in the goldfish optic nerve at the light and EM level . Two types of macrophages are recognized in goldfish optic nerve, granule bearing interfascicular macrophages, and intrafascicular macrophages which resemble conventional macrophages and which phagocytose debris after injury. Neither type is strongly labeled in control optic nerve. Optic nerves were crushed and fish sacrificed 1 hour to 14 wks later. Some labeled interfascicular macrophages are seen by 1 hour and many are labeled by 3 hours PO, before regeneration commences. Labeled intrafascicular macrophages are seen later. The number of labeled macrophages increases to a maximum 4 wks PO, when regeneration is largely complete, and then declines. After 8 wks no labeled macrophages are detected. These results show that the macrophage response is closely related to axonal regeneration in the fish optic nerve, and suggest that the two types of macrophages may have different functions. Supported by ASRI 91-016-1 and NS 16556.

270.11

OXYTALAN FIBRILS ACCUMULATE IN RENAUT BODIES. J. Weis*, M.E. Alexianu, G. Heide, J.M. Schroeder. Inst. of Neuropathol., Technical Univ., D-5100 Aachen, Germany.

Renaut bodies (RBs) are fusiform endoneurial structures found at sites of nerve entrapment, often occupying more than 30% of the cross sectional area of nerve fascicles. Their origin, composition, and function, however, is still incompletely understood.

In this study, the causal relationship between the development of RBs and nerve entrapment was confirmed. RBs were absent in fetal median nerves at the level of the wrist and in adult median nerves above the wrist, but they were found in median nerves within carpal tunnels of 10 year-olds and adults. The number of RBs increased with age. The cells in RBs had long processes resembling fibroblasts. Like perineurial cells, these cells were immunoreactive with antibodies against epithelial membrane antigen (EMA) and were partially covered by a basal lamina reactive with antibodies against laminin and s-laminin. Focally accumulated microfibrils dispersed in an amorphous matrix and bundles of 30-40 nm collagen fibers were major extracellular components of RBs. The diameter of the microfibrils (8-12 nm) corresponded to the size of the microfibrillar component of elastic fibers, the oxytalan fibrils. RBs were stained with antibodies against oxytalan fibrils, several types of collagen, and tenascin.

On the basis of these results, we propose that RBs are composed of fibroblast-like cells that often show perineurial differentiation. These cells produce an extracellular matrix highly enriched in elastic fiber components. Thus, RBs might serve as pressure absorbing cushions for entraped nerves.

270.12

GLIAL PROLIFERATION IN ADULT RAT BRAIN FOLLOWING INJURY. H. Ishiguro, J. Amat, K. Nakamura, and W.T. Norton*. Neurology Dept., Albert Einstein Coll. of Med., Bronx, NY 10461.

It is known that a few immature glia proliferate in the adult CNS, and we have shown that ganglioside GD3+ astrocyte precursors can be isolated from mature brain (Norton and Farooq, J. Neurosci. 9:769-775,1989). We have initiated a study of the fate of such glioblasts in the normal CNS, and whether they contribute to glial proliferation following injury. Adult (250g) rats were subjected to unilateral stab wounds. Proliferating cells were detected by ^{3}H-thymidine autoradiography and by PCNA immunostaining (a marker for S-phase). At 2, 3, 4, 7, 14 and 21 days post-injury (dpi) animals were perfused and sections triple-immunostained for various combinations of the antigens: PCNA, GD3, GFAP, carbonic anhydrase, π and μ isoforms of glutathione-S-transferase, ED-1, and ricin lectin (for microglia). Dividing cells increased dramatically at 2 dpi, reached maximum levels at 4 dpi and diminished considerably by 7 dpi. At 2 dpi these were 1/3rd GD3+/GFAP- and 2/3rds ricin+ (GD3-, GFAP-, ED-1-) , but none were astrocytes. At 3 dpi reactive astrocytes proliferated, some of which were also GD3+. At 4 dpi four populations of dividing cells (ricin+, GD3+/GFAP-, GFAP+/GD3-, and GFAP+/GD3+) were present in the ratio of 43:24:27:6. Thus nearly half of the proliferating cells were microglia (macrophages at the edge of the wound are PCNA-), 33% were astrocytes, and 24% were immature GD3+ cells. GD3+/PCNA+ cells exhibit three different morphologies: small GFAP- cells having few processes and small nuclei , large GFAP+ cells with large round nuclei, and immature subventricular zone cells (also present in controls). As yet we have no evidence that the small GD3+ cells differentiate into either astrocytes or oligodendrocytes. We provisionally conclude that differentiated astrocytes enter the cell cycle at 3 dpi and divide once, and then acquire GD3. (Supported by USPHS grants NS02476 and NS23705)

270.13

NEUROIMMUNE RESPONSE IN THE NEONATAL MOUSE HIPPOCAMPUS FOLLOWING PRENATAL LESIONS TO THE ENTORHINAL CORTEX. C.F. Ide*, U. Chu, D.C. Snyder, B.W. Coltman, and J. Scripter. Dept. of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118.

The nervous system and the immune system cross-regulate each others activities via a series of complex cellular and molecular interactions. Development of specific aspects of this interacting system is of considerable interest, e.g., the ontogeny of reactivity of neuroimmune cells to trauma during late fetal development. To define the capabilities of neuroimmune cells to respond to local damage in the developing fetal cerebral cortex, we lesioned the fetal entorhinal cortex (EC)'using exo utero surgical methods at embryonic day 15-16 (E15-16) in the mouse. At the time of birth (P0), we examined the hippocampal formation, a structure adjacent to the damaged EC and which receives significant prenatal innervation from the EC. Lesioned animals showed increased numbers of GFAP-reactive cells, and increased numbers of cells with macrophage-like staining properties in the hippocampus as determined by immunocytochemistry. Additionally some large bipolar-like cells exhibited an endogenous peroxidase activity. These data indicate that the late fetal cerebral cortex is capable of responding to trauma with basic elements of a more mature neuroimmune response characteristic of older animals.

270.14

CULTURED ASTROCYTES FROM ADULT RATS AFTER PERFORANT PATH TRANSECTION OR ELECTROLYTIC ENTORHINAL CORTEX LESIONS. C. Peterson*, N. Laping, J. Valverde and J. Day. Dept. Gerontology, University of Southern California, Los Angeles, CA 90089

Degenerative changes similar to those in Alzheimer's disease have been observed in rats with entorhinal cortex lesions. Four days post-lesion there is a marked elevation in GFAP immunoreactivity and mRNA in the hippocampus *in vivo* on the side ipsilateral to the lesion. Following surgical or chemical lesions *in vivo* proliferating cultures of astrocytes can be obtained from adult rat brain *in vitro*. Unilateral knife-cut or electrolytic lesions were performed on adult male rats. Four days post-lesion the brains were separated into ipsilateral from contralateral and dissected into various regions (e.g., cortex, hippocampus and striatum). Following enzymatic digestion viable cells from similar regions were plated at identical densities into poly-lysine coated flasks and maintained in Ham's F12/DMEM medium with 10% fetal calf serum. Cells were counted at 1, 3, and 7 days after plating. One day after plating the number of cortical and hippocampal cells that attached was 2 times greater in the lesioned when compared to the non-lesioned side. By 3 and 7 days after plating, the differences had increased to 4 and 6 times greater, respectively. Phase contrast and immunofluorescent antibody identification revealed that all of the cells were astrocytes. Similar results were obtained whether knife cut or electrolytic lesions were used. The differences in the number of cells was not due to an increased proliferation of cells isolated from the lesioned side, because the percentage of nuclei labelled by tritiated thymidine after 24 hours was similar in lesioned and non-lesioned sides. Whether these *in vitro* astrocytes express some of the *in vivo* changes due to the lesion remains to be determined.

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270.15

THE CHARACTERISTICS OF REACTIVE ASTROGLIOSIS CAN BE DIFFERENTIALLY MODULATED BY CYTOKINES. V.W.Yong*, T.Tejada-Berges, E.Wright and V. Balasingam. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4.

There is emerging evidence that cytokines may mediate reactive astrogliosis post-injury. This study addresses whether particular changes of the reactive astrocyte are regulated by different cytokines. Postnatal day 3 mouse pups were subjected to a scalpel blade injury to the cortex, and 20 U (in 2 ul) of cytokines or vehicle applied to the stab cavity. Four days later, brain samples were analysed for GFAP immunoreactivity (IR) or GFAP content. Astrocytic proliferation was assayed in vitro using ^{3}H-thymidine incorporation. GFAP IR was minimal in vehicle-treated pups, in correspondence with reports that gliosis occurs to a lesser extent, if at all, following injury to the neonatal brain. In contrast, all cytokines tested (γ-IFN, IL-1, IL-2, IL-3, IL-6, TNF-α, M-CSF) increased GFAP IR. The specificity of the cytokine effect was shown by the inability of human γ-IFN to evoke gliosis in mice, in accordance with reports that the interaction of γ-IFN with its receptor is species-specific. Content of GFAP is currently being determined. ^{3}H-thymidine measurements revealed that only γ-IFN and TNF-α could alter proliferation. These data suggest that the various parameters of reactive astrogliosis can be differentially regulated, and support the postulate that cytokines are mediators of gliosis post-injury to the CNS.

270.16

AXOTOMY INDUCED MITOGENIC SIGNALS FOR GLIAL PROLIFERATION IN THE ADULT TRIGEMINAL GANGLIA DEPEND ON PROTEIN SYNTHESIS J.Y.M. Wen*, C.M. Morshead and D. van der Kooy. Neurobiology Research Group, University of Toronto, Toronto, Ont., Canada M5S 1A8.

Axotomy of the trigeminal ganglion in adult rats induces a subpopulation of satellite glial cells to proliferate between 15 and 27 hr post-axotomy in explant cultures. In vivo studies support the idea that injured neurons produce signals inducing the glial proliferation. The present study asks whether protein synthesis is required to induce this satellite glial cell population to proliferate, and if so when it is necessary. We demonstrate that blocking neuronal protein synthesis with cycloheximide (1ug/ml) over the first 13.5 hr in culture prevented the glial proliferation seen at 27 hr as measured by scintillation counting after continuous 3H-thymidine uptake. However, cultures exposed to cycloheximide over the last 13.5 hr showed the same amount of glial proliferation as control cultures. Experiments to pinpoint the critical time for protein synthesis inhibition revealed that cycloheximide exposure over the first 7 hr prevented the proliferation seen at 27 hr. Moreover, limiting cycloheximide exposure to the period from 3.5 to7 hr in culture completely suppressed the glial proliferation assessed at 27 hr. It is important to note that this suppression of proliferation was not permanent, since glial cells in similar cultures did proliferate when culture times were extended to 34 hr. However, it is not clear if the proliferation seen at 34 hr in vitro represents a delayed division of the same population of cells normally assessed at 27 hr or rather a new population of glial cells activated only with longer culture times. Experiments designed to double label proliferating cells using independent markers of DNA synthesis at shorter versus longer culture times will help distinguish between these two possibilities. Surprisingly, limiting cycloheximide exposure to the period between 0 and 3.5 hr produced greater glial proliferation compared to 27 hr control cultures. This may reflect the disruption of a constitutive glial inhibitory signal for glial proliferation from neurons or of a constitutive negative signal for proliferation between glial cells. Blocking protein synthesis in the early period after neuronal injury reveals mechanisms that serve to dynamically control glial proliferation.

270.17

CNS CELL CULTURE FROM ALZHEIMER'S DISEASE AUTOPSIES. M.P. McKinley*, J.A. Schaller, L.I. Sue, M.H. Perry, P. Riely, and J. Rogers. Sun Health Research Institute, Sun City, Arizona 85372.

Many attempts to elucidate pathophysiologic mechanisms of Alzheimer's disease (AD) have been limited to observations in postmortem human brain tissue, so that direct evidence of causality, specificity, and temporal sequencing of events has been difficult to obtain. We have begun to develop and characterize primary mixed cultures from human adult brain autopsies from AD and control aged brains. Optimal cell growth was found only if the post mortem interval does not exceed 3 hours before cell plating. Sterility of cultures requires a thorough sterile saline flush of exposed cerebral hemispheres immediately prior to excision of tissue for processing. Various tissue disruption techniques have been tried: a combination of mincing with razor blades, shearing in pipettes, digestion with collagenase and trypsin, and washing through a sieve mesh provides for the best cell growth. Growth of cells is optimal if flasks are pretreated with poly-lysine and cells are immediately resuspended in a medium containing M-199 and OPTIMUM (1:1) plus 10% fetal calf serum and 1% glutamate. Even under these conditions, cells did not become adherent to the flask for more than 24 hours. Numerous GFAP immunoreactive cells have been detected. Additionally, endothelial reactive cells have been identified. Currently we are characterizing the cells in mixed cultures with the intent of developing primary cultures enriched for astrocytes and microglia.

270.18

APOLIPOPROTEIN E EXPRESSION IN ALZHEIMER'S DISEASE AND AFTER HIPPOCAMPAL DEAFFERENTATION IN THE RAT. C. Zarow* and C.E.Finch. Andrus Center, Univ. of Southern California, Los Angeles, CA 90089-0191.

Apolipoprotein E (ApoE) is a low-density lipoprotein receptor ligand, expressed by astrocytes in the CNS. It plays a role in the redistribution of cholesterol between cells, especially during injury and repair. ApoE mRNA is elevated after a bilateral electrolytic entorhinal cortex lesion (ERC lesion) in the rat [Poirier et al., Mol. Br. Res. (1991) 11:97]. Two days after a unilateral ERC lesion ApoE mRNA levels in the ipsilateral hippocampus dropped to half of the control level. These levels increased to control values by 6 days after injury and exceed control values 2-fold at 10 days after ERC lesion. Analysis of hippocampal sections following in situ hybridization revealed a shift in ApoE mRNA expression from astrocytes in the molecular layer to cells with astrocytic morphology in neuronal subfields. Optical density (O.D.) measures were taken over the CA1 neuronal layer and over the molecular layer of CA1 and expressed as a ratio (N:M). There is a shift in the N:M ratio from 0.73 for control to 1.72 at 14 days after lesion (ANOVA, $p<.05$). This analysis was also done for hippocampal sections from 8 Alzheimer and 9 non-Alzheimer brains. There is an increase in the N:M ratio in AD ($1.58 \pm .04$) compared to non-AD ($0.93 \pm .03$) (ANOVA, $p<.0001$). In AD, CA1 suffers the loss of ERC afferents and is subject to neuronal loss and neurofibrillary tangle formation. Our analysis cannot distinguish between neurons with and without tangles. However, we hypothesize that the astrocytes adjacent to these neurons would be recycling the lipids derived from degenerating neurons to those neurons surviving and undergoing dendritic remodelling and axonal sprouting in response to deafferentation. (Supported by AG-07909).

REGENERATION II

271.1

Septo-hippocampal regeneration through biosynthetic bridges containing adult Schwann cells. D. Hoffman* and P. Aebischer. Section for Artificial Organs, Biomaterials and Cellular Technology. Brown University. Providence, R.I.

Schwann cells' production of neurotrophic and neurotropic molecules as well as their complex role in PNS regeneration suggest that they may facilitate regeneration of CNS neurons as well. While embryonic-derived Schwann cells have been shown to aid in CNS regeneration, the present studies utilized Schwann cells cultured from injured adult peripheral nerves. Because of their origin, these cells retain characteristics which may aid regenerating nerve fibers. The purpose of this study was to determine the extent to which a matrix containing purified adult Schwann cells could induce neurite outgrowth from axotomized neurons of the septo-hippocampal system. Schwann cells isolated from adult Fisher rat sciatic nerves were suspended at a density of 80×10^6 cells/ml in an extracellular matrix-containing gel, and surrounded by a hollow permselective poly(acrylonitrile / vinyl chloride) polymer tube. Following a unilateral aspirative lesion of the fimbria-fornix, adult Fisher rats received cell-containing tubes in the lesion cavity, placed so as to abut the septum and the hippocampus. After three weeks, cellular cables bridging the septum to the hippocampus were observed in the implanted tubes. Axons positive for acetylcholinesterase migrated along the longitudinal axis of the cable, throughout the length of the tube, closely associated with the Schwann cells. We surmise that the transplantation of an oriented polymer tube seeded with adult Schwann cells constitutes a favorable milieu for the regeneration of axotomized CNS neurons.

271.2

PERIPHERAL NERVE REGENERATION THROUGH RESORBABLE HYALURONIC ACID ESTER AND SILICONE NERVE GUIDES. G. Favaro, F. Langone§, A. Schiavinato, S. Spini, E. Lini, L. Cavicchioli*, F. Dorigatti and E. Govoni. FIDIA Research Laboratories, 35031 Abano Terme (PD), Italy. §State University of Campinas, 13100 Campinas (SP), Brazil.

Peripheral nerve regeneration successfully occurs through many mm-long gaps in nerve guides made of various resorbable or permanent materials. Here we assessed the efficacy of resorbable nerve guides made of a hyaluronic acid ester (HYAFF11p75) or silicone in promoting rat tibial nerve regeneration through an 8 mm-gap. An autologous nerve graft was used in another group of animals. Electrophysiologic evaluation 90 days after surgery demonstrated that regeneration through the HYAFF11p75 nerve guides was equivalent to that obtained with the silicone ones, and similar to that observed using the grafting technique. The nerve compound action potential amplitude was 0.083±0.037 mV (±S.E.M.) in the autograft group, 0.034±0.01 mV in the silicone group and 0.033±0.01 mV in the HYAFF11p75 group. An ultrastructural study 56 weeks after surgery showed nerves regenerated through silicone tubes with the axonal pathologic alterations described by Le Beau et al. (1988). In addition, the finding of many myofibroblasts in the epineurium-like structure strongly supports the hypothesis that compressive forces are the main factor inducing such alterations. The absence of myofibroblasts and the low percentage of altered axons in fascicles regenerated through the HYAFF11p75 nerve guides suggests that only resorbable materials can be suitably applied for short nerve gaps in severed human nerves. Grants FAPESP(90/4970-0) and FIDIA.

271.3

LONG TERM MAINTENANCE OF AXONAL REGENERATION IN PRIMATES FOLLOWING COLLAGEN NERVE GUIDE REPAIR OF 2CM MEDIAN NERVE DEFICIT. [1]S.J. Archibald*, [4]C. Krarup, [1]L. Wrage, [1]A. Friedman, and [1,2&3]R.D. Madison. [1]Departments of Surgery (Neurosurgery) and [2]Neurobiology, Duke University Med. Ctr., Durham, N.C. 27710, [3]Research Service V.A. Hospital, Durham, N.C. 27710, and [4]Department of Clinical Neurophysiology, Rigshospitalet, Copenhagen, Denmark.

This study follows the course of motor and sensory physiological recovery resulting from the repair of a 2cm median nerve deficit in *Macaca fasicularis*. Adult male macaques received bi-lateral median nerve sections at the wrist and removal of a 2cm nerve segment, followed by either: A) Sural nerve cable graft repair (n=8 nerves), B) Entubulation repair with 2mm I.D. nerve guide conduits (n=8 nerves), and C) No repair performed (negative controls).

Evoked EMG studies of the abductor pollicis brevis (APB) and evoked sensory studies of the median nerve were performed bi-weekly for 154 days following surgery and at monthly intervals thereafter. These assessments of functional recovery have been completed over a period of 546 days to date.

The onset of the first evoked motor response in each group ranged between:98 to182 days in entubulation repairs, 56 to126 days in nerve grafts, and 84 to 350 days in negative controls. The onset of sensory recovery was more delayed and ranged between: 210 to378 days for entubulation repairs, 154 to 266 days for nerve grafts, and 154 to 490 days in negative controls.

At 518 and 546 days the recovery of the compound motor unit action potentials of the APB compared to normal were: 73% for entubulations, 76% for grafts, and 49% for negative controls which had significantly lower emg amplitude compared to the other procedures ($P= 0.01$).

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271.4

DELAYED REPAIR OF A GAP IN A PERIPHERAL NERVE UTILIZING A SCHWANN CELL CABLE FORMED IN A SILICONE CHAMBER. N. A. Azzam,* A. A. Zalewski and L. R. Williams. Lab. of Neural Control NINDS, NIH, Bethesda, MD 20892 and CNS Diseases Research (L.R.W.), The Upjohn Co., Kalamazoo, MI 49001.

A tissue cable containing Schwann cells can form at 4 weeks in a silicone chamber in the absence of regenerating axons. In the present study we investigated whether axons would regenerate through such a preformed cable. Transected stumps of sciatic nerves of adult rats were sutured into the ends of silicone chambers filled with dialyzed plasma, leaving a 10-mm interstump gap. The proximal sciatic nerve was further transected between two ligatures. The proximal nerve stump was reflected leaving a ligated, denervated nerve plug attached to the proximal end of the chamber. After 4 weeks, nerve ligatures were removed and the proximal sciatic nerve stump was anastomosed to the nerve plug-cable-distal nerve stump tissue complex. In some animals, the ligated nerve plug was left undisturbed in order to determine the fate of a chronically denervated Schwann cell cable. Four months later, the cables and segments of nerve proximal and distal to them were removed and examined by light and electron microscopy. All the anastomosed nerve plugs, cables and distal nerve stumps contained numerous myelinated and unmyelinated nerve fibers. Leg muscles were reinnervated and skin sensory function returned. It is interesting that almost all the Schwann cells disappeared from chronically denervated nerve plugs and cables, but not from distal nerve stumps. Since a cable formed by our surgical method promotes axonal regeneration, it has the potential to eliminate the need for a nerve graft to repair a gap in a nerve that requires delayed surgical intervention.

271.5

REGENERATION OF THE RAT OLFACTORY EPITHELIUM AFTER EXPOSURE TO METHYL BROMIDE: THE MICROVILLAR CELL. ML Miller, L Hastings, JE Evans and IA Michaelson*. Department of Environmental Health, Univ. of Cincinnati, Cincinnati, OH, 45267.

Methyl bromide (MeBr) is a toxin which destroys olfactory epithelium while sparing respiratory epithelium. Microvillar cells are depleted from olfactory epithelium immediately after injury but reappear after several months in the late stages of repair.

Male hooded rats were exposed to 200 ppm MeBr 6hr/d for 10 days, and sacrificed 1, 4, 11, 22, 30, 39 and 73 days after exposure. Semi-thin plastic sections perpendicular to the septum were stained with toluidine blue. Microvillar and sustentacular cells were absent from the epithelium until 11 days after exposure.

During regeneration, the distance from the nuclei of these cells to the nasal lumen increased. The ratio of microvillar to sustentacular cells in controls was 1:30; at 39 days after exposure, 1:300. The appearance of the olfactory epithelium was nearly normal at day 39, but microvillar cells remained depressed. The microvillar cells do not degenerate after bulbectomy suggesting they are not sensory in nature (Carr *et al.*, 1991). The presence of microvillar cells may indicate a fully reconstituted epithelium. Their function remains to be determined.

271.6

PERIPHERAL NERVE REGENERATION ACROSS LONG GAPS: A COMPARISON OF ARTIFICIAL NERVE GRAFT AND SUTURED AUTOGRAFT REPAIRS. Jose Padilla, Tanya Atagi, Robert Keeley, Khoi Nguyen, Paula Kadlcik, Eric Sabelman, Albert Yu*, Lawrence Eng, and Joseph Rosen. Dept. of Functional Restoration, Stanford Univ. Sch. of Med., Stanford, CA, 94305.

A study was conducted to compare the regeneration of rat peroneal nerves across 14 mm gaps repaired with artificial nerve grafts (ANG) or sutured autografts (SAG). The ANG models are composed of a synthetic biodegradable conduit made of glycolide trimethylene carbonate (GTMC) filled with either a 1% collagen type I matrix (Coll-ANG) or 0.9% saline (PBS-ANG). Ten rats were implanted with SAGs and Coll-ANGs in contralateral limbs, 10 compared the SAG and PBS-ANG, and 10 compared the Coll-ANG and PBS-ANG. Functional recovery was evaluated by walking track analysis charted throughout the 9-month experiment. Nerves were further analyzed by electrophysiology and histology.

Walking track analysis demonstrated the SAG and Coll-ANG to have equivalent recovery, while both the SAG and Coll-ANG were significantly better than the PBS-ANG ($p<.05$). Electrophysiological values for SAG, Coll-ANG, and PBS-ANG were 88.5±1.9%, 82.9±4.3%, and 81.8±3.1% respectively. The SAG value was significantly higher than the PBS-ANG value ($p<.02$). Myelinated axon counts and average diameters are pending. At biopsy ANGs were resorbed, and reaction to collagen was minimal. Comparison of ANG and SAG peripheral nerve gap repairs demonstrates that a 1% collagen matrix inside a resorbable conduit enhances functional recovery versus a saline matrix, and supports equivalent recovery to a SAG repair.

271.7

Over-riding the outgrowth inhibitory effect of CNS myelin. M.K. Carpenter *, T. Hassinger, L.R. Whalen, S.B. Kater. Program for Neuronal Growth and Development, Dept. of Anatomy and Neurobiology, Colorado State Univ., Fort Collins, CO 80523.

In the adult mammalian brain and spinal cord, myelin is an inhibitory signal for neurite outgrowth. This phenomen has been investigated by culturing neurons on cryostat sections from adult rat brain or spinal cord (Crutcher, K.A., (1989) Exp. Neurol. 104: 39-54; Savio and Schwab, (1989) J. Neurosci. 9(4): 1126-1133). Using this type of culture system, it has been previously shown that neurons seldom adhere to or grow on white matter, but will attach and grow on gray matter.

The experimental conditions developed here *allow neurons to attach and grow on white matter*. Embryonic rat hippocampal neurons were grown on cryostat sections in the presence of glial co-cultures. Under these conditions, neurons will attach and grow on both gray and white matter. In conditioned medium, outgrowth on white matter is approximately 10-fold greater than outgrowth in defined medium, and approximately 3-fold better than outgrowth in serum supplemented medium. Glial cultures condition the media by producing many growth promoting factors (Banker, G. (1980) Science, 209: 809-810). To determine if these growth stimulating factors from the conditioned medium are acting by binding to the substrate, CNS cryostat sections were *pre-incubated* with a glial culture for 2 days. The conditioned medium and the glial cultures were then removed and neurons were plated on the pre-conditioned sections, in defined media. The outgrowth on the pre-conditioned sections was similar to or better than the outgrowth seen on cryostat sections that had neurons growing with glial co-cultures. Therefore, the outgrowth inhibitory signal of myelin is not absolute and, under appropriate conditions, it can be over-ridden.

271.8

SHORTENING OF THE SPINAL COLUMN IN RAT: A METHOD OF STUDYING REGENERATION POSSIBILITIES IN LESIONED SPINAL CORD. K.Trok, L. Olson*. Dept. of Histology & Neurobiology, Karolinska Institutet, Stockholm, Sweden

The mammalian spinal cord has a certain degree of tension under normal conditions. After a complete spinal cord transection, the spinal cord stumps are separated because of this tension, thereby limiting regenerative possibilities. One possible approach to reduce the tension on the spinal cord is to remove a vertebra. The method of removing half of two adjacent vertebrae (rather than an entire single vertebra) gives greater possibility for spinal column stability when the bone is restored. Three different operations were performed in rats: (1) Complete spondylectomy without cutting the spinal cord. The rostral half of T9 and caudal half of T8 were removed.
(2) Complete spondylectomy (as above) with complete transection of the spinal cord. In both cases the remaining parts of T8 and T9 were brought into contact and stabilized with small rods placed on both sides of the column and fixed with non-absorbable thread.
(3) Laminectomy of T8 and complete transection of the spinal cord. Animals were sacrificed after 6 to 10 months. During this period the rats were repeatedly evaluated for motor and sensory deficits. Perfusion was made with PVP (polyvinylpyrrolidone). This method enables cryostat sectioning of undecalcified bone together with soft tissue for immunohistochemistry. After perfusion, the spinal column containing the spinal cord was removed, stored in PVP and then cryostat-sectioned and evaluated immunohistochemically. This method allows lesioned spinal cord stumps to be in physical contact, thereby increasing any possibility of regeneration or sprouting. Functional differences between these three groups can be detected.

271.9

A NERVE REPAIR METHOD THAT ENHANCES COLLAGEN FORMATION AND AXONAL REGENERATION. J.E. Swett* and C.E. Ribak. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA, 92717.

Traditional surgical repairs (epineurial suturing) of severed peripheral nerve (rat sciatic) seldom result in good functional recovery following nerve regeneration partly because only 1/2 to 1/3 of the normal number of neurons regenerate axons distally into the nerve's tributary branches. Experiments were carried out to test alternative methods of neurorrhraphy of the rat sciatic severed at mid-thigh level using fibrin glue to bind nerve ends together in a semipermeable matrix of biocompatible material. Light microscopic analysis revealed that the numbers of sensory and motor neurons, labeled retrogradely with HRP from the common peroneal nerve 10-15 mm distal to the repair site, were close to normal. While large numbers of myelinated axons were present many (25-40%) were from non-peroneal motoneurons. Distally and proximally to the repair the perineural sheath was intact, but, near the repair, it was thin or absent, being replaced by the matrix material. At light and electron microscopic levels large numbers of microfascicles were present within endoneurial spaces and in the outer matrix near the repaired zone along with clear evidence of misdirected axonal profiles. The lamellae of the microfascicles resembled in every respect perineurial cells. Some microfascicles were found in the matrix proximal to the repair site indicating axonal regeneration in inappropriate directions. Collagen formation was particularly enhanced in the endoneurial regions around newly established blood vessels and in the vicinity of the matrix. Circumferentially oriented rings of collagen were separated by longitudinal bands of collagen, their margins often delineated by thin processes of endoneurial fibroblasts. The unusually large accumulation of collagen appears to be induced by the presence of the matrix. The micro-environment thus created appears to favor abundant regeneration of axons into the distal parts of the nerve.

271.10

AN ACTH 4-10 ANALOG, BIM 22015 DECREASES MOTONEURON DENSITY AFTER NERVE TRAUMA. T.S. Lee,*S.J. Lee and F.L. Strand. Department of Biology and Center for Neural Science, New York University, Washington Square, New York, New York 10003.

Previous studies have demonstrated that ACTH 4-10 and its various analogs possess neurotrophic and myotrophic properties[1,2]. This current study was undertaken to determine whether the ACTH 4-10 analog BIM 22015 has an effect on the ventral horn motor cells following peripheral nerve crush.

Male Sprague-Dawley rats (175-200g) were subjected to peroneal nerve crush under ketamine (80mg/kg) xylazine (5mg/kg) anesthesia. A #5 forceps was used for nerve crush, resulting in a 1mm wide lesion, leaving the neural sheath intact. Directly following surgery, the peptide BIM 22015 (40μg/kg/48hrs i.p.) or saline vehicle was administered for 7 days. Animals were perfused transcardiacally with 4% paraformaldehyde and the spinal cords were dissected out. The spinal cord was sectioned and regions L4 to S1 were stained with cresyl violet (Nissl) to observe nerve cell bodies. A decrease in motoneuron density, in the ipsi lateral side was observed with BIM 22015 treatment compared to the control(contra lateral side). We are currently investigating the effect of ACTH 4-10 in this model. This technique may serve to quantify the effects of axonal crush on the soma of the spinal motoneuron. This study was supported by Biomeasure, Inc.

[1] Strand, F.L. *et al.* 1992. Peptide (in press)
[2] Strand, F.L. *et al.* 1991. Physiological Reviews **71:1017**

271.11

THE EFFECTS OF AN ACTH 4-9 ANALOG, ORG 2766, ON 6-OHDA LESIONING OF THE SUBSTANTIA NIGRA. F.J. Antonawich*, H.M. Akbari, E.C. Azmitia and F.L. Strand. Department of Biology and Center for Neuroscience, New York University, New York, N.Y. 10003

ACTH peptide fragments demonstrate potent neurotrophic effects on peripheral nerves in situ, central neurons in culture and have been implicated to affect central neuron *in vivo*. Neurotoxic lesioning of the nigostriatal system, which depletes the striatum of dopamine, provides a feasible model of central regeneration in which to test these peptides. Male Sprague Dawley rats were lesioned unilaterally with 6-hydroxydopamine (8µg/4µl), infused into the substantia nigra over a ten minute period, resulting in unilateral Parkinsonism. They were subsequently treated with 10 µg/kg i.p. of ORG 2766 (ACTH 4-9 analog) or saline every 24 hours starting immediately after the infusion. Since initial behavioral data indicated an effect of ACTH on the nigrostriatal system, a morphological and biochemical evaluation was undertaken. In order to determine the period of therapeutic administration, a time course experiment was performed with morphological analysis being done at various time periods during the experiment. High affinity uptake of the striatum is being performed as a quantitative measure of fiber density.

Evaluation of immunohistochemical staining for dopamine using an anti-tyrosine hydroxylase antibody indicates an enhanced intensity of staining in the ORG 2766 treated tissue as compared to its saline counterpart. High affinity dopamine uptake studies are being conducted to support these immunohistochemical findings. Higher dopamine uptake levels in ACTH treated animals would correlate to a high fiber density level in this group. Therefore, it appears that treatment with the ACTH 4-9 analog ORG 2766 (10µg/kg/ 24hrs.) could offer a protective effect from 6-OHDA lesions in the substantia nigra.

271.12

THE EFFECTS OF α-MSH AND ITS ANALOGS ON NERVE AND MUSCLE REGENERATION FOLLOWING AN INDUCED NERVE CRUSH. K.A. Williams, & F.L. Strand.* Department of Biology and Center for Neural Science, New York University, Washington Square, New York, New York 10003.

This study was undertaken to compare various α-MSH analogs with respect to their effects on nerve regeneration. Sprague-Dawley rats (200-275g) were subjected to crush denervation under ketamine (80mg/kg) and xylazine (5mg/kg) anesthesia. A 1 mm lesion was made on the deep peroneal nerve with #5 watchmaker forceps. Immediately after surgery and every 24h until day 8, the animals were injected i.p. (40µg/kg) with one of three α-MSH analogs, α-MSH (1-13), or physiological saline which served as a control. Footprints were collected from the animals by dipping their hind limbs in non-toxic ink and having them walk up an inclined plane lined with white paper. On day 9 the animals were subjected to electromechanical tests in which twitch response, motor units, tetanic tension at fusion frequency and fatigue at 400Hz of the extensor digitorum longus (EDL) muscle were obtained.

Based on the footprint data the BIM 22029 analog significantly improved the parameters of print area and toespread. Also a trend towards improvement was noted for the the print length parameter using this analog. An improvement of tetanic prameters was seen following treatment with 2 of the analogs. However, it was not significantly different from saline controls. The α-MSH (1-13) did not improve footprint parameters or the electrophysiological parameters. This may be due to the high dosage (40µg/kg) that was used in this study. Future studies will examine varying dosages of α-MSH and its analogs. Subsequent investigations are being undertaken to determine the effectiveness of these analogs as myotrophic agents via the histological examination of the peptide treated EDL muscle.

271.13

REGENERATION OF 5-HT PROJECTIONS TO NEOCORTEX: MAGNITUDE OF p-CHLOROAMPHETAMINE-INDUCED DENERVATION INFLUENCES REINNERVATION. K.J. Axt*, L.A. Mamounas, and M.E. Molliver. Dept. Neurosci., The Johns Hopkins University Schl. Med., Baltimore, MD 21205.

p-Chloroamphetamine (pCA) causes a selective and profound loss of one morphologic type of 5-HT axons in rat forebrain. This neurotoxic effect on *fine* 5-HT axons is dose-related, and exhibits a rostro-caudal gradient at 2 weeks after drug administration. At all doses used, axon loss is greater caudally (occipital pole) than rostrally (frontal pole). After a low dose of 2.5 mg/kg pCA, decreased axon density is most apparent in occipital pole with substantial sparing of fine axons, particularly in frontal pole. At 5 mg/kg, axon loss is more robust, yet many fine axons survive; a notable decrease in fine axon density is observed in the middle layers of parietal cortex dorsal to anterior hippocampus. At 10 mg/kg pCA, the density of fine 5-HT axons in frontal pole is significantly reduced, while the thicker preterminal axons are spared. This dose also produces a marked decrease in the density of fine axons in neocortex, dorsal to striatum. In both 5 and 10 mg/kg-treated rats, the occipital pole is markedly (and comparably) denervated. The pattern of *reinnervation* of neocortex by 5-HT axons was studied at 4 and 8 mos. post-drug. At all doses, the frontal pole was reinnervated earlier and more completely than occipital pole. Four mos. after 2.5 mg/kg, 5-HT axons reach control density in frontal pole, whereas at 8 mos., the axon density in occipital pole remains less than control. After the higher doses, reinnervation of frontal pole is more gradual, and is dose-related. In occipital pole, there is little to no reinnervation at 4 mos., whereas at 8 mos some reinnervation is apparent at the 5 (but not 10) mg/kg dose. These data indicate [1] that the degree of reinnervation is inversely correlated with the magnitude of initial denervation, and [2] target areas closer to the dorsal raphe nucleus are reinnervated sooner and more completely than are more distant areas. The decreased capacity of 5-HT axons to regenerate after a larger dose of pCA may depend on both the insult to the serotonergic neurons (retrograde reaction) and the distance over which the axons must be guided to their targets. [Support: USPHS DA04432 and PMAF]

271.14

ABNORMAL MORPHOLOGY OF REGENERATED 5-HT AXONS IN RAT CEREBRAL CORTEX ONE YEAR AFTER ABLATION BY p-CHLORO-AMPHETAMINE (PCA): ACCELERATED AGING OF SEROTONERGIC PROJECTIONS. L.A. Mamounas*, K.J. Axt and M.E. Molliver. Dept. Neurosci., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

The neurotoxin PCA (10 mg/kg s.c. to 2-mo old rats) causes acute degeneration of fine 5-HT axons in forebrain followed by robust but gradual sprouting of normal-appearing 5-HT axons into denervated regions. However, six months after drug treatment, reinnervation by 5-HT axons remains incomplete, particularly in caudal areas of neocortex; at longer survival times (6-12 months), sprouting terminates, and the density of 5-HT axons in cortex gradually decreases. This delayed axon loss is accompanied by increasing numbers of structurally abnormal 5-HT axons which form thickened, tortuous tangles. In 14-mo old treated rats (1-year after PCA), the pathologic 5-HT axon tangles comprise a large proportion of the 5-HT axons in cortex: they are increased nearly 3-fold (2.9 ± 0.4 axon tangles/mm2 of cortex) relative to age-matched controls (1.0 ± 0.02), coupled with the markedly lower 5-HT axon density 1-year after PCA. Since 5-HT axon tangles of this type are commonly seen in aged, untreated rats (24-mo old: 4.6 ± 0.6) but rarely in young control rats (2-mo old), they are thought to be "degenerative" changes that result from the normal aging process. We further examined whether the emergence of 5-HT axon tangles after PCA treatment (i) constitutes a late phase of the sprouting process or (ii) may be an age-related property, characteristic of axonal sprouting in older (10-14 mo) as opposed to younger (2-6 mo) rats. We found that rats treated with PCA at 10-mo of age and allowed to survive 4 months (to 14-mo of age) exhibit robust sprouting of normal-appearing 5-HT axons, as seen in younger rats. These results suggest that the late degenerative changes seen one year after PCA are more dependent upon long survival-time than age; thus, the occurrence of tangles should not be attributed to advanced age *per se*, but may be due to prolonged 5-HT denervation in forebrain or to a long-lasting effect on 5-HT cell bodies. Although amphetamine neurotoxicity in young rats leads to initial regeneration of 5-HT axons, the late degenerative changes suggest that either axotomy itself or another drug-induced effect may cause accelerated aging of 5-HT projections. [SUPPORT: USPHS NS15199; DA04432]

271.15

THE EFFECTS OF INTRASEPTAL BDNF ON COGNITION IN RATS WITH MS/DB LESIONS. Mary Ann Pelleymounter* and Mary Jane Cullen. Amgen, Inc., Amgen Center, 1840 DeHavilland Dr., Thousand Oaks, CA 91320.

Level of expression for BDNF mRNA is high in the granule cell layer of the hippocampus relative to other brain areas. The granule cell layer contains the nerve terminals of a major cholinergic projection from the medial septal nucleus (MS) and the nucleus of the diagonal band (DB). Lesions in the MS/DB generally produce a 40-60% depletion of hippocampal ChAT and disrupt certain cognitive functions, such as working memory. We have attempted to reverse both the loss of hippocampal ChAT and the decline in cognitive function that results from MS/DB lesions by chronic infusion of BDNF into the MS/DB area one month after the lesions were produced. Lesions were produced by passing DC current (1.2mA; 10 sec) through the tip of an insulated insect pin (000) that was placed stereotaxically into three sites within the MS/DB area. Sham lesions were produced by lowering the electrode without administering current. Following a three week recovery period, rats were tested on the 8 arm radial maze, which has traditionally been used to test working memory. Rats with lesions made significantly more errors than sham controls. After this lesion-induced memory deficit had been demonstrated, rats were implanted with osmotic minipumps that contained BDNF which was infused into the MS area via a 28 g cannula that had been placed stereotaxically. Rats received BDNF (8 ug/day) or PBS vehicle at an infusion rate of 0.5 ul/hour for a period of two weeks, at which time the pumps were removed. Rats were then retested on the radial maze task. MS/DB rats that had received BDNF made significantly fewer errors than their MS/DB counterparts that had received the PBS vehicle. This same group of rats were also tested on tasks that required selective attention or spatial memory. MS/DB rats showed deficits in the selective attention task. MS/DB rats that received BDNF, however, could not be distinguished from their intact counterparts. MS/DB rats did not show deficits in spatial learning. Immnohistochemical studies are currently underway to assess cholinergic/non-cholinergic sprouting or regeneration.

271.16

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) RESCUES MESENCEPHALIC DOPAMINERGIC NEURONS FROM 6-OH DOPA TOXICITY IN VITRO. R. Dal Toso*[1], A. Negro[2] and S.D. Skaper[1]. [1]Fidia Research Labs and [2]Advanced Technology Division, Fidia S.p.A., Abano Terme, Italy.

BDNF supports the survival of sensory neurons as well as retinal ganglion cells, basal forebrain cholinergic neurons and mesencephalic dopaminergic (DA) neurons in culture. Here we examined the ability of recombinant rat BDNF to protect cultured DA neurons from the toxicity of 6-OH DOPA, a metabolite of the DA pathway hypothesized to participate in the pathology of Parkinson's disease. Cells prepared from E14-15 rat mesencephalon were maintained with 10-50ng/ml BDNF for 7 days prior to addition of 6-OH DOPA (10-30uM) for 24hr. BDNF prevented the death of virtually all tyrosine hydroxylase immunoreactive neurons, but the loss of only 25-30% of the overall cell population. Furthermore, the monosialoganglioside GM1 (10uM) acted synergistically with subthreshold amounts of BDNF to rescue DA neurons against 6-OH DOPA toxicity. These results propose that BDNF and gangliosides may provide a new pharmacological approach in the treatment of Parkinson's disease.

271.17

TESTOSTERONE EFFECTS ON SYNAPTIC BOUTON CHANGES AFTER FACIAL NERVE INJURY IN THE HAMSTER. S.K. Jacob[1], T. E. Durica[2]*, K.J. Jones[3].
Department of Anatomy[1,2], Rush Medical College, Chicago, IL 60612 and Department of Physical Therapy[3], University of Illinois at Chicago, Chicago, IL 60612.

Gonadal steroids regulate axosomatic synapses in the normal adult mammalian nervous system. We have previously shown that testosterone propionate (TP) significantly enhances the nerve cell body response to facial nerve injury in hamsters. In this study, we tested the hypothesis that TP achieves this effect by attenuating the synaptic bouton stripping that has been shown to occur after axotomy. Adult intact male hamsters were subjected to right facial nerve axotomies, with one-half of the axotomized animals immediately implanted subcutaneously with 2 TP capsules. At 5 days postoperative (dpo), the animals were sacrificed, and processed for routine ultrastructural examination. The Bioquant System IV was used to assay the percent coverage of somal profiles by synaptic boutons on normal and axotomized neurons from hormone-treated and nonhormone-treated animals, as well as synaptic length. After axotomy alone, there was an 81% decrease in synaptic bouton coverage and a 26% decrease in average synaptic length. Exposure to TP at the time of injury resulted in a 48% decrease in synaptic bouton coverage and a 16% decrease in average synaptic length. Thus, it appears that regulation of synaptic input by TP may play an important role in the accelerative effects of the steroid on facial nerve regeneration.

271.18

ANDROGEN EFFECTS ON FUNCTIONAL RECOVERY FROM FACIAL PARALYSIS AFTER FACIAL NERVE CRUSH IN FEMALE HAMSTERS. A.M. Pucci[1] and K.J. Jones[1,2]*
Departments of Anatomy and Cell Biology[1], and Physical Therapy[2], University of Illinois at Chicago, Chicago, IL 60612.

In adult male hamsters, androgens accelerate functional recovery from facial paralysis after facial nerve crush through an effect on the rate of regeneration. In females, androgens also increase the rate of regeneration, albeit to a lesser degree (30% increase vs. 10%, respectively. In this study, the effects of dihydrotestosterone (DHT), a nonaromatizable form of the steroid, on recovery from facial paralysis following crush axotomy of the facial nerve in female hamsters were examined. Hamsters were subjected to right facial nerve crush axotomies at the level of the stylomastoid foramen, with the left side serving as internal control, and either implanted subcutaneously with 2 or 4 DHT capsules or blanks. The 3 experimental groups were then coded. Signs of recovery from facial paralysis, which included the occurrence of a semi-blink, return of the eye blink reflex and full vibrissae movement, and vibrissae orientation, were then monitored daily. A replicate experiment was also accomplished. In contrast to the males, DHT did not appreciably alter functional recovery from facial paralysis in the females. This may be due, in part, to the reduced effects of androgens on the regeneration rate in females as compared to males. Supported by NIH grant NS28238 (KJJ).

271.19

THROMBOSPONDIN PROMOTES SCIATIC NERVE REGENERATION IN POLYETHYLENE TUBE GRAFTS. J.R. Hoffman*, V.M. Dixit, and K.S. O'Shea
University of Michigan, Ann Arbor, MI 48109

Thrombospondin (TSP) is an extracellular matrix molecule associated with regeneration in both the peripheral (Soc. Neurosci. Abst. 16:338) and central nervous systems (Soc. Neurosci. Abst. 17:48). The ability of TSP to promote nerve regeneration was evaluated *in vivo*. Four mm polyethylene tubes were filled with either 10 ug/ml purified platelet TSP, 20 ug/ml laminin (LN), 100 ug/ml anti-TSP polyclonal antibodies or PBS, and implanted into mouse sciatic nerves leaving a 3 mm gap. When functional recovery was assessed using a sciatic function test, after 23 days, TSP grafts showed improved functional recovery over LN, PBS, and anti-TSP grafts. After 29 days, grafts were removed and analyzed visually and histologically for regeneration. Nerves had grown through all the grafts; however, regenerates through TSP and LN grafts had a larger cross-sectional area than PBS grafts. Nerves in anti-TSP grafts had significantly smaller diameter than other groups. Consistant with its role in supporting neurite outgrowth *in vitro*, TSP appears to support nerve regeneration *in vivo*.

Supported by NIH grant HD-23867

271.20

GM-1 GANGLIOSIDE ENHANCES MOTOR SCORES IN INCOMPLETE HUMAN CHRONIC SPINAL CORD INJURY: RESULTS OF A DOUBLE BLIND CROSSOVER TRIAL. J.B. Walker, M.D., Ph. D.*, H. Gu, Ph. D. M. Harris, PT., Walker Institute, 881 Alma Real Dr., Pacific Palisades, CA 90272.

In a randomized double-blind crossover study, human subjects with chronic spinal cord injury received GM-1 or placebo for 2 months. Sygen (GM-1) administered intravenously at a dose of 100 mg, six days a week, resulted in an improvement of motor scores ($p < .05$) whether given before or after two months of placebo. There was no placebo effect on motor scores, but subjects who received GM-1 before placebo maintained their improvement during the placebo phase (i.e., there was no "washout").

S's who were able to ambulate at speeds greater than 0.5 mph also demonstrated sigificant improvement in efficiency of gait as a result of GM-1 and not of placebo.

These results constitute the first finding that any chemical substance improves function in chronic spinal cord injury.

271.21

ACTION POTENTIALS PASS SEVERED INVERTEBRATE AXON SEGMENTS RECONNECTED BY ELECTRIC-FIELD PULSES. G. Rodziewicz*, P. Qi, A.T. Todorov, J. Fendler, N. Todorova.
Neurosurgery Dept (SUNY Med. Sch.) & Chemistry Dept. (Syracuse U.), Syracuse, NY, 13210, USA.

We have previously shown anatomical evidence of successful axon reconnection in the earthworm (*Lumbricus terrestris*) medial giant axon (MGA) *in vitro* system by the application of 100 μsec, 700-800 V electric field pulses (Todorov, A.T., *et al*: Electric-field-induced reconnection of severed axons, *Brain Res.*, in press). We now report initial results of attempts to pass action potentials (AP's) across the reconnected axon segments.

AP's were generated by stimulating the axon with an extracellular silver-wire electrode pair before and after severing. AP's were recorded with intracellular 3M KCl-filled glass electrodes both proximal and distal to the severed segment. Anatomical reconnection was shown by light microscopy of serial 1-2 μm longitudinal sections.

Of 150 total attempts to record AP passage after reconnection, 11 (7.3%) were successful. Illustrative cases are shown, with evidence of AP passage together with anatomical documentation of reconnection. Control preparations are presented.

Recovery of function in systems such as severed spinal cord and peripheral nerve requires the integrity of the axons transmitting information across the injured segment. Axon reconnection promises the rapid return of axon integrity, a necessary condition for the functional recovery of these systems.

271.22

VIABILITY OF THE OPTIC NERVE FOLLOWING DAMAGE AND SUBSEQUENT CHRONIC TREATMENT WITH D.C. ELECTRIC FIELDS. M.F. Zanakis*, A. Bozack, R.M. Cebelenski, P. Jacovina, L. Guarino, B.H. Hallas. N.Y. College of Osteopathic Medicine, Old Westbury, N.Y. and American BioInterface Corp., New York, N.Y.

Several Previous studies have demonstrated that chronic D.C. stimulation of damaged CNS tissue results in what appears to be either regenerated CNS axons or a "rescuing" of these fibers. Which ever mechanism may be operating, the present study was aimed at further characterizing the extent and degree of viability of these axons in the damaged rodent optic nerve model. Adult rat optic nerves were crushed and a 1.5uA constant current D.C. stimulator (Traxon) was applied so that the cathode was oriented distal to the lesion. Control animals received sham (no current) stimulators. After 5 weeks, animals were intraocularly injected with a 20% solution of HRP and sacrificed 48 hours later. TMB processing of the injected eye, optic nerve and brain followed by histological analysis of the visual system revealed that HRP was transported to the brain in "active" stimulator animals, but not in "inactive" stimulator animals. HRP transport could be demonstrated in virtually all animals that were treated, and in none of the animals that were not. These studies lend further support to D.C. therapy of damaged CNS tissues. Additional studies are aimed at analyzing the underlying mechanisms.

271.23

TIME-DEPENDENT CHANGES IN DNA SYNTHESIS IN NERVE AND DORSAL ROOT GANGLIA (DRG) AFTER CRUSH LESION OF SCIATIC NERVE: THE EFFECTS OF ELECTROMAGNETIC FIELDS. BF Sisken*, J Walker, H Traurig and R Stach. Center Biomed Engr & Dept Anat & Neurobiol; Div Orthop Surg, Univ of Kentucky, Lexington, KY 40506; Dir Res, Univ Mich- Flint, Flint, MI 48502

We have reported that low levels of pulsed electromagnetic fields (PEMF, amplitude 3 gauss, rep rate 2 Hz) increase the rate of sciatic nerve regeneration after a crush lesion (Br Res 485:1989). To understand the mechanisms underlying this stimulation, we are investigating the influence of PEMF on non-neuronal cell proliferation. Four days and 17 days following crush lesion, DRG and sciatic nerve from untreated and PEMF-treated rats were incubated with 25 uCi/ml ^{3}H- thymidine, fixed and processed for autoradiography. Labeled non-neuronal cells in DRG and different areas of the sciatic nerve were counted on autoradiographs. Four days post-lesion, significant ($p<0.001$) increases in numbers of labeled cells in DRG ipsilateral to the injury occurred relative to the unoperated side; PEMF had no significant effect on this response. There was an increase in labeled cells in lesioned nerve and PEMF significantly increased ($p<0.05$) the percentage of cells incorporating label at the crush site. At 17 days post-lesion satellite cells of DRG from PEMF-treated rats maintained a higher rate of proliferation compared to controls. In contrast PEMF did not affect Schwann cell proliferation in sciatic nerve. The PEMF-induced increases in labeled Schwann cells at the crush site at four days and in the satellite cells of DRG at 17 days post-lesion may play a role in the mechanism of PEMF stimulation of nerve regeneration. Supported by NIH NS 29621-01 and by the Orth. Res. Educ. Found.

271.24

LOW-INTENSITY ELECTRIC FIELD EFFECTS ON INJURY CURRENT FLOW IN A MODEL OF MAMMALIAN MYELINATED NERVE. K. Mosallaie and J.D. Sweeney*. The Bioengineering Program, Arizona State University, Tempe, AZ 85287-6006.

It has been reported that steady low-intensity electric fields (LIEFs) can in certain circumstances be used to induce, accelerate and/or guide nerve regeneration. One possible proposed mechanism for this effect is suppression of the axonal 'injury' currents that flow in the early stages of damage and regeneration. Such currents may contribute to axonal 'dieback' [see R.B. Borgens, Adv. Neurol. 47:51-66, 1988]. Our objectives have been (i) to create a computer-based mathematical model of injury current flow in transected mammalian myelinated nerve, and (ii) to simulate the effects of imposed steady LIEFs on injury current suppression. The model is based on iterative solution of a set of coupled finite-difference equations describing intracellular electroneutrality and conservation of ionic current. Nerve fiber nodal and internodal parameters in our compartmental-cable model are based on known or estimated mammalian myelinated nerve values. Ionic conductances of variable type (Ca^{2+}, Na^+, K^+, non-specific) and magnitude are included at the transection site that allow simulation of injury current flow in the early stages following damage. Our results indicate that cathode-distal steady electric fields of low-intensity can be used to suppress injury current entry of calcium and sodium ions at nerve transection sites.

This work supported by a grant from the Whitaker Foundation.

NEUROGLIA AND MYELIN II

272.1

NOVEL ISOFORMS OF MOUSE MYELIN BASIC PROTEIN PREDOMINANTLY EXPRESSED IN EMBRYONIC STAGE. K. Nakajima[1], K. Ikenaka[1], T. Kagawa[1], J. Aruga[2], J. Nakao[1], K. Nakahira[1], C. Shiota[1], S. U. Kim[3] and K. Mikoshiba.[2]* [1]Div. of Regulation of Macromolecular Function, Inst. for Protein Research, Osaka Univ., 3-2 Yamadaoka, Suita 565. [2]Dept. of Molecular Neurobiology, The Inst. of Med. Sci.,The Univ. of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan. [3]Div. of Neurology, Dept. of Med., Univ. of British Columbia, Vancouver, B. C. V6T 1W5, Canada.

Myelin basic protein (MBP) is one of the major proteins of myelin and thought to play an important role in myelin formation. Since myelination occurs postnatally in mice, the expression of MBP has been studied mainly in the postnatal stage. We report here that the MBP gene is expressed in mouse brain from 12th embryonic day (E12), before major gliogenesis occur, and that most of the predominant isoforms expressed in the embryonic stage are not those reported to date. They do not contain the sequence encoded by exon 5, probably due to specific alternative splicing. These isoforms without exon 5 show similar developmental profile with respect to each other, that is, they all peak at the embryonic stage and decrease thereafter. In the dysmyelinating mutant, *jimpy*, the level of these isoforms remain high even in the older ages. These results suggest that MBP has some unknown functions before myelinating stage other than that dedicated for myelination.

272.2

A NEW X-LINKED MYELIN MUTANT MOUSE. S. Billings-Gagliardi, D. Kirschner[1], P. Lane[2], N. Nadon[3], G. Stanhope and M. K. Wolf*, Dept. of Cell Biol., Univ. of Mass. Med. School, Worcester, MA 01655. [1]Children's Hosp. Med. Center, Boston, MA, [2]The Jackson Laboratory, Bar Harbor, ME and [3]Univ. of Oklahoma, Tulsa, OK.

A new mutation resembling jp, designated jp^{4J}, occurred in 1990 in Jackson C3H/HeJ mice. Linkage with Ta supports allelism with jp. The tremor of affected jp^{4J} males is not as marked as in jp but death comes 4-5 days earlier, about P-20. CNS has the least myelin of any known mouse with a single mutation. In 5 mice examined at P-16 to 20, optic nerves, dorsal cervical spinal cord and dorsal brainstem are devoid of myelin with few normal oligodendrocyte-like cells and rare foamy cells. Ventral cervical spinal cord and brainstem have at most 12-15 myelin profiles in 2-3 clusters per total cross-section. Most of that myelin consists of thin sheaths with poorly compacted lamellae. PNS appears unaffected. Immunoblot analyses (4 mice) show no detectable PLP in brain, but perhaps trace amounts in spinal cord. MBP is also significantly decreased but is more abundant than PLP, as in jp. Direct comparisons with jp (restriction enzyme analysis of PCR-amplified fragments) and jp^{msd} (single-strand conformation polymorphism assay) demonstrate that the PLP gene of jp^{4J} differs from both. Supported by NS11425, NS20824, NSF DIR 89 15728, OCAST 4175.

272.3

OLIGODENDROCYTE SURVIVAL AND MYELIN GENE EXPRESSION IN THE OLDER STRAIN OF THE MYELIN DEFICIENT RAT. N. L. Nadon*, I. D. Duncan•, D. Archer•, K. Hoffman•, C. Czisa# and M. Wells∞. University of Tulsa, Tulsa OK 74104, •University of Wisconsin, Madison WI 53706, #New York State Department of Health, Albany NY 12208, and ∞VA Medical Center, Northport NY 11768.

A strain of the myelin deficient (md) rat has been found that lives for up to 70-80 days, three to four times the lifespan of the normal md rat. These animals provide a model for studying the effect of the mutation in the proteolipid protein (PLP) gene on long-term oligodendrocyte (OL) survival. Quantitative Northern blot analysis indicated that in both brain and spinal cord, PLP mRNA levels remain relatively constant from 22 to 70 days of age, as do myelin basic protein (MBP) mRNA levels. The level of GFAP mRNA ranged from 50-180% of control levels, perhaps indicating extreme variability in the response of individual rats to long-term survival in the absence of myelination. EM analysis showed that cells typical of OLs are still present at 80 days of age *In vitro* studies likewise show evidence of mature OLs, as identified by the cell specific antibody 01. In sum, these results demonstrate that OLs survive up to 80 days despite the PLP mutation, and that the expression of the myelin genes does not change significantly with age. (Supported by OCAST #4175 and NIH #NS23124)

272.4

DISTINCT NEUROLOGICAL PHENOTYPES IN MBP-SV40 LARGE-T TRANSGENIC MICE: MYELIN GENE EXPRESSION IN A CLONAL CELL LINE. N.A. Jensen[1], G.M. Smith[2], J.S. Garvey[1], L. Hood[1] and H.D. Shine*[2]. [1]Div. of Biology, Caltech, Pasadena, CA 91125; [2]Dept. of Neurosurgery, Baylor College of Medicine, Houston, TX 77030.

We have targeted the expression of the SV40 large-T antigen to oligodendrocytes and Schwann cells in transgenic mice using the Myelin Basic Protein (MBP) promoter. Two neurological phenotypes, type-A and type-B, appeared in several founders and their offspring. Type-A mice developed action tremors that progressed to convulsions, an early death and had a hypomyelinated CNS. B-type mice exhibited progressive hindlimb ataxia and had either a hypomyelinated PNS or both hypomyelinated CNS and PNS. Immunohistochemical analysis of the CNS of type-A mice revealed T-antigen expression coincident with MBP and O4 positive cells; evidence that oligodendrocytes expressed the T-antigen. Several immortalized cells lines were established from type-A and -B mice. Cells from one cell line, 6E12, established from type-A CNS, were negative for the A2B5 antigen and only a portion were $O4^+$ and $GalC^+$. Treatment with dibutyryl cAMP (DBC) increased the number of $O4^+$ and $GalC^+$ cells. High amounts of MBP mRNA and low amounts of Proteolipid Protein (PLP) were present in cells not treated with DBC. DBC treatment reduced MBP mRNA and increased PLP mRNA. No MBP translation products were detected in the 6E12 despite the high levels of MBP mRNA.

Supported by grants from NIH (PO1 AG07687), the Kleberg Foundation, the Retinal Research Foundation, and the Alfred Benzon Foundation.

272.5

OLIGODENDROCYTE-SPECIFIC EXPRESSION OF BACTERIAL BETA-GALACTOSIDASE IN TRANSGENIC MICE. V.L. Friedrich, Jr. *, A. Gow and R.A. Lazzarini. Brookdale Center for Molecular Biology, The Mount Sinai Medical Center, New York, New York 10029

The mechanisms which restrict expression of the MBP gene to oligodendrocytes and Schwann cells are unknown. To approach this problem we created transgenic mice bearing a chimeric gene (designated MβP) consisting of the *E.coli lacZ* gene flanked upstream by MBP gene fragment -1907 to +36 and downstream by a fragment of the PLP gene containing splice and polyadenylation signals.

Enzyme histochemistry and immunofluorescence of cryostat and vibratome sections showed strong and specific expression of MβP in oligodendrocytes of adult mice and during development. Nuclear run-on assays showed transcription rates approaching 1/3 the native MBP gene. Remarkably, the MβP transgene was not expressed in Schwann cells, which do make MBP.
These results suggest that the MBP promoter/enhancer sequences are at least tripartite: a core promoter, the oligodendrocyte-specific enhancer elements, and a third component that either modifies the action of oligodendrocyte-specific enhancer elements to include Schwann cells or acts independently of it and specifically stimulates transcription in Schwann cells.

272.6

OLIGODENDROGLIAL HETEROGENEITY IN SITU AND IN VITRO. C. Bjartmar*, and C. Hildebrand. Dept of Cell Biology, Faculty of Health Sciences, S-581 85 Linköping, Sweden.

The morphology of myelinating rat oligodendrocytes was examined by light and electron microscopy, using in situ and in vitro preparations of spinal and telencephalic tissue. Light microscopic examination of galactocerebroside (GC) immunoreactive early oligodendrocytes in frozen sections from the initially myelinating cervical spinal cord (neonatal) and the corpus callosum (17 days postnatal) showed that the spinal cells present a generally bipolar morphology, whereas the callosal cells tend to be multipolar. These data were supplemented by serial section electron microscopy, showing that the spinal cells exhibit a few relatively short velate extensions, which provide 2-3 axons with myelin sheaths. The callosal cells reconstructed so far myelinate 6-10 axons through slender processes with varying lenghts. Examination of cultured GC-immunoreactive glial cells prepared from suspensions of neonatal spinal and telencephalic tissue showed that most of the spinal cells develop a fusiform shape, while the telencephalic cells achieve a stellate outline in vitro, in the absence of neurons. The picture is principally similar in oligodendroglial-enriched cultures. The results support the view that oligodendrocytes constitute a structurally heterogeneous population, and that this heterogeneity may be a primary property intrinsic to these cells.

272.7

CNP LABELING OF OLIGODENDROCYTES AND MYELIN IN DEVELOPING RAT BRAIN. N. Nousek-Goebl* and D. S. Grega+. R&D Division, Boehringer Mannheim Corp. and +Indiana Univ. School of Medicine, Program in Medical Neurobiology, Indianapolis, IN 46250.

CNP (2',3'-cyclic nucleotide 3'-phosphodiesterase) is found in high levels in the brain, especially in oligodendrocytes (oligos) and areas rich in myelin. Most in vivo studies have been biochemical in their characterization of CNP distribution, while the most convincing immunohistochemical localization has been reported with cultured cells. We have been interested in the expression of CNP as an oligo marker in the developing rat brain and report a method for the immunohistochemical localization of CNP in rat brain sections using the monoclonal antibody, anti-CNP with an immunoperoxidase method. Brain sections from postnatal day 10, 18 and >4 months (adult) were used as they represent ages of early, peak and mature myelination. Anti-GFAP labeling of astrocytes was performed for comparative purposes. Anti-CNP labeled oligo soma and processes at all ages. Unmyelinated regions first exhibited CNP labeling of highly stellate cells. As myelination progressed, cells displayed fewer labeled processes. There was a shift in the frequency and morphology of labeled cells with age with a peak of the highly stellate cells at PN18. Labeling of myelin increased with age, peaking in the adult, concomitant with a marked decrease in labeled stellate cells. Anti-CNP and anti-GFAP label distinctly different populations of cells. Anti-CNP is an excellent marker for oligos and myelin.

272.8

ISOLATION OF cDNA CLONES FROM RAT OLIGODENDROCYTES CODING FOR ZINC FINGER PROTEINS. U. Pott, N. Schaeren-Wiemers*, M.E. Schwab. Brain Research Institute, University of Zurich, August-Forel-Str. 1, CH-8029 Zurich, Switzerland

The function of the vertebrate nervous system is dependent on the appropriate myelination of its fiber tracts. In the CNS, the myelin sheath around the axons is produced by oligodendrocytes. In order to understand oligodendrocyte differentiation in more detail, we aimed at the cloning of putative transcription factors expressed in this particular glial cell type.

A cDNA library was constructed from highly enriched differentiated rat oligodendrocytes and screened with an oligonucleotide deduced from a conserved region of the Krüppel family of zinc finger proteins (the H/C link). Eleven different cDNAs were obtained, four of which were selected for further analysis. So far, complete sequencing of two clones revealed that they comprise the coding capacity for two new members of the zinc finger gene family; the deduced proteins contain 458 amino acids (with 12 finger repeats) and 503 amino acids (with 13 finger repeats), respectively. While one of the proteins is not complete at its amino terminus the other one seems to be full length. Northern blot analysis was done to investigate tissue specific expression of the corresponding mRNAs. As expected, all four genes are expressed in the spinal cord, the expression of three clones being higher in the CNS than in all peripheral tissues tested (with the exception of testis). Two of the cDNAs recognize RNAs of considerably different sizes, the relative abundances of which vary in different tissues. The expression pattern of all four clones clearly shows that the mRNAs also occur in other cell types in addition to oligodendrocytes.

272.9

MYELIN BASIC PROTEIN mRNA IS ASSOCIATED WITH THE OLIGODENDROCYTE CYTOSKELETON. S.J. Hill* and E. Barbarese, Dept. Neurology, U. Conn. Health Center, Farmington, CT 06030.

Oligodendrocytes synthesize myelin basic protein (MBP), a major component of CNS myelin. In monolayer cultures of oligodendrocytes, MBP is associated with the cell plasma membrane and is localized to the cell body, the processes and their membranous expansions. Extraction of the soluble components of the oligodendrocytes using the protocol of Biegel and Pachter (1991), resulted in an insoluble fraction termed CSK composed mainly of cytoskeletal elements as demonstrated by immunocytochemistry and SDS gel analysis of the proteins. MBP polypeptide and other myelin components such as galactosylcerebroside and cyclic nucleotide phosphohydrolase partitioned with the soluble fraction, in contrast with the results of Wilson and Brophy (1989). Fluorescent in situ hybridization (FISH) was used to localize MBP mRNA in unextracted and CSK extracted cells. The majority of the MBP mRNA remained associated with the CSK of the oligodendrocyte. Combined immunocytochemistry and FISH also indicated that tRNA synthetase and elongation factor I are found with the CSK of the oligodendrocytes and in close proximity to the MBP mRNA. These results support the notion that MBP is translated in the oligodendrocyte processes in association with the cytoskeleton.

272.10

GLUTAMATE IS TOXIC TO OLIGODENDROGLIA BY AN UPTAKE DEPENDENT MECHANISM. A. Oka*, M.J. Belliveau, P.A. Rosenberg and J.J. Volpe. Children's Hospital and Harvard Medical School, Boston, MA, 02115.

We investigated the potential role of excitatory amino acids in white matter injury, using oligodendroglial (oligo) cultures separated from mixed glial primary cultures of dissociated rat cerebral hemispheres. Oligos were highly vulnerable to L-glutamate (GLU) (EC_{50} for 24 hr exposure: 260 μM); no toxicity was observed in astrocytes with 20-fold higher concentrations. Uptake studies and autoradiography with L-[^{3}H]-GLU showed the presence of GLU uptake into oligos. D,L-threo-β-hydroxyaspartate, which inhibited the uptake of L-[^{3}H]-GLU, protected oligodendroglia against GLU. Antagonists for NMDA (MK-801) and non-NMDA (CNQX) GLU ionotropic receptors, respectively, did not prevent toxicity, indicating that cell death is due to GLU transport rather than receptor-mediated mechanisms. Both Na^+-dependent and Na^+-independent uptake was present. A role for cell swelling in the GLU-induced cell death was shown by protection in the presence of hypertonic solutions (100 mM sucrose, raffinose or 50 mM NaCl).

These findings indicate oligos are killed by micromolar concentrations of GLU and that the mechanism of this cytotoxicity involves GLU uptake and cell swelling. Supported by NIH R01-HD-07464 (J.J.V.)

272.11

OXIDATIVE STRESS MEDIATES GLUTAMATE TOXICITY IN RAT OLIGODENDROGLIA. M.J. Belliveau, A. Oka, J.W. Lin, P.A. Rosenberg and J.J. Volpe*. Children's Hospital and Harvard Medical School, Boston, MA, 02115.

We demonstrated that L-glutamate (GLU) induces cytotoxicity in cultured oligodendroglia (oligos) and that GLU transport into the cell is critical for the toxicity (see abstract of Oka et al.). Toxicity was completely prevented by the addition of cystine (CYS; 200 μM). Because glutathione (GSH) is a crucial product of intracellular CYS and an important protective agent against oxidative stress, we measured intracellular GSH levels after exposure to GLU. GSH levels decreased rapidly (60% reduction in 9 hours with 2 mM GLU) prior to the decrease in cell viability, and there was nearly total prevention of GSH depletion at a concentration of CYS that prevented GLU-induced oligo death. Moreover, two free radical scavengers, vitamin E (30 μM) and idebenone (1 μM), protected oligos from the toxicity of 2 mM GLU. Vitamin E did not inhibit uptake of L-[^{3}H]-GLU, and depletion of intracellular GSH occurred in the presence of vitamin E. Protection from GLU-induced cell death by CYS and antioxidants has also been observed with immature neurons by Coyle and co-workers (Murphy et al. (1990) FASEB J. **4**:1624-1633).

Taken together with the results of our previous abstract (Oka et al.), our findings indicate that GLU causes oligo death by uptake into the cell, cell swelling, induction of GSH depletion, and lethal accumulation of oxygen radicals. Supported by NIH R01-HD-07464 (J.J.V.)

272.12

RESPONSES OF OLIGODENDROCYTES ISOLATED FROM THE SPINAL CORD OF EARLY POSTNATAL DOG TO bFGF, NGF, AND SERUM IN A DEFINED MEDIUM K.L. Hoffman, D.R. Archer, and I.D. Duncan*. School of Veterinary Medicine, University of Wisconsin-Madison, 53706

The growth factors bFGF, and more recently, NGF have been implicated in regulating the mitotic cycle and process outgrowth of oligodendrocytes from neonatal rats and adult pigs. We investigated the effects of these growth factors and low concentrations of several kinds serum on the process outgrowth of early postnatal canine oligodendrocytes in a defined medium. Highly enriched oligodendrocytes (95-100% immunoreactive for the O1 anti-galactocerebroside antibody) were obtained from enzymatically and mechanically dissociated spinal cords of early postnatal dogs, using antibody coated dishes to isolate cells immunoreactive for the O1 antibody. The responses of these cells to various environmental conditions were determined in the virtual absence of other cell types. We have found that, although these cells are able to adhere to and extend processes onto a poly-L-lysine coated glass surface within 1-2 days of plating, a very low concentration of serum (0.5%) is necessary for the longer term (7+ days) maintenance of processes. To test the possibility that a component of serum that had adsorbed onto the culture surface was responsible for the maintenance of processes, cells were cultured on poly-L-lysine coated coverslips exposed to FBS for 4 hours, and then rinsed. This modified substrate was not effective at maintaining processes. 2.5S NGF and bFGF were added to serum free medium to determine their effects on the morphology of these cells. Cells exposed to bFGF at 5 and 50ng/ml for 4 days had few processes compared to control and tended to have an elongated, bipolar morphology, whereas NGF slightly enhanced the outgrowth of processes. (Supported by NIH grant NS23124, The Myelin Project, and The Elizabeth Elser Doolittle Charitable Trust.)

272.13

CARBACHOL INCREASES CYTOSOLIC FREE CALCIUM IN CULTURED RAT MICROGLIA. E.R. Whittemore*, A.R. Korotzer, A. Etebari, and C.W. Cotman, Department of Psychobiology, University of California, Irvine, CA 92717

Microglia are resident macrophages in the CNS and may serve as the principle scavenger cells of the brain. In addition, microglia are known to release polypeptide growth factors that can influence neuronal and glial survival and growth. However, the possible signals from neurons to microglia are not well understood. It is possible that select neurotransmitters may signal microglia. Thus, we measured intracellular calcium responses to carbachol in cultured rat microglia using the calcium-sensitive dye Fura-2.

Purified cultures of microglia were prepared from the cerebral cortex of 1-4 day old rat pups, and were plated onto glass coverslips for observation using digital fluorescence imaging microscopy. Microglia were identified by morphological criterion and by using the microglia-specific label, acetylated low density lipoprotein. Cells were loaded with Fura-2/AM, and placed in a flow-through chamber on the stage of a Nikon Diaphot microscope. In these experiments, microglia were found to respond to carbachol (1-100 μM) with an increase in intracellular free calcium that was oscillatory in nature. Some cells responded with robust increases in free calcium, while others showed weaker signals. Generally a second application of carbachol gave a weaker response than the first, or none at all. Carbachol also induced increases in intracellular free calcium in nominally calcium-free medium, suggesting that this response was due to the release of calcium from intracellular stores. The response to 10 μM carbachol was inhibited by 10 μM atropine, consistent with an action of carbachol at muscarinic receptors. These data suggest the possibility that microglia can respond to neurotransmitters, and therefore that there may exist a signaling loop between these two cell types.

272.14

EXTRACELLULAR ATP OPENS CATION CHANNELS IN CULTURED MICROGLIAL CELLS. W.Walz*, R.Banati and H.Kettenmann, Institut f. Neurobiologie, Universität Heidelberg, Im Neuenheimer Feld 345, W-6900 Heidelberg, Germany.

Extracellular ATP is now a well established modulator which is released by neurons, endothelial cells, as well as damaged cells. We studied the effect of ATP on mouse microglial cells in primary cultures using the whole-cell patch clamp technique. If the cells were clamped at their resting potential (-70mV), application of 100μM ATP evoked an inward current of about 100pA, which desensitized rapidly. Application of extracellular ADP, AMP and adenosine did not evoke any responses under these conditions. If during such an ATP response the potential of the cells was clamped stepwise at various voltages between -105 and +35mV, the conductance change evoked by ATP was 2.2nS and the reversal potential of the response was -11mV. Removing extracellular Ca^{2+} and Cl^- did not change the conductance nor the reversal potential of the ATP response. Removing extracellular Na^+ by choline shifted the reversal potential of the ATP response to -43mV and reduced the conductance by 88%. Replacement of extracellular Na^+ by K^+ changed the reversal potential from -10 to +9mV and reduced the conductance by 82%. The results are all compatible with extracellular ATP acting through a P_2-purinoceptor and opening up a cation conductance. The resulting response could well act as an activation signal for microglia.

272.15

BIOLOGY OF ADULT HUMAN MICROGLIA IN VITRO UNDER BASAL AND ACTIVATED CONDITIONS. K. Williams, N.P. Dooley, G.C. Trudel*, V.W. Yong, and J.P. Antel. Dept. of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, P.Q. H3G 2B4.

Microglia participate in multiple types of pathologic reactions occurring in the CNS. We have established highly purified cultures (> 95%) of brain microglia from young adult humans. Microglia under basal culture conditions express Fc receptors I-III, MHC class I and II antigens, and are Leu-M5 and EBM11 immunoreative. In contrast to peripheral blood monoctyes, microglia do not stain with Leu-M3 or anti CD4 antibody and are negative for non-specific esterase. After stimulation with interferon gamma (IFN) (100 U/ml) microglia show increased staining intensity for Fc receptor I and MHC class II antigen, and enhanced ability to present recall antigens (tetanus, candida albicans) to autologous T-cells. Using reverse transcriptase polymerase chain reaction (RT-PCR), levels of IL-1 alpha and IL-6 mRNA expression by microglia were compared with glyceraldehyde-3-phosphate dehydrogenase (GADPH), a house keeping gene. Results show that human microglia express IL-1 alpha and IL-6 mRNA and that the levels of expression are increased after the cells are exposed to IFN. These results indicate that human adult microglial cells can interact with endogenous and exogenous CNS cells via both cell-cell and soluble factor (cytokine) mediated mechanisms.

272.16

INDUCTION OF NITRIC OXIDE SYNTHASE IN RAT BRAIN MICROGLIA. L. J. CHANDLER*, N. GUZMAN, F. CREWS AND C. SUMNERS. University of Florida College of Medicine, Box 100267, Gainesville, FL 32610 USA.

Nitric oxide (NO) is formed in a variety of tissues where it appears to play an important role in a number of physiological and pathological processes. NO is synthesized from L-arginine by nitric oxide synthase (NOS) with formation of citrulline as a end-product of the reaction. Brain neurons possess a constitutive calcium/calmodulin dependent form of NOS, whereas many non-neuronal tissues possess an inducible form of NOS that is calcium/calmodulin independent and can be induced by lipopolysaccharide and cytokines. In the present study, we investigated the possibility that brain may possess both types of NOS. NOS activity was determined by measuring the formation of [^{3}H]citrulline from [^{3}H]arginine in rat primary neuronal cortical, astroglial and microglial cultures. Neurons were observed to possess the constitutive form of NOS that was activated by NMDA, kainate and ionomycin, whereas neither astroglial or microglial cultures exhibited constitutive NOS activity. Exposure of microglial cultures to lipopolysaccharide resulted in a 3-4 fold increase in NOS activity. Induction of NOS by lipopolysaccharide was both dose (maximum at 1 μg/ml) and time (maximum at 24 hrs) dependent and was inhibited by dexamethasone (1 μM), cycloheximide (40 μM) and actinomycin (4 μM). NOS was also induced by the cytokine interferon-γ (100 U/ml), but not interleukin-1β (3 nM) or tumor necrosis factor (200 ng/ml). These studies demonstrate that in addition to possessing a constitutive form of NOS, the brain also contains an inducible form of NOS. Induction of NOS in brain microglia may have important implications in terms of the possible involvement of microglia in many neurodegenerative processes (Supported by NIAAA grants AA10027 and AA06069 and PHS grant NS-19441).

273.1

QUANTAL SECRETION FROM VISUALIZED VARICOSITIES OF SYMPATHETIC NERVE TERMINALS. M.R.Bennett*,W.Gibson,N.A.Lavidis and R.Poznanski of Neurobiology Laboratory, Department of Physiology, University of Sydney, NSW 2006, Australia.

The probability of quantal secretion from sets of one to six varicosities of individual sympathetic nerve terminals on the surface of the mouse vas deferens has been determined. Varicose preterminal axons are observed giving rise to single axons on the surface of the vas deferens using either the FAGLU stain for catecholamines or confocal microscopy following neurofilament antibody staining. Electronmicroscopy shows that the varicosities of these single axons are found up to 4 µm apart and sometimes form close contacts with the smooth muscles. The distribution of current in the three-dimensional bidomain of the smooth muscle syncytium following the secretion of a quantum from a varicosity has been determined using a finite-element model. It is shown that an extracellular electrode of about 4 µm diameter can be used to analyze quantal secretion from single varicosities. In high Ca^{2+} only one quantum per varicosity has been observed using this technique, indicating that a release site is probably only able to secrete at most one quantum on arrival of the nerve impulse.

273.2

NEUROTRANSMITTER RELEASE FROM SYNAPTOTAGMIN-DEFICIENT CLONAL PC12 VARIANTS Y. Shoji-Kasai, A. Yoshida, K. Sato, T. Hoshino, A. Ogura, S. Kondo, M. Takahashi*, Mitsubishi Kasei Institute of Life Sciences. Machida, Tokyo, JAPAN

Synaptotagmin (p65) is an abundant synaptic vesicle protein of neurons and contains regions similar to the regulatory domain of protein kinase C. In PC12 cells synaptotagmin is expressed and located in large dense-core vesicles, containing catecholamines. To assess the functional role of synaptotagmin, we attempted to isolate clonal variants of PC12 cells which are deficient in synaptotagmin. First we elimi nated most of the cells expressing synaptotagmin by selectively killing those cells that reacted with an antibody recognizing synaptotagmin located on the cell surface with the complement. After repeating the selection procedure, we selected three synaptotagmin-deficient clones. No synaptotagmin was detected in the variant cells by immunoblotting with three antibodies of different specificity. Synaptotagmin mRNA was not detected in Northern blotting experiments. Like normal PC12 cells, all of the variants released catecholamine and ATP when treated with medium containing a high concentration of K^+, (60 mM). We conclude that synaptotagmin is not essential for the exocytosis of the large dense-core vesicles of PC12 cells. We are further studying the neurotransmitter release from the variant cells differentiated to neurons by NGF.

273.3

ANTIBODIES AGAINST B-50/GAP-43 AND CALCINEURIN INHIBIT Ca^{2+}-INDUCED CATECHOLAMINE RELEASE. P.N.E. De Graan*, J.J.H. Hens, A.B. Oestreicher and W.H. Gispen. Rudolf Magnus Inst. and Inst. Mol. Biol., Padualaan 8, 3584 CH Utrecht, NL.

Protein B-50 (also known as GAP-43, F1 and neuromodulin) is a nervous tissue-specific protein, which is associated with the presynaptic membrane. B-50 is a well-characterized substrate of protein kinase C (PKC) with a single PKC phosphorylation site at ser^{41}. B-50 dephosphorylation (at least in vitro) can be mediated by a number of phosphatases, including the Ca^{2+}/calmodulin-dependent phosphatase calcineurin. B-50 is an atypical calmodulin-binding protein. Under resting Ca^{2+} conditions (10^{-8}-10^{-7} M) calmodulin is bound, whereas at Ca^{2+} concentrations from 10^{-6} M and higher (concentrations occurring locally during depolarization in the submembrane region) calmodulin progressively dissociates from B-50. The calmodulin-binding domain in B-50 (residues 39-51) includes the PKC phosphorylation site. Phosphorylation of this site prohibits calmodulin binding. Using streptolysin-O-permeated synaptosomes we have shown that polyclonal IgGs against B-50, which interfere with B-50 phosphorylation, inhibit Ca^{2+}-induced noradrenaline (NA) release (Dekker et al., *Nature* **342**: 74, 1989). In this study we introduced monoclonal IgGs directed against N- or C-terminal B-50 epitopes and polyclonal IgGs against calcineurin into permeated synaptosomes to study their effects on Ca^{2+}-induced NA release. We show that only anti-B-50 IgGs inhibiting B-50 phosphorylation, dephosphorylation and calmodulin binding, interfere with Ca^{2+}-induced endogenous and ^3H-labeled NA release. These data confirm that B-50 is involved in the molecular mechanism of catecholamine release after the Ca^{2+} trigger. Since Ca^{2+}-induced NA release cannot be inhibited by PKC inhibitors (PKC_{19-36} and H-7), but is almost completely blocked by anti-calcineurin IgGs, we propose that dephosphorylation (possibly of B-50) by calcineurin, which is stimulated by a depolarization-induced local increase in Ca^{2+} and calmodulin levels, is an important event in the regulation of NA release.

273.4

SPACE AND TIME CHARACTERISTICS OF ACETYLCHOLINE RELEASE IN THE *TORPEDO* ELECTRIC ORGAN. R. Girod, P.Correges, J. Jacquet, M. Schorderet* and Y. Dunant. Departement de Pharmacologie, C.M.U., 1211 Geneve 4, Switzerland.

Spontaneous miniature electroplaque potentials (MEPPs) and evoked electroplaque current (EPCs) were recorded focally with an extracellular electrode in untreated *Torpedo* electric organ. 75 % of MEPPs (fast-MEPPs) had a homogenous and rapid time course. Their amplitude distribution revealed a main bell-shaped population, and a population skewed towards the noise. Both the time-to-peak and the half-decay time of fast-MEPPs showed a positive correlation with the amplitude. 25 % of MEPPs had a much slower time-course. Slow-rising MEPPs with rapid decay were analysed in details. They showed a wide variety of shapes and were in average smaller than the fast-MEPPs. These characterisitics were compared with a bidimensional computer model of synaptic transmission designed to deliver various amounts of acetylcholine (ACh) molecules at the same or at different sites of the synapse, either synchronously or with various temporal patterns. The electric organ MEPPs were found consistent with a model in which a quantum of transmitter is composed of a preferential number of 10 subunits of ACh; the subunits are produced either from a single point or from sites situated at less than 300 nm from each other, so that they act on overlapping receptor fields. The subunits are released in a single synchronous discharge for the majority of events (fast-MEPPs), but with desynchronized shots for a certain percentage of MEPPs (slow-rising MEPPs).

Multiquantal EPCs were elicited by activation of nerve terminals. Their time-to-peak was independent of amplitude but their decaying phase showed a positive correlation with amplitude. Comparison with the computer model suggested that in the electric organ evoked quanta are released from sites separated from each other by 600-1,000 nm.

273.5

VX ENHANCES TRANSMITTER RELEASE IN CULTURED HIPPOCAMPAL NEURONS. E.S. Rocha*, Y. Aracava & E.X. Albuquerque. Dept. Pharmacal. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201; Lab. Mol. Pharmacal., UFRJ, 21944, Rio de Janeiro, Brazil.

VX and (-)physostigmine (Phy), organophosphate and carbamate acetylcholinesterase inhibitors, respectively, have been reported to elicit excitatory postsynaptic potentials and action potentials at the locust neuromuscular junctions (*JPET* 239: 270, 1986). This activity, resulting from an intense neurotransmitter release, was abolished by tetrodotoxin (TTX). To examine whether these compounds also affect the transmitter release at mammalian brain synapses, we used the whole-cell technique to record miniature excitatory postsynaptic currents (mEPSCs) from rat fetal (18- to 20-day-gestation) hippocampal neurons kept in culture for 10-30 days. In the absence of TTX, the spontaneous mEPSC activation was characterized by high frequency bursts of events followed by silent periods. For the VX study, neurons with spontaneous synaptic activity markedly suppressed by TTX (0.3 µM) were used. Addition of VX (10-500 nM) caused a significant increase in the frequency of mEPSCs. In contrast to the TTX-sensitive currents, the VX-induced mEPSCs appeared randomly. The mean amplitude of these currents was dependent on the transmembrane voltage. Antagonists of the N-methyl-*D*-aspartate, nicotinic acetylcholine and GABA receptors were tested, separately, by bath application. All antagonists decreased the number of mEPSCs, the proportion depending upon the contribution of the synapses present on the cell. This indicates a nonspecific action of VX. However, most of spontaneous mEPSCs were blocked by the GABA inhibitor bicuculline. Phy (100 nM-100 µM) had no effect on the mEPSCs, either in the presence or absence of TTX. The present results showed a massive non-specific transmitter release in the presence of VX through a mechanism independent of TTX-sensitive Na^+ channels. Support: US Army Med. Res. Dev. Comm. Contr. DAMD-17-88-C-8119, CNPq, FINEP/UMAB Mol. Pharmacal. Training Program.

273.6

CALCIUM AND NICOTINE INDUCED DESENSITIZATION OF ENDOGENOUS ACETYLCHOLINE RELEASE FROM MAMMALIAN BRAIN CHOLINERGIC NERVE ENDINGS. Tarun Tandon and Enrique L.M. Ochoa * Dept. of Pediatrics, University of California at Davis, Davis CA 95616.

Quantal release of transmitter from presynaptic terminals can be desensitized by calcium injection, reduction of intracellular calcium sequestration, or repetitive electrical stimulation (Miledi, 1966; Katz and Miledi, 1969; Adams et al., 1985; Israel and Lesbats, 1987). Nicotine evokes transmitter release from pharmacologically distinct neurons, but its capacity to induce desensitization has not yet been demonstrated at central mammalian cholinergic synapses. Endogenous acetylcholine release from hippocampal synaptosomes was monitored by a novel continuous fluorometric technique with nanomolar sensitivity using 2'-7'-dichlorofluorescein. Concurrent depolarization of the synaptosomal membrane by (-) nicotine was assesed using the transmembrane equilibrium distribution of tetraphenylphosphonium ion. Nicotine and veratridine depolarized synaptosomes in a tetrodotoxin insensitive and sensitive manner respectively, and κ-bungarotoxin blocked the effects of (-) nicotine. Calcium-dependent acetylcholine release was induced by 30 mM KCl, 30 µM veratridine and 0.001 - 300 µM (-) nicotine. Calcium influx-induced transmitter release in calcium-free media was demonstrated in the presence of gramicidin D (0.1 µM) and the calcium ionophore ionomycin (0.5 µM). A concentration-response curve was constructed for ionomycin-mediated calcium influx (0.05 -10 mM) showing inactivation of release starting at 3.5 mM. Preincubation with femtomolar concentrations of the Ca-ATPase blocker thapsigargin in the presence of external calcium, reduced the amount of acetylcholine release stimulated by 30 µM veratridine. This suggests that the phenomenon is mediated by an elevation of intracellular calcium. Nicotine-induced acetylcholine release (EC50 3 µM) was calcium and κ−bungarotoxin sensitive (0.1 µM) but α−bungarotoxin insensitive (0.1 µM). A 10 min preincubation of the synaptosomes with nM concentrations of (-) nicotine (ineffective in liberating acetylcholine) desensitized nicotine-induced release in the µM range. Desensitization was not due to depletion of trasmitter stores, as shown in control experiments where the synaptosomal membrane was disrupted with Triton X-100. The present data suggest that (-) nicotine may depolarize the synaptosomal membrane and induce acetylcholine release *via* the operation of specific presynaptic nicotinic acetylcholine autoreceptors. Factors in addition to autoreceptors may be instrumental in establishing calcium and (-) nicotine-induced desensitization of transmitter release .

273.7

STRONTIUM IONS HAVE A LESSER EFFECT THAN CALCIUM IONS TO MOBILIZE QUANTA BUT A GREATER ABILITY TO INCREASE PROBABILITY OF TRANSMITTER RELEASE. S.D. Provan* and M.D. Miyamoto, Dept. of Pharmacol., East Tenn. State Univ. Col. of Med., Johnson City, TN 37614-0577.

Extracellular [Sr^{2+}] is less effective than equimolar [Ca^{2+}] in supporting synchronous transmitter release from nerve terminals. We tested whether this was due to a lower efficacy at transmitter release sites by using miniature endplate potentials and sustained 10 mM K^+ stimulation to obtain unbiased estimates of n (no. of functional release sites), p (probability of release), and var_p (spatial variance in p). Data were obtained from single endplates of frogs during 3 changes each in [Sr^{2+}] and [Ca^{2+}], and linear regressions computed for each relationship. The intersection of the 2 lines for m represented the point at which Sr^{2+} and Ca^{2+} were equi-effective in supporting quantal release at a particular junction. Since $m = np$, this could be due to equal n and p, larger n and smaller p, or any combination thereof. For equivalent m's, $n_{Ca} = 14.95 \pm 2.66$ quanta and $p_{Ca} = 0.233$, whereas $n_{Sr} = 10.13 \pm 1.09$ quanta and $p_{Sr} = 0.332$ (8 experiments). If p represents activation of release sites by Ca^{2+} (or surrogate) and n the no. of occupied release sites, this indicates that Sr^{2+} is more effective in activating the release sites and less effective in mobilizing vesicles to these sites. This may be due to the different ionic potentials of Sr^{2+} and Ca^{2+} (Silinsky, Pharmacol. Rev. **37**: 81, 1985). (NIH NS22457.)

273.8

LINOPIRDINE (DuP 996)—A DRUG POTENTIALLY USEFUL FOR ALZHEIMER'S DISEASE—ACTS AT AN EXTRACELLULAR SITE TO INCREASE THE QUANTAL RELEASE OF CHOLINERGIC TRANSMITTER. M.D. Miyamoto* and S.D. Provan, Dept. of Pharmacol., East Tenn. State Univ. Col. of Med., Johnson City, TN 37614-0577.

The actions of linopirdine (3,3-bis(4-pyrindinylmethyl)-1-phenylindolin-2-one) at the frog neuromuscular junction were examined by using miniature endplate potentials (MEPPs) and sustained 10 mM K^+ stimulation to obtain unbiased estimates of n (no. of functional release sites), p (probability of release), and var_p (spatial variance in p). Linopirdine produced an increase in m (no. of quanta released) which was due to an increase in n and p. The increase was dose-dependent between 0.1-100 μM and completely reversible after 15-20 min of wash. At >10 μM, there was a saturation in the increase in p, but not in m or n. There was no change in var_p (believed to reflect Ca^{2+} release from intraterminal stores). Block of presynaptic Na and Ca channels with tetrodotoxin (3 μM) and Co^{2+} (1.8 mM) produced a reversible inhibition in the MEPP frequency increase to linopirdine. MEPP amplitude and time course were not affected, indicating that the drug had little or no anticholinesterase activity. Linopirdine thus enhances cholinergic transmission by a pre- and not a postsynaptic mechanism. This appears to involve an extracellular action to increase Na^+ and/or Ca^{2+} influx at the terminal and not an intracellular action to release Ca^{2+} from cytoplasmic stores. (Supported by NIH NS22457.)

273.9

EFFECT OF CALCIUM CHANNEL BLOCKERS ON PREJUNCTIONAL D_2 DOPAMINE RECEPTOR MEDIATED INHIBITION OF NOREPINEPHRINE RELEASE. D.J. Friedman* and S.P. Duckles, Dept. of Pharmacology, University of California, Irvine, CA 92717

Activation of prejunctional D_2 dopamine receptors mediates an inhibition of norepinephrine release from perivascular nerves of the rat tail artery, an effect which is inversely correlated to stimulation intensity and external calcium concentration. Therefore, the role of calcium channels in the D_2 receptor-mediated inhibition of norepinephrine release was investigated in perfused rat tail arteries preloaded with [^{3}H]norepinephrine. Nifedipine (10^{-7} M) and ω-conotoxin (10^{-8} M) were used to block L-type or N- and L-type calcium channels, respectively. Tritium efflux in response to low intensity (1 Hz/50 pulses) or high intensity (4 Hz/100 pulses) stimulation was measured. At 4 Hz/100 pulses nifedipine alone reduced tritium efflux by 22 ± 6%, while no decrease was observed at 1 Hz/50 pulses indicating that L-type calcium channels contribute more to calcium influx at high levels of nerve stimulation. Together nifedipine and ω-conotoxin reduced stimulation-evoked tritium efflux by 73 ± 8% and 80 ± 3% at low and high levels of stimulation, respectively. The D_2 dopamine agonist N-0923 (10^{-8} M) inhibited stimulation-evoked tritium efflux by 60 ± 9% at 1 Hz/50 pulses and 38 ± 8% at 4 Hz/100 pulses. In the presence of calcium channel blockers the inhibition produced by N-0923 was 73 ± 11% at 1 Hz/50 pulses and 9 ± 15% at 4 Hz/100 pulses. Thus, the effects of D_2 receptor activation appear to be more sensitive to calcium channel blockers at high levels of stimulation.

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273.10

ANALYSIS OF THE AUTOINHIBITORY MECHANISM OF NORADRENALINE RELEASE IN CHICK SYMPATHETIC NEURONS. S.Huck*, S.Boehm, H.Drobny, E.A.Singer, Depts. of Neuropharmacology and Pharmacology, Univ. of Vienna, Waehringerstrasse 13a, A-1090 Vienna, Austria.

Signal transduction mechanisms underlying the α_2-adrenergic modulation of noradrenaline (NA) release from cultured chick sympathetic neurons were studied with patch-clamp techniques and by measuring electrically evoked ^{3}H-NA release.

After 1 day *in vitro*, whole-cell Ca^{2+} currents were recorded with 115mM N-methyl-D-glucamine and 120mM choline chloride as bulk ions of the pipette and the bathing solution, respectively. For release studies, cells were loaded with ^{3}H-NA after 5 days *in vitro* and superfused. ^{3}H-overflow was elicited by 36 monophasic pulses (0.5ms, 3Hz, 40V/cm, 60mA) 76 (S_1) and 96min (S_2) after onset of the superfusion. Test drugs were added to the buffer 16min before S_2, and drug effects were evaluated by calculating S_2/S_1 ratios.

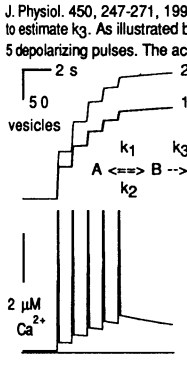
NA (in presence of the reuptake inhibitor cocaine) and the selective α_2-adrenoceptor agonists UK 14304 (UK) and clonidine induced a concentration-dependent, yohimbine-sensitive inhibition of Ca^{2+} currents and of the electrically evoked ^{3}H-overflow. Yohimbine alone affected neither Ca^{2+} currents nor ^{3}H-overflow. A 24h treatment of the neurons with pertussis toxin (100ng/ml) abolished the α_2-adrenergic inhibition of Ca^{2+} currents and of ^{3}H-overflow. ω-Conotoxin greatly reduced Ca^{2+} currents and diminished stimulation-induced ^{3}H-overflow by 95%. The proteinkinase C inhibitor polymyxin B reduced the electrically induced overflow and antagonized the facilitatory action of 4ß-phorbol-12,13-dibutyrate (4ß-PDB). Neither polymyxin B nor a 24h treatment with 4ß-PDB (aiming at down-regulating proteinkinase C) impaired the inhibitory effect of UK. Adenylate cyclase stimulation by forskolin enhanced the evoked release but left the inhibitory action of UK unaltered. Neither the Ca^{2+} current itself nor its inhibition by UK were affected by either 4ß-PDB or forskolin.

Thus, a G-Protein mediated inhibition of ω-Conotoxin-sensitive Ca^{2+} currents underlies the autoinhibitory modulation of NA release in chick sympathetic neurons. Adenylate cyclase and proteinkinase C both control the neurotransmitter release, but are not involved in the α_2-adrenoceptor-mediated autoregulation.

273.11

A TWO-STEP MODEL FOR SECRETORY CONTROL IN NEUROENDOCRINE CELLS. Ch. Heinemann, L. v. Rüden, R. H. Chow, and E. Neher*, Max-Planck-Institut für biophysikalische Chemie. Göttingen, Germany

Recent evidence indicates that neurosecretory cells have at least two functionally distinguishable pools of vesicles -- a small one that is "readily released" ("B", below), and a larger "reserve" pool ("A", below). We examined a model in which these two pools lead sequentially to a "secreted" pool ("C", below). Rates k_1 and k_3 are calcium dependent, and were estimated from membrane capacitance measurements in bovine chromaffin cells: at high fixed Ca step A -->B becomes rate-limiting (after pool B is partially depleted), and k_1 can be obtained (Augustine and Neher, J. Physiol. 450, 247-271, 1992); calcium transients induced by caffeine were used to estimate k_3. As illustrated below, calcium transients were simulated for trains of 5 depolarizing pulses. The accummulation of released vesicles (pool C) elicited by two such trains occurring 90 sec apart are superimposed and numbered 1 and 2. Decreasing steps in pool C size within each train are due to rapid depletion of vesicles in pool B. The increased overall response in the second train is due to a larger initial pool B size, as the moderately elevated inter-train calcium leads to faster supply (k_1) than consumption (k_3) of pool B vesicles. Thus the model simulates secretory depression and augmentation at appropriate time scales. For the simulations illustrated, rate constants were $k_1=0.009s^{-1}\,[Ca]/(1.2\mu M+[Ca])$, $k_2=0.0055s^{-1}$, $k_3=0.023\mu M^{-3}s^{-1}\,[Ca]^3$, and the total number of vesicles in all pools together was 2200.



273.12

PHOSPHATASE INHIBITORS INTERACT WITH PROTEIN KINASES TO REGULATE NEUROTRANSMITTER RELEASE, BUT DO NOT AFFECT CA^{++} HOMEOSTASIS. J.E.Swain*, R. Robitaille, M.P. Charlton. Department of Physiology, University of Toronto, Toronto, Ontario, M5S-1A8.

The purpose of this study is to investigate how protein phosphorylation modulates the synaptic transmission. The phosphatase inhibitor, okadaic acid (OA), causes a reversible, dose- and temperature-dependent increase in neurotransmitter release (NTR) from presynaptic terminals in crayfish and frog neuromuscular junctions (P.N.A.S. 88:1803-1807, J. Neurobiol. 22(8):855-864). Excitatory postsynaptic potentials evoked by electrical nerve stimulation were recorded to monitor NTR at neuromuscular synapses. In addition to OA, we have found that another membrane permeant phosphatase inhibitor, calyculin-A, also enhances NTR.

Since NTR is a function of Ca^{++} entry into the presynaptic terminal, we wondered whether OA affects Ca^{++} homeostasis. Intracellular Ca^{++} was monitored with a confocal microscope and the fluorescent indicator fluo-3. Neither resting nor stimulated Ca^{++} levels were affected by OA. Thus, OA action does not involve altering intracellular Ca^{++} homeostasis. To determine the kinases, whose substrates involved in NTR are dephosphorylated by phosphatases sensitive to OA, kinase inhibitors were used. The general kinase inhibitor staurosporine prevented OA from increasing NTR. Calphostin, a PKC inhibitor, does not affect OA action. We also studied the interaction of kinase stimulators and phosphatase inhibitors in NTR. Serotonin facilitation (involving both PKC and PKA stimulation) was enhanced by OA. The increase in NTR caused by SP-cAMP (a specific PKA stimulator) was larger in the presence of OA and calyculin-A. Therefore, substrates of PKA may be hyperphosphorylated in the presence of OA and cal-A, and increase NTR without affecting Ca^{++} homeostasis. Thus, calcium channels, pumps and endogenous buffers are unlikely to be targets of the inhibited phosphatases.

273.13

THE ROLE OF PROTEIN KINASE C IN THE REGULATION OF GLUTAMATE RELEASE FROM ISOLATED NERVE TERMINALS. E.T. Coffey, T.S. Sihra and D.G. Nicholls* 2+Dept. of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland, UK.

Previous work from this laboratory has indicated that the phorbol ester potentiation of glutamate exocytosis from cerebrocortical synaptosomes can be ascribed to a PKC-mediated inhibition of a presynaptic K^+ channel (Barrie et al., 1991, J. Neurochem. 57, 1398-1404). This would regulate action potential intensity or duration and hence control Ca^{2+} entry through the non-inactivating Ca^{2+} channels closely coupled to glutamate exocytosis (McMahon & Nicholls, 1991, J. Neurochem., 56, 86-94). In this study we further examine the mechanism of this regulation in rat cortical synaptosomes by exploiting the specific inhibitor of PKC, Ro-31-8220. PKC in synaptosomes is partially activated in the absence of depolarization or added agonist since phosphorylation of the PKC-specific substrate 87k is decreased by 10μM Ro-31-8220. The inhibitor also causes a hyperpolarization, a decrease in spontaneous Ca^{2+}-independent glutamate release from the cytoplasm, and decreases in both the 4-aminopyridine (4AP)-evoked increase in cytoplasmic free Ca^{2+} and the 4AP-evoked Ca^{2+}-dependent glutamate exocytosis. Each of these is consistent with a reversal of a tonic PKC-dependent K^+-channel inhibition. Thus little effect is seen on KCl-evoked Ca^{2+} elevation or glutamate release. The effects of Ro-30-8220 are therefore diametrically opposite to those of added phorbol ester. Additionally the inhibitor reverses each of the effects of added phorbol ester. The delayed rectifier inhibitor clofilium mimics the phorbol ester potentiation of 4AP-evoked depolarization. The results are consistent with a major role of PKC-regulated K^+-channels in the presynaptic regulation of glutamate release.

273.14

TWO METHODS TRADITIONALLY USED TO PREVENT MUSCLE CONTRACTION AFFECT STIMULATION-INDUCED CHANGES IN NEUROTRANSMITTER RELEASE AT THE FROG NEUROMUSCULAR JUNCTION. M.A. Sosa* & J.E. Zengel. Depts. Neurosci/Neurosurg., Univ. of Fl. Coll. of Med. & Dept. Vet. Affairs Medical Center, Gainesville, FL 32610.

At the frog neuromuscular junction, repetitive stimulation of the presynaptic nerve can lead to an increase and/or a depression in the amount of transmitter released, depending on the levels of quantal release. To study these stimulation-induced changes in release under normal or high quantal conditions, muscle contraction must be prevented. This has traditionally been done by pretreating the muscle with a glycerol solution or by adding curare. We thought it was important to determine whether these treatments could also affect the release process itself.

End-plate potentials were recorded from frog sartorius muscle under conditions of low (0.6mM Ca^{2+}/5mM Mg^{2+}), normal (1.8mM Ca^{2+}) and high (3.6mM Ca^{2+}) levels of release. Curare (3-5 ug/ml) was added when levels of release were normal or high. Nerves were conditioned with trains of 10 to 200 impulses (20 impulses/sec).

We found that after return to a Ca^{2+} Ringer solution following a one hour exposure to glycerol (0.5-1M), the level of stimulation-induced increases in release was higher than that observed before glycerol. This effect was observed within 30 min after removal of glycerol and continued to increase for up to 3 hr. In experiments done under low quantal conditions, addition of curare (1-2 ug/ml) also resulted in a higher level of stimulation-induced increases in release. Under conditions of normal or high levels of release, increasing the concentration of curare also increased the level of depression of release.

These results indicate that both of these methods of preventing muscle contraction can also affect the process of transmitter release.

273.15

N-(n-PROPYL)-N-(3-FLUORO-4-PYRIDINYL)-1H-3-METHYL INDOL-1-AMINE HYDROCHLORIDE (HP 184): EFFECTS ON THE SPONTANEOUS RELEASE OF ACh AND NE. C.P. Smith*, L.R. Brougham, F.P. Huger, R.C. Effland, and L. Davis. Neuroscience Strategic Business Unit, Hoechst-Roussel Pharmaceuticals, Inc., Route 206, Somerville, NJ 08876.

This report describes the *in vitro* acetylcholine (ACh) and norepinephrine (NE) release properties of HP 184 studied in perfused rat brain slices. HP 184 selectively enhanced the spontaneous release of these neurotransmitters but did not affect electrically-stimulated release. HP 184 caused [^{3}H] release from striatal slices loaded with [^{3}H]choline and was unaffected by the absence of extracellular calcium or the presence of vesamicol, suggestive of either a cytoplasmic or vesamicol-insensitive vesicular source of ACh. Chromatographic analysis showed that the spontaneous release of [^{3}H] correlates with increased release of endogenous ACh and not choline efflux. Similarly, HP 184 enhanced the spontaneous release of [^{3}H]NE from cortical slices in a calcium and sodium channel independent fashion (not blocked by 1 mM EGTA or 5 μM TTX), and was not carrier mediated (occurred in the presence of 10 μM nomifensine). Reserpine treatment prior to [^{3}H]NE loading prevented HP 184-induced release, suggesting that in contrast to its ACh release profile, HP 184 seemed to require intact vesicles to maintain spontaneous [^{3}H]NE release. In summary, the release properties of HP 184 in rat brain slices were found to be novel and of unresolved mechanism. *In vivo* release studies will be necessary to determine if these results are predictive of *in vivo* effects.

273.16

ADENOSINE INHIBITS THE CALCIUM-DEPENDENT ACETYLCHOLINE SECRETION INDUCED BY IONOMYCIN. J.M. Hunt*, H.M. Wilfehrt, and E.M. Silinsky. Dept. of Pharmacology, Northwestern Univ. Medical School Chicago, IL 60611.

The calcium ionophore, ionomycin, was used to stimulate secretion of acetylcholine (Ach) quanta from frog motor nerve terminals. In the presence of extracellular Ca^{2+}, bath application of 5-15 μM ionomycin, or local application 1-20 μM ionomycin via an extracellular pipette (dia $\approx$ 300 μm) positioned adjacent to the nerve terminal produced a rapid, profound increase in the rate of asynchronous Ach secretion. The amount of ionomycin-stimulated secretion depended upon both ionomycin and external Ca^{2+} in a dose-dependent manner. Quantal content of end plate potentials was also augmented by ionomycin. These results suggest that ionomycin transports Ca^{2+} across the plasma membrane to stimulate secretion of Ach quanta.

Adenosine and 2-Chloroadenosine inhibited ionomycin-induced Ach secretion with a similar efficacy to that observed against physiological Ach secretion. These results suggest that adenosine receptor activation inhibits Ach secretion by reducing the ability of Ca^{2+} to promote exocytosis.

273.17

MODULATION OF SYNAPTOSOMAL GLUTAMATE RELEASE, CALCIUM HOMEOSTASIS AND MEMBRANE POTENTIAL BY ADENOSINE ANALOGS AND IDEBENONE. F. Dagani, F. Cattabeni, R.Ferrari, L. Canevari and M.P. Abbracchio* Inst. of Pharmacology, Univ of Pavia, 27100 Pavia (ITALY); Inst. of Pharmacological Sciences, Univ of Milan 20100 Milano (ITALY).

In this study we investigated the effect of two class of drugs, adenosine analogs and idebenone on the modulation of glutamatergic neurotransmission and the related biochemical mechanisms such as energy metabolism, membrane potential and free calcium levels ([Ca^{2+}]i) in synaptosomes isolated from cerebral cortex, hippocampus and striatum of rat brain . Glutamate release was measured by HPLC-ECD or flurometric technique in continuous; [Ca^{2+}]i was measured by Fura-2 and membrane potential by CC5 fluorescence, oxygen uptake (QO_2) and ATP/ADP were also measured by polarographic and luminometric methods. Synaptosomal metabolism and neurotransmitter release were stimulated by addition of both 30-40 mM KCl or 30-40 μM veratridine. Utilizing the binding of A_1-specific agonist [^{3}H]-CHA and by A_2-selective ligand [^{3}H]-CGS 21680, no detectable specific A_2 binding sites was found whereas A_1 binding sites are present: in order of density in the hippocampus>striatum>cerebral cortex. Only in the hippocampus the A_1 specific agonist CPA, in the range of concentrations of 0.01-100 μM, significantly counteracted the KCl-evoked release of glutamate. The same trend was detectable if veratridine was utilysed as depolarizing agent. CPA 10^{-4}M also reduced by 30% the KCl-stimulated increase of [Ca^{2+}]i . Idebenone (in vitro addition at 1-10 μM) was able to inhibit by 30% the veratridine stimulated (QO_2) but no effect was found on ATP levels both in basal conditions and after stimulation with veratridine.

273.18

DUAL ACTION OF D-TUBOCURARINE AND HEXAMETHONIUM ON TRANSMITTER RELEASE AT THE NEUROMUSCULAR JUNCTION OF THE RAT. D. F. Wilson* and A.E. West Zoology Dept. Miami Univ., Oxford, OH, USA 45056.

Previously we reported that d-tubocurarine and hexamethonium enhance transmitter release and we concluded that this was due to blocking the presynaptic nicotinic receptors. In this study we report the effects of d-tubocurarine (0.075 μM) and hexamethonium (400 μM) on transmitter release from the rat diaphragm-phrenic nerve preparation with the calcium level in the bathing medium reduced from 2.0 mM to 0.5 mM. The intent of this study was to reduce the negative feedback action of the transmitter on the autoreceptors and determine if a second action of these nicotinic antagonists could be demonstrated. Intracellular recording techniques were used to monitor end-plate potentials (EPPs) and miniature end-plate potentials (MEPPs) in the isolated cut-muscle preparation. Quantal release in the presence and absence of the antagonists were examined. In the presence of each of these antagonists a significant decrease in quantal release was observed. These results suggest that both hexamethonium and d-tubocurarine have a dual action on transmitter release. It is proposed that the inhibitory effect is unrelated to blockage of autoreceptors and inhibition may dominate at higher concentrations. This may help to explain the divergence in views concerning the role of the autoreceptors. (Supported by NIH grant NS-27260).

273.19

Arachidonic acid potentiates vasopressin release from isolated neurohypophysial nerve endings. E.L. Stuenkel*, G. Dayanithi#, J. J. Nordmann#. Dept. of Physiology, Univ. of Michigan, Ann Arbor MI, and #Centre de Neurochimie du CNRS, 67084 Strasbourg Cedex, France.

Arachidonic acid (AA) and a number of AA metabolites are generated in response to various physiological stimuli. It has been postulated that AA may be important in modulating calcium signaling and neurotransmitter/neurohormone release from nerve endings. Thus, we have evaluated the effects of AA on intracellular calcium ($[Ca^{2+}]_i$) and vasopressin (AVP) release in a preparation of rat isolated neurohypophysial nerve endings. AA was found to increase basal AVP release and potentiate elevated K^+-evoked release in a concentration-dependent manner (minimal effective dose = 25 μM). Sequential AA pulses gave sequential secretory responses. AA was found to dose-dependently (10 - 100 μM) increase $[Ca^{2+}]_i$ as monitored by Fura-2 at the level of single nerve endings. Application of inhibitors of AA metabolism such as BW755C, indomethacin, and PBPB did not inhibit the AA effects on AVP release or $[Ca^{2+}]_i$. Moreover, application of a mixture of HETEs was ineffective at inducing a secretory response. AA could still induce AVP release and evoke, although reduced from control, an increase in $[Ca^{2+}]_i$ in nerve endings bathed in EGTA-containing Ca^{2+}-free medium. A series of experiments also found that AA could evoke a secretory response in streptolysin-O permeabilized nerve endings in the presence of 2 mM EGTA. The data suggest, therefore, that AA can directly increase basal and potentiate evoked AVP release from nerve endings. Furthermore, although AA has strong effects on Ca^{2+} signaling the results on permeabilized nerve endings suggest that AA can induce secretion in a Ca^{2+}-independent manner.

273.20

PURIFICATION AND ANALYSIS OF THE EFFECTS OF SUBSTANCE B ON ACETYLCHOLINE RELEASE. L. B. Pearce*, A. F. Gaillunas and R. MacCallum. Dept. Pharmacology, Boston University Sch. of Med. Boston, MA 02118.

Substance B (SB) is a neuromodulator found in both brain and heart that has been proposed to reverse inhibitory presynaptic receptor function by a mechanism that does not involve direct stimulation of acetylcholine (ACh) release (Pearce et al. PNAS 83:7979,1986). The purpose of this study was to critically examine whether or not the effects of SB can be accounted for by direct effects of this modulator on basal or evoked release of ACh. The effects of purified bovine brain SB on basal and evoked release of ACh were studied in three different preparations. Substance B was purified from an aqueous extract of whole bovine brain by tangential flow filtration, BioGel P2 gel filtration chromatography, methanol extraction, and two step chromatography by HPLC on a silica column using both methanol and H_2O elution. Purification of SB by this procedure resulted in a 3% yield of activity. Superfusion of rat right atria in the presence of HPLC-purified SB did not produce a direct effect on basal or stimulated release of [^{3}H]ACh. However, addition of supernormal amounts (>7 units) of HPLC-purified SB potentiated potassium-evoked release from superfused rat right atria and produced small increases in electrically-evoked contractions without increased baseline tension in guinea pig ileum longitudinal muscle strip preparations. Analysis of the effect of SB (8 units/10 ml superfusate) on brain synaptosomes, indicated that this neuromodulator did not significantly increase basal nor potassium-evoked (50 mM KCl) release of [^{3}H]ACh. The results of these studies describe a procedure for the preparation of highly purified SB. In addition, the actions of SB on basal and evoked release are not inconsistent with the original hypothesis however, high levels of this modulator potentiate evoked release in two of the three systems studied.

LONG-TERM POTENTIATION II

274.1

Ca^{2+}-INDUCED PROTEOLYTIC ACTIVATION OF PKC IN HIPPOCAMPAL HOMOGENATES. J. S. Sessoms, D.M. Chetkovich, J. D. Sweatt *, and E. Klann. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Previous studies by our laboratory demonstrate a persistently activated form of PKC in association with the maintenance phase of LTP in area CA1. Ca^{2+} influx through NMDA receptors contributes to the induction of LTP at these synapses. To investigate potential mechanisms for the LTP-associated persistent PKC activation, we used a model system in vitro: Ca^{2+} addition to hippocampal homogenates. Ca^{2+} (1mM excess over EGTA) was added to homogenates and the mixture incubated at 37°C for two min. The incubation was stopped by addition of EGTA (excess over Ca^{2+}). Kinase activity was then measured using a synthetic peptide fragment of ribosomal S6 protein or the selective peptide substrate for PKC, neurogranin$_{(28-43)}$ (NG$_{(28-43)}$). Ca^{2+} pretreatment caused increased phosphorylation of S6 peptide (ctrl = 2.0±0.49 pmol/min/µg protein, Ca^{2+} pretreated = 4.56±0.76, n=9) and NG$_{(28-43)}$ (ctrl = 7.5±1.5 fmol/min/µg, Ca^{2+} pretreated = 29.5±4.6, n=4). The increased activity was expressed in EGTA, indicating an increase in Ca^{2+}-independent (basal) PKC activity. In fractionation experiments the increased PKC activity was localized to the soluble fraction (for NG$_{(28-43)}$ ctrl = 0.42±0.21 pmol/min/µg protein, Ca^{2+} pretreatment = 5.22±0.56, n=3). Expression of the increased activity could be blocked by the selective PKC inhibitor peptide PKC$_{(19-36)}$. Leupeptin (50 µg/ml) or an inhibitor of Ca^{2+}-activated neutral proteases (0.78 U/ml, Sigma) blocked induction of the increase in basal PKC activity. The persistently activated form of the kinase appeared as a novel peak of activity eluting with 0.4 M NaCl from DEAE columns, as has been previously reported for proteolytically activated PKC. In addition, the persistently activated kinase eluted as a species of lower molecular weight than the native enzyme on HPLC gel filtration columns. Taken together, these results demonstrate robust proteolytic activation of PKC in hippocampal homogenates. Thus, it is reasonable to hypothesize that proteolysis could underlie the persistent activation of PKC in LTP. Supported by the Mcknight and Klingenstein Foundations.

274.2

LTP IS ASSOCIATED WITH REGIONAL AND CELLULAR SPECIFIC ALTERATIONS OF PROTEIN KINASE C ISOFORMS α AND βI. K.Horsburgh*, U. Staubli, E. Masliah, T. Saitoh. Department of Neurosciences, UCSD, La Jolla, CA 92093.

Each of the eight protein kinase C (PKC) isoforms may be differentially involved in modulating synaptic plasticity. We have previously reported biochemical data indicating the involvement of PKC isoforms α, βI and βII in LTP (as a model of synaptic plasticity) using Western blot techniques (Soc. Neurocience Vol.17 S160.16). In order to further characterize these PKC alterations, quantitative immunohistochemistry was applied to localize PKC isoforms α, βI, βII and γ in discrete rat hippocampal areas following LTP.

To induce LTP, five animals received 10 trains of 400Hz pulses to the perforant path. Another group of six animals received the LTP stimulation at 0, 3, 24 and 27 hours (asymptotic LTP). Low frequency stimulation (100 pulses, 1 per 40sec) was given to a control group of five animals. One hour after the last stimulation the animals were sacrificed, the brains removed and processed for immunohistochemistry using affinity purified polyclonal antibodies against peptides specific for each PKC isoform.

PKC(α) was found to be significantly reduced in the granule cell layer of the dentate gyrus from the LTP induced animals by 78% compared to the group of animals receiving low frequency stimulation. However, this dramatic reduction in PKC(α) was not found in the hippocampus of animals in which asymptotic LTP was established. PKC(βI) was unaltered in animals receiving one LTP stimulus but following asymptotic LTP was markedly reduced (30%) in the pyramidal cells of CA3 compared to controls. PKC isoforms βII and γ were not significantly altered in any of the hippocampal regions examined in the LTP groups.

These data would indicate that LTP is associated with specific regional and cellular alterations in PKC isoforms α and βI. Furthermore, these isoforms may be differentially involved in the induction and maintenance of LTP.

274.3

RAPID INCREASE IN CYTOSOLIC PROTEIN KINASE C ACTIVITY AFTER LONG-TERM POTENTIATION, BUT NOT SHORT-TERM POTENTIATION

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We have reported[1] that coupling a short-term potentiation (STP)-inducing weak tetanus with metabotropic (Q_P) receptor stimulation with trans-(±)-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD, 50 µM) induces long-term potentiation (LTP) in rat CA1 slices. We adopted this method to test whether the conversion of STP to LTP involves protein kinase C (PKC) activation. PKC activity was measured in the presence or absence of Ca^{2+}, phosphatidylserine and phorbol ester using the synthetic substrate peptide. Two minutes, but not 30 min, after the coupling, there was a significant increase (>70%) in the cytosolic activator-dependent PKC activity. Either STP alone or ACPD alone was ineffective. There was no change in membrane-associated PKC. When STP was induced by agonist-coactivation of Q_P and ionotropic receptors, there was no change in PKC.

LTP was also induced by strong tetanic stimulation. There was a similar cytosolic PKC increase 2 min, but not 5, 15, 60, 180 min, after tetanus, which was not seen when LTP was reduced by APV. No change in the activator-independent cytosolic PKC was detected, nor was there any change in the membrane-associated PKC during the 3 h.

Q_P receptor involvement in tetanic LTP has been suggested. Our results indicate that Q_P receptor activation may play a part in LTP induction by conjointly activating cytosolic PKC.

1. Otani S & Ben-Ari Y (1991) *Eur.J.Pharmacol.* **205**, 325-326.

274.4

ALTERED STRUCTURAL GENE EXPRESSION AFTER LTE/LTP OF THE SAME PROTEINS (PKC AND F1/GAP-43) WHICH HAVE PHOSPHORYLATION ACTIVITY ALTERED BY LTE/LTP. P.J.Meberg*[1], C.A.Barnes[2], B.L. McNaughton[2] and A.Routtenberg[1]. 1. Cresap Neurosci. Lab., Northwestern Univ. Evanston, IL 60208. 2. Div. Neural Systems, Memory and Aging, Univ. Arizona, Tucson, AZ 85724

Protein kinase C (PKC) activation is critical for the persistence of long-term synaptic enhancement (LTE or LTP). LTE-induced increase in phosphorylation of F1/GAP-43, a PKC substrate, is directly related to LTE persistence. We used quantitative *in situ* hybridization to determine if F1/GAP-43 or PKC gene expression is altered three days after LTE induction in chronically-prepared, conscious rats. Field potentials were recorded from the hilar/CA3 region of the hippocampus following perforant-path stimulation. One hemisphere received high-frequency stimulation (HFS), the other low-frequency (LFS). Results are reported as the difference in labeling between the two sides. In the CA3 subfield a negative correlation was observed between LTE and HFS-LFS hybridization differences for F1/GAP-43 ($r = -0.801$, $p < 0.01$), γ-PKC ($r = -0.781$, $p < 0.01$), and β-PKC ($r = -0.692$, $p = 0.057$), but not for α-PKC ($r = +0.154$, $p > 0.75$). Animals with an increase in EPSP magnitude of greater than 20% (n=6) had less F1/GAP-43 ($p < 0.05$) and γ-PKC ($p < 0.01$) hybridization in CA3 than those with less than a 20% increase (n=6). The decreased F1/GAP-43 mRNA may signify a reduced potential for synaptic plasticity and therefore greater synaptic stability. F1/GAP-43 HFS-LFS hybridization differences were highly correlated with γ-PKC in CA3 ($r = +0.928$, $p < 0.001$), suggesting coordinate regulation. No correlation was observed in any cell field between LTE and hybridization of post-synaptic proteins MAP-2, neurogranin, or the B-flip form of the glutamate receptor. Enduring changes in synaptic efficacy or memory formation may involve phosphorylation of proteins initially, followed by changes in gene expression of the same proteins that participate in the phosphorylation reaction. [Supported by NIMH, AFOSR and ONR grants]

274.5

QUANTITATIVE ANALYSIS OF PROTEIN KINASE C AND PHOSPHORYLATION OF GAP-43 SUBSTRATE FOLLOWING LTP IN HIPPOCAMPAL SLICES. Y. LUO, J. C. LEAHY, C. S. KENT, K. F. MEIRI AND M. L. VALLANO*, Dept. of Pharmacology, SUNY Health Science Center at Syracuse, Syracuse, NY 13210.

Quantal analysis studies suggest that enhanced neurotransmitter release from presynaptic terminals is involved in the maintenance of long-term potentiation (LTP). A majority of the α isoform of protein kinase C (PKC) and some β isoforms are presynaptic, whereas PKC γ is exclusively postsynaptic. Furthermore, phosphorylation of the presynaptic PKC substrate, GAP-43, correlates with neurotransmitter release. Hippocampal slices were used to study the role of presynaptic PKC in the early maintenance phase of LTP in area CA1. The redistribution of PKC isoforms and the state of GAP-43 phosphorylation were examined for up to 1 hour after LTP induction. PKC activation was assessed by quantitative immunoblotting of cytosolic and membrane fractions with monoclonal antibodies recognizing, both the α and β isoforms, the α isoform only, or the single PKC phosphorylation site in GAP-43. Results indicate that tetanization of the Schaffer collateral-CA1 synapse, which induced LTP, causes significant increases in both the translocation of presynaptic PKC isoforms and the PKC-specific phosphorylation of GAP-43. The observed changes were time and frequency dependent, and inhibited by APV, a NMDA receptor antagonist. Bath application of arachidonic acid, one of the putative retrograde messengers for maintenance of LTP, enhanced PKC redistribution to the membrane in hippocampal slices with high potassium depolarization. This redistribution was accompanied by concomitant enhancement of GAP-43 phosphorylation. Preliminary data using hippocampal synaptosomes show similar results. These observations support the hypothesis that presynaptic mechanisms, involving release of retrograde messenger postsynaptically, activation of presynaptic PKC and PKC-specific phosphorylation of GAP-43, contribute to the early maintenance of LTP. (Supported by PHS NS24705)

274.6

TOWARD A FUNCTION FOR THE RC3 PROTEIN; A CALMODULIN BINDING, PROTEIN KINASE C SUBSTRATE LOCATED IN DENDRITIC SPINES. D.D. Gerendasy, K. Wong, J.G. Sutcliffe.* Dept. Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

RC3 is a 78 AA substrate for Protein kinase C which binds calmodulin in the absence of Ca^{2+}. It exhibits a postnatal onset and is significantly enriched in the cerebral cortex, striatum and hippocampus where it is primarily localized to dendritic spines and can often be found associated with post-synaptic densities. These qualities suggest the hypothesis that it plays a postsynaptic role with respect to memory, associative learning and possibly, long term potentiation.

We have cloned, bacterially expressed and purified RC3 to homogeneity. The recombinant protein binds to calmodulin, in the absence of Ca2+ and is able to serve as a substrate for protein Kinase C in vitro. This preparation is suitable for structural studies (NMR and X-ray crystallography), as well as electro-physiological studies (microinjection into hippocampal brain slice neurons).

We have also constructed and bacterially expressed two mutant forms of RC3. One mutant (Ser36 to Ala) fails to act as a substrate for PKC, indicating that *in vitro* phosphorylation of recombinant RC3 is specific (Ser36 is the residue which is normally phosphorylated). This mutant retains the ability to bind calmodulin in a Ca^{2+} manner and can, therefore, be purified in the same manner as the wildtype. The other mutant (Ser36 to Asp) fails to bind calmodulin, indicating that a negative charge at position 36 is sufficient to prevent binding to calmodulin. This is consistent with the fact that the phosphorylated form of RC3 does not bind calmodulin.

Additional mutants have been created and we are investigating the effects of over-expression of RC3 and mutants in transgenic mice and cultured cells. Structural and biochemical studies utilizing purified recombinant RC3 are also in progress.

274.7

INHIBITION OF PROTEIN KINASE ACTIVITY FACILITATES LONG-TERM POTENTIATION OF IPSPs IN HIPPOCAMPAL CA1 NEURONS. B. R. Sastry* and Z. Xie, Neuroscience Research Laboratory, Dept. of Pharmacology & Therapeutics, The University of British Columbia, Vancouver, B. C., Canada, V6T 1Z3.

The activation of protein kinase C (PKC) has been implicated in long-term potentiation (LTP) of excitatory postsynaptic potentials (EPSPs) in the hippocampus. In the present study on guinea pig hippocampal slices, the actions of intracellularly injected K-252b, an inhibitor of protein kinase C (PKC), were examined to determine the involvement of postsynaptic PKC in LTP of gamma-aminobutyric acid (GABA) receptor-mediated fast ($GABA_A$) and slow ($GABA_B$) inhibitory postsynaptic potentials (IPSPs). In control neurons, the IPSPs were recorded with electrodes containing K acetate. A tetanic stimulation (400 Hz, 1 s) of the stratum radiatum induced LTPs of the EPSP and the fast IPSP, but not of the slow IPSP (n=8). If the recording electrode contained K acetate and K-252b (5 μM), a tetanic stimulation of the stratum radiatum did not induced LTP of the EPSP, but LTPs of the fast IPSP and the slow IPSP (n=16) were observed. In some CA1 neurons injected with K-252b, during LTP of the IPSPs a decrease in the height and the duration of the EPSP was observed. This distortion of the EPSP was greatly reduced when the IPSPs were pharmacologically blocked (n=8).

These results suggest that the activation of the PKC is necessary not only for LTP of the EPSP but also to minimize LTP of the IPSPs so that the EPSP is not distorted.

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274.8

PROTEIN KINASE INHIBITORS REVEAL TEMPORALLY DISTINCT PHASES OF LTP. K.M. Huber*, M.D. Mauk and P.T. Kelly. Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77225.

Previous studies have demonstrated the importance of protein kinases in the LTP induction, however, the role of protein kinases in the persistent expression of LTP is unclear, if not controversial. Our studies have systematically examined the temporal requirements for protein kinase activities during LTP induction and expression. We have utilized the broad range and potent protein kinase inhibitor K-252a (Kase, H. et al., J. Antibiotics 39: 1059-1065, 1986) to study protein kinase dependent processes at different times during LTP induction and expression in area CA1 of the rat hippocampal slice. K-252a (0.2-0.6 μM) applied 20 minutes before and after a high frequency tetanus (100 Hz for 1 sec) blocked LTP induction; following post-tetanic potentiation EPSP slopes decayed at a rate of 28.5 ± 6 % per hour. When K-252a (0.4 - 2 μM) was applied for 20 minutes immediately after tetanus, potentiation decayed at a rate of 18 ± 2 % per hour, indicating that decremental LTP occurred under these conditions. When K-252a was applied 20 minutes before and washed out immediately after the tetanus, potentiation decayed at rates (9.6 ± 1 %) that were similar to control slices (7.8 ± 6 %). K-252a (0.2-2 μM) had no effect on basal synaptic transmission, unlike H-7 (400 μM) which attenuates synaptic transmission at the same concentration that it blocks LTP induction (Muller, D. et al., PNAS 87: 4073-4077, 1990). Our results implicate a role of protein kinases during, as well as, minutes after tetanic stimulation. This period of kinase involvement is qualitatively similar to consolidation processes believed to occur during memory formation. Our results suggest that protein kinase activation is required during LTP induction (e.g., NMDA receptor activation) and may be involved in processes during the period after the tetanus (e.g., the production of retrograde messengers and/or presynaptic mechanisms), suggesting a role for protein phosphorylation in the consolidation of the expression of LTP.

274.9

GLUTAMATE-INDUCED PHOSPHORYLATION OF SYNAPSIN I AND MAP2 IN CULTURED HIPPOCAMPAL NEURONS. [1]K. Fukunaga*, [2]T.R. Soderling and [1]E. Miyamoto. [1]Dept. of Pharmacol., Kumamoto Univ. Sch. of Medicine, Kumamoto 860, Japan, [2]Vollum Institute, Oregon Health Sciences Univ., Portland, Oregon 97201, U.S.A.

The role of Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) and protein kinase C has been implicated in the induction of long-term potentiation (LTP) in the hippocampus. However, little is known about how the activation of the protein kinases results in long-lasting change of the synaptic efficiency in LTP. In the present study, we have focused on the target proteins for CaM kinase II in the signaling pathway induced with glutamate in the cultured hippocampal neurons. In the experiments with ^{32}P-labeled cells, the phosphorylation of MAP2 and synapsin I as well as autophosphorylation of CaM kinase II were stimulated by the exposure to 10 μM glutamate. The phosphorylation of MAP2 and synapsin I significantly increased by 128 ± 11 and 126 ± 9 %, respectively, at 1 min and was maintained until at least 15 min in the continued presence of glutamate. The increase in MAP2 phosphorylation and autophosphorylation of CaM kinase II were completely inhibited by the pretreatment with AP5, an antagonist of the NMDA receptor. However, the increase in synapsin I phosphorylation was only partially inhibited with AP5. These results suggest that the activation of CaM kinase II through the NMDA receptor increases the phosphorylation of MAP2 and synapsin I and in turn induces neuronal functions.

274.10

GENETIC ANALYSIS REVEALS THAT THE *FYN* TYROSINE KINASE GENE IS NECESSARY FOR LTP AND LEARNING IN MICE. S.G.N. Grant, T.J. O'Dell*, K. Karl, P. Stein, P. Soriano, and E.R. Kandel. HHMI, Ctr. Neurobiol. & Behav., Columbia P&S, NY, NY 10032; HHMI, Baylor Coll. Med., Houston, TX 77030.

Tyrosine kinase inhibitors block long-term potentiation (LTP) in the CA1 region of the hippocampus (O'Dell et al., 1991). To identify specific tyrosine kinases involved in LTP, we have screened mice with mutations engineered in either of four cytoplasmic tyrosine kinase genes: *src*, *abl*, *yes*, and *fyn*. Although these four kinases are coexpressed in the hippocampus, only the *fyn* mutant mice failed to show normal LTP. With low intensity (25% of max. EPSP) tetanic stimulation (100 Hz/1-sec. x 2) responses one hour post-tetanus were only 108.1 ± 7.6% of control (mean ± sem, n = 3) in slices from *fyn* mutant mice. A modest amount of LTP could be induced using higher intensity (75% of the max. EPSP) tetanic stimulation (133.2 ± 9.3% of control, n = 3), but this potentiation was smaller than that observed in slices from control animals (168.5 ± 11.6% of control, n = 4). Synaptic transmission appeared normal in slices from *fyn* mutant mice and paired-pulse facilitation was not different from that observed in control animals.

The impairment of LTP appears to correlate with impaired spatial learning in the Morris water maze, suggesting a functional link between LTP and spatial memory. In addition to its importance in LTP the *fyn* gene is also necessary for the normal development of the pyramidal cell layer of the hippocampus, since in the CA3 region the cell layer shows structural abnormalities. Together, these data suggest that the *fyn* tyrosine kinase is important for the induction of LTP and implicates a new biochemical pathway contributing to synaptic plasticity.

274.11

NITRIC OXIDE PRODUCES LONG-TERM ENHANCEMENT OF SYNAPTIC TRANSMISSION IN THE CA1 REGION OF HIPPOCAMPUS BY AN ACTIVITY-DEPENDENT MECHANISM. S.A. Small*, T.J. O'Dell, E.R. Kandel, and R.D. Hawkins. Ctr. Neuro. & Behav., Columbia Univ., HHMI, NY, NY 10032.

There is evidence that the membrane-permeant molecule nitric oxide may act as a retrograde message during long-term potentiation (LTP) in hippocampus. A difficulty with the retrograde message idea, however, has been that lateral spread of a diffusible message could lead to potentiation of transmission at inactive presynaptic terminals, which would violate the observed pathway specificity of LTP. A possible solution to this problem would be for the effects of the message to be restricted to recently active presynaptic fibers. We tested this possibility by applying nitric oxide to hippocampal slices either alone or coincident with weak presynaptic stimulation (50 Hz for 0.5 sec). 100 nM nitric oxide alone, presynaptic stimulation alone, or nitric oxide 5 min after presynaptic stimulation ("unpaired" training) produced no significant long-term effects on the field EPSP recorded in the CA1 region. However, when nitric oxide was applied at the same time as the presynaptic stimulation ("paired" training), the synaptic potential was immediately enhanced and remained enhanced for at least one hour ($\bar{x}$ = 184% of Pre, $p < .05$). Paired training still produced significant long-term enhancement of the PSP in the presence of APV, which blocks postsynaptic NMDA receptors and thus blocks the induction of LTP by tetanic stimulation ($\bar{x}$ = 158% of Pre, $p < .05$). Paired training also produced enhancement in the presence of APV plus picrotoxin ($\bar{x}$ = 147% of Pre, $p < .05$). These results are consistent with the hypothesis that nitric oxide acts as a retrograde message with activity-dependent presynaptic effects during LTP. This mechanism would be formally similar to activity-dependent presynaptic facilitation in *Aplysia* and activity-dependent neuromodulation more generally.

274.12

PROTEIN KINASE A INDUCES A PROTEIN SYNTHESIS-DEPENDENT LATE STAGE OF LTP IN HIPPOCAMPAL CA1 NEURONS U. Frey* and E.R. Kandel. Ctr. Neurobiol. & Behav., Columbia P&S, HHMI, NY, NY 10032; Inst. Neurobiol., PG Biotech., Magdeburg, Germany.

Because of its long time course, hippocampal long-term potentiation (LTP) is thought to be an elementary mechanism for certain forms of memory. By using three repeated high-frequency trains separated by ten minutes, it has been possible to generate LTP in hippocampal slices that persists for more than ten hours. In these slices it has been possible to show that LTP has stages similar to those of behavioral memory. A short-lasting early potentiation, which we call E-LTP, lasts 1-3 hours and is independent of protein synthesis. This is followed by a later, longer-lasting, stage, which we call L-LTP, and which requires protein synthesis. Here we report that inhibitors of cyclic AMP-dependent protein kinase (PKA) can specifically block L-LTP without dramatically effecting E-LTP and that analogues of cAMP can induce the protein synthesis-dependent late phase of LTP (L-LTP). The late phase can also be induced by repeated application of dopamine or by the joint activation of the N-methyl-D-aspartate and the dopaminergic D1 receptor in the hippocampal CA1 region in vitro.

274.13

NEURONS CONTAINING NITRIC OXIDE SYNTHASE IN RAT HIPPOCAMPUS

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Histochemistry for NADPH diaphorase was used to study the distribution of nitric oxide synthase (NOS) in the rat hippocampal formation. Animals were perfused with mixed aldehydes; 10-100 µm thick Vibratome sections were stained for NADPH diaphorase and counterstained with neutral red. The distribution of NOS-stained cells was plotted; camera lucida drawings were made to show their morphology. Most of the stained neurons were in the pyramidal layer of the subiculum, stratum radiatum of Ammon's horn, and the subgranular zone of the dentate gyrus. Neurons were also conspicuous in the molecular layer of the dentate gyrus and in the pyramidal layer at CA3. Stained neurons were sparse in the pyramidal layer of CA2 and CA1, and almost absent from presubiculum and parasubiculum. Numerous stained fibers were seen, especially in stratum radiatum and lacunosum-moleculare of Ammon's horn.

Based on their shape and location, the large majority of NOS-positive cells in the hippocampus were probably local circuit neurons. Preembedding and postembedding immunocytochemistry in combination with NADPH diaphorase histochemistry demonstrated that a large fraction of NOS-positive somata also contained GABA. These data suggest that nitric oxide in CA1 may function as a paracrine agent, rather than a spatially-precise retrograde messenger, in long-term potentiation.

274.14

INHIBITION OF LONG-TERM POTENTIATION BY NMDA-MEDIATED NITRIC OXIDE RELEASE Y. Izumi*, D.B. Clifford & C.F. Zorumski
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Recent studies indicate that activation of N-methyl-D-aspartate (NMDA) receptors prior to tetanic stimulation blocks long-term potentiation in the CA1 hippocampal region. In control slices, 38.6 ± 8.1% (mean ± SE, N=7) increase in EPSP slope was detected 60 min after tetanic stimulation. When 1µM NMDA was administered to slices for 5 min prior to the tetanus LTP was inhibited (-4.5 ± 6.9%, N=7).

We have found that NMDA-mediated LTP inhibition can be reversed by coadministration of the nitric oxide inhibitors, L-N^{G}-monomethylarginine (100µM, 36.8 ± 7.1% N=5) or hemoglobin(0.1µM, 42.5 ± 6.2% N=5). When applied alone for 10 min prior to the tetanus, 100µM L-NMMA did not inhibit LTP (30.8 ± 13.3% N=5). Application of 1mM L-arginine with 100µM L-NMMA and 1µM NMDA blocked the development of LTP (1.0 ± 3.8%, N=6). However, 1mM D-arginine did not overcome the effect of 100µM L-NMMA (33.8 ± 11.4 % N=6). Sodium nitroprusside (10µM) mimicked NMDA-mediated LTP inhibition (-5.8 ± 6.9%, N=5). Furthermore, the inhibitory effect of 10µM SNP on LTP could be overcome by 1µM hemoglobin allowing LTP development (47.0 ± 16.5%, N=5). 10µM SNP failed to alter the NMDA component of synaptic potentials (N=2).

These results indicate that the timing of NO release relative to high frequency activation of CA1 synapses may be an important determinant of LTP generation and coupled with earlier studies suggest that NO may play a positive or negative modulatory role in LTP depending on events occurring prior to tetanization.

274.15

Evidence for Nitric Oxide Synthase Inhibitor-Sensitive and Insensitive Hippocampal Long-Term Potentiation *in vitro*.

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Nitric oxide (NO) has been implicated as a possible retrograde messenger in the initiation of hippocampal long-term potentiation (LTP; O'Dell et al., Proc. Natl. Acad. Sci. 88: 11285-11289, 1991). To examine this hypothesis, we have inhibited NO synthase (NOS) to determine if LTP produced by different conditioning stimulus models was differentially affected by reduced NO levels. The models were: 1.) a single 100Hz, 1s conditioning train delivered at half-maximal stimulus intensity; or 2.) two 100Hz, 1s conditioning trains, separated by 60s, delivered at maximal stimulus intensity. The NOS inhibitors N^{G}-nitro-L-arginine (NOARG; 0.1-200µm) and N^{G}-monomethyl-L-arginine (NMMA; 100µm) produced no direct effects on synaptic responses, and in the 1-train model, neither inhibitor was effective in reducing or blocking LTP. In the 2-train model, both NOARG (50 and 100µm) and NMMA (100µm) greatly reduced LTP. Application of the specific N-methyl-D-aspartate (NMDA) excitatory amino acid AP5 (50µm) significantly reduced LTP in both model. These results support the role of NO in the expression of LTP in the hippocampus, but also suggest that NO is not required for the production of LTP *in vitro* under all conditions.

274.16

ACETYL-L-CARNITINE AFFECTS SYNAPTIC TRANSMISSION IN AGED RATS. V. Tancredi*[1], P. Lo Giudice[2], G. D'Arcangelo[1], A. Siniscalchi[1], L. Pacifici[2], M.T. Ramacci[2]. [1]Dept. Medicina Sperimentale, Tor Vergata II Univ. of Rome, Italy; [2]Institute for Research on Senescence, Sigma Tau, Pomezia, Rome.

Acetyl-L-Carnitine (ALCAR), an endogenous substance present in CNS, has been shown to increase spontaneous and evoked electrocortical activity and to improve learning and memory when administered in the aged rat. The aim of the present study was to verify the effect of ALCAR on long-term potentiation (LTP). LTP was examined in the Shaffer collateral/commissural CA1 pathway in hippocampal slices (400 mm thick) of aged (24-26 months) Fischer rats. Recordings of the population extracellular postsynaptic potentials were made in the stratum radiatum of the CA1 subfield, by means of glass microelectrodes filled with 2M NaCl. Orthodromic stimuli (10-500 mA, 20-90 ms, 0.1 Hz) were delivered through an electrode placed in the stratum radiatum. After stable signals were recorded, a short high frequency train (100 Hz, 1s) was delivered at the same stimulus intensity used for the baseline responses. The addition of ALCAR (at doses ranging from 10^{-3} to 10^{-5} M) 30 min before tetanic stimulation enhanced basal synaptic transmission by some 20% and augmented LTP by some 150%, the increase of the latter being only about 125% in controls. These data suggest that ALCAR increases the efficency of excitatory neurotransmission in aged rats.

274.17

IMPAIRMENT OF SPATIAL AND OLFACTORY MEMORY BY L-NITRO-ARGININE. G.A. Böhme, C. Bon, J.M. Stutzmann, A. Uzan*, A. Doble, M. Reibaud, M. Lemaire & J.C. Blanchard, **Rhône-Poulenc Rorer,** Centre de Recherches de Vitry-Alfortville, 94403 Vitry/seine, France.

We (Bon et al., Eur. J. Neurosci., 4 : 420) and others recently confirmed our initial observation that nitric oxide (NO) plays a role in LTP. Our aim here was to determine whether blocking NO production also impairs memory formation in hippocampal-dependent learning tasks. LTP of Schaffer collaterals/CA1 pyramidal cell synapses was dose-dependently blocked in hippocampal slices prepared from rats pretreated for 4 days with the NO-synthase inhibitor L-nitro-arginine (L-NOARG, 25-100 mg/kg i.p., b.i.d.). The same subchronic treatment with 100 mg/kg L-NOARG significantly increased the number of exploration errors made by rats trained to find bait in a radial 8-arm maze having food or water as the reinforcing stimuli. Similarly, L-NOARG at this dose also impaired social recognition of a juvenile by an adult male rat following two consecutive encounters at short (30 min) intervals. These results demonstrate that inhibiting endogenous NO production impairs learning. They also support the hypothesis that LTP may be a cellular substrate of spatial and olfactory memory.

274.18

SYNAPTIC POTENTIATION IN THE HIPPOCAMPUS INDUCED BY PLATELET-ACTIVATING FACTOR (PAF) A. Wieraszko*, G. Li, E. Kornecki[1] and Y.H. Ehrlich, CSI/IBR Ctr. Dev. Neurosci. and Dev. Disab., CUNY, Staten Island, NY 10301 and [1]Dept. Cell Biol. Anat. SUNY, Brooklyn

Platelet-Activating Factor (PAF) is an alkyl-ether phospholipid which has neuroregulatory actions (Kornecki and Ehrlich, Science, 240; 1792,1988). The abilities of PAF to cause increase in intrasynaptosomal calcium levels and to induce vesicular release have suggested that PAF may modulate synaptic efficiency (Kornecki and Ehrlich, Lipids, 26: 1243, 1991). Inhibition of long-term potentiation (LTP) by a PAF antagonist has been reported (Del Cerro, Arai and Lynch, Behav.Neural.Biol. 54:213, 1990) but direct effects of PAF on LTP have not yet been demonstrated. Here we examined the direct effect of PAF on synaptic excitability in hippocampal slices maintained in an interface chamber at 33°C. Stimulating electrode was localized in the Schaffer collateral fibers. Population spike and field EPSP were recorded with two electrodes positioned on the pyramidal cell body layer and on stratum radiatum, respectively. PAF produced an increase in the magnitude of the population spike and of EPSP slope in a concentration-dependent manner (5nM to10μM). The increase in the amplitude of population spike became visible 20-30 min following PAF application and reached maximum within 40-60 min (47% increase with 5nM PAF and over 700% above control levels with 10μM PAF). The potential remained elevated for up to 90 min and then gradually declined to control levels. The potentiating effects of l00nM PAF could be blocked by application of either of two structurally different PAF receptor antagonists, WEB2086 (2μM) or BN52021 (2μM), added 30 min before PAF. These results suggest that PAF may play a role in the modulation of synaptic plasticity. Supported by NIH NS 27866.

274.19

PLATELET-ACTIVATING FACTOR AS A POTENTIAL MESSENGER IN LONG-TERM POTENTIATION K. Kato[1], G.D. Clark*[2], N.G. Bazan[2], D.B. Clifford[3] & C.F. Zorumski[1] [1]Depts. of Psychiatry and [3]Neurology, Washington Univ. Med. Sch, St. Louis MO 63110; [2]Dept. of Neuroscience, Louisiana State Univ. Med. Sch., New Orleans LA 70112

Previously we have shown that platelet-activating factor (PAF) selectively augments EPSCs in cultured hippocampal neurons by a presynaptic mechanism, suggesting that this agent could be involved in hippocampal long-term potentiation (LTP). We have examined the possible involvement of PAF in LTP in the CA1 region of rat hippocampal slices using the hydrolysis-resistant analog, methyl-carbamyl-PAF (MC-PAF), and PAF receptor antagonists. When applied for 30 min prior to the tetanus, 2μM BN-52021, a synaptosomal PAF antagonist, inhibits LTP (N=7) whereas 5μM BN-50730, a microsomal PAF antagonist, has no effect (N=5). A 20 min application of 1μM MC-PAF in the absence of tetanic stimulation produces a slowly developing potentiation of extracellularly recorded EPSPs which persists for > 2h (N=5). In neurons whole-cell voltage clamped at -80mV, MC-PAF produces a small enhancement of EPSCs. However, if MC-PAF is administered immediately following tetanic stimulation, LTP occurs. In the absence of MC-PAF, LTP cannot be produced by tetanic stimulation in neurons clamped at -80mV. This MC-PAF-induced LTP is blocked by 2μM BN-52021 but not 2μM BN-50730 or 50μM APV. Extra- or intracellular administration of MC-PAF also appears to slow the washout of LTP-generating ability which occurs during whole-cell recording, making it possible to induce LTP for as long as 60 min after cell penetration. These data suggest that PAF or PAF-activated processes are important in LTP.

274.20

K⁺-CHANNEL INVOLVEMENT IN INDUCTION OF SYNAPTIC ENHANCEMENT BY MAST CELL DEGRANULATING (MCD) PEPTIDE. T. Kondo[1], K. Ikenaka[1]*, I. Fujimoto[1], S. Aimoto[1], H. Kato[2], K. Ito[2], T. Taguchi[3], T. Morita[3], M. Kasai[3], and K. Mikoshiba[4] [1]Inst. for Protein Research, Osaka Univ., Suita, Osaka 565, [2]Dept. of Physiology, Yamagata Univ. Sch. of Med., Yamagata 990-23. [3]Faculty of Engineering Science, Osaka Univ., Toyonaka, Osaka 560, [4]The Inst. of Med. Sci., The Univ. of Tokyo, Tokyo 108, Japan.

A bee venom, MCD peptide, which induces long-term potentiation (LTP) in hippocampal slices, was found to possess multiple functions. They include [1] binding to a voltage-dependent K⁺-channel in brain membranes, [2] interaction with a lipid bilayer to form voltage-dependent and cation-selective channels by itself, and [3] activation of a pertussis toxin (Ptx)-sensitive GTP-binding proteins. In this study, we prepared several derivatives and analogues of MCD and investigated which function is more closely related to the inducibility of LTP. Another bee venom, apamin, formed ion channels in a lipid bilayers. D-MCD, an optical isomer of MCD, activated a Ptx-sensitive G-protein. However, these peptides did not induce LTP in the hippocampal slices. A snake venom, dendrotoxin-I (DTX-I), bound to the same K⁺-channels as MCD and did induce LTP. These results suggest that the most potent aspect of MCD involved in LTP inducibility is its interaction with the voltage-dependent K⁺-channel.

274.21

MODULATION OF SYNAPTIC PLASTICITY BY ENDOGENOUS ADENOSINE: ADENOSINE ANTAGONISTS BLOCK INDUCTION OF LONG-TERM POTENTIATION AND DEPRESSION IN THE CA1 REGION OF THE HIPPOCAMPAL SLICES. Y. Sekino*, Department of Molecular and Cellular Neurobiology, Tokyo Metropolitan Institute for Neuroscience, Fuchu-city, Tokyo183, Japan.

ATP and adenosine derivatives (ADs) are released and accumulated in the synaptic cleft during repetitive stimulation in the CNS. In order to reveal the roles of the ADs in synaptic plasticity, we have examined the effects of blockers of adenosine receptors (A1 and A2) on synaptic plasticity induced by brief repetitive stimulation in CA1 region of the hippocampal slices. An A2 receptor antagonist (CP-66713) inhibits induction of long-term potentiation by high-frequency stimulation (100 Hz 1s) of excitatory postsynaptic potentials (EPSPs) but not of the population spike (PS) (Y.Sekino et al. ; B.B.R.C.; 181, 1010-1014,1991). Low-frequency stimulation (5Hz 20s) to the Schaffer collaterals caused homosynaptic depression in the PS and field EPSPs evoked by single test stimulation of the tetanized pathway (the Schaffer collaterals) and heterosynaptic depression in the PS and field EPSPs evoked by the non-tetanized pathway (the stratum oriens). The levels of both types of depression were maximal soon after tetanic stimulation and returned to nearly the control level within 5-7 min. Application of an A1 antagonist (8-cyclopentyltheophylline) blocked the induction of homosynaptic depression but not of heterosynaptic depression. This reduction of the synaptic activity only in the tetanized pathway indicates that ADs at the tetanized synapses induce homosynaptic depression through A1 receptors. These results suggest that ADs released from presynaptic terminals during tetanic stimulation can modify these tow forms of synaptic plasticity, long-term potentiation and depression, which originate from different frequencies tetanic stimulation, through different types of adenosine receptors.

274.22

EXCITATORY POSTSYNAPTIC CURRENTS EVOKED IN CA1 HIPPOCAMPAL PYRAMIDAL NEURONS ARE ENHANCED BY DIRECT ACTIVATION OF ADENYLATE CYCLASE. L. E. Chavez-Noriega * and C. F. Stevens, The Salk Institute, Howard Hughes Medical Institute, La Jolla, CA 92037.

We have recently shown that synaptic efficacy of Schaffer/commissural afferents to field CA1 of the rat hippocampus is potentiated by forskolin, a direct activator of adenylate cyclase (Chavez-Noriega and Stevens, 1992, Brain Res, 574: 85-92). Using the whole-cell patch clamp technique, we have now investigated the effect of forskolin on the excitatory postsynaptic current (EPSC) evoked by Schaffer/ commissural activation in CA1 pyramidal neurons of rat hippocampal slices. Transverse hippocampal slices from 2-3 week old rats were prepared using standard procedures. All recordings were carried out in the presence of 10-25 uM bicuculline.

The EPSC peak amplitude was enhanced by forskolin (50 uM) in 5 out of 10 cells by 62 ± 13 % (mean ± SEM). Since the increase produced on the field EPSP slope by forskolin is potentiated in the presence of the phosphodiesterase inhibitor and adenosine receptor antagonist IBMX (Chavez-Noriega and Stevens, 1992), we tested its effect on the EPSC amplitude. We found that in the presence of IBMX (50 uM) the effect of forskolin is enhanced: 7 out of 8 cells manifested an increase in EPSC amplitude of 86 ± 26%. IBMX itself enhanced the EPSC amplitude in 9 out of 10 cells by 76 ± 13%. Changes in the coefficient of variation squared ($c^2 = V/m^2$, V=variance, m=mean peak EPSC), suggest that presynaptic mechanisms significantly contribute to the enhancement produced by IBMX and forskolin in the presence of IBMX. Further studies will be carried out to confirm and extend these observations.

Altogether, our data indicate that the cAMP cascade is an important modulator of glutamatergic neurotransmission in field CA1 of the mammalian hippocampus.

274.23

A ROLE FOR CYCLIC AMP IN LONG-TERM POTENTIATION (LTP)

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Brief application of cAMP analogues or the adenylate cyclase activator forskolin to rat hippocampal slices produces long lasting potentiation of the CA1 population spike. This potentiation is due to increased pyramidal neuron excitability, since the field EPSP is not potentiated. Evidence that a cAMP dependent mechanism underlies the excitability component of activity-induced LTP is provided by occlusion experiments using high frequency stimulation (HFS) of the Schaffer collateral/commissural pathway in combination with a 15 minute bath application of 200μM dibutyryl-cAMP (dbcAMP). Application of dbcAMP after induction of maximal LTP by HFS does not result in any further potentiation of the CA1 population spike. Conversely, HFS applied after dbcAMP induced potentiation does cause further potentiation, but only to the level produced by HFS alone. These results support the the hypothesis that the brief increase in cAMP which can be measured after HFS is the cause of the excitability component (E-S potentiation) of LTP.

274.24

CHOLINERGIC AGONISTS ALLOW OTHERWISE INEFFECTIVE SYNAPTIC STIMULATION TO INDUCE LONG-TERM ENHANCEMENT OF SYNAPTIC EFFICACY. P.T. Huerta and J.E. Lisman*. Dept. of Biology, Brandeis Univ., Waltham, MA 02254.

Central cholinergic systems play a critical role in learning and memory, but the underlying mechanisms are unclear. Here we report that carbachol (CCh), a mixed muscarinic-nicotinic agonist, strongly affects the strength of stimulation required to produce synaptic plasticity at the glutamatergic synapses of CA1 neurons of the rat hippocampus. We used the *in vitro* slice preparation for extracellularly recording evoked responses to low frequency (0.1 hz) stimulation of Schaffer-collateral inputs. This weak stimulation normally produces no change in synaptic efficacy. However, when given during brief (5 min) bath superfusion of CCh (50 μM), both the population spike (PS) amplitude and the field-EPSP slope were enhanced following removal of CCh and remained persistently potentiated (1-2 hr) (PS: 219±14%, n=18; EPSP: 197±13%, n=15 at 1 hr post-CCh). The field-EPSP enhancement was shown to be synapse-specific, e.g. only synapses which were active during CCh superfusion became potentiated while unstimulated synapses stayed unchanged (n=5). This is the first cellular model demonstrating a permissive role for acetylcholine in long-term synaptic plasticity.

Another effect of CCh superfusion was the appearance of rhythmic (4-10 hz) oscillations similar to theta rhythm recorded *in vivo*. The possible connection of these oscillations with the synaptic enhancement is under investigation.

274.25

DIFFERENTIAL EFFECTS OF INTRASEPTAL OXOTREMORINE ON non-NMDA AND NMDA DEPENDENT LTP. K. Pang*, M.J. Williams, P. Hinkle and D.S. Olton, Department of Psychology, Johns Hopkins University, Baltimore, MD 21218.

Long-term potentiation (LTP) in the hippocampus may be utilized in memory formation. LTP of the medial entorhinal to dentate gyrus synapse (MEC-DG) depends on activation of NMDA receptors, whereas LTP of the lateral entorhinal to dentate gyrus synapse (LEC-DG) does not. Oxotremorine (OXO), a muscarinic agonist, infused into the medial septal area (MSA) improved memory and enhanced LTP of the MEC-DG in aged rats. The present study examines the effect of intraseptal OXO on NMDA and non-NMDA dependent LTP in aged F-344 rats. Each rat received five treatments in a pseudo-random order: OXO (2 μg) alone, saline followed by high frequency stimulation (HFS) of the LEC-DG, OXO followed by HFS of the LEC-DG, saline followed by HFS of the MEC-DG, and OXO followed by HFS of the MEC-DG. As in previous studies, preliminary results indicate that LTP of the MEC-DG is enhanced by OXO. In contrast, LTP of the LEC-DG was not enhanced by OXO. High frequency stimulation of the LEC-DG also produced long-term depression of the MEC-DG, which was not altered by OXO. The results suggest that OXO may improve memory by selectively enhancing the NMDA-dependent LTP in the hippocampus.

274.26

EXPRESSION OF LONG-TERM POTENTIATION (LTP) IN HIPPOCAMPAL CA1 IS CONTINGENT UPON SUPPRESSION OF $GABA_A$ RECEPTOR FUNCTION A. Stelzer* and G. Simon. Dep. of Pharmacology, SUNY Brooklyn, Brooklyn, NY 11203.

A number of studies have demonstrated that during LTP orthodromically evoked IPSPs are generally unchanged or increased (cf. Abraham et al., 1987., J. Physiol.) and suppression of GABA-receptor function has been ruled out as a factor in the maintenance of LTP. In the guinea-pig hippocampal slice bath application of $GABA_A$ antagonists bicuculline (bic) or picrotoxin (PTX) at low concentrations results in a potentiation of all orthodromic synaptic potentials in CA1 pyramidal cells similar to changes following tetanization of the Schaffer collaterals. Most notably, partial suppression of $GABA_A$ receptors leads also to a potentiation of the orthodromic early ($GABA_A$-mediated) IPSP. In addition, following tetanization of the Schaffer collateral pathway increased orthodromic IPSPs are found in cells which exhibit a pronounced reduction of the response to iontophoretic GABA (35/39 cells). Bath application of the NMDA-receptor antagonist D-APV (10 μM) prevents both, tetanization-induced increase of orthodromic synaptic potentials and reduction of GABA sensitivity. In the presence of 100 μM PTX, $GABA_A$-mediated IPSPs are not observed, but a remainder of a $GABA_A$-mediated iontophoretic response remains. Both, tetanic stimulation of afferent fibers and further pharmacological suppression of $GABA_A$ receptors results in an enhancement of the orthodromic EPSP in 100 μM PTX. In the presence of sufficient concentrations of $GABA_A$ antagonists to block all $GABA_A$ responses (>100 μM bic, >300 μM PTX, resp.) potentiated synaptic potentials following tetanization recover to control values between 20 to 30 min after the tetanus. These data demonstrate that during LTP the general increase of synaptic efficacy and the activation of the inhibitory circuit are contingent upon suppression of $GABA_A$-receptor function.

274.27

SEROTONIN FACILITATES LONG-TERM POTENTIATION IN AREA CA1 OF RAT HIPPOCAMPUS VIA A 5-HT4 RECEPTOR. S.M.Roychowdhury and E.G.Anderson*. Dept. of Pharmacology, University of Illinois at Chicago., Chicago, IL 60612.

Serotonin produces a dual effect on the evoked population spike in the CA1 area, namely a decrease, followed by a long-lasting increase in its amplitude upon washout. The increase in amplitude, which is mirrored in intracellular recordings as a long-lasting depolarization, and in extracellular single unit recordings as an increase in spontaneous firing, is mediated, in part, by 5-HT_4 receptors. We show here that 5-HT, acting via the 5-HT_4 receptor, can facilitate long term potentiation (LTP).

In the presence of the $5\text{-HT}_{1A/2}$ antagonist spiperone, 10 min. applications of 5-HT increased the pop. spike amplitude often lasting an hour. LTP was induced by delivering a train of pulses at 100Hz for 1 second. Tetanic stimulation applied during the response to 5-HT resulted in an elevated level of LTP at 40 minutes (78±13% vs 44.2±8% increase for controls). In the absence of spiperone, 5-HT induced stimulation upon drug washout. Application of a tetanus after washout also resulted in an increase in LTP (123±10% vs 48±6%). This increase was blocked by 10uM ICS 205-930, but not by 10nM, suggesting the involvement of 5-HT_4, but not 5-HT_3 receptors. Cisapride, a 5-HT_4 agonist, also increased the LTP (110±15% vs 36.5±7%)

Therefore, we conclude that serotonin acting on 5-HT_4 receptors can augment LTP, probably by increasing the degree of postsynaptic depolarization. Since the stimulatory response outlasts the presence of the transmitter, this may be an important modulatory mechanism to incoming synaptic traffic.

274.28

INTERACTION OF CHOLECYSTOKININ AND AFFERENT TETANIZATION IN THE INDUCTION OF SYNAPTIC MODIFICATIONS IN FIELD CA1 OF THE RAT HIPPOCAMPAL SLICE D. Dahl*, E. Rich-Bennett, and B.B. LeCompte III. The Univ. of Texas at Dallas, Richardson, TX 75083.

In the cortex, the ubiquitous neuropeptide cholecystokinin (CCK) is colocalized with GABA, or other neurotransmitters. Little is known of the function of this colocalization, but inference from models of CCK-dopamine colocalization would indicate a modulation of GABA release.

In field CA1, the temporo-ammonic pathway was stimulated, and EPSP and population spike recordings were taken in lacunosum moleculare and stratum pyramidalis, respectively. The sulfated octapeptide of CCK (CCK-8S) was applied by perfusion in doses of *ca.* 500 nM, 750 nM, 900 nM, or > 1 μM. Tetanization (4 trains, 10 pulses, 100 Hz) in the presence of 500 nM CCK-8S was associated with a modest (12-15%) *depression* of EPSPs and population spikes, whereas tetanization in the presence of 900 nM to 1 μM CCK-8S was associated with a modest (15-20%) *potentiation* of responses. Both potentiation and depression persisted for at least 30 min in drug-free ACSF. The 750 nM dosage was not associated with any tetany-induced depression or potentiation. At dosages < 1 μM, CCK-8S perfusion had no apparent effect on field potential responses. However, at dosages > 1 μM, CCK-8S sometimes induced a complete suppression of responses that was probably based on a depolarization block, and was reversible upon washout with drug-free ACSF.

In visual cortex, depression or potentiation of synaptic transmission may be associated with specific levels of postsynaptic depolarization. If CCK modulates GABA release, then a parametric application might affect postsynaptic voltage via a disinhibition and underlie depression or potentiation. The results of this work support such an hypothesis. (Supported by the Whitehall Foundation.)

274.29

HIGH CONCENTRATIONS OF GLYCINE PRODUCE A LONG LASTING INCREASE IN SYNAPTIC EFFICACY IN HIPPOCAMPAL SLICES. K. Shahi*, J. C. Marvizon and M. Baudry, Neuroscience Program, USC, Los Angeles, CA. Glycine has been shown to be a co-agonist of the NMDA receptor. Ligand binding studies have indicated the existence of a high affinity strychnine insensitive binding site with a Kd of about 100 nM. Physiological studies have also shown that this site needs to be occupied for NMDA receptor activation. However, the physiological significance of this site has been questioned as this site is likely to be always saturated under normal conditions. We report here that high concentrations of glycine produce a long lasting increase in synaptic efficacy in hippocampal slices, possibly by acting on the NMDA receptors. Perfusion of hippocampal slices with high concentrations of glycine (5 - 10 mM) for 10 min was followed by a slow increase in the slope and amplitude of EPSPs evoked in CA1 by stimulation of the Schaffer-commissural pathway. The effect reached a plateau within 30 - 45 min after glycine application and represented a 2-3 fold increase in slope and amplitude. Two lines of evidence suggest that the effect may be due to the activation of the NMDA receptors. The repetitive bursting activity recorded in slices incubated in low Mg++ containing medium was potentiated by glycine (5 - 10 mM) and blocked by Mg^{++} and ketamine. High concentrations of glycine were shown to increase ^{3}H-MK-801 binding to extensively washed synaptic membranes. These results suggest that high concentrations of glycine might activate NMDA receptors and produce long lasting changes in synapotic efficacy.
(Supported by Grant BNS 96284 from NSF and N00014-91-J-1796 from ONR).

274.30

CALCIUM RELEASE FROM INTERNAL STORES IS REQUIRED FOR THE INDUCTION OF METABOTROPIC GLUTAMATE RECEPTOR-DEPENDENT LONG-TERM POTENTIATION (LTP) IN DORSOLATERAL SEPTAL NUCLEUS (DLSN) NEURONS *IN VITRO*.
F. Zheng* and J.P. Gallagher. Dept. of Pharmacol. and Toxicol., Univ. of Texas Medical Branch, Galveston, TX 77550.

We have previously demonstrated that induction of LTP in the DLSN was not blocked by the selective NMDA receptor antagonist, D-2-amino-5-phosphonovaleric acid (100μM), but is blocked by a putative metabotropic glutamate receptor (met-GluR) antagonist L-2-amino-4-phosphonobutyrate. Superfusion of (*1S,3R*)-1-aminocyclopentane-1,3-dicarboxylic acid (*1S,3R*-ACPD), a selective met-GluR agonist, resulted in a long-lasting potentiation of synaptic transmission similar to that induced by tetanic stimuli (TS) (Zheng & Gallagher, Soc. Neurosci. Abstr. **16**,653; **17**,950).

In the present study, we demonstrated that another putative met-GluR antagonist L-2-amino-3-phosphonopropionic acid (L-AP3, 50μM) blocked the induction of LTP when it was applied before TS. Superfusion of the same concentration of L-AP3 after TS did not block, but slightly enhanced LTP induced by TS. These data suggest that the induction, not the maintainance, of LTP is blocked by the metabotropic glutamate receptor antagonist. L-AP3 (50μM) also blocked the LTP induced by *1S,3R*-ACPD. Intracellular injection of GTPγS, a non-hydrolysable analogue of GTP blocked both TS-induced and *1S,3R*-ACPD-induced potentiation, suggesting the involvement of a GTP-binding protein in the induction of LTP at the DLSN. Intracellular injection of BAPTA, a calcium chelator, also blocked the induction of LTP, suggesting that the induction of the met-GluR-dependent LTP also required an increase of free calcium concentration during TS in the postsynaptic neurons. The induction of LTP was also blocked by pretreatment with thapsigargin (5μM), which depletes internal calcium stores via inhibition of a Ca^{2+}-dependent ATPase. These data suggest that calcium release from internal stores is required for the induction of LTP at rat DLSN. (Supported by NIMH Grant MH-39163)

274.31

CO-ACTIVATION OF A METABOTROPIC AND N-METHYL-D-ASPARTATE (NMDA) RECEPTORS PRODUCES SUSTAINED HIPPOCAMPAL SYNAPTIC POTENTIATION. M. A. Musgrave* and J. W. Goh. Department of Pharmacology & Toxicology, Queen's University, Kingston, Canada K7L 3N6.

Induction of long-term potentiation (LTP) in the hippocampal CA_1 region is blocked by NMDA antagonists. However, some reports indicate that exogenously applied NMDA to the hippocampus produces only a transient synaptic potentiation. This suggests that NMDA receptor activation is necessary but not sufficient for successful induction of LTP. We report that maintained synaptic potentiation can be elicited by co-application of NMDA and 1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*t*-ACPD), a selective agonist for a metabotropic glutamate receptor (mGluR). Stratum radiatum-evoked CA_1 dendritic EPSPs of rat hippocampal slices showed no change in slope 30 min after bath perfusion (2 min) of either 200 μM *t*-ACPD (93 ± 8% [SEM] of control, n = 11) or 50 μM NMDA (97 ± 5%, n = 11) alone. In contrast, a potentiation at 30 min was elicited by co-application of both drugs (133 ± 9%, n = 11). L-2-Amino-3-phosphonopropionic acid (L-AP3; 500 μM) and L-serine-O-phosphate (SOP; 1 mM), agents which reportedly block mGluR-induced phosphoinositide (PI) metabolism, failed to block tetanus-induced LTP (148 ± 18%, n = 7; 153 ± 20%, n = 8; respectively). Preincubation of slices with Li^+ (20 mM for at least 60 min), which blocks PI turnover as well as function of GTP-binding proteins, resulted in blockade of tetanus-induced LTP (103 ± 5%, n = 13) but not post-tetanic potentiation (PTP) (148 ± 11%, n = 13) measured at 30 and 1 min post-tetanus, respectively. Control slices showed both LTP (136 ± 10%, n = 10) and PTP (159 ± 17%, n = 10). The results suggest that co-activation of mGluR as well as NMDA receptor is required for LTP induction. The subtype of mGluR stimulated by *t*-ACPD may be coupled to a GTP-binding protein and/or PI metabolism that is not antagonizable by L-AP3 and SOP. (Supported by MRC of Canada.)

274.32

PHOSPHATIDYLSERINE INCREASES HIPPOCAMPAL SYNAPTIC EFFICACY. O. A. Ramirez[1], C. M. Borghese[1], R. F. Thompson[2]*. [1]Dept. Pharmacology, Fac. de Ciencias Quimicas UNC, Cordoba Argentina. [2]Neurosciences Program, USC, LA, CA 90089-2520.

In the present study, the effects of phosphatidylserine (BC-PS) on hippocampal synaptic transmission were evaluated. The potential evoked by subthreshold stimulation (0.2 Hz) on perforant path (PP) was recorded from the granulle cell body layer of dentate gyrus in 400 um hippocampal slices perfused with either BC-PS alone (10 µM) or BC-PS in combination with DL-2-amino-5-phosphonovaleric acid (APV, 20 µM) or after previous perfusion with dizocilpine (MK-801, 10 µM) . The evoked-responses potentiation observed after perfusion with BC-PS (173 ± 87 %) was bloked in presence of MK-801 (10 µM but not by APV (20 µM). The increased hippocampal synaptic efficacy produced by BC-PS is discussed in terms of N-methyl-D-aspartate receptors (NMDA) and the associated channels.

274.33

AGE- AND LTP-DEPENDENT CHANGES IN ARACHIDONIC ACID CONTENT OF MEMBRANES FROM DENTATE GYRUS. M.A. Lynch*, K.L. Voss and T.V.P. Bliss, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K. (Spon: BRA)

We have measured the concentration of free arachidonic acid in membranes from the dentate gyrus of old and young PVG hooded rats following unilateral tetanization of the perforant path under urethane anaesthesia. The contralateral dentate gyrus received the same number of stimuli but no tetanus. LTP was induced in 6/6 animals in the 4 month-old group, and in 9/16 animals in the 22 month-old group. Forty-five min after the tetanus, the dentate gyri were dissected on ice and lipids extracted for analysis of fatty acids by HPLC. In the 4 month old group, membrane concentrations of arachidonic acid were significantly higher in the potentiated side than in the control side (0.52 ± 0.05 and 0.43 ± 0.03 nmol/µg respectively; mean ± sem n=6 ; p <.0.05, paired t-test). The arachidonic acid content in the dentate gyrus of aged animals was also increased following the tetanus but only in the group which sustained LTP (0.34 ± 0.05 and 0.24 ± 0.01 nmol/µg for potentiated and control sides respectively; n=9, p<0.05, paired t-test); there was no significant change in old animals in which LTP was not induced (0.22 ± 0.03 nmol/µg and 0.19 ± 0.01 nmol/µg for tetanized and control sides respectively; n=7, p>0.05, paired t-test). There was a significant reduction in the arachidonic acid content of aged compared to young animals (0.43 ± 0.03 (n=6) and 0.22 ± 0.01 (n=16) nmol/µg respectively, p<0.01, unpaired t-test). The age-related decrease in membrane arachidonic acid may be a consequence of parallel decreases in the activities of phospholipases A_1, A_2 and C.

274.34

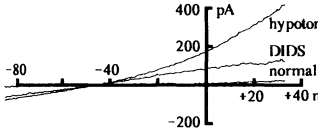
REGULATION OF HIPPOCAMPAL LONG-TERM POTENTIATION BY GLUCOSE: **role of glutamate synthesis and energy metabolism.** M. W. Fleck*, A. M. Palmer, & G. Barrionuevo, *Departments of Behavioral Neuroscience, Psychiatry, & Pharmacology, University of Pittsburgh, Pittsburgh, PA 15260.*

We examined the effects of extracellular glucose concentration on the induction, expression, and maintenance of long-term potentiation (LTP) in area CA1 of rat hippocampal slices. Tetanization of the Schaffer collateral-commissural afferents in normal glucose (10 mM) medium produced LTP of field excitatory postsynaptic potentials (fEPSPs). LTP was not observed after tetanization in glucose-deficient (1 mM) medium. When these slices were returned to normal glucose, LTP appeared, suggesting that LTP expression, but not induction, was prevented by low glucose. To confirm this, slices were tetanized prior to reducing glucose concentration. Glucose-deficient medium had no effect on fEPSPs in control slices but suppressed LTP expression in tetanized slices. Subsequent return to normal glucose restored LTP expression in these slices. These results demonstrate that LTP expression, but not induction or maintenance, is regulated by glucose availability. While fiber volleys and antidromic population spikes were unaffected, potassium-evoked glutamate release was significantly attenuated by low glucose. Addition of 1.0 mM L-glutamine as an alternate metabolic source failed to maintain either LTP expression or glutamate release in glucose-deficient medium. Therefore, glucose regulation of LTP is most likely indicative of enhanced glutamate synthesis not increased energy demand in potentiated Schaffer collateral-commissural synapses. Supported by NS01196, NS24288, AG0897401, and an NIMH Predoctoral fellowship.

275.1

VOLUME-SENSITIVE CHLORIDE CONDUCTANCE IN RETINAL PIGMENT EPITHELIAL CELLS. Lev Botchkin and Gary Matthews*. Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794-5230.

Whole-cell patch-clamp recordings were made from single retinal pigment epithelial cells isolated from neonatal rats (6-10 days); outward K current was blocked with internal Cs and TEA. As shown in the Fig., the resting conductance was small under these conditions. When cells were briefly exposed to hypotonic solution (osmolarity 50-70% of normal), cell volume reversibly increased, accompanied by an increase in conductance (see Fig.). The conductance could also be activated when cell volume was increased by applying positive pressure to the patch pipette, and sometimes developed spontaneously during prolonged recordings. The swelling-activated current reversed at -43 ± 1 mV (mean ± s.e.m.; n=10) with low $[Cl]_i$ and high $[Cl]_o$, and at -2 ± 3 mV (n=9) with symmetrical [Cl]. The current was reversibly reduced by the Cl-channel blockers DIDS (see Fig.) and SITS, but not by the K-channel blocker tolbutamide. This suggests that swelling activates a Cl conductance, which may be involved in regulatory volume changes. Supported by NIH grant EY08673. *Fig. Current-voltage relations in response to voltage ramp (100 mV/sec); no leak subtraction. Internal solution (mM): Cs-gluconate (130), TEA-Cl (10), EGTA (10), HEPES (5), ATP (2), GTP (0.3). All traces were obtained in normal Ringer's solution (2.6 mM K), either in resting conditions ('normal') or after swelling was induced by brief exposure to 50% osmolarity solution ('hypotonic' & 'DIDS'). DIDS concentration: 100 μM.*



275.2

CHLORIDE CHANNELS OF MAMMALIAN AND AMPHIBIAN SKELETAL MUSCLES DIFFER IN THEIR PHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERISTICS. D.Tricarico*, R. Wagner°, S.H. Bryant°, V. Tortorella^ and D. Conte Camerino.Dept. of Pharmacobiology and ^Pharmacochemistry, Fac. of Pharmacy, Univ. of Bari, Italy and °Dept.of Pharmacol. and Cell Biophysics, Univ. of Cincinnati, USA.

The major component of the resting conductance of mammalian and amphibian skeletal muscle is due to chloride permeability, although the absolute magnitude of the resting chloride conductance (GCl) in mammalian muscle is considerable larger than that detectable in frog muscle(Bretag, Physiol. Rev. 67:618, 1987). Recently it has been demonstrated that in mouse fiber the Cl^- channels responsible for the resting GCl are almost exclusively relegated in the T-system(Chua and Betz, Biophys J. 59:1251, 1991); in contrast, in frog fiber the Cl^- channels are mainly distributed on the surface membrane. It has been shown that Cl^- channels of mammalian muscle are modulated by protein kinase C(Tricarico et al., Pflugers Arch. 418:500, 1991), indeed application of 4ß-phorbol 12,13 dibutyrate (4ß-PDB) decreases GCl with an EC_{50} of 23nM. Moreover the application of highly specific Cl^- channels blockers such as anthracene 9-carboxilic acid and S(-) 2(p-chlorophenoxy)propionic acid (S(-) CPIB) produces a drammatic and streospecific block of GCl (De Luca et al., JPET 260:364, 1992). In the present experiments we tested the effects of 4ß-PDB and S(-) CPIB on the component conductances of frog semitendinosus muscle fibers at 25°C by standard two microelectrodes computerized cable technique(Bryant and Conte Camerino, Pflugers Arch., 417:605, 1991). Althought the 4ß-PDB did reduce GCl, even at 30μM it never produced an inhibition greater than 23%, suggesting a saturation for this responce. This effect was prevented by 1μM of staurosporine. Moreover S(-) CPIB at a concentration of 1mM which would block more than 95% of GCl in the rat, did not block GCl in the frog. These results suggest that the frog Cl^- channels are qualitatively distinct from the mammalian Cl^- channels for physiological as well as biochemical and pharmacological aspects.(Telethon-Italy, 1991).

275.3

PRESSURE CLAMP: A METHOD FOR RAPID ACTIVATION OF MECHANOSENSITIVE CHANNELS IN PATCHES AND WHOLE CELLS. D.W. McBride, Jr*. and O.P. Hamill. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

A pressure clamp technique has been developed which enables the application of rapid step changes in suction or pressure to membrane patches or whole cells. The technique is based on a strategy that involves a balance between negative (suction) and positive pressures under feedback control to achieve the desired pressure or suction. This strategy has the advantage of being able to apply both positive and negative pressures. Furthermore, because it involves active processes, fast step changes (~10 ms) in pressure/suction can be achieved. Details of the construction and feedback circuitry of the technique will be presented. Under computer control complex pressure waveforms can be constructed to study the time, pressure and voltage dependence of the dynamic properties of mechanosensitive (MS) channels. Using this technique we have characterized MS channels in a number of different preparations including *Xenopus* oocytes and skeletal muscle cells. In both preparations MS channels display voltage dependent adaptation in response to step changes in suction or pressure. The application of this technique to nonsensory and sensory cells should provide new insights into the molecular mechanisms of MS channels.

275.4

CALCIUM INDEPENDENCE OF RAPID ADAPTATION OF MECHANOSENSITIVE CHANNELS IN *XENOPUS* OOCYTES. O.P. Hamill* and D.W. McBride, Jr. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

The *Xenopus* oocyte expresses a mechanosensitive (MS) channel which displays rapid adaptation in response to step changes in suction or pressure. This adaptation is voltage sensitive but does not require extracellular or intracellular Ca^{++} (Fig. 1). Inclusion of 10 mM EGTA (or BAPTA) in the pipette solution does not eliminate adaptation. Furthermore, adaptation is still present in oocytes preincubated with the membrane permeable form of BAPTA (25 μM) which is sufficient to prevent Ca^{++} activation of Cl^- currents in oocytes Ca^{++} permeabilized with A23187. Our observation of Ca^{++} independence of adaptation contrasts with the current hypotheses concerning the role of Ca^{++} in MS channel adaptation in hair cells in which voltage dependent Ca^{++} influx through the MS channel causes adaptation.

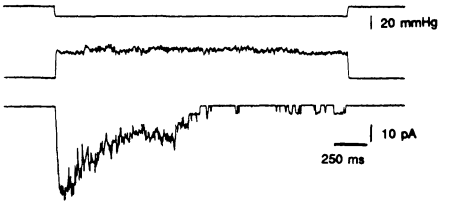


Figure 1. Voltage dependent MS channel adaptation with $[Ca^{++}]_o \neq [Ca^{++}]_i \neq 0$. Pipette solution (in mM): 100 KCl, 10 Hepes, 10 K-EGTA. Bath solution [in mM]: 115 NaCl, 1.5 KCl, 10 Hepes, 25 μM BAPTA AM. (Top trace) Suction waveform, MS current at 100 mV (middle trace), at -100 mV (bottom trace).

275.5

INCORPORATION OF ION CHANNELS FROM THE NEMATODE WORM, *CAENORHABDITIS ELEGANS*, INTO GIANT LIPOSOMES SUITABLE FOR PATCH CLAMP RECORDING. C.A.Leech, J.M.Skeer, C.Tornøe and D.B.Sattelle (SPON: Brain Research Association) AFRC Laboratory of Molecular Signalling, Department of Zoology, Downing Street, Cambridge, CB2 3EJ, U.K.

The nervous system of the nematode worm, *Caenorhabditis elegans*, has been extensively studied and each cell identified and mapped. *C. elegans* provides access to the only known viable mutants of certain ligand gated ion channels, notably nicotinic acetylcholine receptors. The small size of *C. elegans* neurones has limited physiological investigation of such channels in both normal and mutant phenotypes, *in vivo*. Injection of RNA extracted from *C. elegans* into *Xenopus* oocytes results in expression of nicotinic acetylcholine receptor/channels at which levamisole is a potent agonist. With the aim of providing complementary data on native ion channels, we have fused native membrane preparations with exogenous lipids by dehydration and rehydration (Criado and Keller, 1987) to form giant liposomes. These liposomes are suitable for study using conventional patch clamp techniques. Employing this technique, we have obtained single channel recordings from ion channels of *C. elegans*.

Criado, M. and Keller, B.U. (1987). FEBS Lett. **224**, 172-176.

275.6

EFFECTS OF AMITRIPTYLINE ON GABA STIMULATED $^{36}Cl^-$-UPTAKE IN BRAIN MEMBRANE VESICLES OF DOMINANT AND SUBMISSIVE RATS. Irene A. De Leon, Ewa Malatynska*, Doug C. Allen, and Henry I. Yamamura. Dept. of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ 85724.

The *in vitro* effect of amitriptyline (AMI) on GABA-stimulated $^{36}Cl^-$ uptake by cortical membrane vesicles of two behaviorally distinct groups of rats, dominant and submissive, was investigated. Behavioral changes in the dominant-submissive relationship between rats competing for food, as well as the modification of *in vitro* AMI effects on chloride uptake in these two groups after chronic treatment with AMI were also studied.

In vitro $^{36}Cl^-$ uptake assays reveal a dual effect of AMI at the $GABA_A$ receptor chloride ionophore complex for dominant rats; AMI enhances GABA-stimulated chloride uptake at concentrations between 10 nM and 1 μM while inhibiting this effect at 100 μM. AMI shows only one effect in submissive rats. This is inhibition of GABA-stimulated $^{36}Cl^-$ uptake by 10 μM and 100 μM AMI.

The opposite was true for the rats treated *in vivo* (i.p. injections for 21 days) with AMI. The dominant rats show only inhibition of GABA stimulated chloride uptake by 10 μM and 100 μM AMI. The dual effect of AMI was observed in submissive rats; GABA stimulated $^{36}Cl^-$ uptake was elevated at low concentrations (10 nM to 1 μM) and inhibited at 10 μM and 100 μM concentrations.

Both dominant and submissive rats exhibited significant submissive behavior after chronic treatment with AMI. Although *in vitro* studies yielded distinct responses of dominant and submissive rats regarding the stimulation of $^{36}Cl^-$ uptake by AMI, the two groups of rats showed no obvious correlation between this effect of the antidepressant and the behavioral response to AMI *in vivo*. This may be due to the strong inhibitory effect of high AMI concentrations on the GABA-stimulated chloride uptake which is similar for all treatment groups.

275.7

PANNING FOR SELECTION OF TRANSFECTED CELLS IN ELECTROPHYSIOLOGICAL STUDIES. R. F. Margolskee, B. McHendry-Rinde and R. Horn*. Neurosci. Dept., Roche Instit. Molec. Biol., Nutley, NJ 07110.

Panning was used to select transfected cells expressing plasmid-encoded ion channels. Adherent cells were cotransfected, using standard $CaPO_4$ methods, both with the plasmid of choice and with a separate plasmid encoding CD8, a membrane protein. After 1-3 days the cells were suspended, treated with a biotinylated CD8-specific antibody, and placed into streptavidin-coated bacterial petri dishes. After 2 hr these dishes were washed with a saline solution to remove non-adherent cells. Histological assays used a plasmid encoding bacterial β-galactosidase detected by X-Gal. We also expressed either sodium channels or CFTR chloride channels, and assayed for them using whole-cell, perforated-patch, and single-channel recording. The method was tested on TSA-201 and NIH3T3 cells, the latter of which transfected very poorly (usually <4% efficiency) with our standard protocols. By using ratios of ≥8:1 (by molecular weight) of the 'plasmid of interest'-to-the CD8 plasmid, the adherent cells were >50% positive for expression of the co-selected gene. Panning may increase the percentage of positively transfected cells by more than 20-fold.

275.8

CONVULSANT POTENCY OF LAUDANOSINE. R. Thies, F. Dreyer[1], and H. Bigalke*, Med. School of Hannover, Inst. of Toxicology, 3000 Hannover 61, Germany. [1]Univ. of Giessen, Rudolf-Buchheim-Institute, 6300 Giessen, Germany.

Laudanosine (N-methyltetrahydropapaverine), an alcaloid from Papaver somniferum and also a metabolite of the peripheral muscle relaxant, atracurium, elicits convulsant activity in several animal models. Here we report that laudanosine also induced paroxysmal depolarizing events (PDs) in cultured spinal cord neurons while papaverin and atracurium were ineffective. - Electrophysiological experiments were performed in the whole-cell mode. Spontaneous synaptic activity recorded in current clamp consisted of randomly evoked inhibitory and excitatory potentials. The neurons developed PDs when laudanosine was applied. Depending on the concentration, these events became more and more organized. - To elucidate the underlying mechanism the interaction of laudanosine with chemosensitive and voltage-dependent membrane currents was investigated using voltage clamp recording. Glycine and GABA, resp., were applied to single neurons by pressure ejection which caused a strychnine- or bicuculline-sensitive current. Only the glycine-evoked current was dose-dependently reduced by laudanosine (EC_{50}: 34.7 μM). The GABA-evoked current and the voltage-dependent sodium inward current were, if at all, only minimally affected. The appearance of the PDs and the suppression of the Cl^- current were fully reversible after withdrawal of laudanosine.We postulate that an interaction of laudanosine with the glycine-operated Cl^- channel is responsible for its convulsant effects. - This examination also confirms the usefulness of neuronal cells in culture to investigate convulsant and anticonvulsant activity.

275.9

ALTERATION OF ELECTRICAL PROPERTIES AND EXCITATION-CONTRACTION COUPLING IN TAURINE DEPLETED RAT SKELETAL MUSCLE. D. Conte Camerino*, A. De Luca, S. Pierno and R. J. Huxtable[1], Unit of Pharmacology, Dept. of Pharmacobiology, Fac. of Pharmacy, Univ. of Bari, Italy and [1]Dept of Pharmacology, Coll. of Med., Univ. of Arizona, Tucson, Arizona, USA.

Taurine, largely present in mammalian skeletal muscle, has membrane stabilizing effects by increasing membrane chloride conductance (GCl) (Conte Camerino et al. Pharmacol. Res. Comm., 19: 685,1987). To get insight into the role of taurine in the control of membrane properties we tested the effect of taurine depletion in rat skeletal muscle by chronic oral administration of guanidinoethane sulfonate (GES; 1% in drinking water) (Huxtable et al., JPET, 211:465, 1979). The effects of 4 weeks treatment were evaluated in vitro on ionic conductances and membrane excitability of extensor digitorum longus (EDL) muscle with computerized two intracellular microelectrodes technique (Conte Camerino & Bryant, Pflügers Arch, 417: 605,1991). The chronic GES treatment decreased GCl from a control value of 2917±97μS/cm² (n=15) down to 1973±94μS/cm² (n=38) and significantly increased by 80% potassium conductance (GK). Accordingly, the treated EDL had increased membrane excitability. In vitro application of 60mM taurine on depleted muscles complitely restored membrane conductance. The voltage threshold for mechanical activation (MT) of treated and control muscles was evaluated by means of two microelectrodes "point" voltage clamp (holding potential=-90mV), measuring the rheobase voltage at long test pulse durations (200-500ms). The GES treated fibers contracted at significantly more negative potentials (-71.1±2.3mV; n=11) with respect to the normal control (-61.4±2.2mV; n=22). Again, in vitro application of 60mM taurine on depleted muscle brought rheobase voltage to -64.8±2.9 mV (n=10). In vitro application of 60mM of either GES or taurine on untreated EDL did not produce significant changes of MT. Our data corroborate the membrane stabilizing role of taurine via direct control of muscle chloride channels. The GES effects on GK and on mechanical threshold may be related to the ability of taurine to increase Ca^{2+} uptake by sarcoplasmic reticulum (Lake et al., Biophys.J., 59: 64a, 1991) and therefore to modulate cytosolic Ca^{2+} concentration. (Granted by Telethon-Italy, 1991).

275.10

GAP JUNCTION HEMICHANNELS OF CX32 AND CX26 HAVE SLOW VOLTAGE DEPENDENCE OF OPPOSITE SIGN AND FORM JUNCTIONS WITH SOME PROPERTIES OF RECTIFYING SYNAPSES. V.K. Verselis, J.R. Rubin, C. Ginter, M.V.L. Bennett* and T.A. Bargiello. Dept. of Neuroscience, Albert Einstein Coll. of Med., Bronx, N.Y. 10461.

Voltage dependence of homotypic junctions is generally characterized by a slow, symmetric reduction in junctional conductance, g_j, about V_j=0. These symmetric changes in g_j have been shown to result from the pairing of two identical hemichannels in opposite orientation such that each closes for opposite polarities of V_j. A perplexing loss of sensitivity to one polarity of V_j and the appearance of a novel fast V_j dependent rectification were reported in heterotypic junctions formed by two V_j-dependent connexins, Cx32 and Cx26 (Barrio *et al.*, *PNAS* 88:8410, 1991). Domain exchanges of the extracellular loops (E1 and E2) suggested that the altered gating properties were due to interactions of E1 (Rubin *et al.*, *Biophys J* 62:197, 1992). We now report that the loss of slow V_j dependence of one polarity is due to pairing connexins possessing opposite polarities of slow V_j dependence. Site directed mutagenesis of the first two amino acids of E1 produced a characteristic change in slow V_j dependence in homotypic junctions which could be recognized in heterotypic junctions. Thus we could show that Cx32 and Cx26 hemichannels close when V_j on their side is made relatively negative and positive, respectively. All junctions in which the hemichannel polarities were of opposite sign showed one-sided slow V_j-dependence, as well as a marked fast V_j-dependent rectification. All junctions in which hemichannel polarity was of the same sign showed slow changes in g_j for both polarities of V_j, and in some cases a weak fast rectification. These studies suggest that pairing of hemichannels with opposite polarities may underlie the rectification observed at electrical synapses.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY III

276.1

Vulnerability of neurons overexpressing Cu,Zn-Superoxide dismutase (SOD-1) to oxidative stress. Paul J. Schwartz* Yoram Groner[1] and Joseph T. Coyle. Neuroscience Dept., The Johns Hopkins Medical School, Baltimore MD; Psychiatry Dept., Harvard Medical School, Boston MA; and [1]Molecular Genetics Dept., The Weizmann Institute, Rehovot, Isreal

The homology between the 16th mouse and 21st human chromosomes has led to the use of mice trisomic for chromosome 16 (Ts16) as a model of human trisomy 21 (Down syndrome, DS). A consequence of DS is the appearance of pathological and behavioral changes associated with Alzheimer's disease. There are indications that a common pathway for many neurodegenerative disorders is the failure to regulate reactive oxygen species such as the superoxide anion and hydrogen peroxide. The antioxidant enzyme Cu, Zn-superoxide dismutase (SOD-1) is located in the triplicated region of human chromosome 21 requisite for the phenotypic expression of DS. It is unclear whether overexpression of SOD-1 should lead to increased or decreased oxidative stress in the form of highly reactive oxygen radicals. Since both the murine 16th and the human 21st chromosomes contain the gene for SOD-1, we have investigated differences in the vulnerability of neurons derived from Ts16 and euploid embryos to potential oxidative stressors. We use primary cortical cultures from mice trisomic for the 16th chromosome (which encompasses the DS region noted above) and transgenic for human SOD-1 to address questions of the state of antioxidant defense mechanisms in neurons which overexpress SOD-1. We have found that neurons overexpressing SOD-1 are less vulnerable to glutamate analogue-mediated glutathione depletion and cell death (presumably due to blockade of the glutamate-cystine antiporter) than non-overexpressing controls.

276.2

PROTECTION AGAINST FREE RADICAL DAMAGE IN CEREBELLAR GRANULE CELL CULTURES. R.L. Getz, P.S. Puttfarcken, and J.T. Coyle* Dept of Psychiatry, Harvard Sch. of Medicine /MGH, Boston, MA.

Previous studies implicate the generation of free radicals in the progression of excitatory amino acid-induced neuronal death. Both *in vitro* and *in vivo* studies from our laboratory, demonstrated that treatment with lipophilic antioxidants provides protection against the neurotoxic effects of kainic acid (KA). As a result of these observations, we have compared the generation of lipid peroxidation products in cerebellar granule cell cultures (CGC) exposed to 3 different free radical generating systems (FRG); 200 μM ferrous ammonium sulfate (FAS), 20 μM copper (Cu) and 0.01U/ml xanthine oxidase/2.3 mM purine/2.4 μM transferrin (XO), to that observed following exposure to KA (see Puttfarcken, 1992). The effectiveness of the antioxidant, BHT and the lipid peroxidation inhibitor, U78517F, in attenuating these changes was also evaluated. Studies demonstrated a difference in the time course of neuronal death following a 30 minute exposure to each FRG: FAS (90 min), XO (180 min), and Cu (270 min). Despite these time differences, a similar 2-3 fold increase in both lipid hydroperoxide (HP) and conjugated diene (CD) generation was observed. The addition of 100μM BHT significantly attenuated FAS-,Cu- and XO-induced HP and CD formation. BHT reduced the formation of lipid peroxidation products by approximately 60-70%. Unlike BHT, the potency of U78517F depended upon the system utilized to induce lipid peroxidation. Although the protection provided by U78517F appeared to be approximately 50-60%, it was most potent in attenuating FAS-induced HP and CD formation (100 nM), followed by Cu (750nM), and lastly by XO (>2μM).

276.3

KAINIC ACID INDUCED LIPID PEROXIDATION: PROTECTION WITH LIPOPHILIC ANTIOXIDANTS IN CEREBELLAR GRANULE CELL CULTURES. P.S. Puttfarcken*, R.L. Getz, and J.T. Coyle, Dept. of Psychiatry, Harvard Sch. of Medicine/ MGH, Boston, MA.

Previous studies from our laboratory have demonstrated that treatment of cerebellar granule cell cultures (CGC) with lipophilic antioxidants significantly attenuated the neurotoxic effects of kainic acid (KA). In light of these observations, we have investigated the relationship between KA-induced neuronal toxicity and the appearance of lipid peroxidation products. Additionally, to assess the ability of KA to induce membrane damage, we have measured the products of lipid peroxidation following treatment with free radical generating systems (FRG) in the CGC (see Getz,1992). Under conditions designed to examine delayed toxicity, we have demonstrated a dose-dependent increase in KA-mediated conjugated diene (CD) and lipid hydroperoxide (HP) formation which correlates with an increase in LDH release. Maximal doses of KA (500 nM to 1μM), induced both CD and HP formation by approximately 2-4-fold above control. Moreover, the treatment of cells with various antioxidants significantly attenuated KA-induced lipid peroxidation. Treatment with nM concentrations of U78517F, a lipid peroxidation inhibitor, decreased 500 μM KA induced CD formation by approximately 30-40%. The protection provided by U78517F was assessed by examining LDH release. Although U78517F did not alter the release of LDH induced by low concentrations of KA (50-300nM), there was a 30% decrease in the release elicited by maximal doses (350nM to 1 μM) of KA. These data provide further evidence that the generation of free radicals, subsequently leading to membrane disruption, is most likely involved in the process of KA-elicited neuronal death in CGC.

276.4

NITRIC OXIDE SYNTHASE ACTIVATION, OR CYSTINE DEPLETION, MAY NOT BE CRITICAL TO NMDA RECEPTOR-MEDIATED INJURY IN MURINE CORTICAL CULTURES. K. Rose*, D. Liu, and D.W. Choi Dept. of Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Oxygen free radicals may mediate some of the neuronal damage induced by overstimulation of glutamate receptors. Several specific mechanisms might link such overstimulation to the overproduction of free radicals, for example, Ca^{2+} activation of phospholipase leading to increased metabolism of arachidonic acid by lipoxygenase pathways (Soc. Neurosci. Abst. 17:784, 1991). Two other possible mechanisms are Ca^{2+} activation of nitric oxide (NO) synthase, promoting NO and peroxynitrite formation (Dawson et al., PNAS 88: 6368, 1991), or glutamate inhibition of cystine uptake, lowering protective glutathione levels (Murphy et al., Neuron 2: 1547, 1989).

Cultured murine cortical cells exposed to 75 μM NMDA for 5 min developed an intermediate level of neuronal death by the next day. Addition of 100 μM-300 μM N^G-nitro-L-arginine, or 300 μM-1 mM N^G-monomethyl-L-arginine to the exposure solution did not alter resultant neuronal death, even if the cultures were exposed to arginine-free medium for 24 hr prior to NMDA exposure. 24-48 hr exposure to cystine-free medium did not induce cell death.

While neuronal NO production or cystine depletion may be critical to glutamate neurotoxicity in some systems, the role of these two mechanisms is not readily apparent in our system, perhaps because of masking by other, more powerful injury pathways.

276.5

NEUROTOXIC EFFECTS OF SODIUM NITROPRUSSIDE IN RAT HIPPOCAMPAL SLICES A. M. Benz, Y. Izumi, D. B. Clifford*, C. F. Zorumski and J. W. Olney Depts. of Psychiatry and Neurology, Washington Univ., School of Med., St. Louis, MO 63110.

To investigate the involvement of nitric oxide (NO) in the pathogenesis of N-methyl-D-aspartate(NMDA)-induced toxicity we examined the effects of sodium nitroprusside (SNP) using rat hippocampal slices. Incubation of slices with 3mM SNP for up to 2 hours generated a novel pattern of morphological change characterized by nuclear swelling in CA3 and CA1 pyramidal neurons and interneurons, with relative sparing of dendritic fields. The nuclear ballooning was not blocked by lowering the external calcium concentration or co-incubation with MK-801 and/or 6,7-dintroquinoxaline-3,4-dione (DNQX). Several observations indicate that the SNP neurotoxicity is not mediated by NO. Inactivation of SNP by UV light did not prevent the characteristic nuclear ballooning, and it was not attenuated by coadministration of hemoglobin. Additionally, KCN (0.3-15mM) failed to mimic the nuclear swelling.

Electrophysiologically, 100μM SNP diminished the CA1 population spike amplitude and subsequently inhibited the field EPSP irreversibly. The NMDA component of the field EPSP slope was inhibited by 10μM light inactivated SNP (-52 ± 17%, N=6) but not by active SNP 10μM (6 ± 7%, N=6). Consistent with this, the excitotoxicity produced by 100μM NMDA is also blocked by inactivated SNP but not by the active compound.

These results suggest that active SNP and light inactivated SNP have different effects on hippocampal neurons and that SNP induces a characteristic toxicity which differs from that of cyanide or NMDA.

276.6

HYDROXYL RADICAL SCAVENGER, DIMETHYLTHIOUREA, PARTIALLY ATTENUATES NMDA RECEPTOR-MEDIATED TOXICITY. D. E. Supko* and M.V. Johnston. Kennedy Krieger Institute and Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The formation of free radicals and subsequent lipid peroxidation may be factors contributing to glutamate neurotoxicity. To explore this possibility, the effect of a free radical scavenger, dimethylthiourea (DMTU) on glutamate receptor-mediated neurotoxicity was investigated in the rat. Unilateral intrastriatal injections of the glutamate receptor agonist NMDA, were made with or without prior administration of DMTU (500 mg/kg, ip) to post-natal day 7 rats. Damage was assessed one week later by measurement of the change in cytochrome oxidase (CO) activity between the lesioned and control sides of the brain (determined spectrophotometrically by measurement of the rate of oxidation of cytochrome C). NMDA produced a dose-dependent inhibition of CO activity with 5, 10 and 20 nmols resulting in 10.7%, 24.1% and 38.3% inhibition, respectively. Pretreatment with DMTU 1 hour prior to NMDA injection significantly attenuated damage only from 20 nmol NMDA: 19.5% inhibition of CO activity (48.2% protection, $p<0.025$). Intrastriatal injection of AMPA produced a similar decrease in CO activity which was not attenuated by DMTU. The results indicate that treatment with the free radical scavenger, DMTU affords partial protection against NMDA receptor-mediated damage.

276.7

THE EFFECTS OF NO SYNTHASE INHIBITOR ON BRAIN ISCHEMIA IN VIVO. S. Shapira*, R. Brandeis, B-A Weissman and T. Kadar. Dept. Pharmacol., Israel Inst. Biol. Res., Ness-Ziona IL-70450, Israel.

There is vast amount of in vitro data to indicate that NMDA-induced brain damage is mediated by NO activity, and that inhibition of NO synthesis is beneficial to ischemic brain tissue. The purpose of the present study was to investigate the effects of NO on ischemic brain in vivo. Male mongolian gerbils were exposed to 5 min ischemia, 4 hr after administration of nitro-L-arginine (NARG). At 1, 2 and 6 days, spontaneous activity was evaluated, and at 6 days the gerbils were sacrificed and their brain removed for histological assessment. Two days after ischemia, significant increase in spontaneous activity was observed only in NARG pretreated ischemic gerbils, as compared to sham operated NARG pretreated, and to gerbils exposed to ischemia without pretreatment. The usual neuropathology confined to vulnerable areas was observed in animals exposed to 5 min ischemia. NARG pretreated ischemic gerbils displayed neuropathology which extended beyond the vulnerable areas, and was significantly worse on quantitative analysis. It is suggested that through mechanisms yet unknown, NO might be beneficial in cases of massive brain ischemia.

276.8

NITRIC OXIDE AND FREE RADICALS MEDIATE NMDA-INDUCED NEURODEGENERATION. M. Li, A.M. Szczepanik*, K.M. Brooks and C.A. Wilmot. Dept. Biological Research, Neuroscience Strategic Business Unit, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876-1258.

Excessive NMDA receptor activation and subsequent Ca^{2+} entry produce neurodegeneration in many experimental models. The generation of the free radicals nitric oxide ($NO^{\bullet}$) and superoxide ($O_2^{-\bullet}$) may contribute to this process by forming the peroxynitrite anion ($ONOO^-$), which decomposes to the highly damaging hydroxyl ($OH^{\bullet}$) and nitrogen dioxide ($NO_2^{\bullet}$) radicals. The $NO^{\bullet}$ synthase (NOS) inhibitor N-mono-methyl-L-arginine (NMMA) and the free radical scavenger N-tert-butyl- α-phenylnitrone (PBN) were tested for protective effects *in vivo* against NMDA-induced lesions of the striatum in rats. NMDA (150 nmol) or an equiosmolar solution of NaCl was infused into the left or right striatum, respectively. Rats were sacrificed seven days later. Using the contralateral striatum as a control, NMDA-induced deficits of the enzyme markers choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) were determined. When coadministered with NMDA directly to the striatum, NMMA at 1875 and 3750 nmol produced a partial protection. PBN at 100 mg/kg, ip, 30' pretreatment, showed nearly complete protection against NMDA-induced deficits of ChAT and GAD, and was also effective as a 30' posttreatment. These findings support the hypothesis of an involvement of free radicals in NMDA-induced neuronal damage.

276.9

EAA AGONISTS STIMULATE THE FORMATION OF FREE RADICAL SPECIES IN SYNAPTO-NEUROSOMES

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Excitatory amino acid (EAA) agonists are well recognized for their ability to induce excitotoxic-mediated neuronal damage. Although considerable progress has been made in elucidating the early roles of receptor activation and ion influx in the excitotoxic mechanism, much less is known about later steps in the pathological process. In the present study we have examined the ability of EAA agonists to promote the formation of reactive oxygen species (ROS), key intermediates in the process of free radical-mediated oxidative injury. The generation of ROS in the synaptoneurosomes was followed with 2,7-dichlorofluorescin diacetate which, after its intracellular conversion to 2,7-dichlorofluorescin, reacts with ROS to form a fluorescent species (excitation: 488 nm, emission: 525 nm). Kainate (KA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and N-methyl-D-aspartate (NMDA) each stimulated the formation of ROS in a time- and concentration-dependent manner. Of these three agonists, KA proved to be the most potent, with a 60 min exposure to 1 mM KA increasing ROS formation to about 200% of the basal level. Exposure to a similar level of L-glutamate also stimulated ROS formation, but only to about 130% of the basal level. The effectiveness of the L-glutamate may, however, have been limited by its rapid removal by high affinity transport, similar to what has been observed in electrophysiological studies. These findings add further support to the hypothesis that free radical-mediated oxidative injury contributes to the process of excitotoxic neuronal pathology.

276.10

NITRIC OXIDE IS RESPONSIBLE FOR CONTINUED SYNAPTIC TRANSDUCTION FOLLOWING EXCITOTOXIC EVENT

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Nitric oxide (NO), a candidate for retrograde messenger, probably plays a role in cell to cell signaling including Ca^{2+} influx through NMDA receptor-gated ion channels (NMDARC). In addition, NO may mediate glutamate-induced neurotoxicity. Varied actions of NO in the central nervous system are expected under both physiological and pathological conditions.

We have already shown, utilizing a ^{3}H-MK-801 binding technique that NMDARC remains open after ischemic, hypoxic or excitotoxic events in both *in vivo* and *in vitro* systems. In the present study, we examined in rat hippocampal slices (475 μm-thick) the mechanism(s) underlying such a sustained activation of NMDARC. This process must be important to bridge the time gap between initially increased release of excitatory amino acids and delayed cell death dependent on intracellularly accumulated Ca^{2+}. This sustained activation of NMDARC was significantly blocked by 100μM N$^{\omega}$-nitro-L-arginine (NLA), a nitric oxide synthase inhibitor, or by pretreatment of 100μM hemoglobin, which would capture extracellular NO directly. The inhibitory effect of NLA was reversed by 1 mM L-arginine (LA), a substrate for NO-formation. Sodium nitroprusside (SNP) and LA potentiated the activation of NMDARC by glutamate/glycine, although SNP alone dramatically decreased ^{3}H-MK-801 binding. Interestingly, the continued opening of the cationic channels was TTX-sensitive, Ca^{2+}-dependent, but cGMP-independent. Furthermore, this sustained opening of NMDARC was associated with increased release of glutamate and aspartate. Accordingly, NO seems to persistently open the cationic channels linked to NMDA receptors and to increase release of excitatory amino acids.

These findings may account for the participation of NO in excitotoxic neuronal injury and in continued synaptic transduction in LTP.

276.11

IMMUNOLOGIC ACTIVATION OF ASTROCYTES: DISSOCIATION OF NITRIC OXIDE PRODUCTION AND NMDA MEDIATED NEURONOCYTOTOXICITY.

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Addition of bacterial endotoxin, fixed staphylococci, or Mycoplasma *hominis* to 14 day cultures of fetal rat cortex is cytopathic for neurons. These neuronocytopathic effects resemble those observed following addition of toxic concentrations of glutamate, and are blocked by 2.5 μM MK-801, an NMDA-receptor antagonist. Following such immunologic stimuli, nitrite accumulation in cortical culture supernatants, reflecting nitric oxide (NO) production, increased from <0.2 μM/72 h to 1-4 μM/72 h. Nitrite accumulation and neuronocytopathic effects occurred in the absence of microglia, but not in the absence of astrocytes and microglia. Addition of N-methyl-l-arginine (0.5mM) prevented NO production, but not the neuronocytopathic changes. Critically, MK-801, which is neuroprotective, did not prevent NO production. In conclusion, the astrocyte response to immunologic stimuli, which includes IL-1 and TNF release, included NO release (albeit at low levels). However, the associated MK-801-antagonized neuronocytopathic effects appeared to be independent of NO release.

276.12

NMDA RECEPTOR ACTIVATION RESULTS IN HYDROXYL RADICAL PRODUCTION IN PRIMARY MURINE CORTICAL CULTURES

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Free radical damage has long been thought to play a role in ischemic neuronal injury. The hydroxyl radical is one of the more reactive species that may be generated during ischemia and may be one of the species responsible for free radical damage in this setting. Neuronal death can result from excessive N-methyl-D-Aspartate (NMDA) receptor activation. As NMDA receptor activation is an important component of ischemic injury and we have previously shown that antioxidants can diminish NMDA neurotoxicity we attempted to detect the production of hydroxyl radicals in response to NMDA receptor activation. The hydroxyl radical specific metabolite of salicylate, 2,3-dihydroxybenzoate, was measured using an HPLC method (Ingelman-Sundberg et.al. Biochem.J.276:753). We demonstrated an increase in hydroxyl radical production of 15-30% over baseline following 10 min exposure to 500μM NMDA in murine cortical cultures (n=4-8 each condition, three repetitions). This increase was blocked by the NMDA antagonist MK801. Hydroxyl free radical production is detectable in response to toxic exposure to NMDA and may contribute to neuronal damage in the setting of ischemia.

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276.13

BUTHIONINE SULFOXAMINE-MEDIATED GLUTATHIONE DEPLETION EXACERBATES EXCITOTOXIC BRAIN INJURY IN THE NEONATAL RAT.

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L-Buthionine-(S,R)-sulfoxamine (BSO) selectively blocks glutathione (GSH) synthesis when administered to neonatal rats. This results in the depletion of GSH and, thus, the formation of reactive oxygen species are largely unopposed. We tested the effects of BSO administration in a model of NMDA-induced brain injury in the neonatal rat. A number of different BSO dosing paradigms were employed (3 mmol/kg/dose). In pups dosed 5 times over 72 hours and in pups dosed 6 times over 96 hours, a significant exacerbation in hemisphere weight reduction produced by intracerebral injections of NMDA was found. However, in pups dosed only 4 times over 96 hours, no change in injury was observed. In separate experiments, with pups dosed 3 times over 72 hours, GSH was depleted by 93%, and in pups dosed 5 times over 72 hours, GSH was depleted 95%. In a less than optimal dosing paradigm in terms of excitotoxicity potentiation, BSO failed to deplete brain vitamin E. There may be a minimum concentration of GSH (e.g. >5% of normal) above which significant protection from radical enhanced excitotoxicity occurs.

276.14

NITRIC OXIDE IS NOT INVOLVED IN NMDA-INDUCED NEUROTOXICITY IN RAT CORTICAL CULTURES.

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The excitatory amino acid N-methyl-D-aspartate (NMDA) is capable of producing extensive neurotoxicity in cultures of the rat cerebral cortex. NMDA has also been shown to generate the second messenger nitric oxide (NO) from arginine by activation of the calcium-calmodulin-dependent enzyme nitric oxide synthase. The primary role of NO in the cell seems to be activation of the enzyme guanylate cyclase and resultant production of cyclic guanine monophosphate (cGMP). We have therefore investigated the role of nitric oxide in NMDA-induced neurotoxicity.

Neuronal cell death of 30-60% was observed following the 5 min. NMDA exposure. Sister cultures showed a 2.5 to 3-fold increase of cGMP over control. The substituted arginine N-nitroarginine (NNA) has been shown to be a membrane permeant inhibitor of NO synthase. NNA pre and co-incubated at 100uM with NMDA blocked the induction of cGMP, however neurotoxicity was not blocked at any dose of NNA.

Sodium nitroprusside (SNP) generates NO by spontaneous hydrolysis. When added to the culture media for 5 min. SNP induces an 8 to 20-fold increase in cGMP levels and nearly 100% neuronal cell loss. Hemoglobin, which is capable of scavenging the NO radical completely abolishes the SNP-duced increase in cGMP but does not significantly block SNP neurotoxicity.

These observations lead us to postulate that release of NO and subsequent production of cGMP by NMDA represents a metabolic pathway not involved in the excitotoxic chain of events. Further evidence is provided by the observation that 8-bromo-cGMP, which is a membrane permeant and functional analog of cGMP in not neurotoxic to cortical cells.

276.15

EXCITOTOXICITY OF TOPA QUINONE *IN VITRO* AND *IN VIVO*. E Aizenman*, FA Boeckman, C Stafstrom & PA Rosenberg, Dept of Physiol, Univ of Pittsburgh Sch Med, Pittsburgh, PA 15261, and Dept of Neurol, Children's Hosp and Harvard Med Sch, Boston, MA 02115.

We studied whether reduction of the non-NMDA excitotoxin 2,4,5-trihydroxyphenylalanine quinone (TOPA quinone) to its catechol precursor is sufficient to abolish its agonist properties and prevent its neurotoxicity in rat cortical neurons in culture. Also, we examined whether systemic administration of TOPA quinone can produce neurological abnormalities in rats. We first monitored the formation of TOPA quinone from TOPA (30-500 μM) at pH 7.2 spectrophotometrically (O.D. 480). Utilizing glutathione (GSH; 0.05-3 mM) as the reducing agent, we found that the optimal [GSH]:[TOPA] ratio which significantly retards topa quinone formation was 10:1. Thus, 3 mM GSH prevented whole-cell currents induced by a solution of 300 μM TOPA but did not affect currents elicited by 300 μM kainate. In addition, 2 mM GSH protected neurons from the toxic effects of 200 μM TOPA, but was not effective against 200 μM NMDA. In whole animal experiments, i.p. injections of TOPA (50 mg/kg) in 1 mM ascorbate in PBS produced epileptic-like behavior with and EEG correlate in P11-12 rats. These studies suggest that: 1) the presence of endogenous reductants may limit the toxicity of TOPA, and 2) peripheral sources of TOPA may have CNS toxicity.

276.16

PERSISTENT INHIBITION OF GLUTAMATE UPTAKE BY OXYGEN FREE RADICALS: SELECTIVE PROTECTION BY DISULFIDE-REDUCING AGENTS A. Volterra*, D. Trotti, S. Floridi and G. Racagni Ctr. Neuropharmacology & Inst. Pharmacol. Sci., University of Milan, Milan, Italy.

Oxygen free radicals play a role in ischemic brain damage. However, the details of their mode of action are still largely undefined. Here we report that oxygen radicals inhibit high-affinity $Na^+\backslash K^+$-dependent glutamate reuptake in primary cultures of astrocytes from neonatal rat cerebral cortex. Thus, 10 min incubation with either xanthine (500 μM) + xanthine oxydase (10-50 mU\ml) (XA\XOD) or H_2O_2 (500 μM), but not XA or XOD alone, results in a 20-40% reduced uptake. The effect of XA\XOD is prevented by > 80% in the presence of scavenger enzymes superoxide dismutase (SOD, 90 U\ml) + catalase (CAT, 3000 U\ml). Similarly, H_2O_2 inhibition is prevented by CAT. Longer incubations with XA\XOD result in a progressive increase of inhibition. Upon removal of radicals such increase is blocked, but inhibition remains at a steady level for at least 1 h. Reduced glutamate uptake does not correlate with cell damage, since no extracellular lactate dehydrogenase (LDH) activity was detected following 10 min incubation with XA\XOD, while a slight enhancement was observed at 30 min. We tested the possible protective effect of various antioxidants by adding them 10 min prior to exposure to XA\XOD 50 mU\ml: lipophilic Vit. E (20-200 μM) and methylprednisolone (100 μM) and hydrophilic Vit. C (1 mM) did not affect inhibition, while disulfide-reducing agents glutathione (GSH, 4 mM) and dithiothreitol (DTT, 2 mM) both prevented XA\XOD inhibition by 60-80%. This data suggests that radicals likely act by oxidizing functionally relevant SH groups on the glutamate transporter or on a related protein. In summary, oxygen free radicals persistently inhibit glutamate uptake in astrocytes without appreciable cell damage. Such inhibition may be an important early step in the ischemia-induced sequence of events leading to neuronal damage, since it may contribute to elevate extracellular glutamate up to neurotoxic levels.

EXCITATORY AMINO ACIDS: PHARMACOLOGY IV

277.1

IN VIVO ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS BY tACPD POTENTIATES AGONIST EFFECTS AT IONOTROPIC GLUTAMATE RECEPTORS. H. Klitgaard* and P. Laudrup, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Maaloev, Denmark.

Subtypes of glutamate receptors can be differentiated by their coupling to either ion channels (NMDA, kainate, AMPA receptors) or G-proteins (metabotropic receptors). The present study compares the effect of separate or combined in vivo activation of both ionophoretic and G-protein-coupled glutamate receptors using selective glutamate agonists.Male NMRI mice (25+2 g) were infused i.c.v. with different doses of L-glutamate, NMDA, kainate, quisqualate, AMPA or (1S,3R)-tACPD. In another set of experiments the ionophoretic glutamate agonists were co-infused i.c.v. with a fixed dose of tACPD. I.c.v. infusion of L-glutamate, NMDA, kainate, quisqualate and AMPA dose-dependently induced clonic convulsions. In contrast, tACPD elicted dose-dependent grooming and scratching, but was not convulsant. I.c.v. co-infusion of either L-glutamate, NMDA, kainate, quisqualate or AMPA with tACPD markedly reduced the time to onset of convulsions. In conclusion, ionophoretic and G-protein-coupled glutamate receptors induce different behavioral effects. However, the convulsant effects of ionophoretic receptor agonists are markedly potentiated by simultaneous activation of the G-protein-coupled metabotropic receptors.

277.2

BRADYKININ SELECTIVELY RELEASES EXCITATORY AMINO ACIDS FROM CULTURED GLIA IN A CALCIUM-DEPENDENT MANNER. F. Liu*, K. Jeftinija, and S. Jeftinija, Department of Vet. Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011, USA.

Bradykinin (BK) is a nonapeptide that plays a central role in the production of pain and inflammation. It has been shown that BK induces changes in intracellular calcium in neurons and glia but no data are available regarding the capacity of BK to release excitatory amino acids (EAA) from glia. The specific objective of this study was to study the mechanism by which BK releases EAA from cultured peripheral glia. DRG or sciatic nerve from 1 to 8-day-old rats were dissected and cultured on chicken plasma-coated slides for 6 to 8 days. Cultures were mounted in a perfusion chamber and perfused at a rate of 200μl/min, with aerated Ringer solution at 36±1°C. Quantification of amino acids was performed by high performance liquid chromatography utilizing fluorescence detection and pre-column OPA-derivatization. Following a period of culture equilibration, 1min, 200μl samples were collected. Baseline concentrations of Asp and Glu in glia cultures were 6.7±0.6nM (mean±SEM) and 23.6±2.1nM, respectively. Perfusion application of 10nM BK for 1min resulted in an 365±27% increase of Asp and 1038±183% increase of Glu release from cultured glia. A second applications of BK 10 min after first application resulted in an increase in the release of both EAAs that was at the level of 42% of that from the first application. This release of EAA evoked by BK was not abolished in low Ca -EGTA solution, but a second application of BK was without effect. Pretreatment of the glia cultures (30 min) with 50μM BAPTA-AM abolished the effect of BK. Pretreatment of the glia cultures with low Ca-EGTA solution with added ryanodine (10μM) failed to produce depression of BK-evoked release of EAAs. When thapsigargin (1μM) was added to low Ca-EGTA solution for 30 min, BK-evoked release of EAA was abolished. Capsaicin and high potassium were not able to produce any effect on release of EAA from cultured glia. Our results show that BK selectively evokes the release of EAAs from cultured glia in a Ca-dependent manner.

277.3

SELECTIVE EFFECTS OF PROLINE ON GLUTAMATE UPTAKE M.L. Cordero, J.G. Ortiz*, G. Santiago and O. Torres. Department of Pharmacology, University of Puerto Rico, School of Medicine, San Juan, Puerto Rico, 00936-5067

Proline (**PRO**) has been suggested as a possible neuromodulator of glutamate (GLU) transmission (Cordero et al., 1991, Helm et al., 1990, Nadler et al., 1989, Ault et al., 1987). PRO does not affect [^{3}H]GLU uptake in whole brain crude synaptosomal preparation (P_2) from normal (**SPS/SPS**) mice, but increases GLU U_{max} in 15 day-old mice. In audiogenic seizure-susceptible (**SPS/sps**) mice, Pro decreases the apparent K_m (56.3 ± 13.08 μM vs. 15.3 ± 1.47 μM, $p<0.0001$) and the U_{max} (4.46 ± 0.44 vs. 1.5 ± 1.93 ± 0.103 nmoles/mg protein, $p<0.0001$) of GLU uptake while in astrocyte-enriched cultures PRO increases both the K_m (177.9 ± 16.8 μM vs. 27.3 ± 3.01 μM) and the U_{max} values (12.66 ± 0.976 vs. 3.09 ± 0.193 nmoles/mg protein). Dihydrokainate (**DHK**), an inhibitor of GLU uptake, produces similar effects in the astrocytes and normal mice synaptosomes. These results point to the possible use of PRO as a pharmacological tool for examining GLU uptake system(s) in normal and diseased states, such as epilepsy. (Supported by the Institutional MBRS and RCMI Programs and the Animal Resource Center)

277.4

1S,3R-ACPD IS AN AGONIST AT L-AP4 RECEPTORS. W. B. Thoreson* and R. F. Miller. Department of Physiology, University of Minnesota, Minneapolis, MN 55455.

There are two classes of G-protein coupled excitatory amino acid (EAA) receptors: 1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) and L-2-amino-4-phosphonobutyric acid (L-AP4) receptors. L-AP4 receptors can be distinguished from ACPD receptors by their sensitivity to micromolar L-AP4. However, the sensitivity of L-AP4 receptors to ACPD is not known. Using whole cell patch clamp recording techniques, we examined the effects of ACPD on ON bipolar cells in a mudpuppy retinal slice.

Like L-AP4 (5 μM), 1S,3R-ACPD (160 μM) and racemic 1S,3R/1R,3S-ACPD (100 μM; commonly called "trans-ACPD") evoked outward currents accompanied by an increased input resistance and reduced membrane noise. 1R,3S-ACPD (1 mM) had no effect. The response to 1S,3R/1R,3S-ACPD was suppressed by prior activation of the L-AP4 receptor, but unaffected by the ACPD receptor antagonist L-AP3 (1 mM). The inclusion of neomycin (100 μM) in the patch pipette, which should suppress ACPD receptor-stimulated phosphoinositide hydrolysis, also had no effect on the responses to L-AP4 or 1S,3R/1R,3S-ACPD. These results indicate that 1S,3R-ACPD is an agonist at the L-AP4 receptor.

L-AP4 receptors are the only EAA receptors in mudpuppy ON bipolar cells. Using the b-wave of the electroretinogram as an assay for ON bipolar cell responses, we could thus obtain dose/response curves for ACPD enantiomers acting at L-AP4 receptors uncontaminated by effects at other EAA receptors. The rank order potency and IC_{50}'s are L-AP4 (1.1 μM) >> 1S,3R-ACPD (89 μM) > 1S,3S-ACPD (417 μM) >> 1R,3S-ACPD; 1R,3R-ACPD (>5 mM). *Supported by NIH grants EY03014 and EY06213.*

277.5

PHARMACOLOGY OF METABOTROPIC GLUTAMATE RECEPTORS NEGATIVELY COUPLED TO cAMP FORMATION IN THE RAT HIPPOCAMPAL SLICE. B.G. Johnson* and D.D. Schoepp. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285

Metabotropic glutamate receptors (mGluRs) are G-protein coupled to transduction enzymes that produce increased phosphoinositide (PI) hydrolysis and decreased cAMP formation. The pharmacology of PI-linked mGluRs have been well characterized in a variety of CNS tissues (see TiPS 11: 509, 1992). Recently, mGluR(s) negatively coupled to cAMP formation have been cloned (Tanabe et al. Neuron 8: 169, 1992). In this study the pharmacology of cAMP-linked mGluRs were examined in cross chopped slices of the adult rat hippocampus. Various excitatory amino acid agonists and antagonists were examined for effects on forskolin (30 μM)-stimulated cAMP formation. The selective metabotropic agonist (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) was highly potent at inhibiting forskolin-stimulation, producing a maximal effect of 55% inhibition at 30 μM. 1R,3S-ACPD was much less potent, but produced full inhibition at higher concentrations (90% inhibition at 1000 μM). The ionotropic agonists NMDA, AMPA, and kainate did not alter cAMP at up to 500 μM. Non-selective ionotropic/metabotropic agonists that inhibited forskolin-stimulated cAMP included quisqualate (92% at 500 μM), ibotenate (44% at 500 μM), and L-glutamate (41% at 1000 μM). Inhibition of cAMP formation induced by 1S,3R-ACPD (100 μM) was not affected by the ionotropic antagonists MK801 (10 μM), CNQX (100 μM), AP5 (500 μM), or AP7 (500 μM). L-AP3 and L-AP4 have been shown to inhibit the effect of 1S,3R-ACPD at PI-linked mGluRs. However when measuring cAMP formation, L-AP3 (500 μM) and L-AP4 (500 μM) mimicked the effect of 1S,3R-ACPD, producing 64% and 74% inhibition of forskolin-stimulation, respectively. These studies show that mGluRs negatively coupled to cAMP formation can be readily demonstrated in the brain slice preparation. This class of metabotropic glutamate receptors has a pharmacology that is distinct from the ionotropic and PI-linked glutamate receptors.

277.6

(1S,3R)-1-AMINOCYCLOPENTANE-1,3-DICARBOXYLIC ACID (1S,3R-ACPD)-SENSITIVE L-^{3}H-GLUTAMATE BINDING TO METABOTROPIC RECEPTORS IN RAT BRAIN R.A. True* and D.D. Schoepp. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285.

We studied L-^{3}H-glutamate (10 nM) binding to rat forebrain membranes in the presence of saturating concentrations (100 μM) of ionotropic glutamate receptor agonists, NMDA, AMPA, kainate and the uptake inhibitor 4-acetamido-4'-isothiocyano-stilbene-2,2'-disulfonic acid (SITS). Nonspecific binding (≈50%) was defined with 1 mM of the highly selective metabotropic agonist, 1S,3R-ACPD. Samples were incubated on ice 45 minutes, centrifuged @ 50,000 x g for 5 minutes and pellets rapidly washed with ice-cold buffer.

1S,3R-ACPD-sensitive binding was saturable (B_{max} = 2.50 ± 0.27 pmole/mg protein), Cl^--dependent, temperature-independent, reversible and of relatively high affinity, (K_d = 187 ± 60 nM). This binding was differentially distributed in brain regions with the highest levels in hippocampus, striatum and cortex. Like 1S,3R-ACPD, other metabotropic EAA compounds displaced this binding with a pharmacology consistent with known functional metabotropic data.

Compound	Ki (μM)	Hill Coefficient
L-Glutamate	0.092 ± 0.050	1.109 ± 0.049
1S,3R-ACPD	10.3 ± 2.06	0.848 ± 0.064
Ibotenate	36.2 ± 5.40	1.184 ± 0.217
L-AP3	138 ± 23.8	0.866 ± 0.156
1R,3S-ACPD	434 ± 150	0.794 ± 0.049

The glutamate uptake inhibitors, L-cystine and L-AP4, did not significantly affect the specific binding (K_i>300 μM). Quisqualate (QUIS) produced a biphasic inhibition curve (K_{i1} = 0.0087 ± 0.0058 μM; K_{i2} = 59.4 ± 14.3 μM). Low affinity QUIS sites (78% of binding) may represent cAMP-linked metabotropic receptors. The remaining 22% (high-affinity QUIS binding), may reflect binding to phosphoinositide hydrolysis-linked metabotropic receptors.

277.7

PERTUSSIS TOXIN DIFFERENTIATES BETWEEN THE ADENYLATE CYCLASE- AND PHOSPHOINOSITIDE (PI)-LINKED METABOTROPIC GLUTAMATE RECEPTORS IN VIVO. A.J. Sacaan* and D.D. Schoepp. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285

There is evidence for heterogeneity of metabotropic (G-protein linked) glutamate receptors. One type is associated with the stimulation of PI hydrolysis, while the other is negatively coupled to adenylate cyclase. In this study, pertussis toxin was utilized to distinguish between these two types of metabotropic glutamate receptors in vivo. Male Sprague-Dawley rats were anesthetized and placed in small animal stereotaxic instrument. Pertussis toxin (4 μg / 4 μl) or its vehicle (4 μl) was injected into the dorsal hippocampus. At 48 hours after injection, stimulation of PI hydrolysis and inhibition of forskolin-stimulated cyclic AMP formation by 1S,3R-ACPD was assessed in cross-chopped hippocampal slices. In vehicle injected rats, 100 μM 1S,3R-ACPD produced 60.8 ± 4.7% inhibition of forskolin-stimulated cyclic AMP levels. Following pertussis toxin, the inhibitory effect of 1S,3R-ACPD (100 μM) on forskolin-stimulated cyclic AMP levels was completely prevented (4.7 ± 20.0% inhibition). In contrast, there was no significant difference between 1S,3R-ACPD stimulations of PI hydrolysis in vehicle injected (486 ± 58% of basal hydrolysis) and pertussis toxin injected (352 ± 84% of basal hydrolysis) hippocampal tissues. Thus, pertussis toxin can be utilized in vivo as a pharmacological tool to selectively uncouple adenylate cyclase-linked, but not PI-linked, metabotropic glutamate receptors. This may provide an approach to identify which subtypes of metabotropic glutamate receptors are involved in different physiological and pathological events.

277.8

ACTIONS OF PHENYLGLYCINE DERIVATIVES ON METABOLIC AND EXCITATORY EFFECTS OF (1S,3R)-1-AMINOCYCLO-PENTANE-1,3-DICARBOXYLATE. P.J. Roberts[1], E.F. Birse, S.A. Eaton[2], P.L.St.J. Jones, P.C-K. Pook, R.H.P. Porter[1], P.M. Udvarhelyi, B. Wharton, T.E. Salt[2] & J.C. Watkins*, Dept of Pharmacol, The Medical School, Bristol BS8 1TD UK; [1] Dept of Physiol. & Pharmacol, Univ. of Southampton, SO9 3TU UK; [2] Dept of Visual Science, Inst. of Ophthalmology, London WC1H 9QS UK.

(1S,3R)-1-Aminocyclopentane-1,3-dicarboxylate (ACPD) is an agonist at metabotropic excitatory amino acid (EAA) receptors linked to phospho-inositide (PI) hydrolysis and also has excitatory effects on central neurons. We report here that members of a series of phenylglycine (PG) derivatives have differential actions on the metabolic and excitatory effects of ACPD. On chopped cerebral cortex tissue from neonatal rats, (S)-4-carboxy-3-hydroxy-PG and (S)-3-hydroxy-PG stimulated PI hydrolysis in a dose-dependent manner, giving increases of 370% and 507%, respectively, over basal at the highest dose tested (1 mM). These two PGs also potentiated the stimulation of PI hydrolysis produced by submaximal doses of ACPD, but had no effect on the maximal ACPD response (approx 800% stimulation at 100 μM). (R)-4-Carboxy-PG had little or no effect on basal PI hydrolysis but competitively antagonized ACPD (1 μM - 1 mM)-stimulated PI hydrolysis with a K_B of approx 0.6 mM. All three compounds selectively antagonized ACPD- (relative to NMDA and/or AMPA-) induced motoneuronal depolarizations in the neonatal rat spinal cord *in vitro* (bath application) and ACPD-evoked excitatory responses of rat thalamic neurons *in vivo* (iontophoretic application). In motoneurons, the antagonist action of (R)-4-carboxy-PG was competitive, while that of the other two PGs was non-competitive. (S)-3-Hydroxy-PG also showed weak excitatory actions in both types of neuron. Supported by M.R.C. (UK) and US PHS NS 26540.

277.9

A PHARMACOLOGICAL CHARACTERIZATION OF THE $mGlu_6R$-1α AND THE $mGlu_6R$-1β SUBTYPE OF THE METABOTROPIC GLUTAMATE RECEPTOR EXPRESSED IN A MAMMALIAN CELL LINE. C. Thomsen*, E. Mulvihill, B. Haldeman and P.D. Suzdak. Novo Nordisk A/S, CNS Division, DK-2760 Maaloev, Denmark, and ZymoGenetics, Seattle.

Excitatory amino acid receptor subtypes have been classified into two categories based on the signal transduction mechanisms to which they are associated. The "ionotropic" glutamate receptors are directly coupled to ion channels, whereas the "metabotropic" glutamate receptors ($mGlu_6R$) are linked to their effectors via a G-protein-dependent mechanism. The $mGlu_6R$-1α and the $mGlu_6R$-1β cDNA's were subcloned into mammalian expression vectors and transfected into a baby hamster kidney cell line. Two subclones, stably expressing the $mGlu_6R$-1α- and 1β-subunit, respectively, were selected. Both subtypes were coupled to phosphoinositide hydrolysis in this cell line. The relative order of potency was: quisqualate > glutamate > ibotenate ≥ (1S,3R)-t-ACPD for subtype $mGlu_6R$-1α and for subtype $mGlu_6R$-1β: quisqualate> ibotenate ≥ glutamate > (1S, 3R)-t-ACPD. The time course for, and possible mechanism of, desensitization of the $mGluR_6$-1 receptor subtypes following exposure to glutamate was characterized. Regulation of the $mGlu_6R$-1 subtypes by other endogenous modulators was investigated.

277.10

D-ASPARTATE (D-Asp) MAY STIMULATE A NOVEL EXCITATORY AMINO ACID (EAA) RECEPTOR COUPLED TO PHOSPHOINOSITIDE (PI) HYDROLYSIS. L. Littman*, B.S. Glatt and M. B. Robinson. Children's Seashore House; Depts. of Pediatr. & Pharmacol., U. of Penn; Phila, PA 19104

Molecular biological (Tanabe, Neuron 8:169, 1992) and pharmacological studies suggest that there are several subtypes of EAA receptors coupled to the hydrolysis of PI. The effects of quisqualate (Quis), ibotenate, or (±)-1-aminocyclopentane-trans-1,3-dicarboxylate (tACPD) can be non-competitively inhibited by L-aspartate-ß-hydroxamate (L-AßHA) or DL-2-amino-3-phosphonopropionate (DL-AP3) (Littman et al., Neurosci. Abs., 1991). In the current studies, we attempted to identify compounds that are selective for subtypes of metabotropic EAA receptors. D-Asp stimulated PI hydrolysis in hippocampal cross-sections prepared from neonatal (7-12 day old) rats (EC_{50} = 530 ± 80 μM; max. effect = 24,500 ± 3,500 DPM/100,000 DPM incorporated; n=10; mean ± SEM). L-AßHA (100 μM) and DL-AP3 (1000 μM) were much less potent as inhibitors of D-Asp than as inhibitors of tACPD (L-AßHA, 14 ± 7% versus 49 ± 6%; DL-AP3, 13 ± 6% versus 46 ± 4%; n=11, p<0.01), suggesting that D-Asp may activate a subtype of metabotropic EAA receptors. Several approaches were used to further investigate the mechanism of action of D-Asp. Although D-Asp is a substrate for glutamate uptake, the high-affinity uptake inhibitor L-trans-pyrrolidine-2,4-dicarboxylate (100 μM) did not block PI hydrolysis stimulated by D-Asp (9 ± 18%, n=3). D-2-amino-5-phosphonopentanate (100 μM), an NMDA receptor antagonist, atropine (100 μM), a muscarinic receptor antagonist, pirenzepine (100 μM), an adrenergic receptor antagonist, and mianserin (100 μM), a serotonergic receptor antagonist, inhibited PI hydrolysis stimulated by D-Asp by less than 20% (n=4). We have previously demonstrated that a water-soluble forskolin derivative can inhibit PI hydrolysis stimulated by Quis. Water-soluble forskolin (100 μM) inhibited both Quis and D-Asp effects (28 ± 8% and 33 ± 8%, respectively; n=5), suggesting that the intracellular transduction pathways for these two agonists are similar. In neonates, the maximal responses of D-Asp and tACPD were similar. In adults, however, the maximal response of D-Asp was much less than the maximal response of tACPD, adding further support that these compounds activate distinct receptors. (Supported by grant #GM-34781)

277.11

GLUTAMATE TRANSPORT INHIBITORS : EFFECT OF DISTAL ACIDIC GROUP SUBSTITUTIONS ON ACTIVITY R.J. Bridges, W. Daily, F. Lovering, T. Blakely, H. Koch, S. Ho, and A.R. Chamberlin*. Depts. of Chemistry and Neurology, University of California, Irvine, CA 92717.

High-affinity transport of L-glutamate is a key step in the process of excitatory transmission, contributing to signal termination, the recycling of the transmitter, and the regulation of extracellular levels of glutamate below those which will induce excitotoxic pathology. In previous studies we identified L-trans-2,4-pyrrolidine dicarboxylate (L-trans-2,4-PDC) as a potent and selective inhibitor of sodium-dependent glutamate transport in rat brain synaptosomes. To investigate the structure/function relationships of this transporter in greater detail, we have now prepared several novel derivatives of L-trans-2,4-PDC in which the 4-carboxylate group has been replaced with phosphonate and sulfinate groups. In contrast to the activity of L-trans-2,4-PDC, L-trans-4-phosphonopyrrolidine-2-carboxylate (L-trans-2,4-PPC), exhibited little or no ability to inhibit the synaptosomal uptake of ^{3}H-D-aspartate. The structurally analogous 4-sulfinic acid, however, potently inhibited transport. This specificity conferred by the terminal acidic group is also consistent with previous studies using acyclic α-amino diacids. Thus, while serine-O-phosphate does not inhibit uptake, serine-O-sulfate is an effective transport blocker. This narrow tolerance for changes in the distal acidic group provides insight into molecular interactions responsible for binding to the transport protein.

277.12

QUISQUALATE RESOLVES TWO DISTINCT [^{3}H]GLUTAMATE METABOTROPIC BINDING SITES. M.V. Catania*, Z. Hollingsworth, J.B. Penney and A.B. Young. Neurology Service, Massachusetts General Hospital, Boston, MA 02114.

Subtypes of metabotropic excitatory amino acid receptors have been proposed on pharmacologic, signal transduction, and DNA bases. We assessed potential metabotropic binding site subtypes with *in vitro* quantitative [^{3}H]glutamate autoradiography on sections of adult rat brain, in the presence of saturating concentrations of N-methyl-D-aspartate (NMDA) (100 mM) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) (10 mM). Under these conditions, quisqualate(QUIS), a potent agonist, and trans-1-amino-1,3-cyclopentanedicarboxylic acid (t-ACPD), a very specific agonist, were tested as competitors of [^{3}H]glutamate binding to metabotropic receptors. QUIS (1 nM to 1 mM) resolved two distinct binding sites with apparent Ki values of 13±5 nM and 125±35 mM. The two sites were unevenly distributed. The ratio between high and low affinity sites was 3:1 in cerebellar molecular layer, 1:2 in outer cortex, 1:1 in striatum and stratum radiatum of CA1. t-ACPD (2.5 nM to 2.5 mM) competition curves were monophasic with apparent Ki values varying from 33 μM to 110 μM. t-ACPD displaces [^{3}H]glutamate binding in the presence of 2.5 μM QUIS and there is no additivity between maximal concentrations of QUIS and t-ACPD. These results indicate that two metabotropic binding sites with different regional distributions can be detected in adult rat brain on the basis of their different affinities for QUIS and strongly support the existence of distinct and differentially distributed metabotropic receptors in brain.

Supported by USPHS grant NS19613.

277.13

MODULATION OF SYNAPTIC ACTIVATION OF NEOCORTICAL NEURONS BY GLUTAMATE METABOTROPIC RECEPTORS. J.P. Burke* and J.J. Hablitz. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

Cerebral cortex contains high levels of mRNA for metabotropic glutamate receptors (Neuron, 8: 169-179, 1992). Although their function is not yet known in the neocortex, this class of receptors modulates excitability of neurons in hippocampus, septum and thalamus. We studied the actions of trans-ACPD, a potent and selective metabotropic glutamate receptor agonist, on evoked synaptic activity in adult rat neocortex.

Conventional intracellular recordings were obtained from layer II-III neurons. Post-synaptic potentials (PSPs) were evoked via bipolar tungsten electrodes before and after bath application of 20-100 μM trans-ACPD. EPSPs evoked by weak stimulation were reduced by 39% (n=12). Strong stimulation evoked EPSP-IPSP complexes. The EPSP component was reduced by 35%, and the IPSP by 41%. Trans-ACPD caused a membrane depolarization in 7 of 12 cells; input resistance, tested at the original membrane potential, was not altered. All effects were reversible upon washing.

These results show that trans-ACPD has significant effects on synaptic transmission in the adult neocortex and suggest that metabotropic receptors play important modulatory roles in this brain region.

(NS18145, MH10150).

277.14

EFFECT OF THE KAPPA AGONISTS PD117302 AND CI-977 ON POTASSIUM-STIMULATED GLUTAMATE RELEASE FROM NEURONAL CULTURES. M. A. OLESHANSKY*, R. A. GELPI, F. C. TORTELLA and M. A. DECOSTER. Dept. Med. Neurosci., Walter Reed Army Inst. Res., Washington, D.C. 20307-5100.

We have previously reported the effects of the kappa opioid agonists PD117302 (PD) and CI-977 (CI) to protect cell cultures from glutamate neurotoxicity. CI has also been shown to inhibit potassium chloride (KCl)-stimulated glutamate release from slices of rat parietal cortex. Here we evaluated the effect of CI and PD on KCl-stimulated glutamate release from primary rat cortical cultures. Two-week old neuronal cultures were stimulated with 50 mM KCl and glutamate release was determined using fluorometric detection via HPLC analysis. KCl-stimulated glutamate release was 213, 152, and 126% of basal release at 5, 10 and 15 minutes after stimulation, respectively. In cultures stimulated 10 minutes with 50 mM KCl, the presence of 50 nM CI or PD inhibited glutamate release by 27 and 37%, respectively. The ability of these novel kappa opioids to prevent EAA release in culture supports a possible presynaptic mechanism of action mediating their neuroprotective activity.

277.15

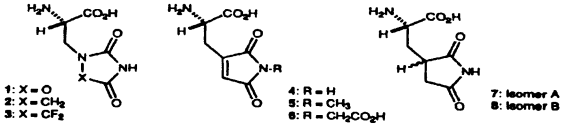
GLUTAMATE DEHYDROGENASE MODIFIED CARBON-FIBER MICROELECTRODES WITH MILLISECOND RESPONSE TIMES. Werner G. Kuhr*, Paul Pantano and Michelle R. Gagnon. Department of Chemistry, University of California, Riverside, CA 92521

The fast scan cyclic voltammetry (FSCV) experiment performed with ultra-microelectrodes has been well established for the measurement of the release and uptake of the easily oxidized neurotransmitters (i.e. dopamine). However, most other neurotransmitters and metabolic intermediates are not oxidized at analytically useful potentials. While many strategies have been developed to immobilize enzymes onto electrodes to improve the selectivity of the electrochemical measurement, few have been constructed that afford both a small probe size and the temporal response required to follow physiologically relevant events. Recently we have developed two such enzyme immobilization techniques for 10μm carbon-fiber microelectrodes that meet these requirements. Dehydrogenases are used in both cases to enzymatically oxidize the non-electroactive species (i.e. glutamate). The concurrent reduction of the enzyme cofactor, NAD+, produces the electroactive species NADH. It is this enzymatically-generated NADH which diffuses back to the carbon surface where the faradaic current generated by its oxidation during the FSCV experiment (100V/s) is proportional to the relative concentration of glutamate. Response times for the indirect detection of glutamate have been observed in the range of milliseconds to seconds, and selectivity has been demonstrated by the lack of signal generated following the introduction of aspartate. The first technique involves the covalent attachment of the enzyme onto the carbon-fiber via a hydrophilic tether using biotin-avidin technology as the coupling technique. The second technique utilizes a micro-dialysis membrane (9,000 MW cut-off, 150μm i.d.) to entrap the dehydrogenase close to the carbon-fiber microelectrode while permitting small molecular weight analytes to diffuse through for enzymatic reaction and subsequent indirect detection. Both approaches have the advantage of having the selectivity of the electrochemical measurement easily changed simply by changing the dehydrogenase used. Work is now in progress to apply these dehydrogenase-modified carbon-fiber microelectrodes to the measurement of glutamate release and uptake in the mammalian brain.

277.16

SYNTHESIS OF QUISQUALIC ACID ANALOGUES AS POSSIBLE SELECTIVE LIGANDS AT QUISQUALIC ACID RECEPTORS. R.J. Roon*, M.K. Schulte, J.F. Koerner, N.L. Subasinghe and R.L. Johnson. Departments of Medicinal Chemistry and Biochemistry, University of Minnesota, Minneapolis, MN 55455

Quisqualic acid (1,L-QUIS) is a potent agonist at multiple excitatory amino acid receptor subtypes in the CNS. In addition to a high affinity for kainate, AMPA, and metabotropic receptors, L-QUIS also inhibits the Ca^{2+}/Cl^- dependent glutamic acid uptake system in brain synaptic plasma membrane preparations and an N-acetyl α-linked acidic dipeptidase which hydrolyzes the brain dipeptide N-acetyl-L-aspartyl-L-glutamic acid. We have previously demonstrated that exposure of slices to L-QUIS also produces a 30-100 fold sensitization of neurons to depolarization by D- or L-2-amino-4-phosphonobutanoic acid (AP4) and related phosphonates (QUIS-effect). At least two binding sites are involved in this effect; a L-QUIS "induction site" and a QUIS-sensitive-AP4 site These are novel sites of action for L-QUIS which are different from the classical AMPA and AP4 receptors. In order to gain additional information regarding the structural requirements of these sites, the quisqualic acid analogues 2-8 have been synthesized and their effects on the various components that make up the QUIS-effect investigated. While none of these compounds induced the QUIS-effect, neurons were sensitized to depolarization by 4,5 and 6 after exposure to L-QUIS. Compound 2 was a potent depolarizer of neurons, but little enhancement of potency was observed after exposure to L-QUIS.



277.17

ATYPICAL NEUROLEPTICS PREFERENTIALLY ALTER GLUTAMATE CONCENTRATIONS IN THE RAT NUCLEUS ACCUMBENS. J. G. Csernansky*, C. T. Wrona, M. E. Bardgett. Psychiatry Dept., Wash. Univ. Sch of Med., St. Louis, MO 63110.

Previous reports have suggested that atypical neuroleptics exert mesolimbic-specific neurochemical changes in comparison to typical neuroleptics. In the present study, the effects of three atypical neuroleptics on glutamate levels in the rat nucleus accumbens (NA) and corpus striatum (CS) were compared to the effects of the typical neuroleptic, haloperidol, after acute and subchronic treatment. Animals received s.c. injections of either saline vehicle, haloperidol (0.18 mg/kg), clozapine (19.4 mg/kg), sulpiride (0.95 mg/kg), or (-)-3-(3-hydroxyphenyl)-N-n-propyl-piperidine (PPP)(65.1 mg/kg) for 1 or 28 days. Relative drug doses were determined by *in vivo* competition for [^{3}H]spiperone binding sites in the NA and CS. All three atypical drugs, but not haloperidol, significantly reduced glutamate concentrations in the NA after acute treatment. Glutamate levels were modestly elevated in the CS after acute haloperidol treatment. Chronic PPP and clozapine treatment were found to slightly increase NA and CS glutamate, respectively, in the left side of the brain only. The results indicate that atypical neuroleptics exert a preferential effect on glutamatergic terminals in the NA.

277.18

A NOVEL METABOTROPIC GLUTAMATE RECEPTOR AGONIST: DEPRESSION OF MONOSYNAPTIC EXCITATION OF MOTONEURONS IN THE NEWBORN RAT SPINAL CORD. M. Ishida*, T. Saito and H. Shinozaki. The Tokyo Metro. Inst. of Med. Sci. Tokyo 113, Japan.

2*S*,3*S*,4*S*-2-(Carboxycyclopropyl)glycine (L-CCG-I) is a potent metabotropic glutamate receptor agonist. From various CCGs, we recently looked for novel agonists which were more potent than L-CCG-I or 1*S*,3*R*-ACPD. DCG-IV, (2*S*,3*R*,4*R*,6*R*)-2-(4,6-dicarboxycyclopropyl)glycine, is so far the most potent metabotropic agonist. DCG-IV contains the moieties of L-CCG-I and (2*S*,3*R*,4*S*)-CCG (L-CCG-IV), which is an NMDA agonist more potent than NMDA. In high concentrations, DCG-IV caused NMDA-like depolarization, but was considerably less potent than L-CCG-IV. Like L-CCG-I, DCG-IV depressed spinal reflexes evoked by stimulation of dorsal roots, in particular, attenuated monosynaptic reflexes without causing postsynaptic depolarization at significantly low concentrations. The threshold concentration of DCG-IV to depress monosynaptic reflexes was lower than 0.1 μM that was markedly lower than that of L-CCG-I. It seems likely that DCG-IV and L-CCG-I act on presynaptic terminal to control transmitter release, and the mode of action of DCG-IV seems different from that of 1*S*,3*R*-ACPD, L-AP4 and L-CCG-I. DCG-IV was more potent than baclofen, which is so far the most potent blocker of monosynaptic reflexes in the newborn rat spinal cord. The action of DCG-IV was slow onset and slowly recovered, and was not blocked by any known pharmacological agents including GABA antagonists. DCG-IV did not depress the depolarizing effect of other excitatory amino acids in the newborn rat spinal motoneuron.

277.19

PHYSICAL DEVELOPMENT AND NEUROCHEMICAL CHANGES FOLLOWING NEONATAL EXPOSURE TO 1-AMINOCYCLOPENTANE-TRANS-1,3-DICARBOXYLIC ACID (TRANS-ACPD) OR D,L-2-AMINO-3-PHOSPHONOPROPIONIC ACID (D,L-AP3) IN ADULT RATS. J. P. Tizzano*, K. L. Griffey and J. A. Johnson. Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN 46140

The metabotropic (met) receptor is a recently discovered receptor of the excitatory amino acid (EAA) class. This receptor is G-protein linked to the hydrolysis of cellular phosphoinositides and is pharmacologically distinguished from ionotropic EAA receptors (NMDA, AMPA or kainate). Met EAA receptor coupling is greatly enhanced during the early period of neonatal development in the rat, suggesting that these receptors play a role in brain development. This study evaluated the postnatal treatment effects of trans-ACPD (met agonist) or D,L-AP3 (met antagonist) on physical development, glutamic acid decarboxylase (GAD) and choline acetyltransferase (ChAT) enzyme activities in various regions in the brain of adult rats. Six litters of CD rats were dosed i.p. (1 pup/sex/litter) on postnatal days (PND) 3-10 with saline, 25 mg/kg/day trans-ACPD, or 400 mg/kg/day D,L-AP3. Body weights were reduced in animals dosed with D,L-AP3 on PND 7-21 and at necropsy (PND 84), while relative brain weights were increased. Retinal dysplasia was found in the D,L-AP3 dosed animals, with severe atrophy of the optic nerve and chiasm. For trans-ACPD treated animals no differences were observed in body weight or gross pathology of the brain and visual system compared to control animals. GAD and ChAT activities were increased in the olfactory region of the brain in animals treated with D,L-AP3, whereas GAD activity was decreased in the striatum and cortex of animals treated with either drug. This study showed that postnatal exposure to met agents can result in persistent neurochemical alterations in the brain and profound degeneration of the visual system that are agonist/antagonist specific.

277.20

GLUTAMATE RECEPTOR MEDIATED PHOSPHOINOSITIDE TURNOVER IN PRIMARY CEREBROCORTICAL NEURONAL CULTURES. G. J. Birrell* and F.W. Marcoux. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48106.

Characterization of glutamate receptor stimulated phosphoinositide turnover was performed in cerebrocortical cultures in vitro. ^{3}H phosphoinositide hydrolysis was measured in cultures pre-incubated with HBSS containing 10 mM LiCl. Test incubations (20 mins) were terminated by addition of 5% TCA.

Quisqualate, 1s, 3r-ACPD, glutamate and Ibotenate evoked dose-dependent inositol phosphate formation (see table below).

	Maximal Effect EC50 (μM)	(% of basal)	n
Quisqualate	0.34	192	28
1s, 3r-ACPD	3.1	224	24
Glutamate	17.3	170	16
Ibotenate	17.8	240	4

Dose-response curves for quisqualate and glutamate were bell-shaped and maximum responses were lower than those for 1s, 3r-ACPD or ibotenate. The selective inotropic receptor antagonists CPP (100 μM) or NBQX (1-100 μM) enhanced the effects of quisqualate and glutamate, causing a left-wards shift in the dose-response curves for these two agonists. These results suggest the occurrence of interaction between inotropic and metabotropic glutamate receptor mediated events.

EXCITATORY AMINO ACIDS: RECEPTORS IV

278.1

COMBINATORIAL RNA SPLICING ALTERS THE SURFACE CHARGE ON THE NMDA RECEPTOR. V. Anantharam*[1], R.G. Panchal[2], A. Wilson[1], V.V. Koltchin[1], S. Cheley[2], S.N. Treistman[1] and H. Bayley[2]. [1]Dept. of Pharmacology, Univ. of Mass. Medical School, Worcester, MA 01655 and [2]Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Recently, a cDNA encoding a 105.5 kD, a subunit of the rat brain NMDA receptor, was isolated by expression cloning (Moriyoshi *et al.*, Nature 354:31, 1991). Using RT-PCR, we now demonstrate alternatively spliced transcripts encoding four related NMDAR1 subunits. NMDAR1-LL contains both the 21 amino acid cassette 1 (positions 191-211) and the 37 amino acid cassette 2 (positions 885-821), while NMDAR1-SL lacks cassette 1, NMDAR1-LS lacks cassette 2 and NMDAR1-SS lacks both cassettes. NMDAR1-SL is the form described by Moriyoshi *et al.* NMDAR1 transcripts are present in rat brain RNA at ratios of LL:SL:LS:SS of about 3:12:2:8. When injected into *Xenopus* oocytes, RNA transcripts derived from cDNAs encoding the splice forms each direct the synthesis of glutamate-activated channels with NMDA receptor pharmacology. The currents elicited by NMDA were dramatically reduced by physiologically relevant concentrations of ethanol suggesting that the NMDAR1 subunits are targets of this drug (see S.N. Treistman *et al.* abstract this meeting). If a commonly used folding model is accepted, the two amino acid cassettes found in the splice variants together increase the extracellular surface charge by +11 units (+55 for a functional pentamer by analogy with the acetylcholine receptor). Hence, the presence or absence of the cassettes may modulate functional properties of the NMDA receptor such as conductance, ion selectivity, the affinity and effects of charged ligands, and voltage-dependent block by Mg^{2+} or phencyclidine. Supported by NIH and DOE (H.B.) and ADAMHA (S.N.T.).

278.2

CALCIUM-INDUCED ACTIN DEPOLYMERIZATION REDUCES NMDA CHANNEL ACTIVITY. C.Rosenmund*† and G.L.Westbrook#. Vollum Institute, and Departments of †Physiology and #Neurology, Oregon Health Sciences University, Portland, OR 97201.

Calcium transients in the postsynaptic spine following activation of NMDA channels are important for the modification of synaptic transmission. However, the calcium-dependent effector site(s) have yet not been identified. The activity of NMDA channels in cultured hippocampal neurons is reduced by [Ca]i, and ATP is required for the maintenance of channel activity in the presence of extracellular calcium. This suggests that the effects of Ca and ATP are interrelated. However, in our experiments this did not appear to be due to kinase or phosphatase activity. We therefore examined alternative Ca- and ATP-dependent processes. One possibility is actin filaments which are highly concentrated in the postsynaptic density and depolymerized by micromolar calcium; reassembly requires ATP, low [Ca]i and other factors.

Whole-cell recordings were made on cultured rat hippocampal neurons. The cell interior was dialyzed with standard intracellular solution buffered to 20 nM [Ca]i, and the NMDA channel activity was measured with pulsed application of 10 μM NMDA/2 mM [Ca]o (see Soc.Neurosci.Abstr. 383.5, 1991). Intracellular dialysis with cytochalasins B, B-dihydro or D (each 1 μM), thought to destabilize actin filaments by enhanced ATP-actin hydrolysis, antagonized the effect of ATP on NMDA currents. Similar effects were observed following 18-30 hr. pretreatment with botulinum toxin C (250 ng/ml) which promotes disassembly of actin filaments via ADP ribosylation. Phalloidin (1 μM), which stabilizes polymerized actin, resulted in well maintained NMDA currents even in absence of ATP, but phalloidin was without effect on kainate currents. Reagents which disrupt (colchicine) or stabilize (taxol) microtubules as well as protease inhibitors (leupeptin and Calpain inhibitor type 1) were also without effect. This suggests that the state of cortical actin filaments has an important influence in the activity of NMDA channel. This could be due to a direct interaction, but perhaps more likely involves another regulatory protein. Supported by USPHS grant MH46613.

278.3

SODIUM NITROPRUSSIDE INHIBITION OF THE NMDA CURRENT IN mRNA-INJECTED XENOPUS OOCYTES A. Omerovic*, and S.R. Kelso. Department of Biological Sciences, University of Illinois at Chicago, Chicago, Il 60680

The effect of sodium nitroprusside (SNP), a compound which spontaneously releases nitric oxide (NO), was tested in Xenopus oocytes injected with total rat brain mRNA. Using two microelectrode voltage clamp technique, NMDA-induced currents were recorded before and after perfusion of the oocytes in SNP (1uM-1mM) for 3-5 minutes. SNP induced a dose-dependent inhibition (EC50 about 3uM) of the NMDA current, with 10uM causing a reduction to 22.9±5.7% of control (9 cells). Currents gradually returned to control level 10-15 minutes after SNP wash out. The effect was specific for NMDA currents: kainate-induced currents remained unchanged after perfusion in 100 uM (5 cells) and 1 mM SNP (5 cells).

Hemoglobin, 10 and 100 uM, coapplied with SNP reduced the SNP-induced effect to 55.8 ±15.8 % (n=5) and 108.8 ± 15.3 % (n=6) of control. Since hemoglobin is a scavenger for NO, this experiment suggests that SNP effect on NMDA currents involved the NO action.

To test the hypothesis that NO may act at the redox modulatory site of the NMDA receptor, SNP was applied after a strong oxidant (DTNB, 500 uM). SNP produced additional inhibition after DTNB-induced reduction of NMDA current. NEM, an alkylating agent commonly used to irreversibly change SH groups of proteins, also failed to block the SNP effect. The dose-response curve for NMDA in the presence of SNP demonstrated non-competitive inhibition. The presence of either the agonist (NMDA) or antagonist (APV) during SNP perfusion did not prevent receptor inhibition. In conclusion, SNP-induced inhibition of NMDA current is most likely a result of interaction of NO, spontaneously released by SNP, with the NMDA receptor. In this oocyte preparation it appears that NO does not act at the redox modulatory site or the agonist binding site.

Supported by NIH grant NS24591 and Klingenstein Fund.

278.4

CLONING OF A NMDA RECEPTOR cDNA VARIANT WITH ENHANCED SENSITIVITY TO PROTEIN KINASE C. G.M. Durand*, P. Gregor[1], M.V.L. Bennett, R.S. Zukin, and G.R. Uhl[1]. Dept. Neurosci., Albert Einstein Coll. Med., Bronx, NY 10461 and [1]ARC/NIDA and Depts. Neurol. and N.S.C.I., J.H.U.S.M., Baltimore, MD 21224.

We used PCR and the published sequence of NMDAR1 to isolate a 3.5 kb cDNA encoding an NMDA receptor (NMDAR1b) from a rat ventral midbrain library. Partial sequencing revealed nearly 100% sequence identity to NMDAR1 at the 5' and 3' ends, but restriction analysis suggested differences from NMDAR1, consistent with the possibility of alternate splicing. NMDAR1b RNA injected into *Xenopus* oocytes directed the translation of functional NMDA channels with electrophysiological properties distinct from those of NMDAR1. NMDAR1b homomeric channels exhibited ~4-fold lower affinity for NMDA ($EC_{50}=74\pm4\mu M$) than did NMDAR1 channels ($EC_{50}=20\pm1\mu M$). Co-expression of the receptor variants generated channels with an intermediate affinity for NMDA ($EC_{50}=36\pm1\mu M$). NMDAR1b and NMDAR1 showed identical affinities for glycine and APV. As for NMDAR1, no response was elicited at NMDAR1b receptors by kainate or quisqualate. Pre-application of the protein kinase C activator phorbol 12-myristate 13-acetate potentiated NMDAR1b responses by ~20-fold; potentiation of NMDAR1 responses was 4.5-fold, comparable to the 5-fold enhancement noted for rat brain message. These findings suggest a role for receptor variants in determining NMDA channel properties.

278.5

Augmentation of NMDA Currents by Cyclic AMP and Forskolin
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The NMDA receptor's role in plasticity and pathology has led to questions regarding its regulation. There is evidence to suggest that protein kinases-specifically protein kinases A and C, may modulate NMDA receptor-mediated currents. This study examines the effect of cAMP and forskolin, both of which lead to activation of PKA, on NMDA and kainic acid (KA) currents expressed in Xenopus oocytes injected with rat brain RNA. Currents were examined using the two electrode voltage clamp technique. Oocytes were voltage clamped at -30mV, which is about the reversal potential for chloride, to eliminate contamination of the NMDA response by the endogeneous Ca^{2}+ activated Cl^{-} current of the oocyte. Experiments were conducted in frog ringer with 5mM Ba^{2+}. Responses to 200 M NMDA or 100 M KA were recorded before and up to 90 min. after incubation with either 5mM 8Br-cAMP (15 min), 50 M forskolin (30 min), 5mM 8Br-AMP (control, 15 min) 50 M 1,9, dideoxy forskolin (control 30 min). 8 Br-cAMP augmented NMDA but not KA currents. The 8Br cAMP-induced increases in NMDA responses persisted for 45 to 60 minutes after washoff of the 8Br-cAMP (n=6). The mean increase in current amplitude was 20% at 45 minutes. Forskolin had similar effects producing an increase in steady-state amplitude in NMDA currents that was shorter lived with the current amplitude returning to baseline levels within 15 to 30 minutes (n=7). 8 BrAMP and 1,9 dideoxyforskolin had no effects on NMDA currents. These data suggest that cAMP-dependent processes, most likely activation of PKA regulates the NMDA receptor-channel complex.

278.6

PROPERTIES OF NMDA-ACTIVATED CHANNELS IN CELL-ATTACHED PATCHES FROM HIPPOCAMPAL CULTURES
K.Z. Haas*, R.C. Araneda & M.V.L. Bennett. Dept. Neuroscience, Albert Einstein Coll. Med.,Bronx, NY 10461.

NMDA receptors play an important role in plasticity and excitotoxicity. Regulation of NMDA receptors by second messenger systems may be involved in these processes. We are studying NMDA-activated channels in cell-attached patches on central neurons to examine possible regulation of the receptors by second messenger systems.

Activity from cell-attached patches was recorded with electrodes containing NMDA (30 μM) and glycine (1 μM) in rat hippocampal cultures (1 to 2 weeks old). NMDA-activated channels had a conductance of 57±6 pS as determined from the slope of I-V relationships. Open time distributions were fit well with a single exponential which gave a mean open time of 4.4±0.74 ms. at -60 mV. At hyperpolarized potentials there was a decrease in the mean open time of the channel (30 % at -130 mV), but opening rate was unaffected. In control experiments when PCP (1 μM) was included in the pipette, NMDA channels were blocked near the resting potential, but depolarization relieved the block. In preliminary experiments we found that serotonin (0.1 - 3 μM) increased the open probability but not the mean open time. However, the PKC activator phorbol 12-myristate 13-acetate (100 -300 nM) produced no change in the NMDA-channel properties.

278.7

PHOSPHORYLATION BY MULTIFUNCTIONAL PROTEIN KINASES OF THE GLUTAMATE 1 RECEPTOR EXPRESSED IN THE BACULOVIRUS/Sf9 CELL SYSTEM.
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Vollum Institute, Oregon Health Sciences Univ. Portland, OR 97201, U.S.A.

The cDNA for the kainate/AMPA glutamate-1 receptor (GluR1) (Hollmann et al., Nature 342: 643-648, 1989) was expressed in the baculovirus/Sf9 cell system. Using Western immunoblot analysis two forms of GluR1 were detected: a 102 kDa protein, which comigrated with GluR from rat brain hippocampal membranes, and a non-glycosylated 91 kDa protein. Immunostaining of the Sf9 cells with GluR1 antibody, generated against the extracellular COOH-terminal 13 residues of the receptor, indicated that some of the GluR1 was properly inserted in the Sf9 cell membrane. GluR1 phosphorylation was studied in vitro in an immunoprecipitate using exogenously added protein kinases. CaM-kinase II (10-100 nM) and protein kinase C (100 nM), but not cAMP-dependent protein kinase (cAMP-kinase), catalyzed rapid phosphorylation, predominantly on serine. These studies indicate that GluR1 is an in vitro substrate for phosphorylation by CaM-kinase II and protein kinase C, but not by cAMP-kinase.

278.8

PROTEIN KINASE A MODULATES A HIGH AFFINITY KAINATE RECEPTOR EXPRESSED IN HUMAN EMBRYONIC KIDNEY CELLS. F.A. Taverna, L.-Y. Wang, X.-P. Huang, J.F., MacDonald, and D.R. Hampson*. Faculty of Pharmacy and Dept. of Physiology, University of Toronto, Toronto, Canada.

Recent cloning studies have revealed a large diversity of non-NMDA receptors (GluR1-GluR7). We and others (L.-Y. Wang et al. (1991) Science 253,1132, P. Greengard, J. et al. (1991) Science 253,1135) previously reported that non-NMDA receptors in neurons are subject to neuromodulation by PKA. Among the cloned receptors, GluR6 possess a potential consensus site for PKA phosphorylation on the loop between third and fourth transmembrane domain. We therefore examined PKA regulation of GluR6 expressed in HEK 293 cells. Membranes from human embryonic kidney (HEK) cells transiently expressing GluR6 showed a high level of ^{3}H-kainic acid binding. Membranes from cells expressing GluR6 were solubilized with Triton X-100 and immunoprecipitated using an antibody raised against a peptide corresponding to the C-terminus of GluR6. A major immmunoreactive band was observed at about M_r=109 kDa. The immunoprecipitated receptor was incubated with the catalytic subunit of PKA and P^{32}-ATP; autoradiography of the sample after electrophoresis and transfer to nitrocellulose revealed a P^{32}-labelled protein corresponding to GluR6.

Application of kainate to GluR6 transfected HEK 293 cells induced inward currents which displayed a dramatic desensitization. This desensitization was blocked by treatment with the lectins WGA or Con A. Direct injection of the catalytic subunits of PKA into Con A pretreated HEK cells significantly potentiated kainate evoked currents with no effect on reversal potential. This potentiation was reversed by subsequent injection of the specific PKA inhibitory peptide. Comparisons of kainate dose-response curves before and after PKA injection showed a marked increase in maximal current amplitude with less change in apparent K_D. These results further suggest that PKA plays an important role in modulating EAA receptors and may potentially contribute to synaptic plasticity. We thank Drs. Egebjerg, Heinemann, and Wenthold for cDNAs and antibodies.

278.9

SUSTAINED ENHANCEMENT OF NMDA RECEPTOR-MEDIATED SYNAPTIC POTENTIAL BY ISOPROTERENOL IN RAT AMYGDALAR SLICES. P.W. Gean*, C.C. Huang and J.H. Lin. Dept. of Pharmacol., Coll. of Med., National Cheng-Kung Uni., Tainan, Taiwan, R.O.C.

Norepinephrine (NE) and isoproterenol (Iso) acting on the beta-adrenergic receptors have been shown to exert long-term regulation of excitatory neurotransmission. One notable example is norepinephrine-induced long-lasting potentiation (NELLP) of perforant path evoked potentials in the hippocampal dentate gyrus. In view of NE induced plasticity requires the activation of NMDA receptors, it became of interest to test the possible effects of Iso on the synaptic potential mediated by NMDA receptors ($EPSP_{NMDA}$).

$EPSP_{NMDA}$ was isolated pharmacologically by application of a solution containing CNQX (10 μM) and bicuculline (20 μM) in the rat basolateral amygdala (BAL) neurons using intracellular recording techniques. Superfusion of Iso (15 μM) produced a long-lasting enhancement of $EPSP_{NMDA}$ (165±11%, n=14). Pretreatment the slices with propranolol (10 μM) completely prevented the effect of Iso (97±4%, n=6) confirming the mediation by beta-adrenergic receptors. Furthermore, the persistent increse in $EPSP_{NMDA}$ induced by Iso was mimicked by forskolin (50 μM) suggesting the possible mediation by c-AMP. These results provide the direct evidence for adrenergic modulation of excitatory amino acid neurotransmission in the vertebrate central nervous system.

278.10

EFFECT OF TRANSMEMBRANE VOLTAGE ON THE NMDA RECEPTOR REDUCTION AND OXIDATION PROCESSES

LH Tang* and E Aizenman, Dept. of Physiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

We have examined the effects of the reducing agent DTT and the oxidizing agent DTNB on the NMDA receptor redox modulatory site (Aizenman et al., *Neuron*, 2:1257; 1989) under different transmembrane voltages. In cultured rat cortical neurons using the whole-cell patch-clamp technique, DTT (4 mM, 2 min) produced a similar level of potentiation of NMDA (30 μM)-induced responses at either -60 mV or +30 mV holding potential (1.8±0.1 fold increase at -60 mV, n=8; 1.8±0.1 fold increase at +30 mV, n=8). In contrast, DTNB (500 μM, 2 min) was a more effective oxidizing agent at -60 mV than at +30 mV: Compared to the DTT-potentiated response, DTNB decreased NMDA responses by 62±3% when the oxidation took place at -60 mV (n=13), while it only decreased these responses by 39±2% at +30 mV (n=13). Interestingly, the NMDA response peak current to voltage relationship became substantially outwardly rectifying when the oxidation took place at a positive holding potential. Furthermore, this rectification was not reversed after holding the cell under study at -60 mV for >5 minutes. The -60/+30 mV response amplitude ratios were: 2.0±0.1 (control, n=13), 1.8±0.2 (oxidation at -60 mV, n=11), 1.4±0.1 (oxidation at +30 mV, n=13). Supported by NIH grant NS29365.

278.11

DIFFERENTIAL BLOCK BY INTRACELLULAR MG^{2+} OF THE NMDA RESPONSE IN WHOLE-CELL AND OUTSIDE-OUT PATCH RECORDINGS. Y.-Y. Li* and J. W. Johnson. Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

The NMDA receptor is regulated physiologically by many mechanisms. Intracellular Mg^{2+} has been shown to block the single-channel current of the NMDA receptor at positive potentials. However, it is unclear how intracellular Mg^{2+} affects the NMDA current at the whole-cell level nor by what mechanism(s). The effects of intracellular Mg^{2+} on the NMDA response in both whole-cell and outside-out patch recordings have been studied.

The whole-cell and outside-out patch responses to 30 μM NMDA plus 10 μM glycine were recorded from cultured neurons of embryonic rat brain cortex at holding potentials from -60 to 60 mV. The current was measured in the absence and presence of various concentrations of MgCl in the pipette solution. The results of the whole-cell recordings show that the I-V relationships with 1, 10 or 30 mM Mg^{2+} are not significantly different from that of control, indicating that intracellular Mg^{2+} seems to have little if any effect on whole-cell NMDA current at these concentrations. In contrast, Mg^{2+} significantly reduces the total charge flowing through NMDA receptors in outside-out patches at positive potentials, as well as reducing the single-channel current. The block is dose-dependent. At a holding potential of +60 mV and with 1, 3 or 10 mM Mg^{2+} in the pipette, the single-channel current is reduced to 64, 34 and 19% and the total charge to 82, 50 and 31% of the control values, respectively. Neither the mean open time nor the burst length was increased by Mg^{2+}, suggesting that the data are not consistent with the traditional open channel block model. It is not clear why the reduction in the whole-cell current is inconsistent with the reduction in the total charge flow of patches. One possibility is that the concentration of Mg^{2+} in the cell was not well controlled. Further studies will address this apparent inconsistency. Supported by NIMH grant R29-MH45817.

278.12

L- AN D-HOMOCYSTEATE ACTIVATE CURRENTS OF NMDA TYPE IN CEREBELLAR GRANULE CELLS IN CULTURE. M. Sciancalepore*, G. Kilic, and E. Cherubini. Biophysics Laboratory, International School for Advanced Studies, Trieste, Italy 34014.

The patch clamp technique (whole-cell and outside-out configuration) was used to study whole-cell and single channel currents activated by L- and D-homocysteate (L- and D-HC) in cerebellar granule cells in culture. Both L- and D-HC (15-50μM) at holding potential of -50 mV, induced inward currents (in the range of 20-50 pA) which after an initial peak slowly declined to a plateau. These currents were reversibly blocked by AP-5 (20μM). AMPA (100μM) and kainate (50μM) induced inward currents which were unaffected by AP-5 (20μM) but were blocked by CNQX (10μM).

In the outside-out configuration L- and D-HC elicited single-channel events the same conductance mean open time and reversal potential of the NMDA-activated single channel currents. In the presence of Mg^{2+}(2mM) L- and D-HC-activated single channel events became voltage-dependent. It is concluded that in cerebellar granule cells in culture, both L- and D-HC activate the same NMDA-receptor channel complex.

278.13

CHARACTERIZATION OF GLUR6(R) GLUTAMATE RECEPTOR CHANNELS EXPRESSED IN HEK-293 CELLS. L.A. Raymond*, C.D. Blackstone and R.L. Huganir, Depts. of Neurology and Neuroscience, Howard Hughes Med. Inst., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

GluR6 is one of a family of recently cloned non-NMDA glutamate receptor subunits with high affinity for kainate, and it contains a consensus site for phosphorylation by cAMP-dependent protein kinase (PKA) in the proposed major intracelluar loop region. Unlike GluR1-4 (A-D), the recombinant GluR6 subunit exhibits desensitizing current responses to kainate when expressed in Xenopus oocytes (Nature 1991;351:745). Using a mammalian expression system, we have further characterized the ion channel properties of the recombinant GluR6(R) subunit. Current responses to glutamate and kainate were recorded using the whole-cell patch clamp technique. After lifting the cell, agonists were applied by a rapid perfusion system; solution exchange was complete within 2 ms. High concentrations of both glutamate and kainate elicited currents which desensitized completely, following a single exponential time course. The time constant for desensitization was 9-11 ms for 4 mM kainate. The desensitization time constant for glutamate-evoked current was dependent on agonist concentration, with values of 7-11 ms for high concentrations (>300 uM) and 80-120 ms for low concentrations (10 uM). The I-V relation for both glutamate (1 mM) and kainate (4 mM) exhibited slight outward rectification. The dose-response curve for glutamate-activated peak currents revealed an EC_{50} of 200-300 uM. Using the HEK-293 expression system, we are now examining the functional effects of PKA phosphorylation of the GluR6(R) subunit. *Supported by the NIH and HHMI.*

278.14

RAT BRAIN GLUTAMATE (AMPA) RECEPTORS RECONSTITUTED IN LIPID BILAYERS EXHIBIT LOW AND HIGH CONDUCTANCE STATES.

V. Vodyanoy[1], B.A. Bahr[2], V. Suppiramaniam[1], R.A. Hall[2], M. Kessler[2], M. Baudry*[3], and G. Lynch[2]. [1]Institute for Biological Detection Systems, Dept. of Physio. and Pharm., Auburn Univ., Auburn, AL 36849; [2]Center for the Neurobiology of Learning and Memory, Univ. of Calif., Irvine, CA 92717; [3]Dept. of Biological Sciences, Univ. of Southern Calif., Los Angeles, CA 90089.

AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) activates a specific subclass of glutamate receptors (AMPA receptors) that mediate most of the excitatory synaptic transmission in the brain. In order to study their channel properties, AMPA receptors were solubilized from adult rat forebrain with *n*-octyl glucoside, partially purified 30 to 60-fold, and incorporated into planar bimolecular lipid membranes (BLMs). The channel conductance of the reconstituted receptors was activated by kainate with an EC_{50} of 600 nM and a Hill coefficient of 2.77 ± 0.35, and by AMPA with an EC_{50} of 174 nM and a Hill coefficient of 2.79 ± 0.54. This suggests that cooperative binding of three agonist molecules is required to induce channel opening. Conductance through the reconstituted AMPA receptors was blocked by the specific antagonist DNQX (dinitroquinoxaline-2,3-dione, 2.5 μM). Conversely, the nootropic compound aniracetam (1-anisoyl-2-pyrrolidinone) significantly increased the conductance activated by AMPA. When the partially purified AMPA receptors were reconstituted by the tip-dipping method in asymmetric saline conditions ('outside-out configuration'), the addition of 300 nM AMPA to the pseudoextracellular solution elicited single ion channel fluctuations. The current activity mediated by AMPA was completely inhibited by 1 μM DNQX. Analysis of currents revealed that AMPA receptors have two distinct conductance levels of 60 ± 16 and 11.7 ± 1.6 pS (mean ± S.D.) and a reversal potential of 6 ± 5 mV. These results suggest that the conductance properties of reconstituted AMPA-type glutamate receptors are similar to those found in patch-clamp studies on hippocampal neurons. This work was supported by grants U.S. Army DAAL03-90-G0009 to V.V. and AFOSR 89-0383 to G.L..

278.15

VOLTAGE-DEPENDENT KINETICS OF CURRENTS ACTIVATED BY GLUTAMATE OR KAINATE. I.M.Raman* & L.O.Trussell Neuroscience Training Program & Dept. of Neurophysiology. U. Wisconsin - Madison, 53706.

The response of neurons to agonists of the AMPA-kainate receptor shows a variable degree of voltage dependence (e.g., Mayer & Westbrook,1984, J.Physiol 354:29). We have examined this effect in isolated, voltage-clamped neurons and outside-out membrane patches from the avian nucleus magnocellularis. Under conditions in which the NMDA receptor is inactive, peak and steady-state responses to glutamate and kainate showed a non-linear I-V (current-voltage) relation. With high agonist concentrations, the chord conductance between +60 and +80 mV was 2-3 times higher than the conductance between -60 and -80mV. Five lines of evidence suggest that the apparent voltage dependence in the conductance change induced by glutamate and kainate is due to a voltage dependence in the kinetics of channel gating.

1) The increase in conductance at positive potentials was associated with an increase in the time constant of desensitization to glutamate.

2) The time course of current decay following removal of kainate lengthened with depolarization, and exactly matched the glutamate desensitization time course at any potential.

3) In the presence of kainate, voltage jumps to positive potentials resulted in a gradual increase in outward current over several milliseconds.

4) Rectification in the I-V relation was much more pronounced with lower agonist concentrations.

5) Non-linearity in the I-V relation persisted in nominally Ca/Mg-free bath solutions.

Mechanisms for voltage dependence which invoke voltage-dependent single channel conductance or channel block either by divalents or by agonist are not sufficient to explain these data. Rather, in the AMPA/kainate receptor, the kinetics of channel gating are sensitive to membrane potential. Supported by NS28901 and the Klingenstein Foundation.

278.16

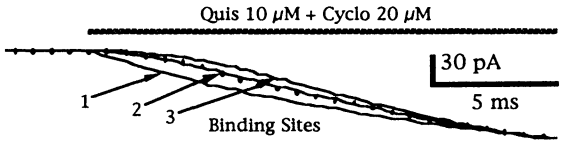
PROTEIN KINASE C MODULATES GLUTAMATE RECEPTORS (KAINATE/AMPA) AND SYNAPTIC TRANSMISSION IN CULTURED HIPPOCAMPAL NEURONS. L.-Y. Wang, E. M. Dudek, M. Browning, and J.F. MacDonald*. Dept. Physiol., University of Toronto, Toronto, Canada and Dept. Pharmacol. University of Colorado, Denver, Co. 80262,

Recent evidence strongly suggests that Protein Kinase C (PKC) plays a key role in modulating excitatory amino acid transmission in the CNS (D. Muller et al. P.N.A.S. 85:6997, 1988, G.-Y. Hu et al. Nature 328:426, 1987). Excitatory amino acid channels are themselves a potential target for functional regulation and phosphorylation by PKC. For example, the non-NMDA receptor clones (GluR1-7) possess a number of potential sites of phosphorylation by this kinase. We therefore investigated the effects of PKC on kainate and AMPA currents and on minature excitatory synaptic currents (mEPSC) recorded from cultured hippocampal neurons. The intracellular injection of the catalytically active fragment of PKC (PKCM) potentiated kainate and AMPA currents and/or reversed the ongoing decline in amplitude following rupture of the patch. Reversal potentials were not altered by PKCM. Furthermore, application of the PKC activator phorbol ester, PMA (100 nM), significantly increased the open probability of kainate (20 μM) channels recorded in the cell-attached configuration. In order to examine synaptic receptors, we evoked mEPSCs from the cell soma by making brief, localized applications of sucrose (0.25 M) in the presence of TTX (500 nM), $MgCl_2$ (1 mM), AP5 (20 μM) and bicuculline (10 μM). These mEPSCs demonstrated a rapid rise time to peak followed by a mono-exponential decay (time constant of about 2 ms). In individual neurons comparisons of mEPSCs were made before and after the injection of PKCM. PKCM significantly increased the amplitude and/or the time constant of decay of the mEPSCs. These data suggest that postsynaptic glutamate (non-NMDA) receptors are under the modulation of protein kinase C in hippocampal neurons and may potentially contribute to synaptic plasticity. Supported By The MRC.

278.17

NUMBER OF AGONIST BINDING SITES ON AMPA/KAINATE CHANNELS. I.D. Clements, Y. Sahara* & G. L. Westbrook. Vollum Institute, Oregon Health Sciences Univ., Portland, OR. 97201.

The number of agonist binding sites on AMPA or kainate channels, and the binding rate of glutamate to these receptors is not known. These parameters are useful in predicting the number of subunits which form the channel, and in estimating the occupancy (saturation) of the postsynaptic receptors following evoked glutamate release. The number of binding sites was estimated by analysing activation kinetics of ensemble currents following rapid application of quisqualate (Quis) to outside-out patches from cultured hippocampal neurons. Cyclothiazide (Cyclo, 20 μM) was used to reduce desensitization of the Quis response. Responses to high concentration of Quis (200 μM + Cyclo) peaked in within 1 ms and desensitized to 70% of peak (τ≈16 ms). At lower concentrations (10 μM) agonist binding was rate limiting and the activation timecourse was sigmoidal. Fits of the activation timecourse suggests that two molecules of Quis are required to open AMPA/kainate channels in the presence of cyclothiazide. However preliminary analysis of domoate responses suggest that >2 molecules of domoate are required for channel opening. Supported by NS26494 and MH46613.



278.18

MODIFICATION OF THE IONIC PERMEABILITY OF AMPA RECEPTORS BY IN VITRO MUTAGENESIS. Bochet P., Curutchet P., Dutriaux A., Lambolez B., Prado de Carvalho L.*, Nalivaiko E., Stinnakre J.* and Rossier J. Institut Alfred Fessard and LNBCM*, CNRS, 91198, Gif sur Yvette, Cedex, France.

The sequences of several, very analogous AMPA receptor subunits have been determined. These subunits form receptors with different electrophysiological properties. In *Xenopus* oocytes GluR1 forms an homomeric receptor permeable to Ca^{2+} ions and displaying a strong inward rectification. GluR2 does not seem to form functional receptors in the oocyte. But the association of GluR1 and GluR2 forms a non rectifying heteromeric receptor impermeable to Ca^{2+}.

Through the construction of chimeras and point mutants, structural determinants of ion flow have been determined. It is now established for instance that a single residue (Glutamine in GluR1 versus Arginine in GluR2) in the putative second transmembrane domain accounts for the different properties of GluR1 and GluR2.

Substitution of this Glutamine in GluR1 by an Histidine at the same position results in a mutant subunit with properties which differ from both GluR1 and GluR2.

	rectification	Calcium permeability
GluR1	+	+
GluR2	no response	
GluR1 + GluR2	–	–
GluR1 + GluR1(Q → R)	–	–
GluR1(Q → H)	–	+

In conclusion the nature of the residue in the Glu/Arg position accounts for both the Ca^{2+} permeability and the rectification properties but different mechanisms are implicated since both properties can be dissociated.

278.19

PENTOBARBITAL INTERACTION WITH SINGLE KAINATE CHANNELS IN MOUSE CORTICAL NEURONS. L.L. Stockbridge* and F.F. Weight. Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Previous whole-cell patch clamp studies in this laboratory have indicated that pentobarbital (PTB) inhibits kainate currents in a concentration-dependent manner (1). We are currently investigating the actions of PTB on single kainate channels in order to elucidate the mechanism of this inhibition.

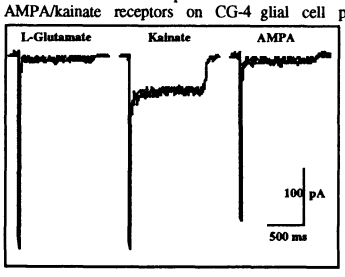
Cortical neurons from day 15-17 fetal mice are plated on collagen/polylysine-coated plates and cultured for 1-4 weeks in an enriched MEM medium prior to use. Single kainate channels are recorded in excised outside-out patches. Data is analyzed with commercial software designed for ligand-gated channels (RC Electronics). This allows observations of changes in charge per unit time as well as changes in amplitude distributions and channel kinetics. Patch pipettes contain (mM): CsCl 155, $MgCl_2$ 2, EGTA 5, HEPES 10 (pH 7.4). The bath solution (HBS) contains (mM): NaCl 150, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, TTX 0.0002. An exchange solution of HBS plus kainate (50 μM) and 2-amino-5-phosphonovaleric acid (100 μM) is applied to initiate kainate channel response while blocking NMDA channels. Under these conditions, at least two types of kainate channels have been distinguished with similar conductance and behavior to those already described for cerebellar granule cells (2). The addition of 100 μM PTB appears to inhibit all kainate channel activity. Concentration-dependency and the mechanism of single-channel current inhibition are being explored.

1. Peoples, RW & Weight FF, Neurosci. Abstr. 16: 1017a, 1990.
2. Howe, JR et al., J. Physiol. 432: 143-202, 1991.

278.20

EXPRESSION OF UNIQUE AMPA/KAINATE RECEPTOR CHANNELS IN GLIAL PRECURSOR CELLS. V. Gallo*, D. K. Patneau, and M. L. Mayer. Lab. of Cellular and Molecular Neurophysiology, NICHD, NIH. Bethesda, MD 20892.

We have studied glutamate receptor channels in a permanent cell line (CG-4) of rat glial cell precursors established from primary cultures of bipotential oligodendrocyte-type 2 astrocyte progenitors (Louis et al., 1992). In voltage-clamp recordings from proliferating progenitor cells, characterized by bipolar morphology, concentration-jump application of L-glutamate, kainate and AMPA evoked large inward currents which rapidly desensitized (see figure); there was no response to NMDA. The kinetics of the onset of desensitization were similar for all three agonists, with time constants of 8.9±2.1, 8.7±2.1, and 9.2±2.4 ms for L-glutamate, kainate and AMPA, respectively. However, the steady-state response to kainate was on average 6 times larger than to L-glutamate. Responses to kainate exhibited less desensitization in cells with multipolar morphology, as well as in oligodendrocytes or type 2 astrocytes derived from CG-4 progenitor cells, suggesting the existence of a developmental switch for AMPA/kainate receptor expression. AMPA/kainate receptors on CG-4 glial cell progenitors exhibit different functional properties than any of the previously characterized glutamate receptors expressed in neurons. The kinetics of kainate responses are similar to recombinant heteromeric GluR5(Q)/KA-2 receptors (Herb et al., 1992), but the sensitivity to AMPA suggests that additional or different subunits may be expressed in CG-4 progenitor cells.



279.1

GABA$_A$ RECEPTORS: EFFECT OF A POINT MUTATION phe-64-->leu IN THE α-SUBUNIT AFFECTING AGONIST-DEPENDENT GATING OF THE RECEPTOR CHANNEL AND CELLULAR EXPRESSION OF RECEPTOR SUBUNIT mRNAs IN HUMAN BRAIN AND SPINAL CORD. P. Malherbe*, E. Sigel#, R. Baur#, S. Kellenberger#, E. Persohn, A. Müller, P. Seeburg± and J.G. Richards. Pharma Division, Preclinical Research, F.Hoffmann-La Roche Ltd, CH-4002 Basel, #Institute of Pharmacology, University of Bern, Switerzland, ±Laboratory of Neuroendocrinology, Heidelberg, FRG.

GABA$_A$ receptors are ligand-gated chloride channels and the targets for a variety of psychoactive drugs. The precise location of binding sites for GABA and competitive antagonists in the primary structure of the receptor is not known. Using *in vitro* mutagenesis, we have found that a single mutation of the phe-64-->leu in the α-subunit - but not homologous mutation in the ß2- and γ2-subunits- strongly decreases the apparent affinity of GABA-dependent channel gating from 6 μM to 1260 μM for the α1ß2γ2 channel expressed in *Xenopus* oocytes. Furthermore, the apparent affinities of the competitive antagonists bicuculline methiodide and SR 95531 were decreased 60 to 200-fold. Our results suggest a close functional and structural association of α-subunits with the agonist-antagonist binding site.

The cellular expression of mRNAs encoding different human GABA$_A$ receptor subunits (α1-3, ß1, γ1,2 and δ) in human brain and spinal cord was investigated by *in situ* hybridization histochemistry. Pronounced differences in hybridization signals in various regions of the CNS -hippocampus, dentate gyrus, visual cortex, substantia nigra and lumbar spinal cord- were obtained with the isoform-selective probes.

279.2

GABA-ACTIVATED Cl$^-$ CHANNELS IN EMBRYONIC NEURONS WITH DIFFERENT GABA$_A$ RECEPTOR SUBUNIT mRNAs HAVE DISTINCT BIOPHYSICAL PROPERTIES .R. SERAFINI*, A. VALEYEV, J.L. BARKER, and M.O.POULTER. Lab. of Neurophysiol., NINDS/NIH, Bethesda, Maryland, 20892.

In situ hybridisation techniques have demonstrated that embryonic day 15 cervical ventral spinal cord (SP.CORD.) and olfactory bulb neuroepithelial neurons (OLF. NEUROEP.) predominantly express GABA$_A$ receptor $\alpha_3\beta_2\beta_3\gamma_2$ and $\alpha_2\beta_2\beta_3$ subunit mRNAs , respectively. We have compared the GABA-activated Cl$^-$ currents in acutely dissociated cells from these two different regions.

	OLF. NEUROEP.	SP.CORD
Whole cell recording dose-response curve		
EC_{50}(μM)	approx. 13	approx. 13
Hill coefficient	>> 1	approx. 1
Fluctuation analysis (GABA 10 μM)		
Elementary conductance(pS)	22.4±3.4(n=9)	15.5±1.2(n=6)
Inferred burst duration:		
τ_{slow}(ms)	180 ± 52	260 ± 42
τ_{fast}(ms)	10 ± 1.8	41 ±2.9
Single channel recording (cell attached)		
Elementary conductance (pS)	19.5 ± 1.8(n=18)	18.6±1.2(n=14)
Mean open time :GABA 2 μM (ms)	7 ± 1(n=10)	6 ± 1(n=5)
Mean open time :GABA 10 μM (ms)	13± 1(n=12)	27 ± 5(n=6)

These data indicate that CNS regions expressing different GABA$_A$ receptor subunit mRNA have GABA-activated Cl$^-$ channels with unique gating properties

279.3

Molecular and Electrophysiological Characterization of an Allelic Variant of the Rat α6 GABA$_A$ Receptor Subunit. F. Tan+, T.P. AngelottiV, K.Kelly*+, and R.L. Macdonald+#. Depts. of Neurology+, PharmacologyV, and Physiology#, Univ. of Mich., Ann Arbor, MI 48104.

A 1.45 kb DNA sequence encoding the rat α6 GABA$_A$ receptor subunit (nucleotides #33-1483) was cloned from a Sprague-Dawley rat brain cDNA library by PCR amplification. Dideoxy sequencing of two individual clones revealed that the nucleotide sequence differed at only one basepair (T^{480} ---> G) from that published previously. This difference altered the deduced amino acid sequence, producing a conservative amino acid substitution (His^{121} ---> Gln). A Gln residue is present at the same location in the bovine α6 subunit. Restriction endonuclease analysis of the total PCR product verified the authenticity of the base pair change and demonstrated that this variant of the rat α6 subunit is the only allele found in this particular rat brain library; the original allele was not present. The α6 subunit with the β1 and γ2S subunits were transiently expressed in L929 cells. Whole-cell recordings obtained from the cells demonstrated that GABA$_A$ receptors with the expected GABA and benzodiazepine pharmacology were produced. Excised outside out single channel recordings from the same cells revealed that GABA elicited brief duration openings to a 33 pS main conductance level and to at least one smaller (approximately 21 pS) subconductance level. Thus this allelic variant of rat α6 subunit could assemble with other subunits to form a functional GABA$_A$ receptor with similar properties to the original allelic form.

Supported by a grant from the Lucille P. Markey Charitable Trust Fund

279.4

Assembly of α1β1 and α1β1γ2S GABA$_A$ Receptor Subunits Produce Unique Ion Channels with Dissimilar Single-Channel Properties. T. Ryan-Jastrow*+, T.P. AngelottiV, R.L. Macdonald+#. Depts. of Neurology+, PharmacologyV, and Physiology#, Univ. of Michigan, Ann Arbor, MI 48109

Various dimeric and trimeric combinations of GABA$_A$ receptor (GABAR) subunits were transiently expressed in mouse L929 cells. Whole-cell (W-C) and single-channel (S-C) electrophysiological techniques were utilized to determine if the subunits assembled into a random heterogenous mixture of receptor configurations or if a preferred form of the receptor existed. Expression of α1γ2S and β1γ2S subunits produced no functional ion channels, whereas α1β1 and α1β1γ2S subunits produced GABARs with dissimilar W-C and pharmacological properties. S-C analysis of α1β1 and α1β1γ2S receptors demonstrated that for each, GABA elicited openings to both a main (15 and 29 pS respectively) and a subconductance level (10 and 21 pS respectively). Open, closed, and burst duration histograms for both recepters were best fit with similar exponential time constants, but the relative proportions of each component differed. The lack of heterogeneity in the S-C properties among different single-channel patches suggest that there most likely exists a single configuration for each of the two assembled ion channels, and furthermore, the lack of α1β1 dimeric receptors upon co-expression with the γ2S subunit demonstrates that the α1β1γ2S receptor is the preferred final form of the GABA$_A$ receptor.

Supported by a grant from the Lucille P. Markey Charitable Trust Fund

279.5

GABA$_A$-RECEPTOR HETEROGENEITY: IMMUNOPURIFICATION OF RECEPTOR SUBTYPES FROM THE BRAIN AND ANALYSIS OF RECOMBINANT RECEPTORS. D. Benke, S. Mertens, F. Knoflach, J.A. Benson* and H. Mohler. Institut of Pharmacology, University of Zürich, CH-8006 Zürich, Switzerland.

The structure of GABA$_A$-receptors is characterized by an extended heterogeneity of constituent subunits classified into five groups (α1-6, β1-4, γ1-3, δ, ρ). To characterize various GABA$_A$-receptor subtypes we developed subunit-specific antisera for their immunoisolation and analyzed their drug binding properties and subunit associations. Four receptor populations with distinct pharmacological properties were identified by immunoisolation with α1-, α3-, α5- and δ-subunit-specific antibodies. The receptor population containing the α1-subunit displayed benzodiazepine receptor (BZR) type I pharmacology whereas receptors containing the α3-subunit corresponded to type II BZRs. Receptors containing the α5- or δ-subunit displayed novel drug binding profiles and thus, appear to contain novel types of BZR binding sites.

The role of receptor heterogeneity with regard to the efficacy of BZR ligands was tested electrophysiologically on recombinant receptors. Two subunit combinations differing in their α-subunits were analyzed ($\alpha3\beta2\gamma2$, $\alpha5\beta2\gamma2$). While bretazenil and divaplon acted as partial agonists on both receptor types, abecarnil displayed differential efficacies acting as full agonist on $\alpha3\beta2\gamma2$ and as partial agonist on $\alpha5\beta2\gamma2$. Thus, subtype-specific variations in the intrinsic activity of BZR ligands may contribute to novel therapeutic profiles.

279.6

CHANNEL PROPERTIES OF A PICROTOXIN-RESISTANT GABA RECEPTOR. H.-G. Zhang, R.H. ffrench-Constant, & M.B. Jackson*, Depts. of Physiology and Entomology, University of Wisconsin, Madison, WI 53706.

A picrotoxin-resistant mutant of *Drosophila* was previously identified on the basis of insecticide resistance, and cloning showed that the resistance gene codes for a GABA$_A$ receptor (ffrench-Constant et al., P.N.A.S. **88**, 7209, 1991). In order to establish the nature of this mutation at the cellular and channel level, we have used patch clamp techniques to study the GABA responses of dissociated cultured fly larvae neurons from the resistant and sensitive strain. Pressure application of 50 μM GABA to fly neurons elicited chloride currents. In neurons from wild-type flies these responses were very sensitive to picrotoxin, with 50% blockade at approximately 0.1 μM. In contrast, the resistant mutant had GABA responses that were 50% blocked by approximately 150 μM. These experiments confirmed the nature of the mutation with a direct physiological assay of the receptor. GABA application to excised outside-out patches elicited single channel currents in neurons from both wild-type and resistant flies. The single channel conductances were 24.6±.4 (n=4) and 27.5±.3 (n=3) for neurons from wild-type and resistant flies, respectively. Thus, a mutation can alter the way in which the channel is blocked, while only slightly altering the channel's permeation properties.

279.7

ACTIVATION OF PKC ENHANCES BENZODIAZEPINE POTENTIATION OF RECOMBINANT $GABA_A$ RECEPTORS. N.J. Leidenheimer*, P.J. Whiting, R.A. Harris. Dept. of Pharmacol., Univ. of Colorado and Denver VAMC, Denver, CO 80262 and Merck, Sharp and Dohme Res. Lab., Essex, U.K.

The $GABA_A$ receptor is a heteromeric ligand gated-ion channel which is the site of action of benzodiazepines (BZs). Recently, this receptor has been shown to be regulated by protein phosphorylation (Leidenheimer, et al. 1991). The present experiments determine if phosphorylation alters the ability of BZs to potentiate $GABA_A$ receptor function. Human cDNAs for $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptor subunits were coexpressed in Xenopus oocytes and $GABA_A$ responses were measured by two-electrode voltage clamp (-70 mV). GABA (10 μM) responses averaged 326±56 nA (n=10, ±SEM) and were potentiated by 300 nM diazepam (181±42% potentiation, n=8, ±SEM). Following bath application of the PKC activator PMA (5-25 nM) GABA responses were decreased to 106±53 nA (n=10, ±SEM, significantly different from control, p≤0.005) and diazepam potentiation was increased to 387±82% (p≤0.05, n=8). The ability of PMA to inhibit GABA responses was significantly correlated with the enhancement of diazepam potentiation (r=0.7, p<0.05, n=9). The inactive phorbol ester PMM (25 nM) did not significantly alter either GABA responses (control=438±90 nA; PMM=513±158, ±SEM, n=4) or diazepam potentiation (control=169±29%; PMM=169±27%, ± SEM, n=4). Furthermore, lowering the clamping voltage toward the chloride equilibrium potential resulted in decreased GABA responses but did not alter diazepam action. Therefore, it appears that PKC-dependent phosphorylation increases the ability of BZs to enhance $GABA_A$ receptor function. We are currently examining the effect of PMA on BZ potentiation on other recombinant receptors.

279.8

HETEROGENEITY OF GABAA RECEPTOR IN THE RAT BRAIN. T. Araki* H.Kiyama and M. Tohyama. Dept.of Anatomy and Neuroscience, Osaka Univ. Med. Sch., Osaka, JAPAN

The GABAA receptor, which is a member of the ligand-gated ion channel family of receptors, has been shown to be comprised of a number of subunits and variants. Using in situ hybridization histochemistry, we investigated the distribution of neurons expressing various GABAA receptor subunits in the mature rat central nervous system and the developmental changes of the expression in the fetal rat brain.

In the fetal brain, no α_1 subunits were detected. Among other subunits, α_4 and β_1 subunits were seen in both the developing neuronal layers and undifferentiated neuroepithelium, while α_3, $\beta_{2,3}$ and γ_2 subunits were seen only in the developing layers.

In the mature brain, differences were found in the distribution of the subunits. Among various subunits, γ subunits showed unique patterns. The distribution of the γ_2 subunit often overlapped with that of neurons expressing glutamic acid decarboxylase, an enzyme synthesizing GABA. On the other hand, the γ_1 subunit was observed in relatively restricted areas, most of which only weakly expressed the γ_2 subunit.

Our results indicate that regulation of expression of GABAA receptor subunits is both region-specific and developmental stage specific, and that some subunits are likely to be involved in neuronal development.

279.9

POSTNATAL DEVELOPMENT OF $GABA_A$ RECEPTORS IN RAT SYMPATHETIC NEURONS. Luis G. Aguayo*, Juan M. Alarcón and Floria C. Pancetti. Neuropharmacology Laboratory, Institute of Chemistry, Catholic University at Valparaíso, Valparaíso, Chile.

It was previously suggested that some physiological and pharmacological properties of $GABA_A$ receptors changed during neuronal development (Smart and Constanti, 1990; Cherubini, et al., 1991). We have examined the effects of several modulators of the $GABA_A$ receptor including Zn^{2+}, benzodiazepines, barbiturates and ethanol on acutely isolated rat (1 day to 4 months) superior cervical ganglion neurons using patch-clamp recordings. Within 12 hours after birth the neurons were sensitive to GABA (1-200 μM). The relationship between the amplitude of the current (I_{GABA}) and the concentration of GABA (EC_{50} = 24 μM; n = 14) in newborn neurons was fitted with a slope (Hill coefficient) close to 1.0. Adult neurons had a similar EC_{50} to that of newborn neurons, but the slope was steeper and better fitted with a Hill coefficient close to 1.5 (n=13, p<0.05).

The concentration of Zn^{2+} that reduced I_{GABA} by 50% (IC_{50}) was 37±16 μM in newborns (n=8) and 43±15 μM in adults (n=12). Consistent with the evidence indicating that $GABA_A$ receptors blocked by Zn^{2+} lack the γ2 subunit (Draguhn et al., 1990), we found that benzodiazepines (flurazepam [10 μM] and diazepam [1-1000 nM]) had minimal effects on I_{GABA} activated in newborn and adult neurons (n= 18). Ethanol (40 mM) reduced the amplitude of I_{GABA} to 87±8% in adult neurons (n=6) and 74±10% of control (n=5) in newborn neurons (<7 days).

In conclusion, we found that the sensitivity of the $GABA_A$ receptor to GABA and Zn^{2+} did not change during *in situ* neuronal development. However, only in newborn neurons the relationship between the amplitude of the response and the concentration of GABA was better described by a single binding site. In addition, the $GABA_A$ receptor was more sensitive to ethanol in newborn than in adult neurons. (Supported by a Grant from NIAAA).

279.10

PHENOTYPIC VARIATIONS IN $GABA_A$ RECEPTOR FUNCTION K.M. Garrett*, I. Niekrasz and T.W. Seale, Depts. of Physiology and Pediatrics, University of Oklahoma HSC, Oklahoma City, OK 73190

The $GABA_A$ receptor contains pharmacologically and structurally discrete binding sites for GABA, benzodiazepines, barbiturates and convulsants acting at the chloride ionophore. Previous behavioral studies have shown that C57BL/6J and DBA/2J inbred mice vary in their responses to drugs which modulate GABAergic transmission. The present study extends the behavioral phenotypic characterization of these two inbred strains to additional drugs acting on the $GABA_A$ receptor. C57BL/6J mice were significantly less sensitive than DBA/2J to tonic convulsions produced by DMCM and picrotoxinin; however, no significant differences were observed in their sensitivity to tonic seizures elicited by bicuculline or NMDA. Diazepam had a significantly larger anxiolytic-like effect in C57BL/6J than in DBA/2J mice in both a mirrored-chamber paradigm (Toubas et al., 1990) and an elevated plus-maze test. CL 218,872 had equal potency in the mirrored-chamber test in the two mouse strains; however, at high doses CL 218,872 was significantly more efficacious in C57BL/6J than in DBA/2J mice. There was no difference in the effects of buspirone in the mirrored-chamber apparatus between these two strains of mice. Anxiogenic-like behaviors were measured by a modification of the mirrored-chamber paradigm. DBA/2J mice were more sensitive than C57BL/6J mice to the anxiogenic-like effects of DMCM. DBA/2J mice were also significantly more sensitive than C57BL/6J mice to decreases in motor activity produced by diazepam. These results suggest that inherent differences occur in specific behavioral responses to drugs that modulate $GABA_A$ receptor function in C57BL/6J and DBA/2J mice, and these differences may be due to polymorphisms that quantitatively or qualitatively alter the subunits of $GABA_A$ receptors.

279.11

QUANTITATIVE AUTORADIOGRAPHY OF [^{3}H]TBOB BINDING TO $GABA_A$ RECEPTORS. S.Y. Sakurai*, D.E.Burdette and R.L. Albin. Dept. of Neurology, U. of MI., Ann Arbor, MI 48109.

We have characterized *in vitro* quantitative [^{3}H]TBOB autoradiography in rat brain. Tissue sections were prewashed in buffer containing 1 mM EDTA. Kinetic and pharmacology experiments were performed at room temperature on 20 μm sections of adult rat brain in 20 nM [^{3}H]TBOB. Binding to the $GABA_A$ agonist site and benzodiazepine site (BZ) was also assessed using [^{3}H]muscimol and [^{3}H]flunitrazepam ([^{3}H]Flu), respectively, in serially adjacent sections.

[^{3}H]TBOB binding reached equilibrium at 60 minutes. Under these conditions specific binding was greater than 80-90% of total binding. Isoguvacine, a $GABA_A$ agonist, inhibited [^{3}H]TBOB binding in a dose-dependent manner. [^{3}H]TBOB binding was inhibited by picrotoxin, a cage convulsant. Bicuculline, a $GABA_A$ antagonist, enhanced [^{3}H]TBOB binding in cerebellar granule cell layer but had no effect on binding in forebrain structures. Clonazepam, a benzodiazepine, enhanced [^{3}H]TBOB binding throughout brain.

The relative distribution of [^{3}H]TBOB binding sites was subiculum = medial cortex > nucleus accumbens > lateral cortex > stratum moleculare of dentate gyrus = cerebellar molecular layer > stratum oriens of CA3 > globus pallidus > striatum > cerebellar granule cell layer. The distribution of [^{3}H]TBOB, [^{3}H]Flu and [^{3}H]muscimol binding in adjacent sections was not closely correlated. Supported by USPHS NS19613, NS01300, NS15655 and NS07222.

279.12

CHARACTERIZATION OF THE GABA-A RECEPTOR COMPLEX IN THE BRAIN OF THE SHARK. M.G. Corda*, M. Orlandi, M.G. Pibiri, M.R. Murgia, D. Lecca, S. Salvadori (1), A.M. Deiana (1) and O. Giorgi.

Dept. of Exp. Biology, Section of Neurosci. and (1) Dept. of Animal Biology and Ecology, Univ. of Cagliari, Italy.

The present study was designed to investigate the phylogenesis of the functional interactions between the different components of the GABA-A receptor complex in primitive vertebrate species. To this aim, we examined the modulatory effects of GABA and benzodiazepine (BZ) receptor ligands on the binding of 35S-t-butylbicyclophosphorothionate (35S-TBPS) in the brain of the shark (Scyliorhinus canicula) and in the rat brain. GABA inhibited 35S-TBPS binding in a concentration-dependent manner both in the shark brain (IC50: 0.5 μM) and in the rat brain (IC50: 2 μM). Diazepam was approximately ten fold less potent at inhibiting 35S-TBPS binding in the shark brain as compared with the rat brain. The convulsant β-carboline derivative methyl 6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM) increased 35S-TBPS binding in the rat brain (Emax: +35 % at 0.1μM) but had no effect in the shark brain (up to 1 μM). In line with the above results, DMCM failed to induce convulsions in sharks in doses up to 40 mg/kg, i.v. Moreover, diazepam (up to 40 mg/kg, i.v.) was unable to induce sedation and to antagonize the convulsions elicited by pentylenetetrazol (150 mg/kg, i.v.) and bicuculline (1 mg/kg, i.v.) in sharks. Our results reflect significant differences in the allosteric interactions between the components of the GABA-A receptor complex in the CNS of mammals and lower vertebrates.

279.13

REPEATED SWIM-STRESS REDUCES $GABA_A$ RECEPTOR α1 SUBUNIT mRNAS IN MOUSE HIPPOCAMPUS. P.Montpied*, A.Weizman, R. Weizman., K. A. Kook, A.L. Morrow, and S.M. Paul. CNB, NIMH Bethesda, MD 20892.

We have recently demonstrated (J.P.E.T. 249: 701,1989) that repeated swim-stress in mice reduces the density and/or functional properties of the GABA A/ benzodiazepine-chloride ionophore receptor complex (GABA/BZD). Using [^{3}H] Ro 15-1788 to label GABA /BZD receptors in vivo, we have observed a reduction in the apparent number (Bmax) of GABA /BZD receptors measured 24 hrs after the last session of repeated swim-stress (7 or 14 days). The most robust reduction in GABA/BZD receptors was observed in hippocampus, an area with both a high density of GABA/BZD receptors and glucocorticoid receptors. Moreover, adrenalectomy prevented the stress-induced reduction in GABA/BZD receptors (Brain Res. 519:341,1990).We hypothesized that stress may induce an alteration of $GABA_A$ receptor expression (perhaps at the level of the transcription) and investigated the effects of repeated stress on $GABA_A$ α subunit mRNA levels in hippocampus. Following 7 daily sessions of swim-stress, there was a trend (non significant) toward a reduced level of α1 subunit mRNAs in the hippocampus. At 14 days, the 4.8 Kb and 4.4 Kb α1 subunit mRNAs were reduced by 47.3% +/- 6.5 (n=16; $p < 0.05$) and 39.8 % +/- 7.6 (n=16; $p < 0.05$) respectively. In contrast, no significant alteration in the levels of GAD (glutamic acid decarboxylase) or ßactin mRNAs were observed suggesting that stress alters the $GABA_A$ α1 subunit mRNAs without affecting mRNAlevels in general.The stress induced reduction in $GABA_A$ receptor α1 subunit mRNA may underlie the reduction in GABA/BZD receptor number observed following repeated stress. The possible role of stress hormones such as the glucocorticoids in mediating the stress-induced reduction in GABA/BZD receptors is currently being studied.

279.14

IMPORTANCE OF INHIBITORY $GABA_B$ AUTORECEPTORS IN MODULATING ENDOGENOUS GABA RELEASE FROM RAT STRIATAL SLICES. R.D. Mayfield* and N.R. Zahniser. Dept. Pharmacol., Univ. of Colorado Hlth. Sci. Ctr., Denver, CO 80262.

Endogenous GABA efflux evoked by multiple periods of electrical field stimulation was examined in rat striatal slices. The GABA uptake inhibitor NO-328 (tiagabine; 10 nM - 50 µM) increased basal outflow (spontaneous release) of GABA from marginally detectable levels to 0.25-0.35 ng/mg/ml at higher concentrations. Electrically-evoked GABA overflow (stimulated release) also increased as a function of NO-328 concentration (EC_{50}=2.0 µM) and was completely Ca^{2+}-dependent. An invariant observation in these experiments was that basal GABA outflow continuously decreased as a function of superfusion time and that stimulation-evoked GABA overflow decreased 25-30% in response to the second of two periods of stimulation (S2/S1 ratios=0.70-0.75). The observed decreases in GABA efflux could not be explained by the amount of GABA lost to the superfusion buffer (percent fractional release), direct depletion of releasable pools of GABA, or slice viability. However, the $GABA_B$ receptor antagonist 2-hydroxy-saclofen (SAC; 316 µM) not only enhanced stimulated GABA release but also resulted in S2/S1 ratios of unity when superfused throughout both periods of stimulation. When SAC was superfused throughout the second stimulation period only, evoked GABA overflow was almost 2-fold greater than that evoked by the corresponding initial period of stimulation (SAC-free) and was approximately 30% greater than S1 responses that were observed in the presence of SAC. These results suggest that $GABA_B$ autoreceptors provide an important negative feedback control of endogenous GABA release from striatal slices. Experiments are currently underway to determine whether similar $GABA_B$ modulation occurs pallidal slices.
(Supported by DA 04216 and AA 07464)

279.15

ONTOGENY OF $GABA_B$ BINDING IN RAT BRAIN. S.M. Turgeon* and R.L. Albin. Neuroscience Program and Dept. of Neurology, University of Michigan, Ann Arbor, Michigan 48109.

Quantitative receptor autoradiography using [^{3}H]GABA under selective conditions was used to characterize the ontogeny of $GABA_B$ binding in rat brain. $GABA_B$ binding was assessed at P1, P3, P7, P14, P21, and P28. $GABA_B$ binding peaked at P3 in the globus pallidus, substantia nigra, and CA3 region of the hippocampus. At P7, $GABA_B$ binding peaked in the deep cerebellar nuclei, striatum, nucleus accumbens, and CA1 region of the hippocampus. $GABA_B$ binding peaked at P14 in the superior colliculus, neocortex, and thalamus, and at P21 in the medial geniculate. At P28, $GABA_B$ binding peaked in the molecular layer of the cerebellum and the regional distribution of $GABA_B$ binding was identical to that seen in adults (Chu et al., Neurosci., 34:341, 1990). Following these regionally specific peaks during the first four weeks of life, binding decreased globally from P28 to adult (P42). The regionally specific ontogenic regulation of $GABA_B$ binding suggests a role for $GABA_B$ binding sites in neuronal development. A developmental function for $GABA_B$ receptors is supported by evidence that $GABA_B$ receptor agonists and antagonists can modulate neurite outgrowth in vitro (Michler, J. Devl. Neurosci., 8:463, 1990). Comparative pharmacology of early postnatal and adult $GABA_B$ binding will be discussed.
Supported by NS01300, NS 19613, and NS07222.

279.16

DOES GABA MODULATE THE EXPRESSION OF IMMEDIATE EARLY GENES IN RAT HIPPOCAMPAL SLICES DURING DEVELOPMENT? I. Massamiri*, K. Bugra, M. Khrestchatisky and Y. Ben-Ari. INSERM Unit 29, 123 Bd. de Port-Royal, Paris, 75014, France.

Induction of immediate early gene (IEG) expression occurs following a variety of stimuli and is characterized by a rapid onset and a transient effect. Induced transcription factors are thought to subsequently alter the expression of effector genes leading to long term changes. Since the neurotransmitter GABA has an excitatory action in the developing hippocampus (Cherubini et al., TINS, 1991 12: 515-519), as opposed to an inhibitory effect in the adult brain, we examined, in a comparative study, the expression of mRNAs for c-fos, c-jun and zif/268 during development.

Using an in-vitro slice model preparation, pooled hippocampal slices were treated with either kainate (200nM), GABA (0.3-3mM) or bicuculline (15µM), prior to RNA extraction. Relative estimations of specific mRNA were determined by a reverse transcriptase coupled polymerase chain reaction followed by southern blots.

In the adult hippocampal slice model, kainate induces the expression of mRNA for c-fos but not c-jun, or zif/268, within 30 minutes of treatment.

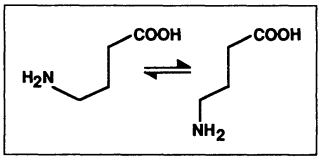
Activation and inhibition of the GABAergic system by GABA or bicuculline, respectively, has no effect on the basal expression of mRNA for zif/268 at the three different ages studied: P0, P4, and P37. Further studies are now in progress to look at the expression of other IEGs upon modulation of the GABAergic system during development.

279.17

GABA RHO 1 EXPRESSION. T. Kusama, C.E. Spivak*, V. Dawson and G. Uhl. Lab. Mol. Neurobiol., ARC/NIDA & Depts. Neurol. & Nsci., JHUSM, Box 5180, Baltimore, MD. 21224.

The GABA rho 1 receptor, highly expressed in retina, readily forms a homooligomeric GABA-gated chloride channel that displays the insensitivity to baclofen and bicuculline that provided the first definition proposed for GABA C receptors by Johnston. GABA rho activities of agonists including CACA and CAMP, whose selective potency defines GABA C receptors in a second fashion, the less specific trans isomers TACA and TAMP, and other rigid GABA analogs were assessed to further characterize the rho 1 site.

Xenopus oocytes expressing GABA rho1 after intranuclear injection of pcDNA1RHO1 were tested under two electrode voltage clamp; COS cells transfected by electroporation were tested by patch clamp. The rank order of agonist potencies was TACA> GABA> muscimol> TAMP> CAMP> CACA> isoguvacine>> piperidine-4-sulfonate(P4S)≥ THIP. Hill coefficients were > 1.5. The predominant channel conductance was 28 pS. Higher TACA than CACA but lower TAMP than CAMP response sizes indicate that GABA rho1 fits the first, and part of the second, definitions proposed for "GABA C" receptors. The low potencies of THIP, isoguvacine, and P4S suggest that the rho 1 recognition site may exclude GABA's folded conformation (figure, left).



279.18

CHRONIC ETHANOL ADMINISTRATION INDUCES DOWNREGULATION OF $GABA_A$ RECEPTOR α SUBUNIT POLYPEPTIDE EXPRESSION IN RAT CEREBRAL CORTEX. M.C. Mhatre and M.K. Ticku*. Univ. TX Hlth. Sci. Ctr., Dept. of Pharmacology, San Antonio TX 78284-7764

Chronic ethanol administration results in the development of tolerance and dependence. The repeated ethanol administration results in a reduction in $GABA_A$ receptor-mediated ^{36}Cl uptake in cortical synaptoneurosomes and primary cultured neurons. We and others have shown that it also results in a 40-50% reduction in $GABA_A$ receptor α subunit mRNA levels in the rat cerebral cortex. In the present study, we investigated the expression of α_1, α_2 and α_3 subunits of $GABA_A$ receptor by immunoblotting using polyclonal antibodies raised against α_1, α_2 and α_3 subunit polypeptides (Zezula et al. Brain Res. 563:325, 1991). Comparison between the control and ethanol treated rats revealed 47% reduction in the α_1 subunit (51 KDa), 37% reduction in the α_2 subunit (53 KDa) and 29% reduction in α_3 subunit polypeptide (59 KDa) and 58% reduction in the lower band (53 KDa) of α_3 subunit. In summary, these data indicate that chronic ethanol treatment results in a decrease in the expression of $GABA_A$ receptor subunits which may underlie alterations in $GABA_A$ receptor function, and could be related to cellular adaptation to the functional disturbance caused by ethanol. Supported by NIAAA grant #AA04090.

279.19

CHANGES IN GABA-A RECEPTOR BINDING AND EXPRESSION FOLLOWING CHRONIC INTERMITTENT ETHANOL IN RATS. **D.W. SAPP, R. TYNDALE, N. KOKKA, A.J. TOBIN AND R.W. OLSEN. Dept. Pharmacology, Brain Research Institute, UCLA, CA 90024.**

As we reported before, there is a decrease in PTZ-induced seizure threshold in rats given chronic intermittent ethanol (CIE) compared to control animals. This decrease persists for at least a month following discontinuation of alcohol administration. A change in PTZ-induced seizure sensitivity suggests that the GABA-A receptor complex is involved in the differences observed between CIE and control animals. In cerebellum the binding of the partial inverse agonist Ro15-4513 to diazepam-insensitive (DZ-IS) sites is decreased in CIE. This change appears to be a lowered affinity and not a change in Bmax. A possible explanation for the change in binding is altered subunit composition. Analysis of mRNA from the cerebellum of CIE and control rats does not show any change in the expression of the $\alpha 6$ subunit which is reported to be responsible for DZ-IS binding. If $\alpha 6$ expression remains unchanged, then some other mechanism must be involved in altering DZ-IS binding in CIE rats. Similar studies on receptors in other brain regions are in progress to attempt to explain increased seizure susceptibility. Supported by AA07680.

OPIOIDS: BEHAVIOR

280.1

CHRONICALLY ADMINISTERED NALTREXONE DUPLICATES THE MAJOR BEHAVIORAL EFFECTS OF ACUTELY ADMINISTERED DRUG IN AUTISTIC CHILDREN. B.H. Herman*, I.F. Borghese, G.S. Asleson, E. Lukens, M.B. Benoit, I. Chatoor, P. Papero, L. Anselmi, & C. Fitzgerald, Brain Res Cen & Depts Psych & Psychiat, Children's National Med Cen & Depts Psychiat & Behav Sci & Peds, GWUSM, Washington, D.C. 20010

There are several lines of evidence implicating a role of opioid peptides in autism (for review see Herman, In J.J. Ratey (ed.), Mental Retardation: Developing Pharmacotherapies, 1991). In a preliminary study of 13 autistic children (3 to 12 y.o.), single doses of naltrexone (0.5 to 2.0 mg/kg, once per week), were found to induce dose-dependent decreases in the severity of autistic symptoms as measured by the Childhood Autism Rating Scales (CARS) and four additional measures of hyperactivity including the Conner's Parent Teacher Rating Scales (CPTRS) (Herman et al., AACAP 7: 52 (1991). Here we show that naltrexone produce similar effects when chronically administered.

Subjects were 15 autistic children (3 to 13 y.o.). Drug included surrounding placebo (P) trials (P1, P2) and four naltrexone doses (0.5, 1.0, 1.5, and 2.0 mg/kg). Each drug dose was administered three days a week, every other day. Results indicated that chronically administered naltrexone significantly decreased CARS scores ($p < 0.005$) and CPTRS scores ($p < 0.05$). For CPTRS,no significant differences were obtained among the 3 drug days. A subgroup of autistics (N =11/15) also showed evidence of reduced extraneous motor behavior scores under naltrexone. Therefore, these data indicate that chronically administered naltrexone significantly decreases the severity of autistic symptoms and the hyperactivity associated with autism, and that repeated administration does not result in an attenuation of these effects. Sponsored by NICHD, FDA and DuPont Pharmaceuticals.

280.2

INHIBITION OF MORPHINE TOLERANCE BY NITRIC OXIDE SYNTHESIS INHIBITOR N^G-nitro-L-arginine. Y.A. Kolesnikov, C.G. Pick and G.W. Pasternak*, The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Departments of Neurology & Neuroscience and Pharmacology, Cornell U. Medical College, New York, NY 10021

The NMDA antagonist MK801 reportedly blocks the development of morphine tolerance in rodents. Nitric oxide (NO) is an important messenger molecule in various physiological functions in the central nervous system and in the periphery which is produced by the enzyme NO synthetase. Activation of MNDA receptors increases the release of NO. In the current study, we examined the effect of the NO synthesis inhibitor N^G-nitro-L-arginine (l-NOARG) on tolerance to morphine analgesia in mice using the tailflick assay. After daily injections of morphine (5 mg/kg, s.c.) for 5 days no mice were analgesic compared to 60% after a single dose. In contrast, 40% of mice receiving combined doses of l-NOARG (2 mg/kg, i.p.) along with the morphine were analgesic after 5 days ($p < 0.05$). l-NOARG did not affect the response to single morphine doses and after 5 days of repeated dosing with l-NOARG a single morphine dose still elicited analgesia in 50% of mice. These results suggest that NO may have an important role in the development of morphine tolerance which may involve an NMDA/NO pathway.

280.3

Differential sensitization and tolerance to the discriminative stimulus properties of *mu*-opioids. C.A. Paronis* and S.G. Holtzman, Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

Analgesic potency of μ-opioids is increased by continuous exposure to naloxone (NX) and decreased by continuous exposure to morphine (MOR) or other μ-agonists. We examined whether similar changes in potency are seen in the discriminative stimulus (S^D) properties of μ-agonists. Male Sprague-Dawley rats, trained to discriminate 3.0 mg/kg MOR from saline in a discrete trial avoidance/escape procedure, were tested for generalization to MOR or fentanyl (FEN) before and 24 hr after 7-day sc infusions of 0.3 mg/kg/hr NX, 0.75 mg/kg/hr MOR, or 0.01 mg/kg/hr FEN. Both MOR and FEN infusions produced tolerance, the magnitude of which varied with both the infused drug and the test drug. The MOR generalization curve was shifted to the right after either a MOR infusion or, to a lesser extent, a FEN infusion; the MOR ED_{50} for generalization increased from 1.43 to 2.23 mg/kg and from 1.39 to 1.73 mg/kg, respectively. The FEN generalization curve, however, was shifted roughly 3-fold to the right following either MOR or FEN, the FEN ED_{50} for generalization increasing from 0.011 to 0.39 mg/kg and from 0.014 to 0.042 mg/kg, respectively. Following NX infusion, a sensitization to the S^D properties of opioids was seen and, as with tolerance, the magnitude of the sensitization varied between the test drugs. Our results show that while both tolerance and sensitization to the S^D properties of μ-opioids develop, a) the decreases and increases in potency are not necessarily proportional for a given drug, and b) the magnitude of potency changes varies between drugs. (Supported by Grants DA00541, K05 DA00008 and Fellowship F31 DA05484.)

280.4

NALTRINDOLE (NTI) IS LESS EFFECTIVE THAN ITS BENZOFURAN ANALOG (NTB) IN ATTENUATING MORPHINE'S DISCRIMINATIVE STIMULUS, STRAUB TAIL AND DEPENDENCE EFFECTS. P. M. Beardsley, O. T. Lopez and J. L. Bloss*. Neurological Diseases Research Dept., G.D. Searle & Co., 4901 Searle Parkway, Skokie, IL 60077.

It has been reported that i.c.v. administration of the *delta* opioid receptor antagonists, naltrindole (NTI), and its non-equilibrium analog, naltrindole 5'-isothiocyanate, could prevent the development of morphine tolerance and dependence (Abdelhamid, 1991). In order to further explore the range of interactions between *delta* opiate receptor antagonists and the pharmacology of morphine, NTI, and its benzofuran analog, NTB, were examined following s.c. administration for their ability to affect naloxone-precipitated jumping in morphinized mice, morphine's Straub-tail effects in mice, and morphine's discriminative stimulus effects in rats. NTB dose-dependently (0.5–3.0 mg/kg) reduced naloxone-precipitated jumping, and (0.1–2.0 mg/kg) morphine's discriminative stimulus effects, although NTI had little effect on either response up to 90 mg/kg. Both NTB (0.5–3.0 mg/kg) and NTI (15–90 mg/kg) were able to attenuate morphine-induced Straub-tail, but only NTB was able to completely abolish it.

280.5

OPPOSITE EFFECTS OF μ AND K OPIOID RECEPTOR AGONISTS ON ABSENCE EPILEPSY IN WAG/RIJ RATS. W. Lason*, B. Przewlocka, E.L.J.M. van Luijtelaar, A.M.L. Coenen and R. Przewlocki. Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland and Department of Psychology, University of Nijmegen, Nijmegen, The Netherlands.

Involvement of endogenous opioids and their receptors in the mechanisms of absence epilepsy has previously been postulated (Snead, '82; Lason et al. '92). In the present study we investigated the effects of opioid receptor agonists on spike-wave discharges (SWD's) in WAG/Rij rats, which are regarded as a genetic model of absence epilepsy. The selective μ opioid receptor agonist DAMGO (0.2-0.7 μg/5μl, icv) increased in a dose-dependent manner the number as well as the total duration of SWD's, and this effect was significantly attenuated by the irreversible μ receptor antagonist ß-FNA (10 μg/5 μl, icv) which was not effective in itself. On the other hand, the κ receptor agonists U50,488H, U69,593 and PD117302 (50-150 μg/5μl, icv) all dose-dependently decreased the number of SWD's and in a higher dose they decreased also the mean duration of the SWD's. The order of the potency of these κ agonists in suppressing SWD's was as follows: U69,593, U50,488H and PD117302.

It can be concluded that the μ and κ receptor activation modulates the number and mean duration of the SWD's in WAG/Rij rats in an opposite manner. Furthermore, the obtained data may point to putative anti-epileptic properties of κ opioid receptor agonists in absence epilepsy.

This research was supported by TNO CLEO grant A 83 of the Research Committee on Epilepsy of the Division for Health Research and to grant KBN 4 1626.

280.6

EFFECTS OF NALOXONE ON SEIZURE SUSCEPTIBILITY IN RATS CHRONICALLY TREATED WITH PICROTOXIN. V.A. Kenigs, W.L. Nores, J. Thomas, R.D. Olson, A.J. Kastin, G.A. Olson, and J.H. Mclean.* Department of Psychology, University of New Orleans, New Orleans, LA 70148.

From previous formal and pilot investigations conducted in our laboratory, there was reason to hypothesize that in a repeated injection paradigm, naloxone (NAL) might have effect on the period of akinesis which follows picrotoxin-induced focal seizures. Male rats received IP injections of saline or NAL 20 min prior to SC injections of picrotoxin (3 mg/kg). Rats were observed for 45 min on 3 test days at 5-day intervals for behavioral signs of seizures. Latencies, incidences, durations, and numbers of episodes were scored for selected categories of seizures. Repeated injection of picrotoxin made NAL pretreated animals more susceptible to generalized tonic-clonic (GTC) and generalized clonic (GC) seizures on the third day of testing, but had no effect on akinetic seizures. The latencies to none of the seizure categories studied were significant. The effects of NAL on GTC and GC seizures suggest NAL has pro-convulsant actions on some types of seizures as well as anti-convulsant effects on focal seizures.

280.7

Tyr-MIF-1 ANTAGONIZES KAINIC-INDUCED, FOCAL SEIZURES AND WET DOG SHAKES. J.Thomas*, W.L. Nores, V.A. Kenigs, R.D.Olson, A.J.Kastin, and G.A.Olson. Dept. of Psychology, University of New Orleans, New Orleans, LA 70148.

The effects of the endogenous opiate antagonist, Tyr-MIF-1, on kainic acid-induced focal seizures and wet dog shakes (WDS) were investigated. Male rats were injected IP with Tyr-MIF-1 (1 mg/kg) or saline 15 min prior to testing with kainic acid (KA) (10 mg/kg, IP). The latencies to focal seizures and WDS, the total number of focal seizure episodes, and the number of WDS in each of three 30-min periods following injection were scored for 90 min after KA injection. Pretreatment with Tyr-MIF-1 increased the latency to the first focal seizure. The mean number of focal seizure episodes over the test session was also lower in animals pretreated with Tyr-MIF-1. The time course of the appearance of WDS was delayed by pretreatment with Tyr-MIF-1, with the largest number of WDS occurring during the third, 30-min interval in Tyr-MIF-1-treated rats, but during the second 30-min period in saline pretreated rats. The results suggest that Tyr-MIF-1 plays a role in the modulation of focal seizures and shaking behavior and should be investigated further.

280.8

NALOXONE CANCELS COMPULSORY APPROACHING SYNDROME IN CATS WITHOUT THE CAUDATE NUCLEI. J.R.Villablanca*, Ch.E. Olmstead and I. de Andrés. Depts. Psychiatry and Anatomy, Mental Retard. Res. Center, UCLA Sch. of Med., LA, CA 90024.

Cats with ablation of the caudate nuclei show a profound behavioral change called compulsory approaching syndrome (CAs; Exper. Neurol. 52:389,76) as well as a changed behavioral response to morphine (Brain Res. 248:159,82). Here we report the effects of naloxone (Nx) upon the behavior of acaudate cats.We used 5 male cats with over 80% bilateral ablation of the caudate nuclei (aspiration, midline approach). The animals received 1.0 to 3.0 mg/kg Nx, either i.m. or through remote i.v. injection. As controls, intact cats received Nx and acaudate cats received saline injections. Prior to Nx, acaudate cats exhibited a full blown CAs: jumped out of a box and approached the investigator; stuck to (the stationary) and followed (the walking) investigator or moving object; came back repeatedly after being pushed away; showed persistant visual tracking as well as tactile and auditory approach; showed "female" lordosis behavior, persistent rooting, purring, paw treading, stereotyped friendliness and hyperactivity. Before and after injection, cats were scored for the above components of the CAs (0-5 point scale; max=49). CAs was no longer present after NX (mean scores, before =32,5; after= 5.3; Wilcoxon $P < 0.001$) but components slowly reappeared after about 60 min and reached preinjection levels after 4-5hr. In addition, signs of precipitated withdrawal were observed in 3 cats (head and tail shakes, salivation, plaintive vocalization, pyloerection, shivering and panting). Results suggests an inbalance of endogenous opiates in acaudate cats (opiate "high"?). Neostriatal dysfunction has been suggested for obsessive-compulsive and Tourette's disorders with Nx having ameliorative effects. Grants. RO1 DA 02518;HD 04612.

280.9

THE NEUROPHARMACOLOGY OF OPIATE-INDUCED MUSCLE RIGIDITY IN THE RAT. M. B. Weinger*, T. Lin, C. Lau, G. F. Koob. Dept. of Anesthesiology, UC San Diego & Dept of Neuropharmacology, Scripps Clinic, San Diego, CA 92093.

Large doses of opiates produce muscle rigidity. While previous studies have identified discrete sites which may mediate this rigidity [Brain Res 544: 181, 1991] and several putative neurochemical mediators of rigidity have been proposed [Anesthesiol 71: 249,1989], the neuropharmacology of this effect remains to be elucidated. After Animal Care Committee approval, anesthetized male Wistar rats were implanted with icv cannulae. The α-2 agonists dexmedetomidine (DEX; 0-10μg) and ST91 (0-100μg), the α-1 antagonist prazosin (PRZ; 0-30μg), the 5HT2 antagonist ketanserin (KET; 0-100μg), or the opiate antagonist methylnaloxonium (MN; 0-4μg) were injected icv in 10 μl by a blinded observer. Left hindlimb electrodes were used to record EMG activity. After a 15 min baseline, ALF (500μg/kg sc) was given, and EMG activity was recorded for 60 min. Time-course data were analyzed using 2-way ANOVA. Log dose data were then corrected to reflect % of baseline rigidity (based on the zero dose for each group) and the lines-of-best-fit were calculated. MN, DEX, and ST91 dose-dependently antagonized ALF rigidity. The slopes of these 3 dose-effect curves were virtually identical; MN and DEX showed similar potency, while ST91 was less. The fact that the slopes of α-2 agonists' dose-effect curves were almost identical to that of MN suggests possible similarity in either site of action or second messengers involved. In contrast, neither KET nor PRZ significantly affected rigidity. The lack of effect of icv KET suggests a peripheral site of action after systemic dosing. PRZ's inactivity contrasts with previous work [Neuropharmacol 28:1163, 1989]; thus the role of α-1 receptors in opiate rigidity remains unclear.

280.10

INDUCTION OF CONDITIONED PLACE PREFERENCE BY ELECTRICAL KINDLING. Raúl G. Paredes*, Verónica Romero and Anders Agmo. Escuela de Psicología,Universidad Anáhuac.México.DF.

Several lines of evidence suggest that opioid peptides are involved in the mechanisms of reinforcement. Intracerebral infusion or systemic administration of opioids have been shown to induce conditioned place preference. Several brain sites including the medial preoptic area (MPOA),are able to sustain opioid reward. Kindling is a model in which repeated subconvulsive electrical stimulation of different brain areas eventually produces generalized motor seizures. A kindling seizure is associated with release of opioid peptides. It could be argued then that the generalized release of opioids could induce a reward state. In the present study this possibility was evaluated. Male wistar rats were implanted with a bipolar electrode in tha right MPOA or right AMG.Once kindling was fully established the animals were tested for place preference. The compartment where the subject spent more time was considered as the preferred compartment. The place preference conditioning was performed with a kindled stimulus as the reinforcing event. At the end of conditioning the animals were tested again for preference. Animals kindled in the MPOA or the AMG showed place preference after conditioning suggesting that the release of opioids produced by a kindled seizure may have reinforcing properties.

280.11

EFFECTS OF MORPHICEPTIN ON SEVERAL PARAMETERS OF MALE COPULATORY BEHAVIOR.
L. Matuszewich* & E. M. Hull. Department of Psychology, SUNY at Buffalo, Buffalo, New York 14260.

As previously reported by Matuszewich & Dornan (1990), morphiceptin, a selective mu opioid receptor agonist, injected into the medial preoptic area (MPOA), delayed the onset of copulation in male rats. Recent experiments tested the site specificity of this effect and whether decreased locomotion, sexual motivation, or genital reflexes contributed to the delay.

Male copulatory behavior was recorded following unilateral injections of morphiceptin into the MPOA or the ventromedial hypothalamus (VMH). Both doses of morphiceptin (100 or 1000 ng) significantly increased mount and intromission latencies following injection into the MPOA, but not into the VMH. After injection of morphiceptin into the MPOA, sexual motivation and locomotion were measured in an X-maze. Morphiceptin did not affect choice of the female or running time to the goal boxes, but did increase the number of trials in which the male did not choose a goal box. In an open field test, morphiceptin did not affect either the number of rears or the number of lines crossed. MPOA injections of morphiceptin had no significant effect on genital reflexes in restrained supine male rats.

These experiments showed: site specificity of the delay in copulation; neither sexual motivation nor genital reflexes were affected by morphiceptin in the MPOA; and there was an impairment of one measure of locomotion.

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280.12

EFFECTS OF OPIATE ANTAGONISTS ON QUINPIROLE-INDUCED PENILE ERECTION AND YAWNING IN THE MALE RAT. P.C. Doherty* and P.A. Wisler The Lilly Research Laboratories, A Division of Eli Lilly and Company, Indianapolis, IN 46285.

Administration of the opiate antagonists naloxone and naltrexone induces erections in men and monkeys, and increases the number of erections observed in rats during the penile erection/stretch-yawn syndrome (PE/SYS) induced by dopamine (DA) agonists. In the present experiments we have compared the efficacy of several phenylpiperidine opiate antagonists with naloxone for their ability to enhance the display of PE/SYS after treatment with the D2 DA agonist quinpirole. Adult male Sprague-Dawley rats, (400-500 mg) were injected s.c. with the antagonists. One half hour later each animal was given an additional injection of saline, 25 or 50 µg/kg of quinpirole. Immediately thereafter, the animals were placed in clear plastic cages and observed over the next thirty minutes for the display of the penile erections and yawns. Naloxone (1-10 mg/kg) induced dose dependent increases in the number of erections induced by both 25 and 50 µg/kg of quinpirole. In contrast, the high level of yawning observed after treatment with quinpirole was inhibited by the opiate antagonist. Two phenylpiperidine opiate antagonists, LY243670, a racemic mixture of four trans isomers, and LY255582, its most active diastereomer at the µ and k receptor subtypes, also showed significant dose dependent enhancement of quinpirole induced erections in doses from 0.03 to 1.0 mg/kg. Quinpirole induced yawning was enhanced by the lower doses and inhibited by the higher doses of LY255582. The peripherally selective opiate antagonist LY280927 had no effect on the occurrence of erections, but significantly enhanced yawning. These findings demonstrate that opiate antagonists can increase the display of quinpirole induced erections in the rat and that this, unlike yawning, occurs through an interaction with opiate receptors sequestered by the blood brain barrier. These findings also support the potential utility of opiate antagonists in the treatment of erectile dysfunction.

280.13

OPIOIDS IN THE VENTRAL TEGMENTAL AREA FACILITATE THE ONSET OF MATERNAL BEHAVIOR IN THE RAT. Alexis C. Thompson* and Mark B. Kristal, Dept. of Anatomy & Cell Biology, Emory Univ. Sch. of Med., Atlanta, GA 30322, and Dept. of Psychology, SUNY at Buffalo, Buffalo, NY 14260.

The influence of opioids in the ventral tegmental area (VTA) on the onset of maternal behavior was investigated. In Exp. 1, maternal sensitization latencies were determined in *virgin* rats given unilateral intra-VTA injections of 1 of 5 doses of morphine sulfate (MS) (0.0, 0.01, 0.03, 0.1, or 0.3 µg) or were given no treatment, on the first 3 days of pup exposure. A dose-dependent decrease in sensitization latencies was found. Rats treated with 0.03 µg MS had significantly shorter latencies (Med = 5.5 days) than did rats treated with 0.0 µg, 0.1 µg, or receiving no treatment (Med ≥ 10 days). Latencies in rats receiving 0.1 µg MS or 0.3 µg MS were intermediate and did not differ significantly from any group. In a follow-up study, we confirmed that the facilitating effect of 0.03 µg MS in the VTA on the onset of maternal behavior was mediated through the opioid receptor (blocked by 1 mg/kg naltrexone hydrochloride, I.P.) and was specific to the VTA (placements dorsal to the VTA were ineffective).

In Exp. 2, *parturitional* rats were used, and the effect of *blocking* opioid activity in the VTA on the onset of maternal behavior was tested. To minimize disruptive effects of drug treatment on labor and delivery, mother rats were unmanipulated until after delivery of the last pup. To minimize maternal behavior during parturition, pup contact was limited by having mothers delivery on a grid floor, and removing each pup from beneath the grid as it was born. Forty min after delivery of the last pup, mothers were given bilateral intra-VTA injections of either the opioid antagonist naltrexone methobromide (quaternary naltrexone, QN, 1 µg), its vehicle, a sham injection, or left untreated. Thirty min after drug treatment, foster pups were introduced and sensitization latencies were determined. Mother rats treated with QN showed significantly longer latencies (Med = 32 hr) than did rats in control conditions (Med for combined control groups = 6 hrs). Therefore, blockade of opioid activity in the VTA disrupted the onset of maternal behavior.

These results suggest that opioid activity in the VTA facilitates the onset of maternal behavior and may be necessary for the natural onset of maternal behavior at parturition.

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280.14

U50,488 AND PENTYLENETETRAZOL, BUT NOT NALTREXONE, ELICIT ULTRASONIC VOCALIZATIONS FROM RAT PUPS IN THE HOMECAGE. S.E. Carden*, A.T. Bortot, & M.A. Hofer. Columbia Univ. & N.Y.S. Psychiatric Inst., New York, NY 10032

Rat pups isolated in an unfamiliar setting emit ultrasonic vocalizations. In the homecage with litter intact, such calls are infrequent. Benzodiazepine and opiate drugs, among others, decrease isolation calls. We now find that U50,488, a highly selective kappa opioid agonist, and pentylenetetrazol (PTZ), an anxiogenic drug that binds at the GABA-benzodiazepine receptor complex, both elicit ultrasonic vocalizations in the home-cage. Ten-day old Wistar pups were injected with saline or PTZ (5 - 45 mg/kg) or U50,488 (1 - 30 mg/kg) then returned to their litter-mates in the homecage. Pre- and post-drug periods were virtually free of vocalization, compared to dose-related increases when animals were given PTZ or U50,488. There were also drug related changes in level of activity and time spent in physical contact with littermates. The opiate receptor blocker naltrexone, at doses up to 5 mg/kg, did not alter rates of vocalization.

280.15

MK-801 BLOCKS MORPHINE-INDUCED ANALGESIA AND TOLERANCE IN MICE. K. Lutfy* and E. Weber, Dept. of Pharmacology, UC Irvine , Irvine, CA 92717.

The present study was aimed to examine the effect of the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801 (dizocilpine) on morphine-induced analgesia and tolerance in Swiss Webster mice. Mice were injected with either MK-801 (0.1 mg/kg, i.p.) or saline. Thirty min later, mice were injected with morphine (2.5-10.0 mg/kg, s.c.) and tested for analgesia (tail flick assay, (TFA)) 45 min following morphine administration. Pretreatment with MK-801 blocked morphine-induced analgesia. In tolerance studies, mice were injected with either saline or MK-801 (0.1 mg/kg, i.p.). Thirty min later, mice were injected with either saline or morphine (10, 20, and 50 mg/kg, s.c. for three consecutive days on day 1, 2, and 3, respectively) and tested for analgesia on day 4. On day 4, a test dose of morphine (2.0 mg/kg, s.c.) was injected and analgesia (TFA) was measured 45 min later. Pretreatment with MK-801 attenuated the development of chronic tolerance to morphine. MK-801 displayed poor affinity for the [3H]naloxone binding site in mouse whole brain homogenates. These data together suggest that the MK-801-sensitive component of morphine-induced analgesia might be responsible for the development of tolerance. Furthermore, these data support the possibility that MK-801 does not act directly via the opioid binding sites but inhibits morphine-induced analgesia and tolerance by some other mechanism. (Supported by NIDA Grant DA 06356)

280.16

INDEPENDENT GENETIC CONTROL OF TWO PHARMACOLOGICALLY DISTINICT BRAINSTEM MU ANALGESIC SYSTEMS. C.G. Pick* and G.W. Pasternak, The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Departments of Neurology & Neuroscience and Pharmacology, Cornell U. Medical College, New York, NY 10021

Morphine injected i.c.v. elicits analgesia alone or synergistically with spinal systems. We now show that the brainstem mu receptors mediating this synergy are genetically and pharmacologically distinct from those responsible for analgesia following only i.c.v. morphine. Mu_1 receptors mediate morphine analgesia supraspinally while mu_2 receptors act spinally. In CXBK mice, a strain deficient in mu_1 receptors, i.c.v. morphine given alone elicits only 20% analgesia at a dose 20-fold greater than the ED_{50} in CD-1 mice. However, the CXBK and CD-1 strains are equally sensitive to i.t. morphine, a mu_2 action. Despite its inactivity given alone, morphine administered i.c.v. potentiates i.t. morphine as effectively in CXBK mice as in CD-1 mice. Additional studies using selective mu antagonists differentiated these two analgesic responses pharmacologically. β-Funaltrexamine, a mu_1 and mu_2 antagonist, given i.c.v. blocks the analgesia following i.c.v. morphine alone as effectively as its potentiation of i.t. morphine, confirming a role for mu receptors in both actions. In contrast, the mu_1-selective antagonist naloxonazine antagonizes the analgesia following i.c.v. morphine alone 6-fold more potently than the potentiation of i.t. morphine. Together our results indicate the presence of two genetically and pharmacologically distinct populations of mu receptors within the brainstem involved with morphine analgesia.

280.17

L-TRYPTOPHAN POTENTIATION OF MORPHINE-INDUCED ANALGESIA IN MICE. K.M. Hull*, D.E. Tolland and T.J. Maher. Dept. of Pharmacol., Mass. College of Pharmacy, Boston, MA 02115.

Opioid administration has been shown to increase the serotonin (5HT) turnover in regions of the CNS. Moreover, drugs that enhance 5HT are known to augment several morphine-induced actions (e.g., analgesia) while pharmacological manipulations which impair 5HT function (e.g., p-chlorophenylalanine) attenuate the morphine-induced response. We examined the ability of the immediate amino acid precursor for 5HT, L-tryptophan (L-TRP), to potentiate morphine-induced analgesia as measured by a hot plate (52 C) test. L-TRP (200 mg/kg) significantly potentiated the analgesic activity of morphine sulfate (2.5, 5, and 10 mg/kg, by 104, 169, and 149%, respectively). L-TRP was without effect when administered alone. The L-amino acids alanine, histidine and lysine, when administered in equimolar amounts, failed to significantly alter the morphine-induced analgesia, nor did they have an effect when administered alone. The pharmacologic use of L-TRP to potentiate morphine-induced analgesia may constitute a rational strategy to reduce the exposure of patients to the opioids if the undesirable effects (e.g., respiratory depression, tolerance development) are not similarly potentiated.

280.18

ROLE OF THE NUCLEUS ACCUMBENS IN THE EFFECTS OF MORPHINE ON SEPARATION INDUCED CRYING IN INFANT RATS. G. A. Barr, M.A. Hofer* and S. Wang. Dept. Psychology, Hunter College New York, NY 10021 and Dept. Develop. Psychobiol., New York State Psychiatric Institute, New York, NY 10032.

Crying following separation from the mother and siblings is a characteristic response of the young of a number of species and is thought to reflect the internal affective state of the infant. In the rat pup, crying is in the ultrasonic range. Opiates modulate this response; μ and δ-agonists quiet the infant and antagonists block the effects of the agonists. Furthermore, following precipitated withdrawal from chronic treatment with morphine, infant rats cry at higher rates than do controls. Little is known of the neural substrates that mediate this fundamental response of the infant. In this experiment, 6 day old pups were implanted with chronic indwelling cannulas aimed at the nucleus accumbens. Sixteen hours later, pups were injected with morphine (3.0 mg/kg i.p.) or saline followed by intracerebral injection of naltrexone (0, 0.3, 1.0, 3.0 μg) or vehicle into the nucleus accumbens. Pups were then tested alone for ultrasonic vocalizations in a six minute test. The peripheral injection of morphine drastically suppressed crying. Intra-accumbens injection of naltrexone reversed the suppression in a dose-dependent manner and had minimal effect on saline injected pups. These results indicate that at least one site of morphine's quieting action is the nucleus accumbens. If crying by the infant rat is a reliable concomitant of its internal state, then the nucleus accumbens is likely involved in the modulation of affect in the infant as it is hypothesized to be in the adult. (Supported in part by DA-06600)

CATECHOLAMINE RECEPTORS: DOPAMINE II

281.1

FUNCTIONAL ANALYSIS OF THE PROMOTER OF THE RAT D-2 DOPAMINE RECEPTOR GENE. T. Minowa, M. T. Minowa and M. M. Mouradian*. Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

The D-2 dopamine receptor is classically recognized to be the primary mediator of the motor, endocrine and behavioral effects of central dopaminergic transmission. To investigate the molecular events leading to the transcriptional regulation of the rat D-2 gene, we have cloned and sequenced the 5' flanking region of this gene. Although multiple transcription initiation sites were identified, strong preference to three consecutive nucleotides were noted. The promoter lacks TATA and CAAT boxes. Transfection of D-2 expressing cells with chimeras of the D-2 promoter deletion mutants / CAT gene indicated a cis-acting enhancer region between nucleotides -75 and -29 relative to the main transcription start site, and a silencer between -217 and -76. DNaseI footprinting revealed Sp1 binding to its consensus sequence at -48 to be crucial for promoter activity of this gene. Lack of Sp1 binding to its consensus sequence at -86 may relate to the negative regulation of this gene. The initiator-like sequence around transcription start site is also discussed. We conclude that the rat D-2 promoter shares with the human D-1A promoter some "housekeeping"-like features, but the D-2 gene has a strong preference for transcription initiation with Sp1 playing a critical role.

281.2

DOPAMINE D2 RECEPTOR GENE EXPRESSION IN MMQ CELLS AND RELATED TRANSDUCING MECHANISMS Carmelo Ventra*, Tullio Florio, Maurizio Grimaldi, Saverio Talia and Gennaro Schettini Dip. di Farmacologia, II Facolta' di Medicina, Universita'degli Studi di Napoli, Italy.

The MMQ cell line, recently isolated from the 7315a transplantable rat pituitary tumor, secretes prolactin and expresses a native and functional dopamine (DA) D2 receptor. These properties make this cell line an useful model to study the D2 receptor, acting via its native "coupling environment". We evaluated a possible functional diversity between this receptor, that expressed in anterior pituitary lactotrophes and that transfected in GH4C1 cells (GH4C1Z-R7). In membranes derived from these cells, we extensively analyzed the transducing mechanisms mediating the effect of DA on basal and stimulated adenylate cyclase activity, focusing on the interaction between this receptor and G-protein/s, using GTP and its non-hydrolyzable analogues. The modulation of DA D2 receptor-gene expression by various stimuli acting via different second messenger-activated pathways is currently under evaluation. (CNR grant n° 9003204 to G.S.)

281.3

RETINOIC ACID MEDIATED INDUCTION OF DOPAMINE D2 RECEPTOR IN HUMAN SHSY-5Y NEUROBLASTOMA CELLS. S. M. Farooqui and C. Prasad*. Laboratory of Neurosciences, Pennington Biomedical Research Center, Baton Rouge, Louisian and Deparment of Medicine LSUMC, New Orleans, Louisiana.

Differentiation of human SHSY-5Y nueroblastoma cells by retinoic acid (RA) is accompanied by the growth of axonal processes, increased electrical activity, induction of many neurotransmitter synthesizing enzymes and catecholeamine transporters and loss of tumorigenicity. The status of dopamine D2 receptor (DR) during differentiation of these cells, however, has not yet been examined. To this end SHSY-5Y cells were treated with increasing concentrations of RA for 15 min-72 hours; RA treatment resulted in to a dose and time dependent increase (4-fold) in the specific binding of [^{3}H]YM09151-2, a DR selective antagonist, to the partially purified cell membranes The level of DR protein (120 kDa), detected by Western blot analysis using a monoclonal antibody to DR peptide, was induced within 30 min of RA treatment. This monoclonal antibody has been characterized in detail elsewhere (Biochem. Biophys. Res. Comm. 1992). The maximal induction of 120 kDa protein was observed after 2 hours of RA treatment and the elevated levels of DR protein was maintained up to 72 hours. The increase in the [^{3}H]YM09151-2 binding, however, appeared several hours after the DR protein induction. In contrast, significant morphological changes in RA treated cells were visible only after 12 hours. Therefore, we conclude that the induction of DR may be one of the early steps in RA-mediated differentiation of SH-SY5Y cells. Supported by US Army grant # DAMD 17-88-Z-8023.

281.4

DISTRIBUTION OF DOPAMINE D_2 RECEPTORS IN SUBPOPULATIONS OF PRIMATE MIDBRAIN DOPAMINERGIC NEURONS. K. Nagamoto,* W.X. Lu, and S.N. Haber. Dept. of Neurobiology and Anatomy, School of Medicine and Dentistry, Univ. of Rochester, Rochester, NY 14642.

The mesencephalic dopamine neurons can be divided into two subpopulations of cells based on their anatomical connections, biochemical compositions, and vulnerability to degeneration under pathological conditions. The ventral tier of dopaminergic cells projects primarily to the dorsolateral striatum, and these cells contain higher levels of tyrosine hydroxylase (TH) mRNA than the dorsal tier (Weiss-Wunder & Chesselet, J. Comp. Neurol., 303: 478-488, '91). In contrast, dopaminergic cells in the dorsal tier including the ventral tegmental area (VTA) receive projections from ventral striatum, and co-contain cholecystokinin (CCK) and calbindin (Lavoie & Parent, Neuroreport, 2: 1601-1604, '91). In order to further characterize the mesencephalic dopamine neurons, we examined distribution of the D_2 receptor (D_2R) in the midbrain of the monkey. We combined *in situ* hybridization histochemistry and immunocytochemistry on the same sections for localization of D_2R and identification of TH-immunoreactive dopaminergic cells, respectively. Our preliminary results show that the dopaminergic neurons in the dorsal tier contain relatively lower levels of D2R mRNA than those in the ventral tier. These results further characterize the mesencephalic dopamine neurons biochemically, suggesting that the D_2R is differentially regulated in the two subpopulations of the dopaminergic cells in primates.
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281.5

THE DISTRIBUTION OF DOPAMINE D2 RECEPTOR HETERONUCLEAR RNA (hnRNA) BY INTRONIC IN SITU HYBRIDIZATION OF THE RAT BRAIN. C.A. Fox*, R. C. Thompson, J. Bunzow, O. Civelli and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720 and Vollum Institute, Oregon Health Sciences University, Portland OR 97201.

Studies evaluating acute changes in brain dopamine D2 receptor mRNA in response to a variety of stimuli indicate that D2 mRNA levels are resistant to change. To more carefully investigate transcription of the D2 receptor gene, we have subcloned a 425 bp Eco RI/Ava II fragment of intron 7 (O'Malley et al., 1990) of the rat D2 gene into pGEM4. This construct was then used as a template to generate a riboprobe for intronic *in situ* hybridization. Since intronic sequences are only included in the short lived heteronuclear RNA (hnRNA), hybridizing to introns reveals the amount of hnRNA in the cell, allowing a more sensitive method for evaluating transcription. The anatomical distribution of the D2 hnRNA is virtually identical to the distribution of D2 mRNA in the rat brain. Signal from the D2 intronic probe is found in the caudate putamen, nucleus accumbens, olfactory tubercle, substantia nigra, ventral tegmental area, and zona incerta. Other regions that contain D2 mRNA, but do not contain D2 hnRNA, include the globus pallidus, prefrontal, cingulate, entorhinal, and piriform cortex, septum, and amygdala. However, these areas have low amounts of D2 mRNA (Meador-Woodruff et al., 1989) and may contain levels of D2 hnRNA that are below detection. This study indicates that D2 intronic *in situ* hybridization may prove to be a useful tool for evaluation of the D2 gene's transcription. Studies are underway to evaluate the ability of specific drugs to alter the activity of the dopamine D2 receptor gene. Supported by NIMH grant PO1 MH42251 and a PMA Foundation Award to C.A.F.

281.6

D_2 DOPAMINE RECEPTOR EXPRESSION ON STRIATONIGRAL NEURONS. *M.A. Ariano,* C.J. Stromski, [1]E. Smyk-Randall, [2]C.David, [2]S. Fuchs, [1]D.R. Sibley. Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT; [1]Chemical Immunology, Weizmann Institute of Science, Rehovot Israel; [2]ETB, NINDS, Bethesda, MD.*

*We have developed specific polyclonal antibodies directed against synthetic peptide sequences unique to the rat D_2 dopamine receptor, enabling detection of the cellular location of this neurotransmitter receptor. The antisera were generated against extracellular and intracellular epitopes, and recognize the native receptor protein, as judged from immunofluorescent expression in stably transfected fibroblasts or Chinese Hamster Ovary cells (**BBRC 179: 824, 1991; Proc. Nat. Acad. Sci. USA 88: 1441, 1991**). The rat substantia nigra was stereotaxically infused with 1 µl of fluorescent labeled latex microspheres. Following a one week survival period allowing for retrograde transport of the label to the striatum in order to mark the striatonigral projection system, the animals were sacrificed and the brain extracted and rapidly frozen. The antisera were applied to 10 µm thick coronal forebrain sections, using antibody dilutions of 1:8000 to 1:20,000 in phosphate-buffered saline (pH 7.2). The fluorescent staining was robust within the neuropil and circumscribing the soma of medium-sized neurons of the striatum. Combining retrograde labeling and immunofluorescence techniques with localization by the various anti-peptide antisera, the D_2 dopamine receptor subtype could be distinguished on as many as 60% of the neurons comprising the striatonigral efferent system. However, some 65% of all D_2 receptor positive cells are represented by other intrinsic neurons in this basal ganglia nucleus, such as the striatopallidal outflow or the aspiny interneuron populations.* *This work was supported in part by USPHS NS 23079 to MAA.*

281.7

PLASTICITY AND ONTOGENY OF MELANOTROPE D_{2A} DOPAMINE RECEPTOR mRNA AND ISOFORM PROTEIN EXPRESSION. B.M. Chronwall*, D.S. Dickerson and K.A. Gary. Biological Sciences, Univ. of Missouri, Kansas City, MO 64108.

Peptide secretion from the rat pituitary intermediate lobe is regulated by D_{2A} dopamine receptors. Chronic antagonist treatment increased D_{2A} receptor binding, B_{max}, but not K_d; chronic agonist treatment did not change either B_{max} or K_d. *In situ* hybridization histochemistry showed a 50% increase of D_{2A} receptor mRNA following chronic antagonist and a 40% decrease following chronic agonist treatment. D_{2A} receptor mRNA levels differed among individual melanotropes.

A short isoform (D_{2A-S}) and a long isoform (D_{2A-L}) of the D_{2A} receptor have been distinguished by biochemical characteristics. Antisera have been generated against D_{2A-L} and the total population of the D_{2A} receptor protein (D_{2A-S+L}) (Dr. D. Sibley, NIH, Bethesda MD) which were used for immunohistochemical localization.

In control pituitaries stained with antibodies to the D_{2A-S+L} receptor protein, brightly labeled melanotropes were randomly distributed throughout the lobe; all other melanotropes showed differing degrees of lesser staining intensity. The D_{2A-L} receptor isoform staining pattern differed substantially from the D_{2A-S+L} receptor staining, showing a few randomly distributed, brightly labeled melanotropes. Antagonist treatment led to an increase in the number and staining intensity of melanotropes positive for the D_{2A-L} receptor antiserum. A concurrent increase in staining intensity of all melanotropes was observed with the D_{2A-S+L} receptor antiserum. Conversely, agonist treatment led to no change in either D_{2A-S+L} or D_{2A-L} receptor immunoreactivity (IR).

Ontogenic studies indicated that prior to postnatal day (PN) 4, D_{2A-L} is the only isoform detected; the numbers of D_{2A-L}-IR cells increased relative to non-labeled cells from PN1 to PN4. After PN6, D_{2A-L}-IR is associated with the axons innervating melanotropes as well as the melanotropes themselves. D_{2A-S+L}-IR appeared after PN4 and then gradually increased to attain a pattern characteristic of the adult around PN14. No axon staining was apparent with the D_{2A-S+L} antiserum. In general, D_{2A-S+L}-IR seems to lag behind the onset of dopaminergic innervation.

281.8

NON-SPECIFIC BINDING CORRECTION IN HUMAN *IN VIVO* ^{123}I-BZM SPECT STUDIES BY MEANS OF ACTIVE AND INACTIVE ENANTIOMERS **R.H. Sexton*, D.W. Jones, A. Braun, K.S. Lee, J.R. Zigun, D.R. Weinberger. CBDB, IRP, NIMH, Washington, DC 20032.**

***In vivo* CNS radioligand-receptor binding studies require correction for non-specific binding. The usual approach is to subtract radioactivity counts in an area of low specific binding (e.g. cerebellum) from counts in an area of high specific binding. An alternative approach is to use an enantiomeric pair of radioligands with one isomer actively binding to a specific receptor type and the other isomer exhibiting no specific binding. We have applied this approach to human SPECT studies with (S)-^{123}I-BZM, a potent D-2 dopamine receptor antagonist, and (R)-^{123}I-BZM, the corresponding inactive isomer. Subjects were injected with 5mCi of (S)-^{123}I-BZM and kinetic SPECT scans were acquired for 3hrs. Acquisition times varied from 20sec/scan initially when rapid changes occur in the uptake kinetics to 30min/scan at later times. We performed a second identical study sequence with (R)-^{123}I-BZM within several days of the active study. Sufficient time was allowed between studies for essentially complete decay of previously injected radioactivity. ROI analyses were performed to generate time-activity curves of the striatum and of frontal, temporal, and occipital cortices. The inactive isomer kinetics for all ROI's are nearly identical as expected for non-specific binding; the active isomer curves display similar behavior for the cortical regions but substantially more accumulation in the striatum. This excess uptake of active isomer in the striatum may be attributed to specific binding to D-2 receptors that are relatively dense in this area of the brain. We have developed a normalization method that allows comparison of active and inactive studies on the same scale. This makes it possible to subtract the non-specific binding, revealed by the inactive isomer study, from the active isomer data thus isolating the specific binding within a particular ROI. This method also confirms the validity of using a low specific binding ROI to define counts related to non-specific binding for IBZM.**

281.9

IN VIVO SPECIFICITY OF N-(2'-[F-18]FLUOROETHYL)BENPERIDOL FOR PET INVESTIGATION OF DOPAMINERGIC D-2 RECEPTOR BINDING. S.M. Moerlein*, J.S. Perlmutter and D. Parkinson. Mallinckrodt Institute of Radiology, Department of Neurology and Neurological Surgery, and Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110.

Benperidol is a butyrophenone that binds to dopaminergic D-2 receptors with high affinity and high selectivity. We have evaluated an analogue of benperidol, N-(2'-fluoroethyl)benperidol (FEB), in which the positron-emitting fluorine-18 ($t_{1/2}$ = 110 min) was attached to the ligand for use as a PET tracer. [F-18]FEB was synthesized in 25-30% radiochemical yield and specific activity exceeding 1000 Ci/mmol within 90 min. Receptor-binding studies *in vitro* using primate brain tissues show that FEB has high affinity for D-2 receptors (K_i = 5.2 nM) and relatively low affinity for serotonergic S-2 receptors (K_i = 31 nM). PET imaging of baboons indicated that the radioligand localized *in vivo* preferentially within D-2 receptor-dense tissues. The free fraction of [F-18]FEB was 2.5 ± 1.3%, and almost all peripherally-generated radiolabeled metabolites were polar. To evaluate the receptor-specificity of radioligand accumulation *in vivo*, receptor-saturating doses of eticlopride (4 mg/kg, i.v.), ketanserin (0.54 mg/kg, i.v.), SCH 23390 (1.1 mg/kg, i.v.) were injected prior to administration of [F-18]FEB. The regional tissue-activity curves after injection of unlabeled S-2 ligand ketanserin or D-1 ligand SCH 23390 were identical to those in the control study. In contrast, pretreatment of the animal with unlabeled D-2 ligand eticlopride resulted in identical striatum and cerebellum tissue-activity curves. For all interventional PET studies, CBF and CBV did not change, neither were the free fraction nor the peripheral metabolism of [F-18]FEB altered. These results indicate that [F-18]FEB is a specific ligand for PET investigation of D-2 receptor binding *in vivo*.

281.10

CHARACTERIZATION OF A DOPAMINE/ANGIOTENSIN II CHIMERIC RECEPTOR: THE ROLE OF THE THIRD CYTOPLASMIC LOOP IN DISTINGUISHING THE AGONIST AND ANTAGONIST BINDING SITES. H. Chen, Y.-H. Hsih, L.-H. Wu, D. A. Downs, D. L. Oxender and F.-Z. Chung*, Parke-Davis Pharmaceutical Research Division, Warner Lambert Company, Ann Arbor, MI 48106-1047.

It has been documented that the third cytoplasmic loop (3 CL) of G-protein coupled receptors played an important role in determining the specificity of receptor G-protein interaction. To determine the importance of the 3 CL of dopamine D2 receptor in affecting receptor binding and in conferring G-protein coupling specificity, the entire loop was replaced by the counter part of the angiotensin II receptor. Angiotensin II receptor (AT1a) has one of the shortest third cytoplasmic loop among all G-protein receptor coupled to phophotidyl inositol (PI) turnover with only twenty four amino acid residues in the loop region. The chimeric receptor gene was transfected into CHO-K1 cells and a cell line producing high levels of the chimeric receptor was isolated and analyzed for receptor binding. Using 3H-labelled spiperone in displacement binding studies, the IC50 values for the two dopamine D2 antagonists spiperone and (+)-butaclamol are 20 nM and 250 nM, respectively. Interestingly, 10 uM concentration of dopamine or (-)-quinpirole displaced only 50% of 3H-labelled spiperone binding, and up to 100 uM concentrations of the two agonists could not displace any of the remaining spiperone binding sites. Our results indicated that in the chimeric receptor, the binding sites for spiperone and (+)-butaclamol are quite different from the binding sites for dopamine and (-)-quinpirole.

281.11

A MOLECULAR MODEL OF THE DOPAMINE D2 RECEPTOR

L.P. Taylor*, A. Mansour, K. De Young, M. Hoversten & H. Akil Mental Health Research Institute, University of Michigan 48109

A framework molecular model of the seven transmembrane domains of the dopamine D2 receptor has been assembled. This coupled with computer simulation using SYBYL software provides a qualitative tool for the analysis of dopamine-ligand interactions. The model was assembled with the following assumptions: invariant amino acid residues within the seven transmembrane family of adrenergic/dopaminergic receptors are critical for receptor function, the transmembrane domains are alpha-helical, the " bends" produced by the Pro residues are essential for defining an interlocked structure of the transmembrane domains, the primary catechol binding sites (based on analogy to the adrenergic receptor) are Asp-114, Ser-194, and Ser-197, and the binding of an agonist must reversibly alter the receptor conformation for signal transduction to occur.

The model was used to examine the putative binding cavity of the receptor with respect to potential interaction with the D4 selective ligand clozapine. Differences in potential steric interactions between clozapine and the D2 or D4 receptor were noted. In order to reduce these potential steric problems, specific amino acids in the D2 receptor were mutated.Mutant D2 receptors were subcloned in a CMV expression vector and expressed in COS-1 cells. Dopamine agonist and antagonist binding to these mutant receptors will be evaluated.

281.12

STRUCTURE-FUNCTION RELATIONSHIP OF DOPAMINE RECEPTORS: A CHMERIC STUDY.

F. Meng*, A. Mansour, M. Hoversten, L.P. Taylor, and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor MI 48109.

The molecular basis of selective ligand binding to D1 and D2 receptors was studied by construction of D1/D2 chimeras. Three pairs of reciprocal constructs were made by ligating transmembrane(TM) domains of the D1 and D2 receptors together using oligo adaptors. These constructs were expressed in COS-1 cells and studied with the selective D1 and D2 ligands (5 nM) listed below.

Contructs	SCH23390	Raclopride	Spiperone	N-0437
Wild D1	27979	268	-	608
Wild D2	508	4724	4441	3217
D1TM1-2/D2TM3-7	620	-	191	900
D2TM1-2/D1TM3-7	408	-	-	542
D1TM1-5/D2TM6-7	2453	-	-	351
D2TM1-5/D1TM6-7	792	-	171	1045
D1TM1-2/D2TM3-5/D1TM6-7	1138	-	-	904
D2TM1-2/D1TM3-5/D2TM6-7	299	206	226	1060

(-) indicates no specific binding was detected

These preliminary results suggest that in the wild type receptors, all TM regions may work synergically with each other to produce selective binding and ligands may differentially interact with transmembrane domains to achieve selectivity. Complete Scatchard analysis and competition studies will follow to better characterize these results.

281.13

THE ROLE OF KEY PHENYLALANINE RESIDUES IN G-PROTEIN COUPLING OF THE D_2 RECEPTOR.

A. Mansour*, K. DeYoung, L.P. Taylor, F. Meng and H. Akil, Mental Health Research Institute, University of Michigan, Ann Arbor MI 48109.

The dopamine receptors are members of a larger family of G-protein coupled receptors that include the adrenergic, muscarinic, and peptidergic receptors. These receptors share a structural motif with rhodopsin which spans the plasma membrane seven times, with the transmembrane domains forming a binding pocket. Site-directed mutagenesis studies suggest that the amino group of dopamine interacts with aspartate 114 of the D_2 receptor, while the meta-hydroxyl of the catechol moiety likely forms a hydrogen bond with serine 197 (submitted). Beyond the anchoring at aspartate 114, it is unclear which amino acid residues are critical in triggering the cascade of events necessary for coupling to G-protein. Computer modeling of the D_2 receptor suggests that there may be a series of phenylalanines (198, 389, 390, 411) that are important in conformationally altering the receptor resulting in an enhanced G-protein coupling. To test this hypothesis, the above phenylalanines were selectively mutated to alanine using site-directed mutagenesis. Mutant D_2 receptors were subcloned in a CMV expression vector and expressed in COS-1 cells where agonist and antagonist binding will be evaluated in conjunction to G-protein coupling.

281.14

CO-TRANSFECTION OF THE DOPAMINE D2 RECEPTOR WITH G PROTEIN α-SUBUNITS ALTERS D2 BINDING.

William Cho*, Alfred Mansour and Huda Akil, Ph.D. Dept. of Psychiatry, University of Michigan, Ann Arbor, MI 48109.

The specific interactions between dopamine receptors and guanine nucleotide regulatory proteins (G proteins) are not well characterized. The D2 dopamine receptor subtype is negatively coupled to adenylyl cyclase, presumably through variants of the inhibititory G-protein, G_i, and may also be linked to K^+ channel activity and PI turnover through other G proteins. COS-1 cells were transiently transfected with the long variant of the D2 receptor (10μg) along with either the α-subunit of G_i ($G_{i\alpha 2}$ or $G_{i\alpha 3}$) or G_o ($G_{o\alpha}$) (20μg). Cells were also simultaneously transfected with a ß-galactosidase construct (5μg) for transfection efficiency corrections. Cells were harvested 48h after transfection and assayed for D2 agonist ([^{3}H]N-0437) and antagonist ([^{3}H]raclopride) binding. In preliminary studies, co-transfection of D2 with $G_{i\alpha 3}$ caused a complete loss of high affinity agonist binding and a marked reduction in low-affinity agonist binding capacity (Bmax=4.1 without $G_{i\alpha 3}$ vs. 0.76 fmol/10^5 cells with $G_{i\alpha 3}$). Similarly, antagonist binding capacity was reduced in the presence of $G_{i\alpha 3}$ (Bmax=2.9 vs. 0.97 fmol/10^5 cells). ß-gal activity did not significantly change between transfections. This phenomenon was relatively specific to G_{ia3} in that transfection of D2 with $G_{o\alpha}$ exhibited a much less dramatic reduction in Bmax, and co-transfection with $G_{i\alpha 2}$ produced no change in D2 binding. Control transfections with D1 and $G_{i\alpha 3}$ failed to show reductions in D1 antagonist ([^{3}H]SCH23390) Bmax.

281.15

SYNTHESIS AND CHARACTERIZATION OF MODEL PEPTIDES CORRESPONDING TO TRANSMEMBRANE DOMAINS OF THE DOPAMINE D_2 RECEPTOR.

Valerie L. Williams*, David I. Schuster, and Randall B. Murphy, Department of Chemistry and Center for Neural Science, New York University, New York, NY 10003.

Site-selective mutagenesis studies have demonstrated that the ligand binding site of the dopamine D_2 receptor is contained within the transmembrane domains, in particular TM III, V, and VII. A substantial degee of homology is observed across these domains which are constitituted of highly hydrophobic amino acid residues. We have designed and prepared a consensus peptide which is based upon the sequences in these domains. The sequence is NH_2-D-Y-A-I-F-V-L-Y-A-S-A-W-L-S-F-L-N-C-P-F-I-V-T-L-N-I-K-COOH. Novel HPLC methodology was developed in order to purify this highly hydrophobic sequence. The secondary conformation was examined in organic solvents and lipid vesicles. Interestingly, this peptide was not found to be substantially α-helical as has been the common assumption, but is relatively extended in structure. Fluorescence studies in lipid vesicles demonstrate that the peptide inserts into the membrane. These experiments suggest that the consensus peptide can be used as a biophysical model for the dopamine D_2 receptor system. (Supported by NSF and New York University).

281.16

PHARMACOLOGICAL CHARACTERIZATION OF A MODEL PEPTIDE CORRESPONDING TO TRANSMEMBRANE DOMAINS OF THE DOPAMINE D_2 RECEPTOR.

Randall B. Murphy*, Valerie L. Williams, and David I. Schuster, Department of Chemistry and Center for Neural Sciences, New York, University, New York, NY 10003

In the dopamine D_2 receptor, the transmembrane domains have been implicated in functional association with ligands. Preliminary work in our laboratory has demonstrated that the type of highly hydrophobic peptides which constitute the transmembrane domains can be synthesized and purified. (see Williams, V.L. et al, This Meeting) We hypothesize that the functional D_2 binding site consists of a multimeric assembly of these transmembrane domains within the lipid bilayer. In order to examine this hypothesis, we have carried out radioligand binding assays to determine if a consensus peptide derived from the transmembrane domains will form a functional binding site in lipid (DMPC) bimolecular vesicles. We observed high-affinity binding with [^{3}H]-spiperidol. The number of binding sites determined indicates that a small percentage of the total peptide which is present forms a functional binding site. These results suggest that molecular engineering of multiple consensus sequences can in principle be used as a model for the dopamine D_2 receptor ligand binding site. (Supported by NSF and New York University)

281.17

REGULATION OF THE D_{2L} DOPAMINE RECEPTOR IN STABLY TRANSFECTED CHO CELLS. L.-J. Zhang*, E.M. Smyk-Randall, F.J. Monsma, Jr. & D.R. Sibley, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

To investigate the regulatory properties of the rat D_{2L} dopamine (DA) receptor, we have stably expressed its cDNA in Chinese Hamster Ovary (CHO) cells. One cell line was characterized using the D_2-selective radioligands [^{3}H]methylspiperone ([^{3}H]MSP) and [^{3}H]YM091512 ([^{3}H]YM). Both radioligands exhibited K_d and B_{max} values of about 50 pM and 2 pmol/mg protein, respectively. DA produced a dose-dependent and pharmacologically-specific inhibition of adenylyl cyclase activity in both intact cell and membrane preparations. DA's EC_{50} for inhibiting forskolin-stimulated cAMP accumulation was about 50 nM with a maximal response at 10 μM which represented a 60-70% inhibition. Pretreatment of the cells with 500 μM DA produced a ~5-fold shift (lower affinity) in the EC_{50} for DA inhibition of cAMP accumulation but no change in the maximum response. Surprisingly, the DA pretreatment resulted in a ~3 fold increase in the maximum binding capacity for both [^{3}H]MSP and [^{3}H]YM with no change in their K_d values. This effect was time-dependent reaching maximal levels after 24 hr with a $t_{1/2} \geq 5$ hr. In a preliminary experiment, prior treatment of the cells with 1 μg/ml of pertussis toxin for 24 hr did not block the DA-induced increase in radioligand binding. Treatment of the cells with various intracellular activators of protein kinases resulted in a general blunting of the D_{2L} receptor responses. Exposure to 1 μM PMA for 24-48 hr resulted in a ~3 fold reduction in the potency of DA to inhibit cAMP accumulation with no change in the maximum response along with a ~25% decrease in receptor B_{max} values. Similarly, treatment with 0.4 mM Sp-cAMPS for 24 hr resulted in a 25-50% reduction in the maximum DA cAMP response with no change in potency along with a 25-50% reduction in B_{max} values. Similar experiments involving the D_{2S} isoform are currently being performed.

281.18

COUPLING OF THE RAT DOPAMINE D2L AND D2S RECEPTORS TO VOLTAGE SENSITIVE CALCIUM CHANNELS IN ATT20 CELLS LA Snyder*[1,3] M. Tsutsumi[1] and S.C. Sealfon[1,2]. [1] Fishberg Center for Research in Neurobiology and [2]Department of Neurology, The Mount Sinai School of Medicine, NY 10029; [3]The Graduate School of the City University of New York, 33 W. 42 St., NY, NY 10036-8099

Alternative splicing of the dopamine D2 receptor gene leads to two D2 receptor isoforms, the D2L and D2S. The receptors differ in the third cytoplasmic loop, a domain implicated in G-protein coupling. Activation of the lactotroph D2 receptor has been shown to inhibit calcium influx through voltage sensitive channels. We have investigated the coupling of each D2 isoform in stably transfected cell lines. Rat D2S and D2L constructs (McChesney et al., Mol Cell Endo 79:R1-R7, 1991) were introduced into AtT20 cells via lipofectin mediated transfection. Cell lines generated containing roughly equivalent levels of receptor expression were selected for study. Cells were loaded with the calcium sensitive dye Fluo-3. Cell suspensions in cuvets were monitored using a spectrofluorimeter equipped with a micro stirrer. 1 uM Bay K 8644, an L-type calcium channel agonist, led to an ~50% increase in intracellular calcium concentration above baseline in transfected and untransfected AtT20 cells. In AtT20 cells expressing either D2L or D2S which were first exposed to Bay K 8644, the addition of 1uM dopamine or quinpirole (a D2 receptor agonist), rapidly decreased intracellular calcium levels to baseline. When introduced just before Bay K 8644, quinpirole was able to block the increase in intracellular calcium levels. Quinpirole had no effect on calcium influx in the presence of the D2 antagonist (+)butaclamol, or in untransfected AtT20 cells. These results indicate that both D2L and D2S stimulation inhibit L-type voltage sensitive calcium channel activation in AtT20 cells. The coupling of each isoform will be further characterized in AtT20 and other cell lines. Supported by NIH grant MH45212

281.19

Potentiation of ATP Stimulated ^{3}H-Arachidonic Acid Release in CHO-D2i Cells as a Measure of Intrinsic Activity. D.L.Evans* and R.A.Lahti. CNS Diseases Research, Upjohn Laboratories, Kalamazoo, MI 49001.

G protein-coupled receptors have been shown to be linked to numerous signal transduction pathways such as adenylate cyclase, phospholipase A_2, and phospholipase C. Felder, et al. (Proc. Natl. Acad. Sci. 88:6477, 1991) found that dopamine D2 receptors which inhibit adenylate cyclase can also amplify ATP stimulated arachidonic acid release. This procedure may provide a reliable means of determining the intrinsic activity of dopamine agonists.

CHO-D2i cells are preloaded with ^{3}H-arachidonic acid by incubating with the radiolabel, rinsed to remove surface radioactivity, and incubated with differing concentrations of dopaminergic compounds in the presence of ATP for 30 minutes. The compound's effect on ^{3}H-arachidonic acid release is measured by counting an aliquot of the media using standard scintillation techniques. The dose dependent ^{3}H-arachidonic acid release is then plotted as a percent response and the maximum response is taken as the compound's intrinsic activity. This procedure provides a quick and simple method for measuring a compound's intrinsic activity via a second messenger system.

Different maximum levels of ATP stimulated arachidonic acid release were found with the full agonist quinpirole (100%), as compared to that of the partial agonists (-)3PPP (72%), terguride (60%), and SDZ-208-912 (22%). These values correlate well with other measures of intrinsic activity such as binding (r=0.85) and electrophysiology (r=0.97) and thus provide a reliable means of determining a drug's intrinsic activity.

281.20

G-protein Coupling and Regulation of Dopamine Release by D_2-like Receptors in the Central Amygdaloid Nucleus. J. E. Lachowicz* and C. D. Kilts, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892 and Department of Psychiatry, Duke University, Durham, NC 27710.

We have previously demonstrated that D_2 receptor activation in the central amygdaloid nucleus (CAN) does not stimulate or inhibit adenylate cyclase activity. To determine whether these D_2-like receptors are coupled to guanine nucleotide binding (G) proteins, the effect of GppNHp on dopamine competition for D_2 radioligand binding was examined. [^{125}I]Iodosulpiride binding in the CAN was assayed by quantitative autoradiography on serial coronal rat brain sections. In the absence of GppNHp, CAN dopamine competition for [^{125}I] iodosulpiride binding conforms to a two site model with a high (IC_{50}=0.14 μM) and low (IC_{50}=4.99 μM) affinity site. GppNHp (10 μM) converts dopamine competition to the low affinity site (IC_{50}=4.60 μM), indicating that these D_2-like receptors exhibit a negative allosteric interaction with a G-protein. D_2 receptor regulation of *in vivo* dopamine release in the CAN was investigated by superfusion of the microdissected CAN with the D_2 receptor antagonist eticlopride to inhibit endogenous dopamine activation of D_2 autoreceptors. Eticlopride produced a 3-fold increase in dopamine release quantified by HPLC and electrochemical detection. These data show that the non-adenylate cyclase coupled D_2-like receptors in the CAN are coupled to inhibition of dopamine release, possibly via G-protein coupling. (Supported by MH 39967)

CATECHOLAMINES: DOPAMINE I

282.1

HUMAN DOPAMINE TRANSPORTER cDNAs: STRUCTURE AND EXPRESSION. D.J. Vandenbergh*, A.M. Persico, A.M. Gonzalez and G.R. Uhl, Lab. Mol. Neurobiol., ARC/NIDA, & Depts. Neurol.& Nsci., JHUSM, Box 5180, Baltimore, MD. 21224.

The dopamine transporter (DAT) removes dopamine from the synaptic cleft to help terminate dopaminergic neurotransmission and may participate in Parkinson's disease pathogenesis, Tourettes syndrome and/or drug addictions. We have isolated human DAT cDNAs that encode a reconstructed 3.9 kb human cDNA with short 5' and long 3' untranslated regions, an open reading frame of 620 amino acids 94% identical to the rat, 3 N-glycosylation sites in the second putative extracellular domain, and 10 copies of a unique 40 bp tandem repeat in the 3' untranslated region. *In situ* hybridization using oligonucleotides complementary to this sequence reveals high levels of expression in neurons of the human substantia nigra compacta and paranigralis nuclei. This cDNA identifies VNTR and RFLP genetic polymorphic markers; *Taq* I RFLPs show race-specific differences in allelic frequencies.

282.2

COCAINE INHIBITS DOPAMINE STIMULATED INTRACELLULAR ACCUMULATION OF SODIUM ION IN STRIATAL SUSPENSIONS OF THE RAT. S. M. Meiergerd and J. O. Schenk*, Dept. of Chemistry, Dept. of Biochemistry and Biophysics, and Prgm. in Pharmacology/Toxicology, Washington State University, Pullman, WA 99164-4630.

Recently, we proposed a multisubstrate transport mechanism, involving Na^+ and Cl^- as cosubstrates in the uptake of dopamine (DA) into striatal suspensions (see McElvain and Schenk, Biochem. Pharmacol., in press). Briefly, it was found that DA and Na^+ bind to the transporter first in random order followed by Cl^- binding last prior to the transport of DA. Cocaine inhibited DA transport uncompetitively by competing with Na^+ for its binding site. The reaction sequence and implication of co-transport of DA with Na^+ and Cl^- was obtained by kinetic studies of the effect of the ions on DA transport and their reaction orders. Thus, co-transport is implied by our findings, but is not proven. The results of the work to be described show that DA stimulates accumulation of Na^+ into the intracellular compartment of striatal tissue as measured by ion selective electrodes. Na^+-accumulation was inhibited ca. 50% by 1.0 uM cocaine, the K_i for inhibition of DA uptake, suggesting that the kinetic implications of the kinetic transport model are valid with regard to Na^+. Supported by the WA Alcohol and Drug Abuse Program (Legislative Initiative 171) and NIDA grant, DA07384.

282.3

DIFFERENTIAL REGULATION OF EXTRACELLULAR DOPAMINE DYNAMICS IN THE BRAIN. P.A.Garris* and R.M.Wightman. Department of Chemistry and Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599-3290.

Synaptic dopamine (DA) overflow in the medial prefrontal cortex (MPFC), basolateral amygdala (BAN), caudate-putamen (CP) and nucleus accumbens (NAc) during transient electrical stimulation of the medial forebrain bundle was monitored using fast-scan cyclic voltammetry in anesthetized rats. Signals were assigned to DA based on electrochemical, pharmacological and anatomical evidence. Despite a 90-fold disparity in terminal density, stimulation in a physiological range of 10 and 20 Hz elicited comparable DA levels in all regions. In contrast, pronounced differences were observed in the dynamics, frequency-dependence and clearance rates of overflow. Rates of DA release and uptake were similar in the MPFC and BAN but approximately 8- and 50-less, respectively, than that in the CP and NAc. When normalized to DA terminal density, relative release was 10-times greater in the MPFC and relative uptake was 10-less in the BAN. In conclusion, sparse mesoprefrontal and mesoamygdaloid DA projections have evolved unique mechanisms to evoke and maintain high concentrations of extracellular DA. The results further define the functional characteristics of mesotelencephalic DA neurons and raise the possibility of the *regio*-specific nature of DA neural transmission in the brain.

282.4

REGIONAL CHANGES IN EXTRACELLULAR NOREPINEPHRINE FOLLOWING FOCAL AND SYSTEMIC COCAINE. D.N. Thomas*, R.M. Post and A.Pert. Biological Psychiatry Branch, NIMH, Bld. 10, Rm. 3N212, Bethesda, MD. 20892

Cocaine prevents the neuronal reuptake of brain catecholamines and indoleamines. Alterations in dopaminergic function have been studied extensively and presumably underlie the majority of the behavioural effects of this psychomotor stimulant. However, considerably less is known about the actions of cocaine on the norepinephrine (NE) system. In the present study using in vivo microdialysis we have characterised the effects of focal and systemic cocaine on extracellular NE in two noradrenergic terminal regions, the hippocampus (HI) and frontal cortex (FC). Male Sprague Dawley rats were anaesthetised with chloral hydrate and dialysis probes were stereotaxically implanted into either the HI or FC. Basal NE concentrations in the HI and FC were 5.8 ± 0.21 pg/sample (mean ± SEM, n=4) and 4.2 ± 0.3 pg/sample (n=4) respectively. Following stabilisation of basal NE, cocaine was applied focally via the dialysis probe at concentrations of 1, 10 and 100μM for 15mins. Sampling was then continued for a further 4 samples prior to application of the successive concentration of cocaine. In the HI cocaine (1 - 100μM) produced a concentration dependent increase in the extracellular NE of 20%, 52% and 142% respectively. However, in the FC only the highest concentration of cocaine (100μM) produced an increase in the extracellular NE (63%). Systemic administration of cocaine (20mg/kg, i.p.) produced no effect on the extracellular NE in either the HI or the FC. These data are consistent with a previous report in which we have shown that desmethylimipramine administered focally into the two regions produces increases in extracellular NE (Thomas et al., 1991 Br.J.Pharmacol. 102), yet systemic administration is unable to alter extracellular NE in either region. The differential effects of cocaine in the two regions may suggest differences in the affinity of cocaine for the NE uptake site.

282.5

SENSITIVITY OF SINGLE DOPAMINE (DA) NEURONS TO IONTOPHORETIC APPLICATION OF SPECIFIC D_2 AGONIST: TIME-DEPENDENT CHANGES DURING WITHDRAWAL FROM CONTINUOUS COCAINE INFUSION. H.Zhang, T.H.Lee, E.H.Ellinwood, and F. Seidler*. Dept. of Psychiatry and Pharmacology, Box 3870, Duke Univ. Med. Ctr., Durham, NC 27710.

Single DA neurons recorded in the substantia nigra zona compacta (SNC) undergo time-dependent changes in their sensitivity to intravenous apomorphine during withdrawal from continuous cocaine infusion (subsensitivity on day 1 followed by supersensitivity on day 7). We have observed in the present study that only supersensitivity on day 7 is observed when firing rate inhibition by bath-applied DA is tested in vitro; single DA neurons are normosensitive on day 1. This lack of subsensitivity on day 1 is consistent with a previous in vivo report that an intact striatonigral feedback is necessary for expression of the decreased sensitivity immediately following daily injections of another stimulant, d-amphetamine. Therefore, soma/dendritic DA autoreceptors in the SNC are probably not subsensitive immediately following cocaine withdrawal. In contrast, the preservation of the supersensitivity in vitro suggest that DA autoreceptors indeed become supersensitive as the withdrawal phase is prolonged. To further examine the time-dependent sensitivity changes, we are currently examining the in vivo sensitivity of antidromically-identified nigrostriatal DA neurons to locally applied D_2 agonists, quinpirole. We will compare and contrast our in vivo and in vitro data. It is hypothesized that DA neurons will be normosensitive to DA on day 1 but supersensitive on day 7, confirming that the supersensitivity is due to a local change within the SNC, probably supersensitive DA soma/dendritic autoreceptors.

282.6

MODULATION OF DOPA DECARBOXYLASE IN THE STRIATUM OF A MOUSE MODEL OF PARKINSON'S DISEASE
M.Hadjiconstantinou[1,2], T.Wemlinger[1], J.P.Hubble[1], C.P.Silvia[1], and N.H.Neff[1]*
Depts of Pharmacology[1] and of Psychiatry[2] and the Neuroscience Program, The Ohio State University College of Medicine, Columbus, OH 43210.

DOPA decarboxylase (aromatic L-amino acid decarboxylase, AAAD) is the rate-limiting enzyme for the production of dopamine (DA) in Parkinsonians undergoing L-DOPA therapy. A change of enzyme activity could have major clinical consequences. Based on our published studies for retina, we found that subchronic treatment (two treatments daily for 7 days) with DA receptor antagonists (SCH 23390, sulpiride & haloperidol) enhanced AAAD activity in the striatum while agonists (L-DOPA, bromocriptine & SKF 38393) depressed activity. Treatment with reserpine also enhanced AAAD activity. Using the mouse MPTP model, we found that AAAD activity could be significantly enhanced to near normal values by treatment with antagonists. From dose-response studies, the MPTP-treated mice were more responsive to the antagonists than normal mice, both by responding sooner after treatment and at lower doses. There was an apparent increase of the Vmax with no apparent change of the Km for L-DOPA or B6. We conclude that AAAD activity is modulated by either DA D1 or D2 receptors and that MPTP mice are more sensitive to modulating drugs. We postulate that L-DOPA, for treating Parkinson's disease, may in the long run actually impede its own conversion to DA by activating receptors that modulate AAAD activity.

282.7

ISOFLURAPHATE ENHANCES THE NEUROTOXICITY OF MPTP
N.H.Neff[1]*, T.Wemlinger[1] and M.Hadjiconstantinou[1,2].
Depts of Pharmacology[1] and of Psychiatry[2] and the Neuroscience Program, The Ohio State University College of Medicine, Columbus, OH 43210.

The irreversible cholinesterase inhibitor isoflurophate (diiso propylfluorophosphate, DFP) is often administered to exaggerate brain cholinergic activity. One consequence is enhanced turnover of dopamine (DA) in the striatum probable because of activation of nigrostriatal feedback loops and intrinsic striatal mechanisms to compensate for enhanced cholinergic tone. MPTP selectively destroys nigrostriatal dopaminergic neurons. To test the hypothesis that active nigrostriatal dopaminergic neurons are more vulnerable to neurotoxins, we administered DFP to enhance DA turnover and then administered MPTP. After a single injection of DFP there was a rise of acetylcholine (ACh) and a concomitant fall of DA and DOPAC. With 7 days of treatment with DFP, however, tolerance developed and DA and ACh content were essentially normal while DOPAC increased suggesting enhanced DA turnover. If MPTP, 30 mg/kg ip, was administered 2 hr after DFP, 1.5 mg/kg sc, for 7 consecutive days there was a dramatically enhanced loss of DA, DOPAC and tyrosine hydroxylase activity from the striatum compared with MPTP treatment alone. The enhanced loss of dopaminergic parameters did not appear to be related to altered MPTP metabolism in the two groups. We postulate that metabolically active dopaminergic neurons are more vulnerable to MPTP.

282.8

PHENYTOIN PREVENTS METHAMPHETAMINE-INDUCED DOPAMINERGIC NEUROTOXICITY IN MICE. L. Manzino* and P.K. Sonsalla. Neurology Dept., UMDNJ-RWJ Med.Sch., Piscataway, N.J. 08854.

Excitotoxicity mediated through stimulation of *N*-methyl-*D*-aspartate (NMDA) receptors is an essential component of methamphetamine (METH)-induced dopaminergic neurotoxicity. Excitotoxicity is also thought to be responsible for neurodegeneration in epilepsy. Anticonvulsants have been reported to attenuate excitotoxin-induced neuropathology in animals. This study was undertaken to determine if anticonvulsants would prevent METH-induced dopaminergic neurotoxicity in mice. Neostriatal tyrosine hydroxylase activity and dopamine content were decreased by 67% and 80%, respectively, 7 days after METH treatment (3 i.p. injections, 10 mg/kg, 2-h intervals). Phenytoin (10-50 mg/kg) prevented these METH-induced changes in a dose-related manner although it did not prevent dopaminergic neurotoxicity produced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). METH-induced neuropathology was not prevented by two other anticonvulsants (valproic acid or diazepam) under the treatment conditions used. These results suggest a novel form of protection against METH-induced neurotoxicity by phenytoin.

282.9

CORRELATION OF POSTMORTEM STRIATAL DOPAMINE LEVELS WITH NIGRAL CELL COUNTS AND UPTAKE OF 18F-FLUORODOPA IN PREMORTEM PET SCANS. E.G. McGeer*, D. Calne, B. Snow, I. Tooyama, H. Takahashi and T. Yamada, Kinsmen Lab. for Neurological Research and the Neurological Disorders Centre, U. B. C., Vancouver, B.C., Canada V6T1Z3

Brains of 1 Parkinson (PD), 1 Alzheimer with some PD pathology, 1 ALS and 3 PSP cases PET scanned during life with [18F]-6-fluorodopa (FD) have come to autopsy. Dopamine (DA), serotonin (5HT) and their metabolites were determined by HPLC on samples of the left midcaudate and midputamen and averaged to get a mean for the striatum (CP). In this small group, only the postmortem DA levels were significantly correlated with FD uptakes (R = 0.96). Correlations between FD uptake data and levels of individual DA metabolites, or with 5HT and its metabolite (5HIAA), were positive but insignificant with very low Rs. In another series of 5 PD and 5 age-matched controls, the number of pigmented neurons counted at the level of the oculomotor nerve correlated significantly with the mean striatal DA level (R = 0.86). The postmortem DA levels probably reflect DA bound in vesicles within endings. These data are consistent therefore with the hypothesis that the FD uptake measured by PET is an index of the number of surviving DA terminals in the CP, and probably reflects in most cases the number of surviving DA neurons. (Supported by the MRC).

282.10

IN VIVO STRIATAL DOPAMINE TURNOVER IS INCREASED IN RATS WITH HALOPERIDOL-INDUCED ORAL DYSKINESIA. Ronald E. See* and Cathy E. Murray, Department of Psychology, Washington State University, Pullman, WA, 99164-4820.

We have previously found that chronic haloperidol (HAL) administration in rats produces unique changes in oral movement patterns that are not seen after short-term treatment. The present study examined changes in oral movements and dopamine (DA) activity in rats treated with chronic HAL. Female, SD rats were administered HAL or no drug via subcutaneous implants for 32 weeks. Oral movements were recorded with a video analysis system and analyzed for total number and form of movements. After 24 weeks, HAL-treated rats began to show persisting, significant increases in oral activity at 1-3 Hz and decreases at 5-7 Hz when compared to control animals. DA release and metabolism were assessed at the end of the chronic HAL administration period using in vivo microdialysis. Bilateral guide cannulae were implanted into the striatum (A +0.2, L +3.4, V -5.0). Following one week of recovery, during continuous HAL treatment, dialysis probes were unilaterally inserted and perfusion initiated. The same procedure was conducted 3 days after drug withdrawal on the contralateral side. Perfusates were injected into an HPLC-EC system and analyzed for DA, DOPAC, and HVA. Basal extracellular levels of DOPAC were significantly increased in the HAL-treated rats during continuous administration, while HVA and DA showed non-significant increases. Following withdrawal, there were no differences between groups in basal levels of any of the analytes. Increased DA turnover, manifested during continuous HAL administration, may be related to the development, but not the maintenance of orofacial dyskinesia. Supported by National Institutes of Health Grant DE09678.

282.11

CHANGES IN THE CENTRAL DOPAMINERGIC SYSTEMS IN THE STREPTOZOTOCIN -INDUCED DIABETIC RATS. D.K. LIM*[1], B. HOSKINS[2] AND I.K. HO,[2] School of Pharmacy[1], Chonnam Natl. Univ., Kwang Ju, 500-757 Korea and Dept. Pharmacol. & Toxicol.[2], Univ. MS Med. Ctr., Jackson, MS 39216

Dopamine metabolism and characteristics of D-1 and D-2 receptors after chemically inducing the diabetic state were studied in rat striatum. Streptozotocin (STZ) was administered on three consecutive days (40 mg/kg, ip) to induce diabetes. Twenty-four hours after the last administration of STZ, the occurrence of catalepsy produced by an injection of SCH-23390 (0.05 mg/kg, sc) were monitered. After some rats were sacrificed 24 hr after the last treatment with STZ, changes in dopamine metabolism and in D-1 and D-2 receptors were determined using HPLC-ECD and radioligand binding ([^{3}H]SCH-23390 and [^{3}H]Sulpiride) assays, respectively.

The latency to onset of and the duration of SCH 23390-induced catalepsy were significantly increased (2 times) and decreased (0.4 times), respectively, in the diabetic rats. Striatal concentrations of DOPAC and HVA and their ratios to DA were significantly decreased (0.65 - 0.72 times) in the diabetic rats. Also the affinities of striatal D-1 receptors were significantly increased (18 %) without changes in the densities, while the densities of D-2 receptors were significantly increased (11 %) without affecting affinities. These data indicate an upregulation of dopamine receptors that may be due to decreased dopamine metabolisms. Furthermore, the results suggest that central dopaminergic activities is altered in the diabetic states.

282.12

BORNA DISEASE VIRUS CAUSES DOPAMINE DISTURBANCES IN RATS. Marylou V. Solbrig, George F. Koob, Sandra Loughlin, William Tsai and W. Ian Lipkin*

*University of California, Irvine, CA 92651 and The Scripps Research Institute, La Jolla, CA 92037

Borna disease virus (BDV) causes neurologic dysfunction in a wide variety of animal species. To define motor and behavioral features of Borna disease, infected adult rats were studied using video, activity cage monitors and EEG. Rats showed a syndrome characterized by myoclonus, athetosis, dystonias, dyskinesias, autostimulation, self-mutilation, cannibalism and seizures (automatisms or behavior arrest). The pharmacology of these behaviors was analyzed using direct- and indirect- dopamine (DA) agonists, D1 and D2 receptor antagonists and a DA synthesis blocker. Amphetamine increased stereotypy and locomotion. SCH23390 decreased self-mutilation and, at higher dosage, suppressed all movement. Low dose apomorphine had a presynaptic autoreceptor effect rendering rats akinetic. BDV causes a syndrome of apparent DA excess with behavioral supersensitivity to amphetamine and apomorphine.

282.13

KINETICS OF ASCORBATE'S EFFECTS ON DOPAMINERGIC AGONIST BINDING. L.C. Tolbert*, S.C. Ashe, and J.J. Spollen. Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama, Birmingham, AL 35294.

Experiments conducted over the last decade or so using a variety of approaches ranging from in vitro and in vivo animal work to clinical trials in subjects with schizophrenia or autism have produced results consistent with the hypothesis that one of the roles of this vitamin in the CNS might be that of a dopamine neuromodulator. One of the approaches this lab has been exploring to investigate this hypothesis is that of in vitro ligand binding studies. Our previous results indicated that ascorbate inhibited dopaminergic agonist binding whether [^{3}H] dopamine or the more selective and stable, D_1 or D_2 receptor specific ligands, [^{3}H] SKF 38393 or [^{3}H] N-0437 were used. Further, this inhibition was not explained by a simple "redox" effect on either ligand stability or at the level of the receptor(s) because the effect of ascorbate was not mimicked by structural analogues with essentially identical redox properties. The purpose of the present experiments was to explore possible mechanisms of this inhibition of dopaminergic agonist binding by ascorbate.

The inclusion of the non-hydrolyzable GTP analogue, Gpp(NH)p, to shift the affinity state of the receptors "right shifted" the IC_{50} for ascorbate inhibition of both D_1 and D_2 agonist binding. Kinetic experiments of both D_1 and D_2 agonist binding in the presence and absence of ascorbate failed to demonstrate any effect of ascorbate on the association rate. Conversely, the dissociation rate constants for both the D_1 agonist, [^{3}H] SKF 38393, and the D_2 agonist, [^{3}H] N-0437, were effected by the inclusion/exclusion of ascorbate at physiologically relevant concentrations.

These results are consistent with the hypothesized dopamine neuromodulator role for ascorbate and the relationship to an allostearic mechanism will be discussed.

282.14

REPEATED HALOPERIDOL AND ASCORBATE TREATMENTS INCREASE BASAL ASCORBATE BUT NOT BASAL DOPAC IN RAT NEOSTRIATUM. R. C. Pierce*, L.A. Shapiro, A.J. Clemens and G.V. Rebec, Prog. Neural Science, Dept. Psychology, Indiana Univ., Bloomington, IN 47405.

Growing evidence suggests a neuroleptic-like action of neostriatal ascorbate (Pierce et al., Neuroscience, 1991, 45: 373-378; Rebec et al. Science, 1985, 227: 438-440). Because neuroleptics exert maximal therapeutic effects with chronic treatment, we monitored extracellular ascorbate and DOPAC levels voltammetrically (via electrochemically-modified carbon-fiber electrodes) in the neostriatum of freely moving rats 24 hrs after the last of 21 daily treatments (s.c.) of 0.5 mg/kg haloperidol. Our results revealed that repeated haloperidol treatments increased basal neostriatal ascorbate to 350 μM (relative to the control value of 200 μM). Similarly, repeated daily administrations of ascorbate (500 mg/kg for 21 days, i.p.) significantly increased the extracellular concentration of neostriatal ascorbate (100 μM above control). Neither chronic haloperidol nor chronic ascorbate treatment altered basal neostriatal DOPAC. Taken together, these results suggest that increased basal neostriatal ascorbate may contribute to the antipsychotic effect of chronic haloperidol treatment.

Supported by NSF grant BNS 91-12055.

282.15

OPPOSING EFFECTS OF d-CPPene AND HALOPERIDOL ON ASCORBATE AND DOPAC LEVELS IN THE NEOSTRIATUM AFTER LONG-TERM AMPHETAMINE TREATMENT. M.E. Taylor*, C.T. Carter, and G.V. Rebec. Prog. Neural Science, Dept. Psychology, Indiana University, Bloomington, IN 47405.

Glutamate appears to regulate the amphetamine (AMPH)-induced release of ascorbate (AA) in the neostriatum (Basse-Tomusk and Rebec, Pharmacol. Biochem. Behav., 35:55, 1990). To assess the mechanisms underlying this process, we examined the effects of d-CPPene (SDZ-EAA-494), a competitive NMDA antagonist, on extracellular levels of AA and DOPAC in the neostriatum after acute or long-term AMPH treatment. Rats were prepared for freely moving voltammetry and received 7 days of either 2.5 mg/kg d-AMPH or saline treatment. On the following day, electrochemically modified carbon-fiber electrodes were lowered into the neostriatum and the rats received either d-CPPene (2.0 or 5.0 mg/kg), 0.5 mg/kg haloperidol, or saline. After 20 min all animals then received 2.5 mg/kg d-AMPH. Our results indicate that whereas acute and long-term AMPH treatment induced comparable increases in AA, CPPene potentiated this effect but haloperidol reversed it. In contrast, DOPAC was decreased in both the saline and CPPene treatments, but increased by the haloperidol treatment. Amphetamine-induced behaviors were reversed by haloperidol, and in CPPene animals, accompanied by ataxia. Collectively, this evidence suggests that AMPH-related changes in extracellular AA, which are reversed by haloperidol, are enhanced by competitive blockade of NMDA receptors.

Supported by NSF grant BNS 91-12055.

282.16

SEROTONIN INTERACTIONS WITH ASCORBATE INDUCED LIPID PEROXIDATION IN POSTMORTEM HUMAN BRAIN. A. C. Andorn* and R. Strong. Depts. of Psychiatry and Pharmacology, St. Louis Univ. Schl. of Med. and St. Louis VAMC.

Ascorbate (0.1 mM) induces lipid peroxidation (LP) as measured by the production of thiobarbituric acid positive reactants (TBAR) in particulate membrane fragments (PMF) derived from postmortem human brain. Using a standard photometric assay for TBAR with malondialdehyde as the standard, incubation of PMF from prefrontal cortex (PFC) at 0.2 mg protein with Na-Hepes (34.4 mM) and 0.1 mM ascorbate for 120 min at 37°C produces 19.4 ± 4.0 nmoles MDA per mg protein in young (< age 45) specimens (N = 12). Adding increasing concentrations of serotonin (5HT) to the incubation medium reduces the amount of MDA produced in the presence of ascorbate. No dose of 5HT enhances ascorbate stimulated LP in PFC. The logIC_{50} for 5HT inhibition of ascorbate stimulated MDA production is -5.14 ± an SEM of 0.07 (N = 3) for an IC_{50} of 7.1 x 10^{-6}M. In caudate and cerebellum, however, the dose-response relationship of 5HT and ascorbate induced LP is changed. Nanomolar doses of 5HT actually enhance ascorbate stimulated LP, even though the IC_{50} for the inhibition of ascorbate induced LP remains nearly the same in both caudate (2.7x10^{-6}M) and cerebellum (7.4x10^{-6}M). The PFC is an area rich in $5HT_1$ and $5HT_2$ receptors, while the caudate has a paucity of $5HT_2$ and the cerebellum, a virtual absence of both of these types of 5HT receptor. We hypothesize that there may be an interaction between 5HT receptors and LP in human brain.

282.17

ASCORBATE AND GLUTATHIONE ACT SYNERGISTICALLY TO MAINTAIN BRAIN SLICE ASCORBATE LEVELS AND TO PROTECT TISSUE FROM OXIDATIVE DAMAGE. M.E. Rice*, E. Lee & M.A. Pérez-Pinzón, Dept. Physiology & Biophysics, New York University Medical Center, New York, NY 10016.

Ascorbate, like glutathione, is an endogenous, water soluble antioxidant and free-radical scavenger. Ascorbate is rapidly lost from mammalian brain slices incubated in ascorbate-free media (Schenk et al. *Brain Res.* 253: 353, 1982). This loss leaves the tissue vulnerable to damage from reactive oxygen species in the hyper-oxygenated *in vitro* environment generated by equilibration with 95% O_2 / 5% CO_2. Here we describe incubation conditions necessary to maintain tissue ascorbate content, determined by HPLC-ED, in slices of rat cerebral cortex. Vibratome cut slices (400 µm) were incubated at room temperature for 6 hours in physiological saline with 0, 200 or 400 µM ascorbate (normal extracellular ascorbate concentration is 200-400 µM), with an equivalent level of thiourea, sodium bisulfite or glutathione. Intact cortex had an ascorbate content of 2.34 ± 0.07 µmol/g tissue wet weight (S.E.M., N = 26). With incubation in ascorbate-free media, ascorbate content fell to 23% of the intact value. This loss was inhibited by including ascorbate in the incubation medium, which maintained a 50% ascorbate level. The addition of a second antioxidant did not alter these levels, with one exception. Ascorbate content was maintained at nearly 100% of intact values when 400 µM glutathione was included with 400 µM ascorbate. Preliminary histological data suggested that maintenance of ascorbate content helped protect tissue from oxidative and glutamate-induced damage *in vitro*. These data demonstrate a synergism between ascorbate and glutathione and the benefit of their inclusion in *in vitro* media.

This study was supported by NIH Grant NS-28480.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION: CRF

283.1

PREFERENTIAL INDUCTION OF THE INTERMEDIATE-EARLY GENE, NGFI-B, IN HYPOTHALAMIC NEUROSECRETORY NEURONS IN RESPONSE TO STRESSES OR CORTICOSTEROID WITHDRAWAL. E.R. Brown*, R.K.W. Chan, K. Kovacs and P.E. Sawchenko, The Salk Institute, P.O. Box 85800, La Jolla, CA 92037.

The promoter regions of the rat CRF, oxytocin (OT) and vasopressin (AVP) genes contain sequences similar to the response element identified for NGFI-B, an intermediate-early gene structurally related to the steroid receptor superfamily. Combined immuno- and hybridization histochemical approaches were used to determine whether challenges that target CRF, OT and/or AVP secretion and expression result in alterations in expression in neurosecretory neurons of NGFI-B, and another intermediate early gene, *c-fos*, which is widely used in the neuroendocrine and other systems as a marker for functionally activated neurons. Unlike *c-fos*, NGFI-B mRNA was found to be expressed at constituitively high levels in the telencephalon, but not in the endocrine hypothalamus, of unperturbed controls. NGFI-B and *c-fos* mRNAs and Fos protein were induced with a similar time course and in similar cell types in response to acute hypotensive hemorrhage (15% blood volume), salt loading (7 days on 2% saline) and adrenalectomy (at 6, 12, 24, but surprisingly not at 48 h or longer). Fos mRNA and protein were readily demonstrable in afferent pathways we have implicated as mediating the neuroendocrine responses in the two stress paradigms; these include medullary catecholaminergic cell groups in response to hemorrhage, and cell groups lining the lamina terminalis in response to salt loading. By contrast, NGFI-B mRNA was expressed at much lower levels, if at all, in these relevant afferents. Unlike *c-fos*, NGFI-B appears to be a preferential marker for synaptic and/or transcriptional activation in aspects of the magno- and parvocellular neurosecretory systems.

283.2

EFFECTS OF ANDROGEN ON HYPOTHALAMIC CORTICOTROPIC-RELEASING HORMONE (CRH) AND CRH mRNA. E.W. Bingaman*, R.J. Handa. Dept. of Cell Biology, Neurobiology, and Anatomy, Loyola University, Chicago. Maywood, IL 60153.

The plasma corticosterone and ACTH response to novelty or foot shock stress is greater following long term gonadectomy of male rats. To further characterize the role of androgens in the regulation of the hypothalamo-pituitary-adrenal (HPA) axis we gonadectomized (gx'd) male Fischer 344 rats and treated them with dihydrotestosterone (DHT). Control animals were either gx'd and sham treated or left intact. After 3 weeks of treatment animals were sacrificed immediately following removal from their home cage. Radioimmunoassay of CRH in the hypothalamus showed a significant increase ($p < .05$) in CRH concentration in gx'd, sham-treated rats compared to intact animals (529.3±42.3 and 217.0±23.7 pg/mg protein, respectively). DHT-treatment of gx'd rats prevented this increase (220.5±58.0 pg/mg protein). CRH mRNA was examined by *in situ* hybridization histochemistry (ISHH) using an ^{35}S-labelled oligonucleotide probe complemented to nucleotides 496-543 of rat CRH mRNA. Image analysis of hybridization density in the paraventricular nucleus (PVN) showed no significant differences between the treatment groups. These data suggest the influence of androgens on the HPA axis occurs, at least in part, at the level of the hypothalamus, and changes in CRH content seen after 3 weeks of castration cannot be explained by concurrent changes in CRH mRNA in the PVN. Supported by NSF BNS9109226.

283.3

NEUROTENSIN AND IT'S ANALOGUE NT 8-13 ACTIVATES THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS: A ROLE FOR THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS.
W. Rowe*, M.J. Meaney, R. Quirion. Douglas Hospital Research Center, Dept. of Psychiatry, McGill University, Montreal, H4H 1R3, Canada.

Central administration of neurotensin (NT) has been shown to activate the hypothalamic-pituitary-adrenal (HPA) axis in rats as evidenced by increases in circulating ACTH and corticosterone (B). We investigated the effects of NT analogues (NT 1-8, and NT 8-13) on HPA activity as well as the possible site of action of NT. Intraventricular (icv) injection of the biologically active NT 8-13 into freely moving adult male rats caused significant increases in plasma ACTH and B compared to NT 1-8 and saline-treated controls. The temporal profile revealed peak activation of ACTH and B for NT 8-13 at 60 min following icv administration. This time point coincides with that previously reported for the parent compound NT 1-13 (Rowe et al. *Annals NYAS*. in press). Since NT 8-13 is capable of causing the same activation of ACTH and B as NT 1-13 , this suggests that it is acting via the well known pharmacologically characterized NT receptor. We found that irCRF content to be higher in NT-treated animals 60 min following injection. However, there was no change in irAVP. Taken together these findings suggest that NT's stimulatory effects on HPA activity occur, in part at the level of the paraventricular nucleus (PVN) of the hypothalamus to regulate the synthesis/release of CRF.

Eight days following electrolytic bilateral lesions of the PVN, NT (6 ng/μl) was administered icv to freely moving animals. PVN lesioning significantly attenuated ($p< 0.05$) the NT induced activation of ACTH and B compared to sham lesioned controls up to 4 hrs following injection: ACTH; 96.7 $\pm$ 5.1 vs 143.1 $\pm$ 19.6 pg/ml/min, respectively, B; 5.7 $\pm$ 1.3 vs 13.2 $\pm$ 2.9 μg/dl/min. This suggests that the PVN is required for the stimulatory action of NT on HPA activity.

283.4

CALCIUM DEPENDENCY OF *IN VIVO* RELEASE OF CORTICOTROPIN RELEASING FACTOR FROM RAT MEDIAN EMINENCE.
R. W. Gabr[*], D. L. Birkle[†] and A. J. Azzaro[*‡]. [†]Departments of Pharmacology and Toxicology/ [‡]Neurology/[*]Behavioral Medicine/Psychiatry, WVU, Morgantown, WV 26506.

Corticotropin releasing factor (CRF) has been characterized as a major neurotransmitter in many autonomic, neuroendocrine, and behavioral responses to stress (Nemeroff, 1991). The characteristics of endogenous CRF release from the anterolateral median eminence/arcuate nucleus were investigated using the technique of in vivo microdialysis. Male Sprague-Dawley rats (250-300 g) were acutely implanted with microdialysis probes in the anterolateral median eminence under chloral hydrate anesthesia (David Kopf stereotaxic coordinates: +6.1 anterior to interaural line, +.3 lateral to saggital sinus, and -9.8 from dura). A solution of artificial CSF containing 0.5% BSA was perfused through the probe at a flow rate of 1 μL per minute. Inclusion of ascorbate (200 μM) and aprotinin (25 μg/mL) in the perfusion media optimized recovery of CRF for analysis. Standards spiked into a pooled dialysate matrix were found to parallel the standard curve when subjected to centrifugal ultrafiltration. Release of CRF-like immunoreactivity into the probe was quantified by RIA with a detection limit of 2.5 pg per sample. Basal levels of CRF at 2 hours post-implantation averaged 12.1 pg/20 μL/20 min (n=9). Addition of 100 mM KCl to the perfusate caused a 3.5 - fold increase in CRF release. Because levels of CRF tended fluctuate following K^+-depolarization, a period of two hours between stimuli was necessary for basal levels to stabilize. K^+-evoked release was found to be inhibited by the L-type Ca^{++}-channel blocker verapamil (100 μM). In addition, a perfusate containing 100 μM of the calcium ionophore A23187 caused a significant increase in CRF release over basal levels. This demonstration of in vivo calcium dependency of evoked CRF release supports the findings of in vitro (Smith, 1986) and in vivo (Merlo Pich, 1991) experiments performed in other laboratories.

283.5

COORDINATE REGULATION BY THE VENTRAL LAMINA TERMINALIS OF OSMOTIC INFLUENCES ON CRF EXPRESSION IN MAGNO- AND PARVOCELLULAR NEUROSECRETORY NEURONS.
K. Kovács and P.E. Sawchenko*, The Salk Institute, La Jolla, CA 92037.

Chronic hyperosmolality results in a marked down-regulation of corticotropin-releasing factor (CRF) expression in parvocellular neurosecretory neurons and an up-regulation in magnocellular oxytocin (OT) neurons, such that after as few as 3 days of maintenance on 2% saline as a sole source of fluids, the pattern of CRF expression is virtually indistinguishable from that of OT. A variety of in situ approaches were used to identify the afferent pathways that mediate these effects. Five days of salt-loading resulted in prominent Fos protein induction in cell groups associated with the lamina terminalis, including the subfornical organ (SFO), the median preoptic nucleus (MePO) and the vascular organ of the lamina terminalis (OVLT). Unilateral knife cuts in the coronal plane, just caudal the OVLT and extending 2 mm laterally from the midline and 2 mm dorsally from the base of the brain, abolished the effects of 5 days of salt loading on CRF mRNA in both neurosecretory cell types on the ipsilateral side. Cuts in the horizontal plane that sever projections from the SFO and/or MePO to the hypothalamus did not alter the effects of salt loading on hypothalamic CRF mRNA. Finally, ibotentic acid lesions of the OVLT and immediately adjoining aspects of MePO abolished the effects of salt loading on CRF mRNA levels in the magno- and parvocellular neurosecretory systems. The ventral lamina terminalis is either the seat of, or an obligate relay for, osmosensitive neurons that appear capable of coordinately influencing CRF expression in two neurosecretory cell types.

283.6

EFFECT OF A GLUCOCORTICOID RECEPTOR ANTAGONIST (RU-38486) ON CORTICOTROPIN RELEASING HORMONE GENE EXPRESSION IN THE NEONATAL HYPOTHALAMUS.
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Mechanisms controlling the synthesis of corticotropin releasing hormone (CRH) in neonatal rat hypothalamus and the ontogeny of glucocorticoid (GC) feedback control of hypothalamic CRH have not been defined. Stress-induced up-regulation of CRH gene expression during the first postnatal week may be impaired, and GC feedback role at the hypothalamic level in the stress-hyporesponsive period is unclear.

We studied the ontogeny of the negative feedback regulation of CRH gene expression by GC in the paraventricular nucleus (PVN). We implanted chronic cannulae containing a GC-receptor antagonist, RU 38486, in neonatal rats on postnatal days 3, 4, 5, 7, 9, 10, 12 and 15. Three days later, animals were sacrificed, and brains were analyzed for CRH-messenger RNA (CRH-mRNA), using semi-quantitative in situ hybridization. Animals implanted with cholesterol-containing cannulae served to evaluate the stressful effect of implantation on CRH-mRNA abundance. The presence of GC receptor messenger RNA (GR-mRNA) in the PVN of neonatal rats was also determined.

RU 38486 did not increase CRH-mRNA abundance during the first postnatal week, despite the presence of GR-mRNA in the PVN. Chronic-implantation stress also failed to increase CRH synthesis during this period. CRH gene expression in the PVN was enhanced in infant rats implanted with either RU-38486 or cholesterol on postnatal day 9 or later. CRH-mRNA is enhanced by chronic blockade of GC receptors, compared to cholesterol, only subsequent to the eighth postnatal day. Furthermore, such blockade does not affect the response of CRH-mRNA to chronic stress in the neonatal rat.

283.7

REPEATED ECS PRODUCES A LONG-LASTING INCREASE IN CRH mRNA EXPRESSION IN RAT HYPOTHALAMUS.
Linda S. Brady*, Allison B. Lynn, John R. Glowa, Dung Q. Le, and Miles Herkenham. Section on Functional Neuroanatomy, Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

Electroconvulsive shock (ECS) therapy is effective in treating depressed patients that do not respond to antidepressant drugs. We used *in situ* hybridization histochemistry to examine the effects of repeated ECS in rats on the mRNA expression of CNS genes thought to be dysregulated in major depression. Treated rats received ECS (80 mA, 0.5 s) via ear-clips daily for 1, 3, 7, or 14 days. Control animals were treated similarly but received 0 mA. Rats were sacrificed 24 h after the last treatment. A single ECS treatment increased tyrosine hydroxylase (TH) mRNA levels in the locus coeruleus (LC) (200% of control), but did not alter corticotropin-releasing hormone (CRH) mRNA levels in the hypothalamic paraventricular nucleus (PVN). Repeated ECS (3, 7, or 14 daily treatments) increased TH mRNA levels further, with a peak effect at 7 treatments (367% of control). CRH mRNA levels in the PVN rose to their peak at 3 days (210% of control) and plateaued. Unlike TH mRNA which dropped, this elevation persisted at 4 weeks after discontinuation of ECS. The data suggest that alterations in activity of the hypothalamic CRH and LC-norepinephrine systems may be responsible for the therapeutic efficacy of ECS in the treatment of human depression.

283.8

IN SITU HYBRIDIZATION STUDIES OF AVT, IST AND CRF mRNAs IN THE GOLDFISH NEUROHYPOPHYSEAL SYSTEM
J.N. Fryer* and Y. Okawara. Department of Anatomy, University of Ottawa, 451 Smyth Road, Ottawa, Ontario, Canada K1H 8M5.

In goldfish, secretion of adrenocorticotropic hormone is stimulated by corticotropin releasing factor (CRF), as well as by arginine vasotocin (AVT) and isotocin (IST). Immunocytochemical studies of the goldfish hypothalamus have revealed that CRF and AVT are co-localized in perikarya of the nucleus preopticus (NPO).

We have established techniques to examine the regulation of CRF, AVT and IST gene expression in the goldfish hypothalamus by the localization of the messenger RNAs (mRNAs) for each of these peptides with *in situ* hybridization histochemistry. The structure of the goldfish genes and of the mRNAs encoding CRF, AVT and IST have not, as yet, been characterized. As hybridization probes, so-called guessmer oligonucleotide sequences were synthesized, based upon sequences deduced from precursor cDNA sequences determined for another Cypriniform fish, *Catostomus commersoni*. The oligonucleotide probes were non-radioactively labelled at the 3'-end with digoxigenin-11-dUTP using terminal deoxinucleotidyl transferase and the probe-mRNA hybrids visualized enzymatically with alkaline phosphatase conjugated to a digoxigenin antibody.

AVT mRNA was localized in some parvocellular (pc) and many magnocellular (mc) perikarya of the NPO with intermediate staining intensity. IST mRNA was distributed ubiquitously in pc and mc cell bodies with high staining intensity. AVT and IST mRNAs were co-localized in some mc perikarya. CRF mRNA was localized in scattered pc and mc perikarya with low staining intensity, and was co-localized with AVT or IST mRNA in some mc perikarya.

The present investigations lay the foundations for investigation of gene expression for hypothalamic neuropeptides regulating pituitary-adrenocortical function in the goldfish, a well established teleost endocrine model. (Supported by the M.R.C.)

283.9

PRIMARY CULTURES OF FETAL RAT AMYGDALA CONTAIN CORTICOTROPIN-RELEASING FACTOR (CRF) THAT IS RELEASED BY DEPOLARIZATION. M.S. Cratty, D.L. Birkle. Dept. of Pharmacology and Toxicology, West Virginia University, Morgantown, WV 26506.

CRF is a 41 amino acid peptide found in many brain regions. This peptide appears to play a pivotal role in stress, anxiety and depression. A limbic brain region that is of particular interest is the amygdala, which has high concentrations of CRF and is important in emotional and autonomic responses to stress. Few studies have examined the release of CRF from the amygdala. We used primary neuronal cultures prepared from amygdala/piriform cortex from embryonic (E18-20) rat brains to study the regulation of CRF release. Amygdala neurons were dissociated and plated in 35 mm wells. In these cultures, the presence of CRF-containing neurons was confirmed using indirect immunocytochemistry. CRF-like immunoreactivity was distributed in a bead-like fashion in about 1% of the neurons. After 15-20 days in culture, CRF release experiments were done by collecting samples of incubation media at various times. CRF was measured by radioimmunoassay. Basal CRF release was undetectable. Time dependent CRF release in response to 56 mM K^+ was detectable at one minute and reached a plateau at ten minutes. These data suggest that primary cultures are an effective model system to study the regulation of CRF release in the amygdala.

283.10

ELECTROPHYSIOLOGICAL ACTIONS OF CORTICOTROPHIN-RELEASING HORMONE (CRH) ON NEURONES OF THE PARAVENTRICULAR NUCLEUS (PVN) OF THE RAT *IN VITRO*. D.W.F. Porter* & Q.J. Pittman, Dept. Med. Physiol. & Neurosci. Res. Group, Univ. of Calgary, Alberta, Canada.

The PVN, pivotal to the neuroendocrine regulation of the HPA axis, contains both magno- and parvocellular neurones immunoreactive for CRH and/or AVP. These two neuropeptides act synergistically on pituitary corticotrophs to encode ACTH secretion in response to specific stress-related stimuli. Anatomical and functional studies suggest there is also an interaction of the two neuropeptides within the PVN itself. We therefore investigated the electrophysiological actions of CRH on PVN neurones in the in-vitro hypothalamic slice. Male Sprague-Dawley rats (80–100 g) were perfused intracardially under pentobarbital anaesthesia with oxygenated ice-cold high-sucrose, high-Mg^{2+} aCSF. The hypothalamus was excised and coronal slices (400 μm) containing the PVN were cut. Electrophysiological recordings were made from submerged slices perfused with aCSF (2–2.5 ml/min). Intracellular electrodes (~100–200 MΩ; 2 *M* K^+ acetate, containing 2% Neurobiotin) were directed visually to the PVN. Recordings of cells characterized as type-I (magnocellular; Tasker & Dudek, '91) on the basis of electrophysiological and immunocytochemical evidence showed that bath-application of CRH (10^{-6} *M*; 5 min) resulted in a long-lasting, but reversible, depolarization (10–25 mV), accompanied by an increased membrane resistance and firing of action potentials. CRH appeared to act directly on the recorded cells as the depolarization persisted in the presence of TTX (1 μg/ml). In a second group of cells, identified as type-II (parvocellular) cells, application of CRH resulted in a long-lasting hyperpolarization (~10–15 mV) with increased membrane resistance. Thus, CRH has contrasting actions on putative magno- and parvocellular neurones within the PVN.

Supported by the Medical Research Council of Canada.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION: POMC, ACTH, OTHER FACTORS

284.1

ENHANCEMENT OF TRANSIENT OUTWARD (A) CURRENT BY DEXAMETHASONE IN MOUSE CORTICOTROPE TUMOR (AtT-20) CELLS: CORRELATION WITH HORMONE RELEASE. A. J. Pennington, M. D. Woods, J. S. Kelly and F. A. Antoni (*SPON: Brain Research Association*) Department of Pharmacology and MRC Brain Metabolism Unit, Univ. of Edinburgh, Edinburgh, EH8 9JZ, U.K.

Glucocorticoids suppress stimulated corticotropin (ACTH) release in AtT-20 cells by inducing new protein(s). The aim of the present study was to investigate whether glucocorticoid-mediated inhibition is brought about by changes in membrane currents. The nystatin-perforated whole-cell patch configuration was used to record K^+ currents from AtT-20 D16:16 cells at room temperature. In separate studies, hormone secretion at 37°C was measured by radioimmunoassay. Four types of K^+-current could be discerned: 1)A transient outward (A-type) current that was fully blocked by 1mM 4-aminopyridine (4-AP) and partly suppressed by 30 mM tetraethylammonium (TEA); 2) a delayed rectifying component sensitive to TEA, 3) a delayed rectifying component blocked by apamin and 4) a residual delayed rectifying component not blocked by the combination of 30mM TEA and 100nM apamin. Treatment with 100 nM dexamethasone (DEX) for 2h markedly increased the proportion of cells with A-current (Contr:10/33 cells; DEX:13/19 cells, $P< 0.005$, chi^2 test), moreover, the size of A-current evoked by a -60mV to +50 voltage step was enhanced (Contr: 30.7±4.5 n=8 ; DEX: 44±4 pA/pF, n=12 mean±SE, $P<0.05$, t-test). In secretion studies, ACTH release induced by corticotropin releasing-factor (CRF) was strongly inhibited by DEX (EC_{50} 3 nM, 70-100 % inhibition at 100 nM). Hormone release was also stimulated by TEA (5-25 mM). DEX inhibited the response to TEA (EC_{50} 50 nM) much less potently than that to CRF. 4-AP potentiated the ACTH releasing effect of CRF and also reduced the potency of dexamethasone (2-3-fold). Hormone release induced by 50mM KCl or by the combination of TEA and 4-AP was largely resistant to inhibition by dexamethasone. These data suggest that treatment with dexamethasone enhances potasssium currents in AtT-20 cells, and that these changes are instrumental to the inhibition of hormone secretion.

284.2

EARLY GLUCOCORTICOID INHIBITION IN MOUSE CORTICOTROPE TUMOR (AtT-20) CELLS: INDUCTION OF CALMODULIN BUT NO CHANGE IN LIPOCORTIN (ANNEXIN) I mRNA OR PROTEIN. M.J. Shipston, E.L. Mullens^, S.L. Lightman^*, M.D. Woods, and F.A. Antoni. MRC Brain Metabolism Unit, Department of Pharmacology, University of Edinburgh, Edinburgh, U.K. and ^Neuroendocrinology Unit, Charing Cross and Westminster Medical School, London, U.K.,

Early inhibition (<2h) of secretagogue-stimulated adrenocorticotropin (ACTH) release by glucocorticoids involves the induction of new mRNA and protein. In the present study we have investigated whether calmodulin and the purported "second messenger" protein of glucocorticoid action, lipocortin I, may be involved in this process. AtT-20 D16:16 cells (passage 15-35) were used in all experiments, ACTH was measured by radioimmunoassay. Corticotropin-releasing factor (CRF, 10 nM) stimulated ACTH release to 2-3-fold above basal. Preincubation with dexamethasone dose-dependently inhibited CRF-induced ACTH release (EC_{50} ca.3nM) within 45 min, maximal effect was evident at 120 min. The inhibitory action of dexamethasone was prevented by blockers of mRNA and protein synthesis. Using a ^{32}P-labeled 0.4 kbp fragment of mouse calmodulin (pCAM2) Northern blots of total RNA revealed that treatment with dexamethasone increased the level of a ca. 1.6kb mRNA to about 5-fold within 45 min. No change in the level of lipocortin I mRNA was observed using a ^{32}P-labeled 1.37 kbp human lipocortin I cDNA probe. Immunoblotting for lipocortin I with a monoclonal antibody showed no expression of this protein before or after treatment with dexamethasone in D16:16 cells. In contrast, both lipocortin I protein and mRNA could be readily detected by these methods in AtT-20 D1 cells, but no induction by dexamethasone could be found. In summary, lipocortin I mRNA or protein were undetectable in AtT-20 D16:16 cells at a time when glucocorticoid mediated inhibition was maximal. In contrast, a marked induction of calmodulin mRNA occurred, the significance of which requires further study.

284.3

MICROINJECTIONS OF GALANIN ALONG THE LATERAL BORDER OF THE LOCUS COERULEUS INHIBIT PLASMA ADRENOCORTICOTROPIN IN CATS. D. E. Carlson* and D. S. Gann. Departments of Surgery and Physiology, University of Maryland School of Medicine, Baltimore, MD 21201.

The locus coeruleus (LC) has been implicated in the control of adrenocorticotropin (ACTH) release in cats. In the rat, galanin-containing processes that originate from the dorsal medulla have been identified in the LC and the laterally adjacent parabracheal nucleus (PBN). To determine if galanin-like activity is present in these areas in the cat, immunocytochemistry was employed. Several areas of the PBN had dense distributions of reactive processes. In contrast, a less dense distribution of such processes was found to extend medially into the lateral locus coeruleus. Eleven acutely prepared, chloralose-anesthetized cats were then tested with microinjections of galanin (10 μM, 100 nl/min, 2 min) into these nuclei. Injections at five sites along the lateral border of the LC elicited significant inhibition of plasma ACTH ($P<0.01$, ANOVA) at 2 (-17.8±4.6 pg/ml), 4 (19.0±7.0 pg/ml), and 6 min (-19.6±6.8 pg/ml) after the onset of injection. This response differed significantly from that to vehicle at the same 5 sites ($P<0.05$) and from that to galanin at 10 sites that were remote to the lateral LC ($P<0.01$). Furthermore, mean arterial pressure did not change during the injection of galanin along the lateral LC. The results suggest that the galanin-containing processes in the lateral LC drive an ascending pathway to the hypothalamus that modulates the release ACTH. Supported in part by DK-26831.

284.4

ELEVATED ANTERIOR PITUITARY PROOPIOMELANOCORTIN (POMC) GENE EXPRESSION AT LIGHTS OFF IN AGED RATS WITH SPATIAL MEMORY IMPAIRMENT. N. Levin*, N. Bengani, W. Rowe, M.J. Meaney, J.L. Roberts. Fishberg Res Ctr Neurobiol, Mount Sinai Sch Med, NY, NY 10029 USA & Douglas Hosp Res Ctr, Montreal, Quebec, Canada.

Several studies have reported elevations in basal or stress-induced plasma corticosterone (Cort) levels in aged rats. The present studies were undertaken to determine the effects of age-related elevations in plasma Cort on anterior pituitary (AP) POMC gene expression. A population of 24-26 month-old Long-Evans rats was subdivided into aged-impaired (AI) or aged-unimpaired (AU) groups based on behavioral screening using a test for spatial memory. See Sarrieau et al, abstract submitted to the 1992 Neuroscience Meeting, for a complete description of this paradigm and the hormonal status of these animals. AU, AI and young (Y) rats were decapitated under basal conditions at the nadir (AM) or peak (PM) of the circadian rhythm in HPA axis activity. Steady state levels of AP POMC nuclear RNA primary transcript and cytoplasmic mRNA were determined by solution hybridization/nuclease protection analyses.

Group, time (n)	POMC primary transcript pg/0.5 AP (±SEM)	POMC cytoplasmic mRNA pg/μg RNA (±SEM)
Y, AM (11)	67.7±5.8	318.1±89.2
Y, PM (2)	62.2±3.2	105.8±39.5
AU, AM (7)	68.0±3.4	267.1±62.1
AU, PM (2)	70.4±4.8	110.3±11.7
AI, AM (7)	71.1±10.5	311.6±67.5
AI, PM (5)	117.8±22.6**	196.5±24.7

There was a significant increase in AP POMC gene expression in the PM in AI rats (**$p<0.05$ versus AU, PM and Y, PM), consistent with an increase in ACTH secretion in the PM in AI rats, with respect to AU or Y rats (see Sarrieau et al). From these initial data it appears that the decrease in cytoplasmic mRNA levels in the PM in Y and AU rats is not achieved in AI rats, despite elevated PM plasma Cort levels in AI rats (see Sarrieau et al). These data suggest that there is a greater than normal increase in POMC gene expression in the PM in AI rats, possibly due to a decreased sensitivity of the HPA axis to Cort feedback inhibition in AI rats.

284.5

HYPOTHALAMIC-PITUITARY-ADRENAL ACTIVITY AND CORTICOSTEROID RECEPTOR EXPRESSION IN AGED, COGNITIVELY-IMPAIRED AND COGNITIVELY-UNIMPAIRED RATS. A. Sarrieau[1], W. Rowe[1], D. O'Donnell[1], S. LaRocque[1], N.P.V. Nair*[1], N. Levin[2], J.R. Seckl[3], and M.J. Meaney[1]. [1]Douglas Hosp. Res. Ctr., Depts. of Psychiatry, and Neurology and Neurosurgery, McGill Univ., Montreal, Canada H4H 1R3. [2]Dept. of Neurobiology, Mount Sinai Medical Ctr., New York, NY 10029. [3] Dept. of Medicine, Western Gen. Hosp., Univ. of Edinburgh, Edinburgh EH4 2XU, Scotland.

Increased glucocorticoid levels have been associated with hippocampal neuron loss and cognitive impairments in rats. Among 24-26 month old rats increased hypothalamic-pituitary-adrenal (HPA) activity is selectively associated with hippocampal dysfunction. Aged, cognitively-impaired (AI) rats show increased glucocorticoid secretion, while aged, cognitively-unimpaired (AU) rats do not differ from young animals. In the present study we found that AI animals show elevated plasma levels of both ACTH and ß-endorphin compared with both AU and young animals, but only in the dark phase of the cycle. In addition, AI animals show increased median eminence levels of both CRF and, to a greater extent, AVP; again only in the dark phase of the cycle. Previous studies (Sapolsy et al. Neuroendo., 1990; Herman et al., J. Neurosci., 1989) have shown that glucocorticoids (via both mineralocorticoid and glucocorticoid receptors) feedback at the level of the hypothalamus and hippocampus to regulate CRF and AVP synthesis. Interestingly, AI animals show decreased hippocampal mineralocorticoid and glucocorticoid receptor density; receptor mRNA levels are currently being analyzed from in situ hybridization studies. These studies suggest that the loss of hippocampal neurons and the decreased sensitivity to circulating corticosterone result in increased synthesis of ACTH secretagogues and elevated pituitary-adrenal activity in AI animals.

284.6

A COMPARISON OF HYPOTHALAMIC ACTH SECRETAGOG CONTENT IN THE MEDIAN EMINENCE AFTER COLCHICINE BLOCKADE TO PORTAL SECRETAGOG RELEASE. L. Michael Romero,*[1] Paul M. Plotsky,[2] Steven W. Sutton,[2] and Robert M. Sapolsky[1] [1]Depart. of Biological Sciences, Stanford Univ., Stanford CA 94305; [2]Peptide Biology Lab, Salk Institute, La Jolla, CA 92037.

Hypothalamic portal cannulation studies indicate that release of ACTH from the anterior pituitary is controlled by several secretagogs, primarily corticotropin releasing factor (CRF), arginine vasopressin (AVP), and oxytocin (OT). The precise patterning of each of these secretagogs appears to be determined by the stressor applied, indicating that various stress afferents are integrated at the median eminence (ME) to produce a coordinated ACTH response to variety of different stressors. Unfortunately, the invasiveness of portal vessel cannulation precludes measuring secretagog patterns in an awake animal undergoing psychological stressors. To circumvent this, we modified the technique of Berkenbosch and Tilders (Brain Research 442:312, 1988). We injected colchicine into the lateral ventricles of freely-behaving rats to disrupt secretagog resupply to the ME and compared decreases in the ME content of CRF, AVP, and OT to various stressors. In first comparing this technique to published portal data we discovered that awake rats show a different secretagog output to insulin-induced hypoglycemia than that seen with portal cannulation. In agreement with Berkenbosch *et al.* (Endocrinology 125:28, 1989), we found that both CRF and AVP content declined in the ME during hypoglycemic stress. In a novel observation, we see no changes in OT content. This contrasts with our prior findings using the portal cannulation technique (Plotsky *et al.* Endocrinology 117:323, 1985) showing that only AVP is released into the portal vasculature in response to insulin-induced hypoglycemia. Although it would seem *a priori* that measuring actual secretagog output into the portal vasculature would be preferable to measuring changes in ME content, the invasiveness of portal cannulation surgery makes results difficult to interpret. It is unclear, therefore, which technique produces the more physiological results. It is, however, impossible to assess ME integration of a psychological stress response with a portal preparation. Preliminary data of ME secretagog content indicate that the psychological stressor of restraint has a distinct pattern of secretagog output.

284.7

THE ROLE OF TYPE I AND TYPE II ADRENAL STEROID RECEPTORS IN MAINTENANCE OF BASAL ACTH LEVELS. R.L. Spencer*, A.H. Miller, H. Moday, M. Stein and B.S. McEwen. Lab. of Neuroendo., Rockefeller Univ., and Dept. Psychiatry, Mt. Sinai School of Med., New York, NY.

Adrenalectomy of rats results in an increase in basal ACTH levels, which can be normalized by glucorticoid treatment. The subtype and location of the adrenal steroid receptors responsible for maintaining normal ACTH levels have not yet been fully determined. We have treated adrenalectomized (ADX) male Sprague-Dawley rats (250-300 g) with the selective Type I receptor agonist, aldosterone (ALDO), or the selective Type II receptor agonist, RU28362, (1, 4, or 10 µg steroid/h, s.c., Alzet miniosmotic pump, n = 5). After 7 days of steroid replacement, ADX rats and sham ADX rats were sacrificed 2 h into their light period. Trunk blood was collected for RIA determination of ACTH levels, and the hippocampus, hypothalamus, and pituitary were removed for measurement of available cytosolic Type I and Type II adrenal stereoid receptor binding. Plasma ACTH levels of 7 day ADX rats were 438±92 pg/ml compared to 55±7 pg/ml in sham ADX rats. RU28362 normalized ACTH levels, whereas none of the doses of ALDO (high dose plasma ALDO = 3.2±0.7 µg/dl) reduced ACTH from ADX levels. The high dose of ALDO occupied/activated more than 90% of the Type I receptors in the hippocampus and the pituitary. RU28362 occupied/activated Type II receptors in a dose-related manner while having no effect on Type I binding. These data suggest that activation of the Type II receptor is sufficient to maintain normal basal ACTH levels. The prospect that Type I receptor activation by corticosterone may have effects on basal ACTH secretion that were not apparent with Type I activation by ALDO is under investigation. (Supported by MH47674)

284.8

MICROINJECTION OF NICOTINE INTO BRAINSTEM REGIONS ELEVATES PLASMA ACTH. B.M. Sharp, F.J. Wilson* and S.G. Matta, Endocrine-Neuroscience Lab and Depts. of Med., Hennepin County Med Center and Univ of Minn, Mpls, MN 55404 and UMDNJ-RWJohnson Med. Sch., Piscataway, NJ 08854.

Nicotine is a potent secretagogue for ACTH; this response appears to be mediated by central catecholamine secretion. We have previously shown that fourth ventricular administration of nicotine rapidly elevated plasma ACTH levels and that a nicotinic cholinergic antagonist, mecamylamine (MEC), instilled into the fourth ventricle, inhibited the ACTH response to i.v. nicotine. Thus, the present investigations sought to identify nicotine-responsive regions in the brainstem which result in ACTH secretion and give rise to the ascending catecholaminergic afferents. Chronic brain and jugular cannulae were implanted and nicotine (50 nl over 30s) was infused into the locus coeruleus (LC), nucleus of the solitary tract (NTS), C_1, or A_1 cell regions of freely moving adult male rats. Injection of nicotine (free base; 0.1-10 µg) into NTS resulted in a dose-dependent increase in plasma ACTH. Injection into A_1 or C_1 cell groups only showed responses to the highest dose of nicotine in C_1. In LC, nicotine induced a dose-dependent elevation of plasma ACTH in response to 2.5, 5.0 and 10.0 µg. MEC (0.1 mg/kg bw, i.v.), administered 2 min prior to nicotine (10 µg into LC), inhibited the 7 min peak ACTH response (60% inhibition of the net response). Taken together with our previous reports, both the LC and NTS appear to mediate the ACTH response to systemic or fourth ventricular nicotine. (Supported by DA 03977)

284.9

INDUCTION OF c-FOS PROTEIN BY NICOTINE IN THE RAT BRAIN. S.G. Matta* and B.M. Sharp, Endocrine-Neuroscience Lab and Depts. of Medicine, Hennepin County Medical Center and University of Minnesota, Mpls, MN 55404.

Nicotine is a potent secretagogue for ACTH and this response appears to be mediated by central catecholamine secretion. We have previously shown that fourth ventricular (IV) administration of nicotine rapidly elevated plasma ACTH levels and that a nicotinic cholinergic antagonist, instilled into the IV, inhibited the ACTH response to i.v. nicotine. The present investigations sought to identify brain sites which were responsive to nicotine as evidenced by the induction of c-Fos protein. Rats received nicotine sulfate [2.0 mg/kg bw i.p., 0.05 or 0.10 mg/kg bw i.v. (via indwelling jugular cannulae)] or saline, 60 min prior to cardiac perfusion with 2.5% acrolein + 4% paraformaldehyde in phosphate buffer, pH 7.2. Free-floating, frozen sections (20µ) were incubated for 48h with c-Fos antiserum (Oncogene Science; 1:2500) and stained by the ABC method. The c-Fos response showed regional specificity and varied with dose. In the hypothalamus, paraventricular nucleus (predominantly parvocellular) and supraoptic nucleus were positive compared to saline. In brainstem, locus coeruleus and nucleus of the solitary tract (NTS) were positive, whereas A_1 and C_1 regions were negative. The greatest staining in the brain, in response to i.v. nicotine, was evident in the dentate gyrus, followed by CA_3 region of the hippocampus. In cortex, cingulate gyrus was positive after i.v. and i.p. nicotine, whereas piriform cortex only stained in response to i.p. nicotine. These findings suggest that locus coeruleus and NTS may be directly activated by systemic nicotine, leading to stimulation of cells within the paraventricular nucleus which depend on ascending catecholaminergic afferents from these brainstem regions. (Supported by DA 03977)

284.10

PERIPHERAL AND CENTRAL SEROTONIN$_2$ RECEPTOR ACTIVATION DIFFERENTIALLY REGULATES ADRENOCORTICAL SECRETION. Jon E. Welch and David Saphier*, Dept. of Pharmacology, LSU Medical Center, P.O. Box 33932, Shreveport, LA 71130.

Serotonin (5-HT) is generally considered to exert a facilitatory effect in the regulation of adrenocortical secretion in the rat, with several studies indicating that central 5-HT$_{2\backslash 1C}$ receptor activation mediates such responses because agonists increase plasma corticosterone (CS) concentrations. 20 min following either intraperitoneal (ip) or intracerebroventricular (icv) injection of selective 5-HT$_{2/1C}$ receptor agonists or antagonists, blood was collected for measurement of plasma CS concentrations. IP administration of the 5-HT$_2$ agonist (±)DOI increased plasma CS concentrations, as did icv administration at the higher doses tested. ICV administration of *m*-CPP, an agonist with greater selectivity for 5-HT$_{1C}$ receptors, failed to alter adrenocortical secretion. ICV administration of the 5-HT$_2$ antagonist ketanserin dose-dependently increased adrenocortical secretion, suggesting that 5-HT tonically inhibits adrenocortical secretion through 5-HT$_2$ receptors. Central administration of ketanserin did not block the plasma CS increases observed after ip (±)DOI, suggesting that the adrenocortical increases were not due to central 5-HT$_2$ receptor activation. In sodium pentobarbital anesthetized rats, icv (±)DOI decreased adrenocortical secretion at all doses tested, whereas, ip administration continued to increase adrenocortical secretion. ICV ketanserin again did not block the plasma CS responses to ip (±)DOI in anesthetized rats. 6-OHDA-induced lesions of the ventral noradrenergic ascending bundle abolished the ip response to (±)DOI suggesting that (±)DOI interacts with central noradrenergic systems to increase adrenocortical secretion. These results suggest that central and peripheral 5-HT$_2$ receptors differentially regulate adrenocortical secretion, the central component being sensitive to pentobarbital anesthesia and the peripheral component dependent upon central noradrenergic systems.

284.11

EFFECTS OF COCAINE ON ACTH SECRETION, STRIATAL C-FOS EXPRESSION, AND LOCOMOTOR SENSITIZATION IN AWAKE AND ANAESTHETIZED RATS. German Torres,* Catherine Rivier, and Friedbert Weiss*. The Salk Institute, The Clayton Foundation Laboratories for Peptide Biology, and *The Scripps Research Institute, Department of Neuropharmacology, San Diego, CA 92186.

The response of ACTH secretion, striatal immunoreactive c-fos expression and locomotor activity to cocaine exposure was studied in awake rats and in animals anaesthetized with a combination of ketamine hydrochloride, acepromazine, and xylasine. In the absence of anaesthesia, acute cocaine exposure significantly increased plasma ACTH secretion and the expression of c-fos within neurons of the caudate putamen. In contrast, the aforementioned neurochemical or neuroanatomical effects were not observed in rats anaesthetized with ketamine. Daily injections of cocaine administered to awake rats for 5 days caused the well documented locomotor sensitization. However, when the rats were anaesthetized before each daily cocaine injection, the behavioral effects normally associated with the progressive and potent enhancement of locomotor behavior was absent. By itself, ketamine did not measurably alter pituitary or striatal metabolic function as reflected by the integrity of responsiveness of these loci to subsequent pharmacological challenges. These observations suggest that the effects of cocaine in the rat brain may depend at least in part on sensorimotor mechanisms, and on the participation of common excitatory neurochemical systems in both hypothalamic and striatal systems. *(Supported by NIDA DA 05602)*

284.12

WITHDRAWN

284.13

THE ROLE OF c-*fos* IN THE CELLULAR REGULATION OF POMC. B.B. Ruzicka* and H. Akil, Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan, 48109-0720.

While the integrative function of the hypothalamic-pituitary-adrenal (HPA) axis during stress is fairly well understood, less clear is how the pituitary corticotroph integrates the signals it receives during stress to coordinate the secretion and biosynthesis of relevant peptides. The objective of this work was to study the role of the proto-oncogene, c-*fos*, in the cellular regulation of pro-opiomelanocortin (POMC). Mouse anterior pituitary tumor (AtT-20) cells were plated (10^6 cells/100 mm plate) in DMEM (+2.5% FCS) and grown until 70% confluent. The cells were then exposed to corticotropin releasing factor (CRF) (10nM) for various times (5min-48h) and analyzed for levels of ß-endorphin (ßE) and ß-lipotropin (ßLPH) secretion, content and POMC and c-*fos* mRNA. CRF increased ßE release at all time points when compared to cells not exposed to CRF. The maximal response, representing a 4-fold increase in ßE release, occurred after 0.5h of CRF stimulation. Generally, CRF evoked only small, if any, increases in ßLPH release, with the maximal response (2-fold increase) occurring at 1h. However, CRF produced biphasic and parallel changes in the contents of ßE and ßLPH: these changes, which were more pronounced with ßE, reflected an initial (at 5-15 min) decrease (~50%) in peptide content followed by an increase towards control levels at 0.5-1h of CRF exposure. A second decline in peptide content, observed at 2-4h, was followed by a gradual increase to beyond control levels (1.5-2-fold) by 12h. Thereafter, peptide levels returned to control levels. c-*fos* mRNA was elevated as early as 5min after CRF treatment, was maximally induced (3-fold) at 1-2h and returned to control levels by 12h. The temporal relationship between CRF-induced changes in c-*fos* and POMC mRNA are currently under investigation. The present data show that CRF alters the secretion and content of POMC-derived peptides and c-*fos* gene expression. Experiments conducted in the presence of anti-sense c-*fos* RNA should provide more conclusive data regarding the role c-*fos* may play in the cellular regulation of POMC. Supported by NIMH grant PO1MH42251. B.B.R. is the recipient of a Medical Research Council of Canada post-doctoral fellowship.

284.14

INTERLEUKIN 1 INDUCES POMC VIA c-FOS AND c-JUN

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We have demonstrated that IL-1 after a long period of treatment stimulates ß-endorphin release and potentiates the effects of other secretagogues in AtT-20 cells, a mouse anterior pituitary cell line. The mechanism of transduction of this effect was examined. IL-1 markdly increases the precursor of ß-endorphin, proopiomelanocortin (POMC), over a period of 24 hours. The role of intermediate early genes c-fos and c-jun on the ability of IL-1 to induce POMC was determined. We found that an early signal triggered by IL-1 in AtT-20 cells involves the enhancement of c-fos and c-jun mRNA expression. The treatment of the cells with antisense oligonucleotides to c-fos and c-jun had no effect on the ability of IL-1 to induce POMC synthesis. However when antisense to c-fos and c-jun were added together to AtT-20 cells, IL-1 no longer induced POMC. Nifedipine, a calcium channel blocker abolished the ability of IL-1 to increase POMC. These findings show that the effect of IL-1 on c-fos and c-jun is a necessary step in IL-1 inducing POMC mRNA.

284.15

EFFECTS OF CORTICOSTERONE ON HIPPOCAMPAL 5HT AND SPATIAL MEMORY. V. Luine*, R.L. Spencer and B.S. McEwen. Dept. of Psychology, Hunter College, and Lab. of Neuroendocrinology, Rockefeller University, NY, NY 10021.

The effects of chronic corticosterone (CORT) treatment on spatial memory performance and serotonergic function in the hippocampus were investigated in ten month old, male Fisher 344 rats. Rats initially received 20 training trials on the 8-arm radial maze and then were split into a control group and a group that received CORT in their drinking water (400 µg/ml) for 8 weeks. Serum CORT levels were 25±6 µg% midway through lights off. Two weeks following treatment, another 20 trials of maze testing were given, and then 5HT and its metabolite, 5HIAA, and NE were measured by HPLC in CA1, CA3 and dentate gyrus (DG) of the hippocampus, frontal cortex (FCtx) and n. basalis. Post treatment maze testing showed no overall effect of CORT on trials to criterion or choice accuracy measures. However, performance in the CORT group was heterogeneous and several rats were impaired relative to all subjects. These impaired rats had consumed the most CORT during treatment. CORT treatment increased 5HT (44%) in the DG and decreased 5HT and NE in the FCtx (50%). In addition, levels of 5HT AND 5HIAA in some hippocampal areas was correlated with performance. Performance of both groups combined was negatively correlated with 5HT levels in CA1 ($p<0.05$), and in the DG, higher 5HIAA levels in the CORT group were correlated with lower correct choice scores and earlier mistakes ($p<0.01$). Thus, prolonged CORT ingestion is associated with altered forebrain and hippocampal 5HT, and some of these changes correlate with impaired spatial memory performance. (Supported by NS07080 and HD12011).

284.16

SEPTAL LESIONS INCREASE SUBMISSIVE BEHAVIOR AND HPA ACTIVITY IN HAMSTERS. B.N. Bunnell*, M.H. Hebert, B.R. Metcalf, E.H. Mougey[1], and J.L. Meyerhoff[1]. Dept. of Psychology, U. of Georgia, Athens, GA 30602 and [1]Walter Reed Army Inst. Research, Washington, DC 20307.

Submissive hamsters have elevated levels of POMC-derived hormones. Septal lesions increase submission by increasing approach behaviors toward aggressive opponents. To see if lesion-induced increases in submissive behavior would result in increased HPA activity, 12 male hamsters received septal lesions and 8 served as operated or normal controls. After 14 days recovery, jugular catheters were placed in all males. 48 hours later they began five days of social tests against ovariectomized female opponents. Each test lasted 15 min. and blood samples were obtained immediately after the tests on days 1, 3, and 5. Plasma was assayed for cortisol and ACTH. Social behavior was scored by two observers, using a 30 category inventory. Each male faced 1 of 2 females on different days. All males became submissive to their female opponents. Those with lesions of the medial septal nuclei made significantly more submissive responses per aggressive act by their opponents and had significantly elevated cortisol and ACTH compared to controls. The magnitude of the hormone changes reflected the increases in frequency of submissive behaviors. There was no indication that the lesions themselves alter normal HPA activity in stressful situations.

284.17

REGULATION OF BRAIN VASOPRESSIN RECEPTORS BY GLUCOCORTICOIDS. V.K. Patchev[1], O.F.X. Almeida[2] and G.P. Chrousos[1*]. [1]SPE, DEB, NICHD, Bethesda, MD 20892 and [2]Max Planck Institute of Psychiatry, Munich, Germany.

Arginine-vasopressin (AVP) plays significant roles in neuroendocrine regulation and cognitive information processing. AVP, along with CRH, is a major regulator of ACTH and glucocorticoid secretion. Hippocampus (HIPP) and hypothalamus (HT) are principal target sites of glucocorticoid neuroregulatory effects and contain significant concentrations of AVP receptors. We investigated the effects of bilateral adrenalectomy (ADX) and subsequent replacement with supraphysiological doses of corticosterone or dexamethasone on the binding characteristics of AVP receptors in HIPP and HT of male rats. ADX significantly decreased AVP receptor concentrations in both structures. Interestingly, chronic glucocorticoid treatment was associated with further suppression of receptor concentrations in HIPP, but had no effect on the receptors in HT. Thus, AVP receptors in the brain are regulated by glucocorticoids, however, HIPP and HT receptors differ in their responsiveness to chronic glucocorticoid administration.

284.18

CALCIUM-MEDIATED METABOLIC STIMULATION OF NEUROENDOCRINE STRUCTURES BY CENTRAL ENDOTHELIN-1 IN RATS.

D.S. Wainman, D.F. Weaver, F.J. Espinosa and P.M. Gross*, Departments of Surgery, Medicine, Chemistry & Physiology, Queen's University & Kingston General Hospital, Kingston, Canada K7L 3N6

Although the cerebral distribution of binding sites for the endothelium- and brain-derived peptide, endothelin-1 (ET), is known (Kohzuki et al., *Neuroscience* 42:245, 1991), functional responses in the nervous system to systemic or central ET have not been well characterized. We reasoned that the effects of ET would be particularly distinct in neuroendocrine structures of the hypothalamo-hypophysial system in which ET appears to be a native molecule (Yoshizawa et al., *Science* 247:462, 1990). We applied the quantitative autoradiographic [^{14}C]deoxyglucose method to assay focal rates of cerebral and pituitary glucose metabolism (GM) in conscious male Sprague-Dawley rats given saline or ET either intravenously (14 nmol/min, iv) or centrally (9 pmol in 3 µl, icv) via a lateral cerebral ventricular cannula. Compared to values for saline-infused rats, iv ET produced no significant alterations in GM among forebrain neuroendocrine structures, except for substantial increases (106-219%) in the pituitary anterior and intermediate lobes (*Endocrinology* 129:1110, 1991). Compared to saline effects, icv injection of ET evoked diverse neuroendocrine stimulation, including the subfornical organ, median eminence, paraventricular n., supraoptic n., periventricular n., ventromedial n., and all lobes of the pituitary gland (range of GM increases was +30-109%). These stimulatory effects of ET were inhibited by central pretreatment with the Ca^{2+} L-channel antagonist, nimodipine (72 nmol in 1 µl, icv). The results identify that central, but not peripheral, ET produces a Ca^{2+}-mediated hypermetabolism of several forebrain neuroendocrine structures. ET, therefore, is a neuroendocrine stimulant centrally, whereas its functional effects as a circulating hormone acting on neuroendocrine activity may be important only in the intermediate and anterior lobes of the pituitary gland.

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284.19

EFFECT OF DEXAMETHASONE ON THE CONDITIONED ENHANCEMENT OF NATURAL KILLER CELL ACTIVITY. **C. Hsueh, R. Hiramoto, S. Christian*, and V. Ghanta. Departments of Biology, Microbiology, and Psychiatry, Univ. of Alabama at Birmingham, Birmingham, AL 35294.**

The involvement of hypothalamic-pituitary-adrenocortical (HPA) axis in conditioned enhancement of natural killer (NK) cell response was examined. Dexamethasone (Dex, at 100 µg/kg) was injected intraperitoneally into conditioned animals prior to the conditioned stimulus/uncoditioned stimulus (CS/US) association or before reexposure to the CS. Conditioned enhancement of NK cell response was blocked by Dex given at recall (test) but not at the association. The results indicated that activation of the HPA activity was required to elicit the conditioned NK cell response at recall but not at the association level. However, the central stimulatory effect of methionine-enkephalin (Met-Enk) on NK cell activity was not blocked by prior injection of Dex. Methionine-enkephalin either exerts its effect independently of the HPA axis or it acts through the HPA axis by overriding the inhibitory effect of Dex on the HPA axis.

Supported by NIH grant CA37570 and ACS grant IM-509.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION: LHRH AND LH II

285.1

RAPID SUPPRESSION OF GLUCOSE AVAILABILITY REDUCES FOS EXPRESSION IN GnRH NEURONS IN SYRIAN HAMSTERS. S.J. Berriman* and G.N. Wade. University of Massachusetts, Dept. of Psychology, Neuroscience and Behavior Program, Amherst, MA 01003

The availability of metabolic fuels controls Syrian hamster ovulatory cycles. Food deprivation during days 1 and 2 of the estrous cycle prevents the next expected estrus and ovulation. In rats, sheep, and other species, food deprivation results in cessation of pulsatile luteinizing hormone (LH) secretion. Treatment with drugs such as 2-deoxy-D-glucose (2DG) and insulin which rapidly reduce availability of glucose, inhibit ovulatory cycles in hamsters and pulsatile LH secretion in lambs (Hileman, et al. Biol. Reprod. 44 (Suppl. 1):138, 1991). We have shown previously that food deprivation blocks the Fos expression normally seen on day 2 in caudal preoptic (POA) GnRH neurons. To determine whether inhibition of glucose availability affects the same subset of GnRH neurons, hamsters were injected with 10 U Lente insulin (n = 3), 2DG (2000 mg/kg body weight) (n = 3), or saline (n = 3) on day 1 of the cycle and killed 6 h later. A double-label immunoperoxidase technique was used to localize Fos and GnRH in hamster brains. The percent of Fos/GnRH colocalization was determined in the diagonal bands, medial septum, and rostral and caudal POA. Inhibition of glucose availability significantly reduced Fos expression in caudal POA GnRH cells; 6% of the GnRH neurons expressed Fos in the insulin- and 2DG-treated groups compared to 43% in the saline group. These results support the idea that glucose availability affects a site-specific population of GnRH neurons in the caudal POA. Since caudal POA GnRH neurons are the only GnRH neurons that express Fos on the days of the estrous cycle when LH is secreted in a pulsatile pattern, it is possible that glucose availability controls those GnRH neurons responsible for pulsatile LH secretion.

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285.2

GnRH GENE EXPRESSION IS DECREASED WITH FASTING IN THE MALE RAT BRAIN. D.A. Gruenewald and A.M. Matsumoto*, Univ. of Washington Sch. of Med. and VA Med. Ctr., GRECC, Seattle, WA 98108

Fasting causes a decrease in serum gonadotropin and testosterone (T) levels in the male rat. However, the mechanism underlying this decrease is unclear. We hypothesized that the fasting-induced decrease in serum gonadotropins is due to decreased GnRH gene expression. Prepro (pp-) GnRH mRNA and serum gonadotropin and T levels were compared in 90 day old male Wistar rats fed ad lib or fasted for 60 hours (n=8/group). pp-GnRH mRNA was quantitated by in situ hybridization and computerized image analysis in 18 anatomically matched 20 µm coronal sections from the medial preoptic area (MPOA) and diagonal band of Broca (DBB), using a 35S-labeled 48 base oligodeoxynucleotide probe complementary to rat pp-GnRH mRNA. Trunk blood was assayed for LH, FSH and T by RIA. LH, FSH and T levels were lower in fasted vs. fed rats (LH: 0.17 ± 0.03 vs. 0.36 ± 0.06 ng/ml, $p<0.01$; FSH: 6.7 ± 0.5 vs. 8.7 ± 0.6 ng/ml, $p<0.05$; T: 1.34 ± 0.36 vs. 2.21 ± 0.30 ng/ml, p=NS [0.08]). In both the MPOA and DBB, the number of neurons expressing GnRH was lower in fasted vs. fed rats (6.6 ± 0.7 vs. 11.1 ± 1.4 cells/section, p=0.01), while cellular GnRH mRNA content was unchanged with fasting (83 ± 1 vs. 80 ± 2 grains/cell). These data support the hypothesis that in the Wistar rat, fasting-related decreases in gonadotropin secretion are due, at least in part, to a decrease in GnRH gene expression, which is manifested by a decrease in the number of neurons expressing the GnRH gene.

285.3

LONG-TERM FOOD RESTRICTION DOES NOT ALTER LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) GENE EXPRESSION IN SHEEP HYPOTHALAMI T. McShane *, S.L. Petersen, S. McCrone, and D.H. Keisler. University of Missouri, Columbia, MO.

Long-term food restriction decreases secretion of LH, presumably as a result of central inhibitory influences on secretion of LHRH. We tested the hypothesis that long-term food restriction results in decreased biosynthesis of LHRH. Ten female lambs were ovariectomized at 18 wks of age, and received 100% nutritional requirements for metabolizable energy and crude protein (FED; n=5) or 30% requirements (R; n=5), fed between 18 and 25 wks of age. At 25 wks of age, blood samples were taken every 10 min for 6 h, and serum assayed for LH. Fewer than 2 pulses in 6 h were detected in 3/5 R lambs, and FED lambs averaged 6.2 ± 0.6 pulses/6 h. Four days following blood sampling, brains were collected and hypothalami dissected and stored at -80°C prior to sectioning. Coronal sections (12 μm) were taken through the hypothalamic preoptic region. Using in situ hybridization histochemical techniques, relative levels of mRNA encoding LHRH were determined. Mean levels of LHRH mRNA per neuron were not different between R and FED lambs. Individual mean concentrations of LH did not correlate with mean cellular levels of LHRH mRNA. These results provide evidence that the inhibitory effects of long-term food restriction on secretion of LH probably do not involve changes in LHRH biosynthesis.

285.4

FASTING REDUCES SERUM LH AND MODULATES LHRH RELEASE IN THE RAT. J.A. Farr, P.G. Harms, and N.H. McArthur*, Depts of Animal Science and Vet Anat and Public Health, Texas A&M Univ, College Station, TX 77843.

To evaluate the influence of fasting on serum LH and hypothalamic (HYP) LHRH, intact or castrate adult male rats were allowed ad libitum access to food (CONT) or fasted for 96 hours (FAST). Serum LH and HYP LHRH release was measured by RIA. Serum LH (ng/ml) was reduced ($p<0.05$) in the intact FAST animals (CONT=1.66±0.10, FAST=1.16±0.15). Preopticohypothalamic (POA-HYP) explants were placed in culture tubes containing 0.6 ml of Krebs-Ringer bicarbonate medium (4.7mM potassium, K^+, 10mM glucose, 0.1% BSA). The explants were incubated for 3 hours at 37°C in a 95%O_2:5%CO_2 atmosphere using a vortex mixer. At 30 min intervals, 0.5 ml of incubate was removed from each tube and replaced with fresh media. All explants were challenged, after the fourth 30 min sampling period, with 60mM K^+ in KRB to quantitate (mean difference ± SE between fourth and fifth samples) depolarization induced LHRH release. Explants from FAST rats released more ($p<0.05$) LHRH than CONT when K+ challenged (CONT=90.61 ±16.8, FAST=267.13±37.4). When POA-HYP explants from similarly treated intact rats were acid extracted, there was no difference ($p>0.1$) (CONT= 6.71±0.69, FAST= 6.80±0.43) in total POA-HYP LHRH content (ng/ml extract); however, POA HYP explants from FAST castrate rats had a greater ($p<0.05$) content of LHRH than the castrate controls (CONT=2.09±0.10, FAST=2.64±0.13). These data demonstrate that fasting reduces serum LH and suggest that LHRH release is modified in intact and castrate male rats.

285.5

A HYPOTHALAMIC SITE OF ACTION FOR SYSTEMICALLY ADMINISTERED EXCITATORY AMINO ACIDS. M.M. Fahy* and H.F. Urbanski. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Excitatory amino acids (EAAs), such as *N*-methyl-D-aspartate (NMDA), can stimulate the secretion of LH through a supra-pituitary pathway involving LHRH. The aim of the present study was to determine whether specific EAA receptors exist in the vicinity of LHRH neurons, within the mediobasal hypothalamus, and whether they are accessible to systemically administered EAAs. Brains from adult rats were perfused with 4% paraformaldehyde and sectioned coronally using a cryostat (10 μm). They were then hybridized with a ^{35}S-labeled NMDA receptor cRNA probe (NMDAR1; Moriyoshi *et al.*, 1991). Intense hybridization was observed in the cerebral cortex and hippocampus, a finding that is in harmony with previous radio-ligand autoradiographic studies. Interestingly, intense hybdridization was also found within the hypothalamus, especially in the arcuate nucleus and ventromedial region. In a parallel study, involving the systemic administration of [^{3}H]-glutamate (200 μCi/kg BW), autoradiography revealed the accumulation of glutamate in the mediobasal hypothalamus, but not in other brain regions. These results demonstrate that EAA receptor mRNA is expressed in a brain area that is associated with the neuroendocrine control of the reproductive axis. Moreover, since this area appears to lie outside of the blood-brain barrier the findings raise the possibility that exogenous EAAs (e.g., of dietary origin) are capable of influencing the secretion of LHRH.
(Supported by NIH Grants HD-24312 and RR-00163)

285.6

NMDA-INDUCED PUBERTY AND ACTIVATION OF GnRH NEURONS IN THE MALE DJUNGARIAN HAMSTER. S.M. Yellon*. Div Perinatal Biol, Depts Physiol, Peds and Anat, Loma Linda Univ Sch of Med, Loma Linda, CA 92350

The excitatory amino acid NMDA (N-methyl D-aspartate) induces precocious puberty, presumably by the activation of GnRH neurons that control gonadotropin secretion. Following NMDA treatment, GnRH cells express the proto-oncogene FOS (*Dev Br Res 56: #294, 1990*), an indication of increased transcriptional, hence neuronal activity (*Soc Neurosci Abstr 17: #361.10, 1991*). In the Djungarian hamster at the onset of puberty, an increase in the number of unipolar, but not bipolar GnRH-immunoreactive neurons in the medial preoptic area (MPOA) and diagonal band of Broca (DBB) is associated with increased gonadotropin secretion and rapid testes growth. Since GnRH cell subtypes may be differentially regulated, the present study tested the hypothesis that NMDA activates the unipolar subpopulation of GnRH neurons to induce puberty. Males were shifted from long (16L:8D) to short days (10L:14D) at 18 d of age and injected each morning with NMDA (1.6 mg ip; n=4) or saline (n=4). At 25 d, brains were obtained following intracardiac perfusion with Zamboni's fixative 1 h after treatment. Brain sections (60 μm) from the MPOA and DBB were stained by immunocytochemistry for FOS (Iaderola antiserum) and GnRH (Benoit LR1 antiserum). Testicular development was induced by daily NMDA treatment but blocked in saline-treated males; mean testes weights (±SE) were 245±16 and 43±11 mg, respectively. The number of GnRH-stained cells (both subtypes) was significantly increased in NMDA-treated males compared to that in saline controls (152±21 vs 73±7; $p<0.05$, ANOVA). Although both unipolar and bipolar GnRH cells expressed FOS, less than 14% of each subtype, irrespective of treatment, were FOS-labelled. In this experimental paradigm, the data suggest that NMDA-induced puberty coincides with increased numbers of both unipolar and bipolar GnRH perikarya but reproductive development is not necessarily associated with activation of a specific subpopulation of GnRH neurons. (Supported by NIH HD22479)

285.7

EFFECTS OF NMDA ON LH RELEASE AND LHRH MRNA LEVELS IN ESTROGEN-TREATED OVARIECTOMIZED NORMAL AND ANDROGEN-STERILIZED RATS (ASR). C.A.Barraclough* and J-J Liaw, Dept. of Physiol., Sch. Med., Univ. Maryland, Baltimore, MD 21201.

The present studies examined the temporal changes which occur in plasma LH and LHRH mRNA levels following the iv injection of NMDA (30 mg/kg b.wt.) to control and ASR. In both groups, plasma LH increased significantly within 10 min, peaked between 20-30 min and declined thereafter. However, all LH values were significantly less in ASR. No differences in LHRH mRNA levels were detected in OVLT versus rostral (r), medial (m) or caudal (c) preoptic area (POA) neurons of controls nor were these levels different in LHRH neurons of controls versus ASR. 1 h after NMDA treatment of controls, LHRH mRNA levels were significantly elevated but only in rPOA and OVLT and they remained high to 4 h. In contrast, LHRH mRNA levels in these same brain regions of NMDA-treated ASR did not differ from untreated control or ASR values in any of the brain regions analyzed. Thus, although NMDA induces LHRH release it doesn't increase transcription of LHRH mRNA in ASR. We conclude that *if* NMDA acts directly on LHRH neurons to evoke LH release, then the intracellular mechanisms responsible for increasing transcription of LHRH mRNA have been damaged by neonatal androgen treatment. Supported by HD-02138.

285.8

ACUTE EFFECT OF NMDA ON GONADOTROPIN SECRETION IN MALE SYRIAN HAMSTERS. H.F. Urbanski* and M.M. Fahy. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

The inhibitory effects of short days on the reproductive axis of Syrian hamsters can be overcome by daily administration of *N*-methyl-D-aspartate (NMDA), an excitatory amino acid analogue. In the present study, the acute effect of NMDA (15-20 mg/kg BW, i.v.) on gonadotropin secretion was determined both in intact and orchidectomized hamsters of the LSH/Ss Lak strain, maintained under either long or short days. Although the intact animals showed a significant increase ($P<0.05$) in plasma LH levels within 10 min of NMDA injection, under both photoperiods, the orchidectomized animals showed a significant increase ($P<0.01$) only under short days. On the other hand, NMDA had no acute effect on FSH secretion in any of the treatment groups, a surprising finding in view of previous reports that chronic NMDA treatment of short-day hamsters dramatically increased plasma FSH levels. Therefore, in contrast to the immediate stimulatory effect of NMDA on LH secretion, this long-term enhancement of plasma FSH levels might result from NMDA-induced suppression of melatonin release; in turn, this would cause a more sustained alteration in the pattern of LHRH secretion. To investigate this possibility, short-day hamsters were given a single injection of NMDA (25 mg/kg BW, s.c.) and the effect on plasma melatonin levels was determined by RIA. The nocturnal peak of melatonin release, however, remained unperturbed by the NMDA treatment.
(Supported by NIH Grants HD-24312 and RR-00163)

285.9

SIZE AND AGE-RELATED CHANGES IN GNRH-CONTAINING NEURONS OF THE TERMINAL NERVE AND PREOPTIC AREA IN JUVENILE *HAPLOCHROMIS BURTONI*. R.C. Francis*, H. Lee and R.D. Fernald. Neuroscience Program, Stanford University, Stanford, CA 94305.

Soma size of irGnRH neurons in the preoptic area (POA) of the teleost fish, *H. burtoni*, varies according to social status and reproductive condition. GnRH neurons in the terminal nerve (TN), however, show no such plasticity. We monitored changes in the number and size of irGnRH neurons in the TN and POA in juvenile (sexually immature) fish in order to determine whether these two irGnRH populations exhibit different developmental trajectories. We sampled from two broods that exhibited markedly different growth rates. Two to four subjects were removed from each group at weekly intervals, and processed for ICC with an antibody to salmon GnRH. In both the TN and POA, irGnRH neurons first appear at age 2-4 weeks when the size of the fish reaches approximately 1 cm. Thereafter, cell number remains constant in the TN but continues to increase in the POA throughout the juvenile phase. Both number and size of irGnRH neurons were more highly correlated with body size than with age. However, comparisons of individuals of the same size from the slow- and fast-growing broods revealed that size rank within broods, not absolute size, accounts for most of the variance in soma size of POA irGnRH neurons. The TN population of irGnRH neurons shows no such effect of relative body size. Relative size is an important determinant of social rank. Hence, size of the POA neurons, but not the TN neurons, may be socially modulated. These results provide further evidence for the functional independence of the irGnRH neurons in the TN and POA. Supported by NIH HD23799 and MH09485.

285.10

ABSENCE OF FOS EXPRESSION IN GNRH NEURONS IN POA-GRAFTED HYPOGONADAL MICE (HPG). T.J. Wu*, G.M. Miller, M.J. Gibson and A.-J. Silverman, Dept. of Anat. Cell Biol., Columbia Univ., New York, NY 10032 and Dept. of Medicine, Mt. Sinai Sch. of Med., New York, NY 10029.

Fos oncoprotein expression is widely accepted as a marker for neuronal activation. We have shown that in normal steroid-primed ovariectomized mice paired with an ejaculating male, Fos expression occurs in greater than 40% of GnRH neurons and is maintained at this level for a longer time than similarly treated females paired with an unsuccesful male. HPG's lack a functional GnRH gene; females can enter persistent estrus when a preoptic area (POA) graft is placed in the third ventricle. The expression of Fos in these grafted GnRH neuron was determined in HPG/POA introduced to experienced males at 1400 h and sacrificed 50-60 min post-ejaculation. All mice were anesthetized and prepared for combined Fos and GnRH immunocytochemistry. Increased Fos expression in response to sexual behavior occurred in neurons widely distributed in the brain of HPG/POA. However, under these conditions, only 1 GnRH neuron (1.5% of 26-41 neurons/ animal) contained Fos. Experiments are in progress to determine the overall capacity of grafted GnRH cells to synthesize this oncoprotein. (HD10665, NS20335 HD19077, T32 NS07062, T32DK07645)

285.11

GONADOTROPIN-RELEASING HORMONE (GNRH)-CONTAINING NEURONS CHANGE SIZE WITH REPRODUCTIVE STATE IN FEMALE FISH. S.A.WHITE* & R.D.FERNALD. Neuroscience Program, Stanford University.

GnRH plays a vital role in regulating reproduction. In mammals, hypothalamic release of GnRH into the portal vasculature causes secretion of pituitary gonadotropins which stimulate gonadal production of sex steroids. In the teleost fish, *Haplochromis burtoni*, GnRH-containing neurons in the preoptico-hypothalamic area (POA) send direct projections to the pituitary. Here we show that in female *H. burtoni*, GnRH soma size depends critically on reproductive state. We selected animals prior to, during, and after the reproductive portion of their life history, in both brooding and spawning states. Immunocytochemical staining of brain sections showed that the two-dimensional areas of POA GnRH neurons are up to twice as large in spawning females than in females carrying broods, a transformation which occurs within two weeks. At a younger age, GnRH neurons are larger in females which have never spawned than in brooding age-mates. Older, post-reproductive females have the largest GnRH neurons and little to no ovarian tissue. Enlargement of GnRH cells in these fish may be due to a lack of negative feedback of sex steroids, comparable to that reported for castrated male conspecifics (Francis *et al.*, 1992). In intact male *H. burtoni*, social stimuli regulate changes in GnRH soma size (Francis & Fernald, 1992). Since reproducing females have no social hierarchy and are exposed to similar external stimuli, the primary factor(s) controlling GnRH neuron size appears to be internal reproductive state. Thus, while GnRH neuronal size is plastic in both males and females, its regulation is dramatically different in each. Cyclical changes have been described for neurons in female rat hypothalamic nuclei (*cf.* Naftolin *et al.*, 1990; McEwen, 1991). The present finding of soma size change in a non-mammalian vertebrate reveals a novel way in which neuronal plasticity underlies dynamic regulatory processes in adult brain. *Supported by HD23799, MH09986, and the Scottish Rite Foundation.*

285.12

BEHAVIOR-RELATED ALTERATIONS IN DISTRIBUTION OF BRAIN GNRH-IR MAST CELLS. **X. Zhuang and R. Silver*, Columbia University and Barnard College, New York, N.Y. 10027.**

We have previously described the appearance of GnRH immunoreactive (ir) mast cells in doves that had been courting for 2 hrs (Silver and Silverman, Soc Neurosc Abstr 1992; Silver et al. J Neuroendocrinol. 1992). The present study describes the overall distribution of mast cells in the dove brain, and examines the effects of behavioral cues on the distribution of brain mast cells. GnRH-ir mast cells were observed in or around circumventricular organs (OVLT and SSO), the pia mater, blood vessels, and in the parenchymal tissue of the habenula. Four groups of adult male birds were studied. In the courtship group, males were paired with sexually active females and were permitted to court for 2 hours. In the aggression group, males were paired with a squab for 2 hours, during which time they displayed aggressive behavior towards the squab. The third group were visually isolated from other birds for at least 3 weeks. The fourth group were long term castrates housed communally. In the first three groups, GnRH-ir mast cells were observed in all the areas described above, with differences among groups in numbers of habenular mast cells. As previously reported, there were significantly fewer mast cells in the habenula of isolated animals than in courted animals. Animals displaying aggressive behavior were intermediate between these two groups. The most dramatic effect was seen in castrated animals where virtually no mast cells were seen in the habenula and few in the pia mater. These results suggest a mechanism for interactions among the nervous, endocrine, and immune system in the brain during specific behavioral activities. Supported by NIMH grant 29380 (to RS).

285.13

MAST CELLS WITH GnRH- IMMUNOREACTIVITY OCCUR IN THE BRAIN AFTER COURTSHIP. R. Silver[1] and A.J. Silverman*[2], Barnard College of Columbia University[1], N.Y., N.Y. 10027 and Department of Anatomy and Cell Biology[2], Columbia College of Physicians and Surgeons, N.Y. 10032.

Interest has recently been focused on interactions between the nervous and immune systems. We report here a new relationship between environmental stimuli that activate the reproductive axis and cellular elements of the immune network. We have previously described the migration of non-neuronal, GnRH immunoreactive cells into the brain of the ring dove following a brief period of courtship. Ultrastructurally the cells contain large vacuoles, a highly condensed nucleus and numerous filamentous processes. These characteristics suggested either a monocyte or mast cell lineage. The current experiments were destined to distinguish between these two classes. Two markers for macrophages, the OX42 antibody and the lectin, *Bandetraea simplifolia* B4 isolectin, did not label these cells. When markers for endocytotic activity (fluorogold and I125 GnRH) were either ICV or IP prior to courtship initiation, the cells did not capture any label. We conclude that the cells are not of the monocyte lineage. On the other hand, the habenula cells were metachromatic with acidic thionin and immunoreactive for histamine. Double label experiments indicated that all habenular GnRH positive cells of this morphology contained histamine. Future studies will explore the nature of the signal that elicits the movement of mast cells into the brain and the role that the mast cells might play in altering nervous system function. Supported by NIMH grant 29380 (to RS) and HD 10065 (to AJS).

285.14

EXPRESSION OF THE INTERMEDIATE FILAMENT PROTEINS PERIPHERIN AND VIMENTIN IN GONADOTROPIN RELEASING HORMONE PRODUCING NEURONS. L. Jennes*. Dept. Anatomy & Neurobiology, University of Kentucky, College of Medicine, Lexington, KY 40536.

Immunofluorescent double stainings for gonadotropin releasing hormone (GnRH) and peripherin, vimentin or neurofilament-70 were used to determine the identity of the intermediate filaments in GnRH neurons of the adult rat. The results show that GnRH cells are unique neurons in the septum-diagonal band-rostral hypothalamus in that they express peripherin and vimentin but not neurofilament-70. Both peripherin and vimentin form dense perinuclear networks from which peripherin extends only into the proximal neurites while vimentin can be seen in certain GnRH terminals in the median eminence. The presence of peripherin and vimentin and the absence of neurofilament-70 in GnRH neurons as well as in olfactory receptor neurons suggests close embryological ties between these cell types and supports the view that both cell types arise from a common ancestor. Supported by USPHS Grant HD24697.

285.15

DIFFERENTIAL EFFECTS OF UNILATERAL LESION IN THE PREOPTIC AREA (POA) ON COMPENSATORY OVULATION (CO) AND COMPENSATORY OVARIAN HYPERTROPHY (COH) IN ADULT HEMISPAYED RATS. J.L. Morán , M.E. Cruz , R. Salceda*and R. Domínguez . ENEP-Zaragoza and Instituto de Fisiología Celular, UNAM, México.

There are several indications that POA exerts an asymmetrical control on the regulation of ovulation and ovarian weight. Our interest was to analyze in unilateral lesions in POA affects CO and COH in the same way. Animals with unilateral lesions of POA in the day of estrus (E), after three consecutive 4-day cycles were hemispayed on the day of E, right of left ovary extirpated. In another experiment rats hemispayed on E received a unilateral lesion in POA 12 days later. All the animals were autopsied on E. No differences in ovulation rate, CO and COH in right or left hemispayed rats were observed. Only 7/19 rats with lesion in the right side of POA ovulated, while 16/22 with left side lesion did ($p<0.05$). The left ovary did not show CO when the animals had a lesion in either side of POA (0 and 33% vs. 107%, $p<0.05$). The COH of the right ovary was lower in rats with a ipsilateral lesion of POA (17 and 2% vs. 58%, $p<0.05$).

Data present herein support the existence of asymmetry in the regulation of ovulation by POA. In both, hemispayed and intact animals, the lesion of the right side of POA partially blocks ovulation while it does not occur when the left side was lesioned.

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285.16

COMPARISON OF PULSATILE AND CONSTANT TESTOSTERONE INFUSION ON LUTEINIZING HORMONE SECRETION IN THE RAM. T.J. Rhim, D.J. Schaeffer, D. Kuehl, L. Lubbers, G.L. Jackson*. Department of Veterinary Biosciences, University of Illinois, Urbana, IL 61801.

Although testosterone (T) is secreted in an episodic pattern in males, previous studies have used constant T administration to study the effect of T on luteinizing hormone (LH) and/or LH-releasing hormone secretion. Thus, the objective of the present study was to compare the effectiveness of a pulsatile versus a constant pattern of T to suppress LH secretion in the ram. The study was conducted as a cross over experiment in a 4 x 4 Latin square design. Adult castrated rams (n=8) were subjected to each of four different i.v. infusion regimens for 3 days: (1) constant diluent; (2) constant T; (3) pulsatile diluent (one pulse every 4 h); (4) pulsatile T. Equal amounts of T (768 ug/kg/24 h) were delivered by the pulsatile and constant regimens. Blood samples taken at 10-min intervals for 4 h prior to infusion (Day 0) and during the last 4 h of the infusion (Day 3) were analyzed for T and LH. Neither mean, pulse amplitude, nor inter-pulse interval of LH differed ($p>0.1$) among treatment groups at Day 0. Compared to diluent, T-treatment decreased ($p<0.001$) mean LH and increased ($p<0.001$) LH inter-pulse interval. LH inter-pulse interval was increased more ($p<0.005$) by constant T than by pulsatile T. Mean LH was slightly more suppressed ($p=0.052$) by constant T than by pulsatile T. None of the treatments affected LH pulse amplitude. These results indicate that constant T is more effective than pulsatile T in suppressing LH secretion in the ram. (Supported by USPH Grant 1R01 HD 27453)

285.17

ENDOCRINE DEREGULATION AND FERTILITY OF SPINAL CORD INJURED MEN. N.L. Brackett*, C.M. Lynne, M.J. Amador and W.E. Bloch. The Miami Project and Dept. of Urology, Univ of Miami School of Medicine, Miami, FL 33136

Following spinal cord injury (SCI), most men are infertile because of poor semen quality, most notably, poor sperm motility. The etiology of impaired semen quality in SCI men is poorly understood. Several mechanisms have been hypothesized, including deregulation of the hypothalamic-pituitary-gonadal axis, but little is known about this possibility. The objectives of the present study were 2to determine the prevalence and severity of endocrine deregulation in SCI men, and to determine if endocrine deregulation in SCI men is related to impairments in their semen quality.

Serum levels of testosterone (T), follicle-stimulating-hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) were measured in fifty SCI adult male volunteers. Within two weeks after hormone determination, semen was obtained either by masturbation, vibratory stimulation, or electroejaculation.

More than half of the subjects (Ss) (26 out of 50) had abnormal hormone profiles. The remainder had a high prevalence of hormones in the low-normal range. The most common alteration occurred in levels of FSH, which were below normal in 13 subjects and above normal in 5 subjects. T, LH, and PRL were below normal in 5, 8 and 0 Ss and above normal in 0, 3 and 3 Ss, respectively. 12 of the above Ss had more than one abnormal hormone level. The incidence of hormonal abnormality was significantly higher at levels T8-12 compared to levels T3-4, which had the lowest incidence of abnormality. 5 out of 6 Ss with a C5 injury had abnormal FSH levels. Semen quality was impaired in all Ss, but the impairments did not relate to any specific hormone abnormality. For example, of 5 Ss who were azoospermic, three had high FSH, and two had normal FSH. Sperm motility and viability were low in all Ss, and did not relate to a particular endocrine profile. We conclude that endocrine deregulation is a likely consequence of SCI in men, but is not the sole mechanism contributing to impairments in their semen quality.

285.18

LOCALIZATION OF NEURONS CONTAINING LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) mRNA IN THE HUMAN HYPOTHALAMUS AND BASAL FOREBRAIN. N.E. Rance*, W. S. Young, III, B.O. Parks, N.T. McMullen. Depts. of Pathology and Anatomy, Univ. of Arizona Col. Med., Tucson, AZ 85724 and Lab. Cell Biol., NIMH, Bethesda, MD, 20892.

In the present study we used in situ hybridization and computer-assisted microscopy to map the distribution of neurons expressing LHRH gene transcripts in the human hypothalamus. Every 20th section from the hypothalami of five adult males was incubated with an [^{35}S]-labeled cDNA probe complementary to LHRH mRNA. Specificity was confirmed by hybridization of adjacent sections with a probe targeted to the gonadotropin-associated protein region of the LHRH mRNA. Maps were manually digitized with the aid of an image-combining computer microscope. We report a much greater number of LHRH neurons than previously described in the human brain. Three morphologic subtypes were observed: 1) Small, oval and sparsely labeled neurons which were numerous in the septal-preoptic region (20 to 40 neurons/section) and also scattered from the bed nucleus to amygdala ("extended amygdala"); 2) Heavily labeled oval or elongated cells located in the base of the infundibular nucleus, the infundibular stalk, lateral hypothalamus immediately dorsal to the supraoptic nucleus, and rostral periventricular hypothalamus (6 to 12/section); and 3) Large neurons (> 500 μm^2 cross-sectional area), intermediate in labeling density which were distributed in the extended amygdala, substantia innominata, ventral pallidum, putamen and nucleus basalis of Meynert (20 to 50/section). The pronounced differences in staining intensity, morphology and location of these three subtypes suggest distinct functional subgroups of LHRH neurons in the human brain. (Supported by NIH Ag-09214 and the Arizona Disease Control Research Commission)

NEUROENDOCRINE REGULATION: OSMOTIC REGULATION I

286.1

ENDOCRINE FACTORS DIFFERENTIALLY MODULATE PERIPHERAL BENZODIAZEPINE RECEPTORS IN MALE AND FEMALE RATS. R.C. Drugan* and P.V. Holmes. Schrier Research Lab, Dept. of Psychology, Brown Univ., Providence, R.I. 02912

The peripheral benzodiazepine receptor (PBR) has been shown to be under tonic hormonal control. Rapid alterations in renal PBR have also been reported in response to acute hormone administration and stress. Thus, the renal PBR is regulated both tonically and phasically. We reported a sexual dimorphism in the stress-induced reduction in renal PBR. Ovariectomy and castration had no effect on this response. We recently discovered a rapid hormonal regulation of renal PBR by Angiotensin II (ANG II, see Holmes and Drugan. this volume). We examined the impact of ANG II and Atrial Natriuretic Peptide (ANP) as possible mediators of this gender effect. Both male and female rats received either a 25 ug injection of ANG II and were sacrificed 5 min. later or 3 X 25 ug injections of ANG II at 0, 30 and 60 min. and were sacrificed at 80 min. Male rats showed a significant reduction (25%) in renal PBR binding at both time points while female rats exhibited a significant reduction only after 80 min. Preliminary data also indicate gender differences 5 min. following a single, 30 ug injection of ANP. Females show an increase in renal PBR, while males show no effect at this dose. A sexual dimorphic response to both ANG II and ANP may be important causal factors in the gender differences in renal PBR stress reactivity. Supported by a Sloan Research Fellowship (R.C.D)

286.2

ANGIOTENSIN II REGULATES THE PERIPHERAL BENZODIAZEPINE RECEPTOR IN RATS. P.V. Holmes* and R.C. Drugan. Schrier Research Lab., Dept. of Psychology, Brown Univ., Providence, RI 02912

The peripheral benzodiazepine receptor (PBR) is an outer mitochondrial membrane receptor found in both peripheral and CNS tissues. Recent evidence links the PBR to steroidogenesis in adrenal, gonads, and glia. The PBR is rapidly regulated during stress. Mild or brief stressors are generally reported to cause increases in PBR densities in several tissues whereas intense or prolonged stressors cause decreases. This biphasic response suggests that PBR alterations during stress reflect an endocrine feedback regulatory mechanism. Previous research has suggested that the renal PBR response to stress occurs independently of the hypothalamo-pituitary-adrenal axis or sympathetic nervous system. The roles of angiotensin II (ANG II) and atrial natriuretic factor (ANF) in the PBR stress response were examined. The stress-induced decrease in [3H]Ro5-4864 binding to renal PBR was attenuated by captopril pretreatment, and acute ANG II administration (25-125 µg s.c.) alone rapidly modulated the PBR in a fashion similar to stress. Decreases in PBR binding in kidney, heart, and cortex occurred within 5 minutes following a single 25 µg dose of ANG II, and the decrease observed in these tissues persisted for two hours following the final 25 µg injection of a 125 µg cumulative dose. Scatchard analysis revealed that the ANG II-induced decrease in renal PBR binding was due to a reduction in Bmax. Possible interactions between ANG II and ANF are being explored. These experiments suggest that ANG II may act as the extracellular signal to regulate PBR activity in several tissues during stress. Supported by Alfred P. Sloan Fellowship #Br 2852 to R.C.D.

286.3

C-TYPE NATRIURETIC PEPTIDE (CNP) AFFECTS VASOACTIVE INTESTINAL PEPTIDE (VIP)-PEPTIDE HISTIDINE ISOLEUCINE (PHI) NEURONS IN THE RAT HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM. N.Minamitani, A.Kawaguchi, I.Nagai, K.Chihara, K.Sakai*. Med. Ctr. for Students, Third Div. Dept. Med., Dept. Psy. Kobe Univ. Sch. Med., Kobe Merc. Ma. Univ., Kobe 650 Japan.

Recently identified CNP has been demonstrated to possess pharmacological spectrums and amino-acid structure very similar to those of atrial NP and brain NP. It, however, remains to be clarified whether CNP affects VIP-PHI neurons in the hypothalamus (HT) and neurohypophysis (NL). Using the perifusion study, as previously reported we investigated the effect of CNP on basal levels and osmotic-stimulated VIP and PHI releases from isolated NL and two different hypothalamic slices of adult male rats; one, frontal section (HT) without median eminence (ME), including paraventricular nucleus and suprachiasmatic nucleus, the other, horizontal section (ME). CNP induced a significant increase of VIP and PHI releases in 20 min-perifusates from HT, ME and NL in the range of doses from 10^{-12} to 10^{-7}M. The rises by osmotic pressure (310 mOsm/kg) of VIP and PHI release from these tissues were significantly suppressed by the addition of CNP with the range of 10^{-12} to 10^{-7}M.

These results suggest that CNP acts on the VIP-PHI neurons in the NL as well as in the hypothalamus to stimulate the basal secretion and to attenuate the osmotic-stimulated releases of VIP and PHI.

286.4

ROLE OF NATRIURETIC PEPTIDES IN THE REGULATION OF MAGNOCELLULAR NEUROENDOCRINE NEURONS. K.M. Hurley* and C.B. Saper. Depts. of Pharm. & Physiol. Sci. and Neurology, Univ. of Chicago, Chicago, IL 60637

Natriuretic peptides oppose the effects of vasopressin systemically, and may play a role in regulating vasopressin secretion as neuromodulators in the brain. To explore the basis for this interaction, we examined the origin of atrial natriuretic peptide- (ANP-) and brain natriuretic peptide- (BNP-) like-immunoreactive (-ir) fibers innervating magnocellular neurons in the supraoptic nucleus (SON) in the rat using retrograde tracing in combination with immunocytochemistry. Most ANPir neurons that innervate the SON were found in the anteroventral periventricular nucleus, whereas most BNPir neurons projecting to this target were found in the tuberomammillary nucleus.

In order to determine the functional significance of this projection, we recorded intracellularly from magnocellular neurons in the *in vitro* hypothalamic slice during the application of ANP (10^{-7} to 10^{-10} M). Bursting neurons responded with a hyperpolarization of the membrane potential, associated with an increased conductance and enhancement of the afterhyperpolarizing potential, consistent with potentiation of the $IK^{+}_{Ca^{++}}$. This effect was abolished in the presence of low Ca^{++} and high Mg^{++} medium. Non-bursting neurons showed more heterogeneous responses: Among irregularly firing neurons, 83% showed a hyperpolarization (63% with membrane conductance increased, 33% decreased) and 16% demonstrated a depolarization associated with increased conductance. Among continuously firing neurons, 43% were hyperpolarized, 14% depolarized and 43% did not respond. Silent neurons generally did not respond (67%), although 33% were depolarized. The effects of natriuretic peptides on ion currents in vasopressin cells may sculpt their characteristic bursting activity, thereby reducing the secretion of hormone.

286.5

HYPOPHYSIAL-PORTAL PLASMA LEVELS OF VASOPRESSIN AND OXYTOCIN INCREASE AFTER COMPRESSION OF THE PITUITARY STALK. G.B. Makara, S. Sutton, S. Otto, and P.M. Plotsky*, Institute of Experimental Medicine, Budapest, Hungary and The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037.

Compression of the pituitary stalk has been used to induce posterior pituitary gland denervation (PPD) in the rat and to study the role of the magnocellular neurosecretory system in regulation of the anterior pituitary gland (AP). Earlier studies demonstrated that PPD resulted in a loss of arginine vasopressin (AVP) from the neural lobe and an accumulation of immunoreactive AVP and oxytocin (OT) in fibers of the stalk median eminence (SME). In the present experiments, we studied the time-course of alterations in hypothalamic-pituitary peptide systems from one to four weeks following PPD. SME content of AVP and OT increased sharply while CRF content was unchanged one week after PPD. Assay of peptide levels in hypophysial-portal blood collected from anesthetized (urethane, 1.1 g/kgBW, ip) rats via a ventral transpharyngeal approach revealed an incremental 4- to 11-fold elevation of portal AVP levels above controls from one to four weeks following PPD; portal OT levels were increased 8- to 14-fold over the same time period. In contrast, hypophysial-portal plasma levels of corticotropin-releasing factor (CRF) were decreased following PPD. Evaluation of AP sensitivity to CRF and AVP was performed one week post-PPD by measuring *in vitro* release of ACTH from incubated AP fragments exposed to various secretagogue concentrations. CRF-induced (0.1, 1, 10 nmol/l) ACTH release was similar in sham and PPD animals, while ACTH release elicited by AVP (0.5, 5, 50 nmol/l) was markedly reduced in PPD rats as compared to shams. The large and prolonged elevation of AVP and OT content in hypophysial-portal plasma and in the SME was probably due to peptide secretion from sprouting fibers of the magnocellular system in the SME above the level of the compression. The sustained elevation of portal AVP concentration following PPD was associated with desensitization of corticotropes to the actions of AVP on ACTH secretion. This model may be useful for studying the consequences of chronic hypersecretion of AVP and OT into the hypophysial-portal system.

286.6

BAROREFLEX CONTROL OF VASOPRESSIN SECRETION DURING PREGNANCY IN CONSCIOUS DOGS. V.L. Brooks* and L.C. Keil, Oregon Health Sciences University, Portland, OR 97201 and NASA-Ames Research Center, Moffett Field, CA 94035.

To test the hypothesis that baroreflex regulation of vasopressin (VP) secretion is altered during pregnancy, pregnant (P) dogs (n=8) were surgically prepared with femoral (arterial and venous) and atrial (right and left) catheters. On different days, one of 3 doses of nitroprusside (NP; 1,2 or 4 $\mu g \bullet kg^{-1} \bullet min^{-1}$) was infused for 30 min in P dogs, and the NP series was repeated >1 month after delivery (non-P dogs). Compared to non-P dogs, P dogs had lower basal arterial pressure (BP; 102±3 vs 112±2 mmHg; $p<0.01$), higher heart rate (113±9 vs 74±3 b/min; $p<0.01$), lower left atrial pressure (LAP; 7±0.9 vs 10.2±1.1 cm H_2O), lower right atrial pressure (RAP; 2.5±1.0 vs 5.5±1.0 cm H_2O; $p<0.01$), but similar VP (2.2±0.4 vs 2.3±0.2 pg/ml). In non-P dogs, the highest NP dose decreased BP by 21±2 mm Hg, LAP by 6.3±1.3 cm H_2O and RAP by 5.7±0.7 cm H_2O, and increased VP by 19.1±2.8 pg/ml. In P dogs, NP produced similar decreases in BP (23±3 mmHg) and LAP (3.3±0.9 cm H_2O), but smaller decreases in RAP (2.6±0.9 cm H_2O; $p<0.05$). The increase in VP was less (9.7±4.9 pg/ml; $p<0.05$). As a result, the relationship between BP and VP was shifted downward ($p<0.05$) in P dogs compared to non-P dogs. These results indicate that the cardiovascular system is altered during pregnancy in dogs as in other species, and suggest that reflex increases in VP may be blunted. Supported by NIH HL 39923.

286.7

PASSIVE REGULATION OF POSTERIOR PITUITARY VASOPRESSIN CONTENT. M.D. Fitzsimmons*, M.M. Roberts, and A.G. Robinson. Department of Medicine, Division of Endocrinology Univ. of Pittsburgh PA 15261.

In these experiments, we sought to determine whether the increasing pituitary vasopressin (AVP) content in the young adult male rat is actively regulated. Both hypo- and hypernatremia decrease AVP content relative to age-matched controls: hyponatremia by decreasing AVP synthesis and hypernatremia by increasing release faster than synthetic mechanisms can compensate. We postulated that the patterns of recovery from these conditions would indicate how pituitary AVP content is controlled; if pituitary content is actively regulated, after both perturbations, pituitary content should return to that of age-matched controls. To test this, we examined pituitary recovery at 1 and 3 weeks after 1 week of hyponatremia or hypernatremia (2% NaCl for 21 hours/day) beginning at age 7 weeks. Six male Fischer 344 rats were sacrificed at each time and assayed for pituitary AVP. Whole pituitary AVP content (ng ± SE) is shown.

age(weeks)	Control	Hyponatremia	Hypernatremia
7	773 ± 44	773 ± 44	773 ± 44
7.5		778 ± 31	592 ± 49
8	952 ± 36	759 ± 56	599 ± 100
9	1065 ± 78	782 ± 36	998 ± 108
11	1466 ± 94	973 ± 109	1412 ± 98

Diminished AVP content in the hyponatremic animals is not the result of weight maintenance because, in another study, hyponatremia with weight gain still blocked age-related increase in pituitary AVP content. Both hyper- and hyponatremia reduced pituitary content (versus age-matched control) but pituitary AVP during recovery caught up to controls only in the hypernatremic animals. These results support our hypothesis that AVP mRNA transcription (which increases during hyper- but decreases during hyponatremia) controls AVP synthesis and that posterior pituitary content passively reflects the balance between synthesis and release.

286.8

A SEXUAL DIFFERENCE IN VASOPRESSIN-IMMUNOREACTIVE NEURONS WITHIN THE NEUROENDOCRINE SYSTEM OF GOLDEN HAMSTERS. C.F. Ferris* and Y. Delville. Physiol. Dept., Univ. of Mass. Med. Ctr., Worcester, MA 01655.

In mammals, large neurosecretory neurons localized within the hypothalamus, particularly the supraoptic (SON) and the paraventricular (PVN) nuclei are the source of vasopressin (AVP) released in the neurohypophysis. Although this system appears to be insensitive to gonadal hormones in rats, we now report the existence of a sexual difference in golden hamsters. In males, a larger number of AVP-immunoreactive perikarya was counted in all hypothalamic neurosecretory cell groups than in females. The difference was largest (50%) within the SON. In addition, the concentrations of AVP extracted from the hypothalamus were twice as high in males than in females. It is unclear whether these differences are related to any sexual difference in endocrine function. However, a relation with sexual differences in brain function is also possible, as a large number of magnocellular AVP-immunoreactive neurons do not project to the neurohypophysis.

286.9

VASOPRESSIN IS PRESENT IN THE ENDOCRINE PANCREAS OF THE RAT: IMMUNOHISTOCHEMICAL LOCALIZATION C.L. Cream, A.S. Friedmann, V.A. Memoli, W.G. North* Depts of Physiology and Pathology, Dartmouth Medical School, Lebanon, NH 03756 USA

Earlier work by others demonstrated immunoreactive vasopressin (irVP) in human and rat pancreatic extracts. Subsequent studies revealed that VP can stimulate insulin release from pancreatic beta cells. In the present study, irVP was localized to cells of the rat pancreas using immunohistochemistry and our rabbit polyclonal antibodies (Gonzo3) specific for vasopressin. Pancreas from Long Evans rats were removed, fixed in Bouin's solution, embedded in paraffin, and sections of 2 microns prepared for use in immunohistochemistry. Vasopressin immunoreactivity was demonstrated in small cells throughout the endocrine pancreas. These cells were located at the periphery of the islets and contained dendritic-like processes. VP and insulin immunoreactivity failed to colocalize in serial sections. This demonstration of irVP in cells of the endocrine pancreas, raises the possibility of a paracrine role for this peptide in the regulation of insulin release. Future studies aimed at determining the characteristics of the irVP-containing cells should lend further insight into the role this peptide plays in the endocrine pancreas.

286.10

DUAL ACTION OF C-TYPE NATRIURETIC PEPTIDE (CNP) ON ARG-VASOPRESSIN (AVP) RELEASES FROM RAT HYPOTHALAMIC SLICES AND NEUROHYPOPHYSES PERIFUSED IN VITRO. I. Nagai, N.Minamitani, A.Kawaguchi, K.Chihara, K.Maeda*. Med. Ctr. for Students, Third Div. Dept.Med. Dept. Psy. Kobe Univ. Sch. Med., Kobe Merc.Ma.Univ., Kobe 650 Japan.

Very recently, CNP was found to be the third member of the mammarian NP family identified from porcine brain following atrial NP and brain NP. CNP mRNA was also detected mainly in the brain. Its physiological function in the brain remains to be elucidated. In order to clarify both the site of action and the effect of CNP on basal levels of and osmotic- stimulated AVP releases, the perifusion studies were performed in such a way as previously reported, using isolated neurohypophyses (NL) and two different hypothalamic slices of adult male rats; one, frontal section (HT) without median eminence (ME), including paraventricular nucleus and supraoptic nucleus, the other, horizontal section (ME) containing ME. The addition of CNP to artificial cerebrospinal fluid for perifusates, ranging in doses from 10^{-12} to 10^{-7}M caused a significant increase of AVP release from basal levels in 20 min-perifusates from HT, ME and NL. CNP, however, significantly blunted osmotic- stimulated AVP release from these tissues with the range of 10^{-12} to 10^{-7}M.

These results indicate that CNP has a dual action on AVP release in the NL as well as in the hypothalamus ; stimulatory on its basal release, and inhibitory on its rise by the osmotic stimulation.

286.11

C-TYPE NATRIURETIC PEPTIDE (CNP) ATTENUATES OSMOTIC STIMULATED SOMATOSTATIN (SRIF) RELEASE FROM RAT HYPOTHALAMIC SLICES AND NEUROHYPOPHYSES PERIFUSED IN VITRO. A. Kawaguchi, N.Minamitani, I.Nagai, K. Chihara, H.Kaneda*. Kobe Merc.Ma.Univ.,Med.Ctr.for Students, Third Div.Dept.Med.Dept.Psy.Kobe Univ. Sch Med., Kobe 650 Japan.

CNP has been identified from the porcine brain with its structure similar to atrial NP and brain NP. It remains to be settled whether CNP affects hypothalamo-neurohypophysial SRIF neuron system. Using the perifusion system, as previously described, we sought to find the site of action and the effect of CNP on basal levels of and osmotic-stimulated SRIF releases from isolated neurohypophyses (NL) and two different hypothalamic slices of adult male rats; one, frontal section (HT) without median eminence (ME), including periventricular nucleus, the other, horizontal section (ME). A significant rise of SRIF from basal levels in 20 min-perifusates from HT, ME and NL was induced by addition of CNP to artificial cerebrospinal fluid used for perifusates in the range of dose from 10^{-12} to 10^{-7}M. The increase by osmotic pressure (310 mOsm/kg) of SRIF release from these tissues were significantly inhibited by CNP with the range of 10^{-12} to 10^{-7}M in a dose dependent manner.

These results suggest that CNP acts on the SRIF neurons in the NL as well as in the hypothalamus to stimulate the basal secretion and to suppress the osmotic-stimulated releases of SRIF.

286.12

PHASIC FIRING ACTIVITY IN RAT SUPRAOPTIC VASOPRESSIN NEURONS IS BLOCKED BY THE NMDA RECEPTOR ANTAGONIST KETAMINE. **R. Nissen*, B. Hu, L.P. Renaud.** *Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Ontario, Canada. K1Y 4E9*

A potent NMDA receptor system has recently been characterized in rat supraoptic vasopressin (VP) and oxytocin (OT) neurons in vitro. The physiological function of the NMDA receptor complex expressed in these neuroendocrine cells remains unknown. In this study we used ketamine, a non-competitive NMDA open channel blocker, to examine whether NMDA receptor blockade can affect the patterned spontaneous activities observed in VP/OT neurons in vivo. Extracellular recording of phasic activity or irregular continuous discharges was obtained from identified VP or OT neurons, respectively, in pentobarbital anesthetized Long-Evans rats. In 13/15 VP cells, systemic administration of ketamine (<1.5 mg/Kg) caused a complete cessation of spontaneous phasic discharge. This blocking effect was dose-dependent and fully reversible, but did not affect the threshold of antidromic activation of the same cells. Most (5/6) continuously firing OT neurons recorded from the same preparation remained unaffected by ketamine. We further tested ketamine applied directly to an additional 8 VP cells using multibarrelled electrodes. In these neurons, locally applied ketamine (1mM intrabarrel concentration) consistently blocked ongoing phasic activity in a similar manner and repetitive application caused complete suppression of phasic discharges. The same concentration of ketamine was also effective in blocking the responses evoked by local application of NMDA. Our data suggest that NMDA receptors play a critical role in controlling the physiological activity in VP neurons in this species. (Supported by MRC).

286.13

NMDA AND NON-NMDA RECEPTORS MEDIATE SYNAPTIC ACTIVATION OF SUPRAOPTIC VASOPRESSIN NEURONS FOLLOWING ORGANUM VASCULOSUM LAMINA TERMINALIS (OVLT) STIMULATION IN VITRO.

C.R. YANG*, V.V. SENATOROV AND L.P. RENAUD. Neuroscience Research, Loeb Research Institute, Ottawa Civic Hospital, Canada, K1Y 4E9.

The OVLT is a circumventricular organ that projects to the supraoptic nucleus (SON). Neurones from both of these areas are osmosensitive and functionally involved in body fluid homeostasis. We previously reported that electrical stimulation of the OVLT evokes GABAergic inhibitory and glutamatergic excitatory post-synaptic potentials in SON neurones in rat superfused hypothalamic explants (Yang & Renaud, SN Abst. 1991). The present study further evaluates the role of NMDA and non-NMDA receptors in mediating this excitatory response.

In media containing bicuculline (10-20 μM) to abolish all IPSPs, low intensity repetitive stimulation of the OVLT (≤80 μA,10 Hz,1 sec) evokes a voltage-dependent 3-7 mV *slow depolarization* (duration, 1.5-3 sec) upon which are superimposed fast EPSPs and burst firing. This *slow depolarization* is markedly enhanced in media containing 0-Mg^{2+} and is reversibly blocked by an NMDA antagonist (-)APV (10-20 μM). The *fast EPSPs* are reversibly blocked by a non-NMDA antagonist CNQX (10-30 μM). The SON cells activated by OVLT stimulation fired phasically and demonstrated VP-immunoreactivity. These data suggest that a glutamatergic input from the OVLT activates both NMDA and non-NMDA receptors on SON cells. The latter may depolarize the VP cells sufficiently to remove the voltage-dependent Mg^{2+} block of the NMDA receptor-channel complex and thus permit the NMDA receptor-mediated *slow depolarization*. A possible role of this OVLT input to SON VP cell in response to osmotic stimulation remains to be defined. (Supported by MRC)

287.1

CIRCANNUAL RHYTHM IN THE EFFECTS OF FOOTSHOCK ON HUMORAL IMMUNE RESPONSE IN RAT. J. Amat, A. Torres and H. Vanegas*. Lab. Neurofisiología, Inst. Med. Exp., Univ. Central de Venezuela, Apartado 50587, Caracas, Venezuela.

Many immunological mechanisms are subjected to large seseasonal fluctuations. Such phenomenon result from the circannual rhythm of neurotransmitter systems modulating hypothalamic function. Our study tested the possibility that stress might affect the circannual rhythm of immunity. Adult female sprague Dawley rats were kept in doors, under natural cycles of light and temperature. Twenty min of intermitent footshocks were delivery though the floor of a plexiglass box, the mornings of 4 consecutive days. After the last stress session, stressed and control animals were injected 10^7 sheep red blood cells ip. Afterwards, blood was drawn from the tail every 3 days, and the sera titrated by microhemagglutination. This was repeated once a month between July 1990 and March 1992. Stressed animals showed higher antibody titers than controls the two Jul-Aug periods studied, and lower ones both Sept-Feb periods. This opposite effect of stress is in phase with the annual rythm shown for humoral immunity in "unstressed" animals (1). Therefore, we suggest that the circannual rythm reported for immune mechanisms results from an interaction between the central effects of the minimal stress to which both animals and humans are normally exposed, and the hypothalamic effects of internal clocks with a circannual period.

1) Pati, A., & col., Cell. Immunol., 108 (1987) 227-234

287.2

CLASSICAL CONDITIONING AFFECTS CHEMICALLY-INDUCED TUMORIGENESIS. Joan M.C. Blom, Lawrence Tamarkin and Randy J. Nelson*. Dept. of Psychology, Johns Hopkins University, Baltimore, MD 21218 and Assay Research Inc., College Park, MD 20742.

Traditionally, the immune system has been regarded as an autonomous physiological entity responsible for host defense. Nonetheless, complex interactions exist between the central nervous system (CNS) and the immune system. Classical conditioning, which requires the CNS, of immune function has been demonstrated in several studies. Furthermore, classical conditioning effectively modifies host resistance to the growth and development of transplantable tumors. Suppressed immune function has been associated with increased risk of cancer in humans and non-human animals. Based on the hypothesis that certain aspects of the CNS and immune system interact and that altered immune function affects carcinogenesis, we examined the effects of learned immunosuppression on the development of chemically-induced tumors in mice. Cyclophosphamide (CY) (100 mg/kg ip), an immunosuppressive agent, was paired with saccharin in the drinking water (0.1 %) of adult female mice (CF-1). Conditioned mice were exposed to saccharin twice in the absence of CY, on days 4 and 7 after the first exposure (day 1). All mice were injected with the chemical carcinogen 9,10 dimethyl- benzanthracene (DMBA, 50 mg/kg sc) on day 4 of conditioning. Two subsequent exposures to saccharin substantially increased the risk of developing DMBA-induced tumors (91%), as compared to control animals (36%) that had not received this pairing. Mice that received all agents (i.e., CY, DMBA, and saccharin) in a slightly different order did not display elevated tumor incidence (36-44%). Three separate exposures to CY also significantly increased the number of animals developing tumors in response to the carcinogen (75%). These results indicate that suppression of the immune system enhances susceptibility to chemically-induced tumors, and that learned immunosuppression can increase the risk of developing cancer if coincident with a carcinogenic event.

287.3

SOCIAL STRESS DURING IMMUNE DEVELOPMENT: AN AVIAN MODEL. J. E. Cunnick*, L. Kojic, and R. A. Hughes. Dept of Microbiology, Immunology, and Preventive Medicine and Dept. of Psychology, Iowa State University, Ames, IA 50011.

Young domestic fowl present a useful model to examine the effects of stress on a developing immune system. They express signs of distress during social isolation. Those indicators of stress include increased vocalization and increased body temperature.

We used social isolation paradigm to induce stress in 12 day old birds. We then measured functional immune parameters in the spleen, blood, and thymus after 0, 15, 30, 60, and 90 min of isolation. The immune parameters examined included mitogen responses to Concanavalin A (ConA) and Poke Weed mitogen (PWM). There was a time dependent change in blastogenesis with the greatest change occurring at 60-90 min. In particular, the thymus and peripheral blood showed the most significant suppression of blastogenesis.

Thus, this research demonstrates that stress can alter immune function in developing young birds. Moreover, this paradigm will prove very useful for an examination of acute and long term effects of stress on a developing immune system.

287.4

IN VITRO ADRENOCEPTOR LIGANDS APPROXIMATE STRESS-INDUCED MITOGENIC SUPPRESSION OF HUMAN BLOOD LYMPHOCYTES. J.C.Cochran*, E. Bachen, and B.S.Rabin. Brain, Behavior, and Immunity Center, Univ. Pittsburgh, Pittsburgh, PA 15213.

It is well known that stress can cause the lymphoid system to undergo functional alterations resulting in a decreased response to stimulation by nonspecific mitogens. The mechanism of this suppression in humans is thought to involve the sympathetic nervous system. We have found in high reactor subjects that the stress generated during performance of the Stroop Test reduced lymphocyte mitogenesis to phytohemagglutinin (0.5-5.0 ug/ml) in whole blood assay by an average of 10.5%, while mean catecholamines increased from 1.32×10^{-10} to 2.64×10^{-10} M for epinephrine, and from 7.35×10^{-10} to 8.40×10^{-10} M for norepinephrine. We now confirm the role of catecholamines in mediation of the altered functional response. Human blood lymphocytes were cultured, in whole blood or isolated lymphocyte assays, with 0.5, 1, or 5 ug/ml phytohemagglutinin. Norepinephrine, epinephrine, or the adrenoceptor agonist, isoproterenol, were added to these in vitro cultures at 10^{-10}, 10^{-8}, or 10^{-6} M concentrations. Separate analyses of variance with epsilon correction ($p \leq .01$) were performed on the data of the two assays. In the whole blood assay, the suppressive effect was significant at all concentrations (9.8%, 10.5%, and 14.9% at 10^{-10}, 10^{-8}, or 10^{-6} M, respectively), whereas in the isolated lymphocyte assay suppression was significant only at the highest concentration, suggesting the involvement of accessory cells in the whole blood assay. Thus, with in vitro addition of concentrations of catecholamines comparable to the increases measured in the in vivo stress paradigm, the whole blood assay produced comparable suppression. These data indicate that in vitro addition of physiologic levels of catecholamines or agonist can approximate stress-induced immunosuppressive effects and point to future studies to examine the possible participation of other mediators of the mitogenic suppression.

287.5

ENHANCED HUMORAL AND CELL-MEDIATED IMMUNITY INDUCED BY ELECTRIC FOOT-SHOCK STRESS IN RATS.

Paul Wood, Alex Kusnecov, Larry Wechsler*, Bruce S. Rabin. Brain, Behavior, and Immunity Center, University of Pittsburgh, Pittsburgh, PA. USA

Rats were given electric foot-shocks at either day -1, 0, +1, or +3 in relation to a 50µg I.P. injection of Keyhole Limpet Hemocyanin (KLH). Fourteen days later the rats received an intradermal challenge with antigen and 72 hours later the rats were sacrificed and the response of spleen lymphocytes to stimulation with KLH and the IgG and IgM antibody levels determined. The mean diameters of the delayed hypersensitivity skin test reactions were measured and histology of the skin test reactions was determined. Although the mean diameters of the skin test reactions did not differ between groups the animals stressed on day 0 or day +1 had a large lymphocytic infiltrate in the dermis whereas the non-stressed rats had a minimal infiltrate and the day -1 or +3 stressed rats had an intermediate amount of infiltrate. The largest lymphocyte mitogenic response to KLH was present in spleen lymphocytes from the day 0 or day +1 rats (peak response of 40,000 CPM in comparison to 10,000 CPM in the non-stressed rats). Rats stressed on day -1 or +3 had elevated mitogenic responses in comparison to the non-stressed rats, but lower than the rats stressed on days 0 or +1. The response of the spleen lymphocytes to non-specific mitogen did not differ between groups. The highest IgM antibody responses were in the rats stressed on days 1 or 3 and the highest IgG antibody responses were in the rats stressed on days 0 and +1. Thus, immunologic augmentation can be produced in rats to specific antigen challenge and several functional aspects of the immune system are each found to be affected. As KLH is a T-dependent antigen, it is possible that all immune parameters found to be enhanced are related to T-helper cell function.

287.6

OPIOID SYSTEM REGULATION OF STRESS INDUCED IMMUNE ALTERATION.

M.R. Shurin, A.W. Kusnecov, B.S. Rabin*. Brain, Behavior, and Immunity Center, University of Pittsburgh, Pittsburgh, PA 15213.

The opioid system is believed to participate in the stressor induced modulation of immune system function. The aim of this study was to (1) evaluate the role of delta opioid receptors in stressor induced suppression of cellular immunity, and, (2) quantitate the changes of endogenous opioids in lymphoid organs after a stressor. The study was performed in male Lewis rats and the stressor used was 16-5 second electric foot shocks over 64 minutes. When the shock was preceded by the I.P. injection of 10ug/kg of the delta opioid agonist Leu-Enk (D-Ala^2, Arg^5), there was an amelioration of the suppression of spleen lymphocyte proliferation to the non-specific mitogens PHA and Con-A. The PHA response was ameliorated by 98% and the Con-A by 80%. The amelioration was blocked by pretreating the rats with the delta opioid antagonist Naltrindole (50ug/kg I.P.). This suggests that agonists of the delta opioid receptor can inhibit the stressor induced suppression of lymphocyte responsiveness to non-specific mitogen. Following stress the activity of opioids was measured by displacement using a radioreceptor assay and acetic acid extracts of rat tissue. The opioid activity increased approximately 30% in the spleen and decreased approximately 15% in the thymus, 60% in the pituitary, and 70% in the adrenal. The composition of the opioid subtypes have not yet been determined. These studies support the hypothesis that opioid metabolism is changed in lymphoid tissue in association with a stressor and that the delta opioid receptor can antagonize stressor induced immune suppression.

287.7

EFFECTS OF FOOTSHOCK STRESS UPON SPLEEN AND PERIPHERAL BLOOD LYMPHOCYTE MITOGENIC RESPONSES IN PARAVENTRICULAR NUCLEUS (PVN) LESIONED RATS. M.A. Pezzone*, J. Dohanics, J.G. Verbalis, and B.S. Rabin. **Departments of Pathology and Medicine, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.**

Physically-aversive stressors such as mild electric footshock suppress the mitogenic responsiveness of peripheral blood and splenic lymphocytes as well as other measures of immune function. Although, the pathways mediating these responses have not been fully characterized, neuroendocrine and autonomic components have both been implicated. Using c-Fos as a marker of stimulated neuronal activity, we have previously reported that cells of the hypothalamic PVN (some of which contain corticotropin-releasing hormone, CRH) where strongly activated following an immune-modulating, footshock stimulus. Because neuronal projections from the PVN can influence both the neuroendocrine axis and the autonomic nervous system, the PVN may thus be responsible for mediating many of the effects of stress. To further assess the immunomodulatory role of the PVN, the following study was performed. Male Lewis rats were subjected to either sham or bilateral PVN lesions using a triangle-shaped rotating knife. Six days following surgery, lesioned and sham animals were subjected to either a one hour session of footshock stress or home cage conditions. Subsequently, spleen and peripheral blood samples were collected, and lymphocyte responses to the T-cell mitogens, Concanavalin-A (Con-A) and Phytohemagglutinin (PHA), were measured with the following results. In the peripheral blood, footshock induced a marked suppression of lymphocyte mitogenic reactivity to both PHA and Con-A that was significantly attenuated by the PVN lesions. In the spleen, however, the footshock-induced suppression of lymphocyte mitogenic reactivity to both PHA and Con-A was further suppressed in the PVN lesioned animals. Thus, the PVN has different roles in mediating stressor-induced immune alterations in the spleen and peripheral blood.

287.8

MAJOR DEPRESSIVE DISORDER AND IMMUNE FUNCTIONING IN INNER-CITY ADOLESCENTS: BLOCKADE OF LYMPHOCYTE PROLIFERATION AT G_2.
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Our previous work has demonstrated decreased mitogen induced lymphocyte proliferation in healthy adolescents who met criteria for Major Depressive Disorder (MDD) compared to matched controls without MDD. The rate of entry of stimulated lymphocytes into the various stages (G_1, S, G_2) of the cell cycle is an important determining factor in the proliferation response. We investigated the percent of cells in G_1, S, and G_2 at 24, 48, and 72 hours post-stimulation for both PHA stimulated and media only samples. Ten sets of MDD and control subjects were analyzed and analyses revealed a trend for a G_2 block in the MDD subjects; MDD subjects had a higher percent of cells in G_2 than controls at 48 hours for both PHA stimulated ($F=3.4$, $p<0.09$) and non-stimulated samples ($F=7.1$, $p<0.02$) and at 72 hours for the non-stimulated samples ($F=4.0$, $p<0.06$). This suggests that decreased response to mitogen in adolescents with MDD may be due to cells remaining in the G_2 stage of the cell cycle. Future research to confirm these findings and explore mechanisms of G_2 block, such as blockage of the signal to begin anaphase, is warranted.

287.9

EFFECTS OF STRESS ON PULMONARY NATURAL KILLER (NK) CELL ACTIVITY IN THE RAT N. Quan*, S.K. Sundar, K.M. Boericke & J.M. Weiss. Department of Psychiatry, Duke University Medical Center, Durham, NC 27710.

NK cells in the lung are potentially important for destroying tumor cells, pathogenic microbes, and virus-infected cells at this privileged site. This study investigates the effects of acute stress on pulmonary natural killer (NK) cell activity in Charles-River CD-VAF and Fisher 344 rats. Rats were sacrificed either from their home cage (control) or immediately after an 8 hr session of intermittent tail shock (one per minute, 20 seconds' duration, 1.2 mA intensity). Pulmonary cells, free of blood cell contamination, were collected by the method of Thivierge and Rola-Pleszyczynski (Am. Rev. Respir Dis. 1991; 144: 272-277), and then purified. Lymphocytes from blood and spleen were also extracted. NK activity in these various compartments was measured by assaying the ability of their lymphocytes to lyse YAC ATCC cells. In control rats, NK activity of lymphocytes from the lung and spleen were not different in both CD-VAF and Fisher 344 rats. In CD-VAF rats, the NK activity in the lung, blood, and spleen cells was depressed by the stress to a similar extent (30-45% decrease as compared with the corresponding controls). In Fisher 344 rats, NK activity in the lung, spleen, and blood immediately after the stress was depressed by 50%, 20%, and 13%, respectively; these decreases had not completely recovered 24 hrs after the stress session. These results, therefore, indicate that the activity of resident pulmonary NK cells are also depressed by this form of stress as is NK activity of blood and splenic lymphocytes.

287.10

VOLUNTARY EXERCISE EFFECTS ON IMMUNITY IN RATS.
K. J. Coleman, D. R. Rager and C. J. Karwoski*. Dept. of Psychology, University of Georgia, Athens, GA 30602.

Most studies of effects of exercise on immune function in rodents have employed forced exercise paradigms which may be confounded by stress associated with either involuntary activity or the aversive methods used to induce exercise. To avoid these potentially confounding variables, we examined effects of voluntary wheel running on immune function in male Long-Evans rats. Exercised rats were allowed access to running wheels during the dark period of a 12:12 hr light/dark cycle daily for eight weeks. Five weeks into the study, exercised animals and non-exercised control rats were immunized with Keyhole Limpet Hemocyanin (KLH), and antibody responses to KLH were monitored during the subsequent three-week period. At the end of the study, rats were sacrificed, and spleens and whole blood were assayed for proliferation in response to Concanavalin A. In exercised rats splenic lymphocytes showed enhanced proliferation, and whole blood proliferation was suppressed, while antibody response did not differ from controls.

287.11

EFFECTS OF STRESS ON THE IMMUNE RESPONSE OF SPLEEN CELLS ARE MEDIATED BY THE SPLENIC NERVE. D.M. Nance*, W. Wan, C.Y. Vriend, L. Wetmore, J.G. Gartner and A.H. Greenberg. Depts. of Path. and Physiol. and Inst. of Cell Biol., Univ. of Manitoba, Winnipeg, MB., R3E 0W3, Canada

Intermittent footshock (FS) suppresses immune function of spleen cells. To determine if the autonomic nervous system mediates this immunosuppression in splenic cells, we tested whether cutting the splenic nerve, which depletes splenic norepinephrine and eliminates catecholamine fibers, blocks the effects of stress. Splenic nerve sections, sham operation or no surgery was performed on male S/D rats. Ten days later, rats were injected with sheep red blood cells (SRBC). Three days later, rats were placed in a chamber equipped with a shock grid. FS (1.6 mA) was administered for 5 sec on a VI 3.5 min schedule for 60 min. Each FS was preceded by a 15 sec warning tone. Controls were treated identically except for the FS. The next day spleen cells were harvested and the number IgM plaque forming cells (PFC's) calculated. For the sham and unoperated control animals, the number of PFC's was reduced for the stressed animals, relative to the nonstressed controls and there was no effect of the sham surgeries. In contrast, there was no difference between the stressed and nonstressed groups in which the splenic nerve had been sectioned and their PFC response was comparable to the controls. Next we compared the effects of FS on the proliferative response to mitogens (PHA and ConA) following splenic nerve sections or sham operations. One week following surgery, animals were given a 60 min session of FS or exposed to the chamber/tone without FS. Rats were then killed, spleens harvested and the proliferative response to mitogens determined. FS suppressed the response to mitogens in the sham rats, relative to the sham operated animals not exposed to FS. In contrast, there was no difference between the stressed and nonstressed groups in which the splenic nerve had been cut and their proliferative responses were comparable to the nonstressed-sham animals. Thus, the effects of stress on the PFC response and the proliferative response of spleen cells is mediated via the splenic nerve. These results indicate that the sympathetic nervous system is a fundamental and significant mediator of brain-immune interactions. Supported by MRC, MHRC and HSCRF.

287.12

STRESS-INDUCED REDUCTION IN MLR IS DEPENDENT ON MACROPHAGES BUT NOT ON CHANGES IN PHENOTYPES.
M. Fleshner*, L. R. Watkins, M. L. Laudenslager, and S. F. Maier. Dept. of Psychology, University of Colorado, Boulder, CO 80309.

Exposure to inescapable shock (IS) has been shown to alter many aspects of immune function. We have previously reported that one possible mechanism of stress-induced decreases in anti-KLH antibodies is a shift in CD4/CD8 ratio. The following experiments examined whether 1) exposure to IS would result in changes in another measure of immune function, mixed lymphocyte reaction (MLR), 2) whether this change was associated with changes in phenotype, and 3) whether macrophage depletion would effect any stress-induced changes in MLR. Rats were exposed to either 100 1.6 mA inescapable tail shocks or remained in their home cage (HCC). Spleen (SPL), cervical nodes (CN), and mesenteric nodes (MS) were removed and weighed immediately after IS termination. CN, MS and nylon wool macrophage depleted lymphocytes were tested in an MLR. CD5, CD4, and CD8 positive cells were labeled and assessed using flow cytometry. IS was associated with a decrease in spleen weight ($p<.05$), a decrease in MLR in both CN and MS ($p<.05$), an increase in the % CD4+ cells ($p<.05$) only in MS, and an increase in the CD4/CD8 ratio ($p<.05$) only in MS. Depletion of macrophages eliminated the stress-induced reduction in MLR. These results suggest that IS induces a reduction in MLR and that this reduction is not associated with changes in phenotype as seen with *in vivo* antibody production, but is instead associated with alterations in macrophage function. The specific role of macrophages in the stress-induced reduction of MLR is currently being examined.

287.13

ONTOGENY OF CORTOCOTROPIN-RELEASING FACTOR GENE EXPRESSION IN THE HYPOTHALAMUS, SPLEEN AND THYMUS OF IMMATURE RATS. F. Aird* and E. Redei. Psychiatry Department, University of Pennsylvania, Philadelphia, PA 19104.

Corticotropin-releasing factor (CRF) initiates stress-induced immunosuppression via the hypothalamic-pituitary-adrenal axis and is known to have direct effects on immune cells. We have previously demonstrated the presence of CRF mRNA and bioactive peptide in rat spleen and thymus. In this study we analysed CRF mRNA expression in the rat hypothalamus, spleen and thymus at 7, 14, and 21 days of age. CRF mRNA levels were measured by the semi-quantitative reverse transcription-polymerase chain reaction. In the hypothalamus, the CRF transcript increased at day 14 and decreased at day 21. CRF mRNA levels in the spleen remained constant from day 7 to 21, and in the thymus decreased from day 7 to 21. The role of maternal glucocorticoids in regulation of CRF gene expression was also investigated . Our results suggest that the ontogeny and regulation of "immune" CRF are different from hypothalamic CRF. We propose that the effects of stress on immune function may be mediated through changes in expression of splenic and thymic CRF acting as an immunomodulator. Supported by ADAMHA grant AA07389.

287.14

SUPPRESSION AND ENHANCEMENT OF THE IMMUNE RESPONSE TO CHOLERA TOXIN.

Alex W. Kusnecov, Harry Fowler* and Bruce S. Rabin. Brain, Behavior and Immunity Center, University of Pittsburgh, Pittsburgh, PA. USA

Studies were initiated to investigate the influence of a footshock stressor on antigen-specific immune responses in rats. Experiment 1: Male Lewis rats (n=5) were immunized ip with 1μg cholera toxin (CT) and 7 days later exposed to footshock stress. Spleen cells were assayed immediately for PHA- and CT-stimulated proliferation. Home cage (HC) rats served as controls (n=5). Whereas PHA-induced proliferation was significantly suppressed by greater than 50% in shocked animals, the response to CT was significantly enhanced. Experiment 2: Rats were allocated into 4 groups (n=5/gp) prior to immunization with CT as above. Group D0 received a single session of shock immediately before immunization; Group D5-7 received 3 daily shock sessions on Days 5, 6, and 7 after immunization; Group D7 was shocked on Day 7 as in Expt 1; Group HC was immunized but never shocked. All rats were sacrificed on Day 7, with Groups D5-7 and D7 being sacrificed immediately after footshock exposure. Spleen cells were cultured with CT, and serum was assayed for anti-CT IgM and IgG by ELISA. The results replicated those of Expt 1 in that the D7 group, but not the D5-7 group, had significantly higher CT-stimulated proliferation. The proliferative response of the D0 group was significantly depressed, as were the serum IgM and IgG responses. Similarly, the D5-7 group showed a depressed IgG response, with the IgM response showing a relatively modest reduction. These results suggest a complex interaction between the timing of stressor exposure, immunization, and assessment for antigen-specific cellular and humoral immune function.

287.15

EVIDENCE FOR THE CONDITIONING OF IMMUNE REACTIONS TO A CHOLERA TOXIN. B.J. Kucinski*, L.E. Nelson, A.W. Kusnecov, and H. Fowler. Dept. of Psychology and Pathology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We previously demonstrated that a conditioned stimulus (CS) for a foot-shock unconditioned stimulus (US) suppresses the proliferation of splenic and whole-blood lymphocytes to T-cell mitogens. To investigate whether this conditioned effect would also interfere with immunologic reactions to a specific antigen, we first gave rats Pavlovian conditioning involving a clicker CS and a foot-shock US. Then, after an 11-day recovery in their home cages, the rats were given daily exposures to the conditioning chamber for 13 days and were sacrificed on the next. During this phase, all rats were immunized with a cholera toxin (CT) immediately after the 7th session. In addition, three experimental groups received CS presentations during every other session, starting with the day before, the day of, or the day after immunization. A control group was exposed to just the training context. To our surprise, the results of both a CT-stimulation assay of splenocyte proliferation and an ELISA of IgM and IgG responses indicated significantly enhanced reactions for the group that first experienced the CS on the day of immunization. That suggests that the CS induced an interoceptive effect which functioned as a conditioned stimulus for the unconditioned antibody response induced by the antigen, and that subsequent presentations of the CS during the incubation phase elicited conditioned antibody responses.

NEURAL CONTROL OF IMMUNE FUNCTIONS

288.1

SEX HORMONES AND AGE: EFFECTS ON NATURAL KILLER CELL ACTIVITY S. BEN-ELIYAHU,* G.G. PAGE, S.A. BOUN, J. Hasiano, J.C. LIEBESKIND & A.N. Taylor Brain Research Institute, and dept. of Psychology UCLA; and West LA VAMC, Los Angeles, CA 90024.

It has been suggested that sex hormones and estrous cycle can affect immune function. We undertook a longitudinal study comparing NK activity between males and females at pre- and postpubescence (36 and 63 d, respectively), and studied the relationships between estrous phase and NK activity in the Fischer 344 rat. A group of 70 d old males was used in each assay as an interassay reference for age-related changes. NK cytotoxicity was assessed using a whole blood 4 h ^{51}Cr release assay. Estrous phase was established using vaginal cellularity smears for 10 consecutive days. At 36 d (prepubescent), females exhibited higher NK activity than the age-matched males, and both exhibited less NK activity than the reference group. At postpubescence (63 d), however, males exhibited higher NK activity than females, nearly reaching the reference group level. Females in metestrus exhibited high NK activity, only slightly lower than age-matched males, whereas females in proestrus, estrus, and diestrus, exhibited low NK activity, similar to their pre-pubescent level. The increase in NK activity from pre- to postpubescence in males may be related to increased circulating androgens or to other age related changes, while the similar increase evident in females, only during metestrus, seems to reflect estrous cyclicity. Supported by NIH-HD07228, NIH/NS 07628, VA Medical Research Service (ANT), and the UCLA Psychoneuroimmunology Task Force.

288.2

ETHANOL INCREASES METASTATIC COLONIZATION AND DECREASES LUNG CLEARANCE BY SUPPRESSING NK ACTIVITY IN VIVO G.G. Page,* S. Ben-Eliyahu, R. Yirmiya, J. C. Liebeskind and A. N. Taylor Depts. of Anat. & Cell Biol. UCLA; and West LA VAMC, Los Angeles, CA 90024.

We have previously shown that ethanol increases metastasis in the Fischer 344 rat. We now provide direct evidence that suppression of natural killer (NK) cell activity mediates this ethanol effect. Male rats were injected i.p. with either 2.5 g/kg ethanol or saline. Whole blood cytotoxicity was assessed 2 h later using a 4 h ^{51}Cr release assay in which serum was or was not replaced by RPMI 1640 medium (media). Additionally, we used in vivo methods shown to sensitively reflect NK activity: Lung clearance and metastatic colonization of the MADB106 tumor cell line. To assess the role of NK cells in mediating ethanol effect on lung clearance, a group of rats was selectively depleted of LGL/NK cells using the mAb 3.2.3. NK cytotoxicity per ml blood was significantly suppressed when serum was replaced by media; an even greater suppression was evident when serum was not replaced by media. Lung clearance was suppressed by ethanol in normal rats but not in LGL/NK depleted rats, demonstrating that this effect of ethanol is mediated by its effect on LGL/NK cells. Metastatic colonization was enhanced only at times in which the MADB106 is known to be sensitive to NK activity and blood ethanol levels were elevated. These findings demonstrate that by suppressing NK activity in vivo, ethanol increases the metastatic process. Supported by VA Medical Research Service (ANT), NIH/NS 07628, and the UCLA Psychoneuroimmunology Task Force.

288.3

ALCOHOL IMMUNOTOXICITY F. CHIAPPELLI*, L. HANSON and T. WYLIE Psychiatry, Harbor-UCLA Med. Ctr., Torrance, CA 90509; BRI, Psychoneuroimmunol, Public Health, UCLA; West Los Angeles VAMC.

Alcohol is immunotoxic. The mechanisms of alcohol-mediated immunotoxicity remain to be described. Because of the crucial role of T lymphocytes in the initiation, propagation and regulation of antigen-driven immune competence, we study the mechanisms of alcohol toxicity upon T cells early during lymphocyte activation. We use established human T cell lines (HUT-78) or peripheral blood T lymphocytes from normal healthy donors. The cells are stimulated with anti-CD3 antibody or in a mixed leukocyte culture. Alcohol alters the growth patterns of HUT-78 cells plated in AIM-V medium: Day 4 (control: 7±5% $X10^4$ cells; 0.1% alcohol: 11±4% $X10^4$ cells, $p<0.05$); Day 6 (control: 14±4% $X10^4$ cells; 0.1% alcohol: 10±3% $X10^4$ cells, $p<0.05$). These preliminary data suggest that alcohol favors an initial burst in cell metabolism and growth, that is transient and reversed by prolongued incubation. Alcohol (0.1%) also impairs the expression of membrane activation markers (CD25, CD71) following activation. Experiments now test the effects of alcohol upon the PKC, PKA and phosphatase transmembrane pathways in the presence or the absence of glucocorticoids because alcohol consumption leads to an activation of the HPA axis and elevated cortisol levels. (funded by Fetzer Foundation & REI-BRSG).

288.4

MITOGEN-STIMULATED LYMPHOCYTE PROLIFERATION ACROSS THE ESTROUS CYCLE OF THE RAT. D.R. Rager*, K.J. Coleman, T.E. Parsons, and J.D. Newbern. Dept. of Psychology, Univ. of GA, Athens, GA 30602.

Although a number of studies have shown that gonadal hormones can affect immune function, few have investigated whether immune responses vary as a function of estrous stage in intact cycling females. This experiment explored whether a standard *in vitro* measure of immune function (i.e., mitogen-stimulated lymphocyte proliferation) varies across the estrous cycle in the adult female Long-Evans rat. Estrous cycles were monitored by daily vaginal lavage, and peripheral blood samples were obtained from tail veins of normally cycling rats during either proestrus/estrus or metestrus/diestrus. Proliferation of peripheral blood lymphocytes (PBLs) in response to concanavalin A was determined using a radiolabeled thymidine incorporation assay. PBLs derived from females during proestrus/estrus exhibited lower proliferative responses as compared to PBLs derived from females during metestrus/diestrus. These data are discussed with regard to hormonal changes that may mediate estrous stage differences in immune responses.

288.5

EFFECTS OF CGRP AND TACHYKININS ON RAT LYMPHOCYTE PROLIFERATION. J.G. Hamra*, X.-Y. Hua and T.L Yaksh. Dept. of Anesthesiology, Univ. of California San Diego, La Jolla, CA 92093.

Sensory nerve fibers containing immunoreactive calcitonin gene-related peptide (CGRP), substance P (sP), and other neuropeptides have been demonstrated in both primary and secondary lymphoid organs. A number of studies have shown that CGRP and peptides of the tachykinin family have potent effects on immune responses. However, the effect of these peptides appears to vary with species and cell type. In this study, we evaluated the effect of CGRP and selective NK receptor agonists on rat lymphocyte proliferative responses. Rat spleens were removed and lymphocytes isolated by density gradient centrifugation. The lymphocytes were incubated with a combination of mitogens, Concanavalin A (ConA) and pokeweed mitogen (PWM), and peptides. CGRP enhanced lymphocyte proliferation in the absence of mitogens (10^{-8}M, $P<0.001$) as well as in PWM stimulated cells (10^{-6}M, $P<0.01$). Substance P and [Sar^9, $Met(O_2)^{11}$]sP, a selective NK1 receptor agonist, had no effect on proliferative responses. Senktide, a NK3 receptor agonist, also had no effect. Neurokinin A (NKA) enhanced proliferative responses to suboptimal concentrations of PWM (10^{-6}M, $P=0.05$). However, [Nle^{10}]Neurokinin A_{4-10}, a highly specific NK2 agonist, suppressed both ConA and PWM stimulated proliferation as well as proliferation in the absence of mitogens (10^{-6}M, $P<0.001$). As sP, CGRP and NKA are costored in and corelеased from the peripheral terminals of sensory afferents, these results suggest a direct immunodulatory effect mediated by antidromic afferent activity, possibly through a NK2 site. These results also suggest that the cell population involved (i.e., T or B lymphocytes) may determine the net effect on proliferative responses. (Supported by NIH NS07329 - JGH, TRDRP 1KT-19 - XYH).

288.6

CALCITONIN GENE-RELATED PEPTIDE (CGRP) INHIBITS EARLY B CELL DIFFERENTIATION BY A RECEPTOR MEDIATED MECHANISM J.P. McGillis*, V. Rangnekar, and J. Ciallela. Micro-Immunol., Univ. Kentucky, Lexington, KY 40536-0084

CGRP receptors and the effect of CGRP on 70Z lymphocytes was examined. 70Z cells are pre B cells which have rearranged heavy (μ) and light (κ) immunoglobulin (Ig) genes. Following LPS treatment 70Z cells differentiate into immature B cells which express Ig on the cell surface. Binding studies with ^{125}I-CGRP found that both differentiated and undifferentiated 70Z cells express high affinity CGRP receptors. ^{125}I-CGRP binding was specific in that it could only be displaced by CGRP and CGRP analogs. The 70Z CGRP receptor protein was affinity labelled by crosslinking ^{125}I-CGRP to the receptor with DSS. SDS-PAGE analysis of the affinity labelled 70Z CGRP receptor revealed that it is a single protein of 103,000 mw. CGRP also stimulated cAMP in a dose dependent manner. The effect of CGRP on LPS induced differentiation was examined by FACS analysis of surface Ig expression and northern blot analysis of μ and κ mRNA. LPS treatment increased the % of cells expressing surface Ig from less than 5% to greater than 90%. CGRP reduced the level of LPS induced surface Ig expression by about 30%. This was due to a decrease in the levels of both μ and κ mRNA. These studies suggest that CGRP inhibits early B cell differentiation by binding to an adenylate p cyclase linked receptor.

288.7

ACUTE AND CHRONIC EFFECTS OF N-METHYL-D, L, ASPARTIC ACID (NMA) INJECTION INTO THE ANTERIOR HYPOTHALAMIC AREA (AHA) ON THYMUS GLAND AND PERIPHERAL CORTICOSTERONE (CORT), PROLACTIN (PRL) AND LYMPHOCYTE SUBTYPES. C.P. Phelps*, L.T. Chen, L.L. Poole, J.E. Miley, J.M. Oliver, B. Muffly and R.A. Menzies. Depts. of Anatomy and Psychiatry and Behavioral Medicine, Coll. of Med., Univ. South Florida, Tampa, FL 33612

We have studied the acute (hrs) and longer (wks) term effects of NMA on endocrine and immune functions after bilateral NMA injections (0.6M in 0.1µl and 0.15µlaCSF) into the AHA of male rats (350-400g). Animals received atrial cannulas 5-7d before (0 day, AM, PM), over 2 hrs and at 3 and 4d intervals 3-28d after NMA. Plasma Cort and Prl were assayed by RIA and peripheral thymus-origin lymphocytes (T cell) were separated into %CD4+ (helper) and %CD8+ (cytotoxic/suppressor) using fluorescent antibody cell sorting antibodies (facs). Brains and thymus glands were removed and studied histologically. NMA produced 4 small ($0.1mm^3$) conical-shaped cellular lesions, with aCSF damage restricted to needle tracks. Plasma Prl showed very little change during the first 2 hr after either aCSF or NMA. Plasma Cort was elevated 5X from Od(50-70 ng/ml) by pentobarbital (PB) and 8X from Od by PB plus stereotaxic surgery. Although NMA resulted in no additional acute Cort changes, there were subsequent chronic 2X elevations during the first few wks after NMA. Associated Prl levels were one-half basal levels (6-8 ng/ml). Thymus glands after NMA were reduced in size by approximately 50% when compared to glands from control rats at all intervals (8-10d, 14-18d, 20d and 28d) studied. The percentage of circulating CD8+ T cells in peripheral blood also fluctuated after NMA, whereas CD4+ T cells remained relatively constant. In summary, Cort and Prl changes after NMA injection into the AHA were associated with smaller thymus glands and a shift in CD8+ T cells. Supported by MH46808.

288.8

IN VIVO EFFECTS OF ACUTE AND CHRONIC NICOTINE ON THE IMMUNE SYSTEM OF RATS

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Cigarette smoke alters several measures of immune function. However, since cigarette smoke contains at least 3800 products, its immunologic effects cannot necessarily be attributed to the actions of nicotine per se. We recently reported a dose-dependent decrease in the proliferative response of blood T lymphocytes to Con A in male rats 1 hr after s.c. nicotine (Caggiula et al, 1992). In the present study, an **ACUTE** injection of nicotine bitartrate (1.32 mg/kg, free base) suppressed the blood lymphocyte response to Con A and PHA but had no effect on spleen lymphocytes. After **CHRONIC** (10 daily) injections of nicotine, tolerance developed to the suppressive effects in blood whereas the response of spleen lymphocytes actually increased above controls. The relative proportions of major cell types did not change after any of these treatments, so the differences between spleen and blood cannot be attributed to a change in the trafficking of the major cell types. In summary, nicotine can significantly alter functional measures of the immune system in rats. The effects are complex, depending on the duration of exposure and compartment sampled.

288.9

ANESTHESIA-INDUCED IMMUNOMODULATION OF *IN VIVO* ANTIBODY PRODUCTION. L.L. Lockwood*, L.H. Silbert, L.R. Watkins, ^M.L. Laudenslager, and S. F. Maier. Department of Psychology, University of Colorado, Boulder, CO 80309. ^Dept. of Psychiatry, Univ. of CO-HSC, Denver, CO 80204.

Surgery and anesthesia alter immune function. These changes in immune function may have implications for the potential development of disease after surgery and complicate the study of the neural control of immune function. Further elucidation of anesthesia-induced changes in immune function is important to our understanding of the interplay between surgical stress, anesthesia, and the immune system. Previous research has demonstrated that anesthesia alone can alter lymphocyte proliferation and recirculation, reduce phagocytosis and accelerate metastasis of tumors. These were all measured soon after anesthesia administration. We sought to determine if various anesthetics would have long term immunomodulatory effects on an *in vivo* measure of antibody production. In a series of experiments, Sprague Dawley rats were injected intraperitoneally with 100 ug of antigen, Keyhole Limpet Hemocyanin (KLH), three weeks after exposure to surgical doses of pentobarbital, methoxyflurane, halothane, ketamine/xylazine, or chloral hydrate. Serum samples to measure anti-KLH IgG antibody production were taken 7, 9, and 14 days after antigen injection. When antigen was introduced three weeks after anesthesia exposure, animals that had been anesthetized with chloral hydrate or pentobarbital showed significant suppression of antibody production as compared to home cage controls. No significant differences were observed for halothane, ketamine/xylazine or methoxyflurane. Further studies are being done to investigate the range of effects of these anesthetics and potential interactions between surgery, anesthesia and immune function.

288.10

IMMUNE AXIS ACTIVATION UP- AND DOWN-MODULATES BETA-ADRENERGIC RECEPTOR MESSENGER RIBONUCLEIC ACID LEVELS IN THE RAT THYMUS. M.C. Morale*, F. Gallo, N. Batticane and B. Marchetti. OASI Inst. Mental Retard. and Brain Aging, Troina (EN) and Dept. Pharmacology, Medical School, University of Catania, 95125 Catania, Italy.

Important alterations of noradrenergic acitivity are known to occur in specific brain regions and in different lymphoid tissues during the course of an immune response (IR). Lymphokines and other soluble substances produced by the activated immune system in addition to changes in corticosterone production have been suggested as possible mediators in this crosstalk between the neuroendocrine and immune cells. The aim of the present study was to assess whether products of activated lymphocytes interact with the thymic beta-adrenergic receptor (BAR) signaling system. Thymic BAR gene expression was measured using a human BAR cDNA as a probe, at different time intervals after immunization. Northern blot analysis of thymic RNA and iodocyanopindolol binding measurements revealed almost 70% decreases of mRNA concentration and BAR numbers 3 days after immunogenic challenge. This effect was accompanied by a significant decrease in the ability of isoproterenol to stimulate adenylate cyclase activity in membranes preparations of thymic tissue. On the other hand, 15 days following immunogen administration, a marked increase of BAR mRNA content and BAR numbers, with a parallel stimulation of adenylyl cyclase activity, was observed. The up- and down-modulation of BAR gene expression coupled to the alteration of adenylyl cyclase-cAMP signaling pathway may represent feedback mechanisms to down regulate the immune response.

288.11

HYPOTHALAMIC CONTROL OF PERIPHERAL IMMUNE FUNCTIONS MEDIATED BY THE SYMPATHETIC NERVOUS SYSTEM IN RATS. T. Katafuchi*, S. Take, T. Ichijo and T. Hori. Dept. of Physiology, Kyushu Univ., Fac. of Med., Fukuoka 812 Japan.

ICV injection of recombinat human interferon α (IFNα) in rats suppressed the cytotoxic activity of splenic natural killer (NK) cells, depending on central opioid receptors and intact splenic innervation. ICV IFNα (1500-6000U/rat) produced a dose-dependent and long-lasting (more than 1 hr) excitation of the electrical activity of the splenic sympathetic nerve, which was transiently suppressed by naloxone (iv). Since an electrical stimulation of the nerve suppressed the NK activity in the spleen, the IFNα-induced suppression of NK activity is brought about by an opioid receptor-mediated activation of the splenic sympathetic nerve.

In the present study, we investigated the central mechanisms of the immunosuppression mediated by the splenic sympathetic nerve. In urethane-αchloralose anesthetized rats, lesion of the preoptic/anterior hypothalamus (POA) but not the thalamus induced a significant suppression of NK cytotoxicity, which was completely blocked by the splenic denervation. Ablation of the POA enhanced the splenic nerve activity, while electrical and glutamate stimulations of the POA suppressed it. Glutamate infusion into the PVN increased the activity. Furthermore, microinjection of IFNα (200U/rat) into the POA produced an excitation of the nerve activity, while an injection into the PVN had no effect. We have previously demonstrated that IFNα inhibited the POA neuronal activity in tissue slices. The present findings, taken together, suggest that the POA may play an important role in the INFα-induced suppression of splenic cellular immunity mediated by the splenic sympathetic nerve.

288.12

HYPOTHALAMIC INTERFERONα (IFNα) REDUCES SPLENIC NK CYTOTOXICITY. S.Take, T.Katafuchi, T.Ando, D.Uchimura, Y.Kanemitsu, T.Ichijo, N.Shimizu, T.Hori and T.Kosaka*. Dept. Physiol. & Anat.*, Kyushu Univ., Fac. Med., Fukuoka 812, Japan

The brain produces high levels of IFNα during viral infections. We showed that ICV injection of rhIFNα (>1000U) reduced the cytotoxicity of splenic natural killer (NK) cells through brain opioid receptors. This immunosuppression was abolished by splenic denervation, but not by adrenalectomy. ICV rhIFNα increased the activity of the splenic sympathetic nerve. Thus, brain IFNα seems to stimulate opioid receptors and thereby reduces the NK activity through sympathetic innervation.

To determine the site of action of rhIFNα to reduce the NK activity, rhIFNα was injected through an implanted cannula into different brain sites of male Wistar rats (150-200g). After 30 minutes, the spleen was extracted and the NK activity was measured by a standard chromium release assay. An injection of rhIFNα of 200U into the preoptic area (POA) induced a decrease of NK activity by about 60% which was of the same degree to that observed after ICV rhIFNα of 20000U. The other hypothalamic sites (PVN, the posterior hypothalamus) appear to be less sensitive to microinjected rhIFNα in affecting the NK activity. The injection into thalamus had no effect. Pretreatment with α-helical CRF (25μg, ICV) completely abolished the IFNα-induced suppression of NK activity, while CRF (1-2.5μg,ICV) resulted in an increase of splenic nerve activity and noradrenaline release as determined by an in vivo microdialysis which accompanied by reduced NK activity. The results suggest that brain IFNα reduced the NK activit through activation of the CRF system and that the POA seems to be one of the sites of action of IFNα.

288.13

INTERFERON-INDUCED EFFECTS ON CA3 PYRAMIDAL CELLS IN RAT HIPPOCAMPAL SLICE CULTURES: MEDIATED BY HYDROGEN PEROXIDE? Michael Müller*, Adriano Fontana, Gerhard Zbinden and Beat H. Gähwiler. *Brain Research Institute, University of Zürich, 8029 Zürich, Switzerland.*

Neuronal dysfunction observed in viral infection may be due to lytic effects of the virus and/or the antiviral host response including production of cytokines such as interferons (IFNs). We have therefore studied the effects of recombinant murine IFN-γ and IFN-α/β on functional properties of pyramidal cells in hippocampal slice cultures. Bath application of IFNs induced an excitatory effect on CA3 pyramidal cells, and a decrease in IPSP amplitude eventually leading to epileptiform bursting. These effects were slow in onset (several minutes), suggesting an indirect mechanism of action. The release of hydrogen peroxide (H_2O_2) from non-neuronal cells may be in part responsible since IFN effects were found to be blocked by the free radical scavengers catalase and superoxide dismutase, and H_2O_2 mimicked the effects of IFN on pyramidal cells: it reduced evoked inhibitory synaptic transmission, leading to epileptiform bursting, and induced a hyperpolarizing K^+-current. These data indicate that production of IFNs in the CNS may contribute to neurologic disease in virus infection.

288.14

DEGRADATION OF MET-ENKEPHALIN BY HUMAN GRANULOCYTE MEMBRANE PEPTIDASES. M.K. Leung*, R. Flowers, S. Houston and G.B. Stefano. Departments of Chemistry and Biological Sciences, SUNY/Old Westbury, Old Westbury, NY 11568-0210.

Met-enkephalin, (ME; YGGFM), as a immunoregulator, is capable of inducing changes in adhesion and morphology of human granulocyte. These changes are potentiated by peptidase inhibitors suggesting the inactivation of ME is mediated by membrane-bound peptidases. To identify these peptidases, human granulocyte membrane was washed free of all soluble peptidases and dissolved in PBS with 2% Triton X-100. ME (50 uM) was incubated with the solublized membrane preparation in the presence or absence of peptidase inhibitors at 37°C. The incubation mixtures were inactivated by the addition of an equal volume of 20% CH_3CN in 0.2% trifluoroacetic acid at the end of the incubation periods and analyzed by HPLC. Time-course study, showed half of the ME was degraded by membrane peptidases in 10 min and this was accompanied by an increase in the degradation fragments, GGFM, FM and F. The formation of GGFM was inhibited by bestatin while FM, by phosphoramidon indicating the presence of aminopeptidase and neutral endopeptidase 24.11. The formation of F was inhibited by both bestatin and phosphoramidon suggesting F was formed by the action of aminopeptidase on the degradation product, FM. The role of these peptidases in the inactivation ME is currently under investigation.

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288.15

BIDIRECTIONAL SIGNALS BETWEEN TRYPANOSOMA BRUCEI BRUCEI AND DORSAL ROOT GANGLIA NEURONS. K.Kristensson, A. Eneroth*, M. Bakhiet and T. Olsson. Clinical Research Center and Department of Neurology, Karolinska Institutet, Huddinge Hospital, S-141 86 Huddinge, Sweden.

The extracellular haemoflagellate Trypanosoma brucei brucei (T.b.b.) releases a factor (TLTF), which can trigger CD8+ T-cells to interferon-γ (IFN-γ) production, and IFN-γ derived from these cells promotes proliferation of the T.b.b. We now report that T.b.b. can interact with small neurons in rat dorsal root ganglia (DRG) cultures, which contain a IFN-γ-like immunoreactive (IR) molecule. Cultures of DRG could promote both the proliferation and survival of T.b.b. and this growth promoting effect by the cultures was completely blocked by monoclonal antibodies against TLTF, IFN-γ and CD8+ and their Fab-fragments. Living T.b.b. and TLTF induced an increase in major histocompatibility complex (MHC) class I antigens expression and a decrease in the levels of IFN-γ IR in the cultures. The MHC induction was blocked by Fab fragments of the IFN-γ antibody, indicating that it is mediated by release of the neuronal IFN-γ like IR molecule. These data strongly suggest, that the IFN-γ like IR molecule in small DRG neurons mimicks certain physiological effects of lymphocyte derived IFN-γ. Furthermore, T.b.b. and small sensory neurons seems to interact bidirectionally by the TLTF and the IFN-γ like molecule, whereby perhaps neuronal disturbances may be elicited.

288.16

THE NUDE MOUSE AS MODEL TO STUDY THE IMMUNOMODULATORY EFFECT OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH)

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Nude mice contain many T cells but cannot generate pathogen-specific T-cell responses because they have only a thymic rudiment. While there are many T cell marker positive cells in peripheral lymphatic tissues of nude mice and they contain alloreactive cytotoxic T-cell precursors as well as T-helper cell activity for antibody production, they do not generate protective MHC restricted pathogen-specific T cells. Thus, the question of how nude mice can select and propagate precursor T cells remains unknown, and the specific components of the thymic microenvironment involved in T cell development have not been well characterized. Since our recent studies have postulated a major role for the hypothalamic neuropeptide LHRH in thymus physiology, the purpose of this work was to investigate the ability of LHRH and one of its potent agonistic analog, D-Trp^{6}LHRH, to influence thymus-dependent immune functions in this model. Using flow cytometry as a tool to analyze intrathymic differentiation pathways, this study shows that pulsatile administration of the neuropeptide markedly stimulates thymocyte differentiation and maturation, suggesting that LHRH via an interaction with the thymic microenvironment may promote thymocyte development.

PAIN MODULATION: BRAINSTEM AND THALAMUS

289.1

BEHAVIORAL CHARACTERIZATION OF THE INTERACTION BETWEEN THE NUCLEUS RAPHE MAGNUS AND THE LATERAL RETICULAR NUCLEUS: ROLE OF NORADRENALINE. A.Z. Murphy and M.M. Behbehani. Dept. of Physiology, Univ. of Cincinnati College of Medicine, Cinti., OH 45267-0576.

Using electrophysiological techniques we have recently shown that lateral reticular nucleus (LRN) receives a major input from the nucleus raphe magnus (NRM). In order to determine the role of this pathway in the descending analgesia system, we examined the effect of blocking noradrenergic activity within the LRN on NRM stimulation produced analgesia. Male Zivic-Miller rats were anesthetized and 25 gauge guide cannulas were stereotaxically placed within the NRM, and bilaterally within the LRN. Following a 7 day recovery period, the effect of bilateral yohimbine (YOH) administration into the LRN on the analgesic response produced by d,l-homocysteic acid (DLH) administration into the NRM was determined using the tail-flick (TF) test . Microinjection of DLH (2mM; 0.5 ul) into the NRM increased TFL's 140% of baseline at 20 min post-injection and remained stable for up to 2 hrs. Pretreatment with YOH (3ug/.5ul), bilaterally within the LRN, consistently potentiated the effects of DLH alone at all time periods tested, with an average increase in basal TFL of 160%. This effect was most notable within the first 10 mins following DLH administration into the NRM in which YOH pretreatment produced a 30% increase in TFL in comparison to DLH treatment alone. Administration of YOH alone (YOH + saline) had no effect on baseline TFL. These results concur with our previous studies and further show that this region is an important relay in the NRM-spinal cord descending system. Supported by PHS grant NS20643.

289.2

THE OPPOSITE EFFECTS OF CHOLECYSTOKININ (CCK) AND MET-ENKEPHALIN (ENK) ON PERIAQUEDUCTAL GRAY (PAG) NEURONS MEASURED BY CONVENTIONAL AND PATCH CLAMP METHODS. M.M. Behbehani*, H. Liu, S.D.Chandler and M.T. Shipley. Dept. of Physiol and Anatomy U. of Cincinnati, Cincinnati, OH, 45267-0576

To determine the cellular basis by which CCK antagonists potentiate morphine's anagesic effect, in intact rats the effects of both CCK and ENK on the same PAG neuron was examined. ENK inhibited 84% (12/14) of cells that were excited by CCK. To further analyze these actions, extracellular procedures were used to measure the effects CCK and ENK on PAG neurons maintained in vitro. CCK excited 40/56, inhibited 7/56 and had no effect on 9/56 cells. The excitatory response to CCK was blocked by CR1409 in 32/36 cells and by proglumide in 25/27 cells. Conventional intracellular recording were made from 16 cells. In 13 cells CCK produced a 5-14 mv membrane depolarization and increased the firing rate by as much as 72%. An inhibitory response to CCK was noted in 2 cells and CCK had no effect on one cell. The response to both CCK and ENK was compared in 25 cells. CCK excited 15/25 and inhibited 5/25 cells. ENK inhibited 15/25 and inhibited 4/25 cells. In the whole cell patch clamp studies, the effects of CCK on 10 cells were examined. In these cells CCK produced a decrease in resting membrane conductance and shifted the IV curve to a more depolarizing potential. It is concluded that 1) The excitatory effect of CCK is due to depolarization of the membrane more likely due to increased Na conductance 2) The majority of cells that are inhibited by ENK are excited by CCK. Supported by PHS NS20643.

289.3

EFFERENT PROJECTIONS TO RAT PERIAQUEDUCTAL GRAY MATTER AND PARABRACHIAL NUCLEI FROM TRIGEMINAL NUCLEUS ORALIS. L. A. Smith* and W. M. Falls. Dept. Anatomy, Michigan State University, East Lansing, MI 48824.

Trigeminal nucleus oralis (Vo) is involved in the reception and central processing of non-noxious orofacial sensory information and plays a significant role in the reception and central processing of nociceptive sensory information from oral and dental structures and cranial vasculature. Electrical stimulation of the midbrain periaqueductal gray (PAG) inhibits cutaneous nociceptive responses of brainstem trigeminal neurons. This study examines the overall course, distribution, organization and morphology of efferent axons and their terminal arborizations originating from neurons in dorsomedial (DM), ventrolateral (VL) and border zone (BZ) subdivisions of rat Vo and innervating brainstem structures contributing to the intrinsic analgesia system, i.e., PAG, nucleus parabrachialis (PB). Unilateral deposits of the anterograde tracer PHA-L were made iontophoretically into each Vo subdivision. Axons of Vo projection neurons form terminal arborizations of a single type in PAG and more than one type in medial and lateral PB. The greatest density of Vo efferents originate from DM neurons followed by BZ and VL cells. Vo efferent projections to PAG may play a role in relaying noxious information to initiate inhibitory feedback interactions through PAG innervation of nucleus raphe magnus which directly inhibits nociceptive trigeminal neurons. Supported by NIH/BRSG to C.O.M.

289.4

CHRONIC NOCICEPTION CAUSES AN INCREASE IN BASAL ENKEPHALIN IN THE PERIAQUEDUCTAL GRAY: A MICRODIALYSIS STUDY. F. G. Williams*, W. M. Renno, and A. J. Beitz. Department of Pathobiology, University of Minnesota, 1988 Fitch Ave., St. Paul, MN 55108

Opiate-induced antinociception has been postulated to involve GABAergic interneurons in the periaqueductal gray matter (PAG). In an animal model for chronic nociception, we have examined the functional, dynamic release of enkephalin in the ventral-lateral PAG. Extracellular enkephalin levels were determined using microdialysis followed by radioimmunoassay. Complete Freund's adjuvant (CFA) or mineral oil (120 ul) was injected into one hindpaw, and the ventrolateral PAG on the contralateral side was dialysed either 24 hours or 7 days later. Twenty-four hours following hindpaw injection, enkephalin recovery in the dialysate of animals receiving CFA was increased nearly five-fold over those that received oil: from 1.29 to 6.25 pg/12 minute fraction ($p < .01$). The increase was attenuated 7 days following CFA or oil injection: basal enkephalin levels in the dialysate dropped to approx. two-fold higher in animals that received CFA. Veratridine (75uM) induced the release of additional enkephalin from the PAG of all experimental groups. Veratridine-induced release was greater in oil-injected control rats than in CFA-injected experimentals. These release data correlate with behavioral data (paw withdrawal latencies) and increased enkephalin biosynthetic capacity (determined using quantitative hybridization histochemistry for pro-enkephalin A mRNA) to suggest that the enkephaliergic system in the PAG is activated by nociception. The inverse relationship between GABA and enkephalin levels in PAG dialysates supports the hypothesis that endogenous enkephalin inhibits GABA release in the PAG. Supported by NIH grants NS28016, DA06687, and DE06682.

289.5

GABAERGIC INPUTS TO TWO TYPES OF PAIN MODULATORY NEURONS OF THE NUCLEUS RAPHE MAGNUS OF THE ADULT CAT SHOWN BY ELECTRON MICROSCOPIC IMMUNOCYTOCHEMISTRY.

K Skinner*[1], P Mason[1], AI Basbaum[2,3] and HL Fields[1,2]. Depts of Neurology[1], Physiology[2] and Anatomy[3], University of California, San Francisco, CA, 94143

The regulation of a GABAergic input to on and off cells of NRM has been implicated in opioid analgesia (see Fields *et al*, Ann. Rev. Neurosci. 14:219, 1991). In this study we used postembedding immunocytochemistry to reveal the distribution and characteristics of direct GABA-immunoreactive (ir) inputs to two NRM neurons. Electrophysiologically characterized NRM cells were intracellularly labelled with horseradish peroxidase. After reconstruction of the cells, selected regions were processed with an immunogold procedure using antisera directed against glutaraldehyde-conjugated GABA.

GABA-ir boutons contacted the soma, proximal and distal dendrites of both on and off cells, however the distribution over the two cell types differed. Immunoreactive boutons were distributed uniformly over the entire on cell soma and dendrites, but we found no GABA-ir boutons on the proximal 50μm of the off cell dendrites sampled and only two boutons were found on the off cell soma. Bouton density on the off cell progressively increased over distal dendrites. GABA-ir terminals covered 15.6% and 8.9% of the total synaptic membrane of on and off cells respectively. Furthermore, GABA-ir boutons constituted 16.9% of the total number of boutons apposed to the on cell and 10.5% of the boutons apposed to the off cell.

This study provides anatomical confirmation that there is a GABAergic regulation of both on and off cells in the cat NRM. The more uniform input to the on cell may provide the anatomical basis for a tonic inhibition. The regionally distributed input to the off cell may subserve a selective inhibition. Supported by NS21445 and NS14627.

289.6

SEROTONIN IMMUNOCYTOCHEMISTRY OF PHYSIOLOGICALLY IDENTIFIED NEURONS IN THE RAT ROSTRAL VENTROMEDIAL MEDULLA. S. Potrebic*H.L. Fields, and P. Mason, Dept of Neurology, UCSF, San Francisco, CA 94143.

The rostral ventromedial medulla (RVM) is the major source of serotonin (5-HT) in the dorsal horn. A large body of evidence suggests that RVM serotonergic neurons inhibit nociceptive transmission and mediate the antinociceptive effects of supraspinal opioids. Three physiological cell classes in RVM, off-, on- and neutral cells, putatively have different effects on nociceptive transmission. The aim of this experiment was to determine which RVM cell class contains 5-HT.

RVM cells were characterized by their responses during a nociceptive withdrawal reflex in the lightly anesthetized rat. Cells were intracellularly labeled and then processed for 5-HT immunocytochemistry. Labeled cells were examined with epifluorescence and imaged using a confocal microscope.

A total of 24 RVM neurons were intracellularly labeled. Only neutral cells (4/8) contained 5-HT immunoreactivity. No off- (N=9) or on- (N=7) cells were immunoreactive for 5-HT.

Since opioids alter the firing of on- and off-, but not neutral cells in RVM, these studies indicate that opioid anti-nociception does not require a change in serotonergic RVM cell activity.

289.7

Interconnections between rostral ventral medullary nuclei in the rat: an anterograde tract tracing study A. Zagon* University Dept. of Pharmacology, Oxford, U.K. and Dept. of Physiology, University of California, San Francisco CA 94143

The rostral ventral medulla oblongata is involved in both blood pressure control and the central modulation of nociception. Physiological and pharmacological data show that the area might be an important site where integration between these functions occur. Our aim was to reveal the anatomical connections among various nuclei of the rostral ventral medulla that could provide a basis for such functional interactions. Discrete injections of Phaseolus vulgaris leucoagglutinin (PHA-L) were placed into the rostral ventrolateral reticular (RVL), the lateral paragigantocellular (LPGi), the raphe magnus (RMg) and/or pallidus (RPa), obscurus (ROb) and gigantocellular pars alpha nuclei (GiA). After 12-14 days, the tracer content of the medulla oblongata was visualized immunohistochemically. The distribution of labelled terminals was mapped contralateral to the injection centre.

Following PHA-L injections into the RVL, labelled terminals were most abundant in the RMg, ROb, RPa, GiA and RVL. Projections from the LPGi targeted the RMg, ROb, RPa, GiA, ventral gigantocellular nucleus (GiV) and LPGi. Terminals from the GiA and rostral RMg-RPa areas were focused in the medial aspects of the ventral medulla oblongata, in the GiA, GiV, LPGi and GiA, ROb, LPGi respectively. A dense, widespread projection to all parts of the ventral medulla originated from the ROb, while only a small number of labelled efferents could be observed following injections of the RPa.

The observed widespread projections of the ROb suggest a coordinative role of this nucleus in the rostral ventral medulla. As inputs from the majority of the ventral medullary nuclei reached the LPGi and GiA, the data point to the LPGi and GiA as major sites of local information integration.

289.8

THE DISTRIBUTION OF BRAINSTEM AND SPINAL CORD NUCLEI ASSOCIATED WITH DIFFERENT FREQUENCIES OF ELECTROACUPUNCTURE ANALGESIA. J. H. Lee*and A. J. Beitz, Dept. of Vet. PathoBiology, Univ. of Minnesota, St. Paul, MN55108.

Immunocytochemical localization of the c-*fos* primary gene protein, Fos, was utilized to identify spinal cord and brainstem sites activated by either 4Hz or 100Hz electroacupuncture(EA) applied to the Zusanli acupuncture points of both hindlimbs in lightly anesthetized rats. The number and distribution of Fos immunoreactive neurons in the brainstem and spinal cord of 4Hz and 100Hz EA treated rats were compared with those in anesthesia and room control rats. Compared to nonstimulated control rats, both 4Hz and 100Hz EA treated groups exhibited a significantly greater number of Fos labeled neurons in the dorsal horn of the L2 spinal cord segment, lateral parabrachial nucleus, substantia nigra, raphe pallidus, dorsal raphe nucleus, locus coeruleus, posterior pretectal nucleus and the ventrolateral periaqueductal gray. In the 4Hz treated group, significant increases in Fos labeling were also observed in the cuneiform nucleus, dorsal and dorsolateral subdivisions of the periaqueductal gray, habenular nucleus, arcuate nucleus, and the ventrolateral and lateral hypothalamic nuclei as compared to nonstimulated controls. The only brainstem nucleus that exhibited significantly increased Fos immunoreactive neurons in 100Hz but not 4Hz EA was the rostroventrolateral nucleus of the medulla. These results indicate that many brainstem regions are activated by both 4Hz and 100Hz EA but additional brain stem regions are selectively activated by 4Hz EA which may relate to the opiate sensitivity of 4Hz EA. In sum, these data identify several distinct brainstem nuclei that may play a role in acupuncture mediated analgesia. Supported by NIDA grant DA 06687 and NIH grants DE 06682 and NS 28016.

289.9

INFLUENCE OF MIDAZOLAM AND PROPOFOL ON ACTIVITY OF THALAMIC NEURONS IN RAT. V.M. Tronnier*, N.M. Pham, M. Lis-Planells and P.C. Rinaldi, Dept. of Neurosurgery, University of California, Irvine, CA 92717

Functional stereotactic procedures performed in patients for relief of chronic pain are often accompanied by neurophysiological recordings from neurons to map appropriate thalamic targets. Recent recordings of neuronal hyperactivity and bursting in medial and lateral somatosensory thalamic nuclei may relate to chronic pain mechanisms. During fixation of the stereotactic frame and cranieotomy, local anesthetic and sedation is often employed. Drugs include the short-acting benzodiazepine, midazolam, and the hypnotic, propofol. Both agents modulate cortical EEG, however little is known about their effects on subcortical areas. Of particular concern for our studies of thalamic neurons and chronic pain is the possibility that these drugs might be responsible for the bursting of neurons recorded during surgery in these patients. To study this hypothesis naive rats were sedated and neurons stereotactically localized and conventionally recorded from medial (MD,CL,Re,PC) or lateral (VPL,VPM) nuclei for computer analysis. Pre- and post-IV midazolam (0.014mg/Kg) or propofol (2.5mg/Kg) multiple and isolated single unit activity were compared. Custom interval histogram and burst analyses revealed varied degrees of suppression of neuronal activity following the administration of either drug. Midazolam (n=9) produced a depression of activity (49%) in all rats with a mean depression of 27% and 66% in intervals of lateral and medial thalamic multiple units respectively. Propofol (n=6) produced a similar depression of activity (47%) in all rats with a mean of 69% and 26% in lateral and medial multiple units respectively. Analysis of single units (n=9) showed depression of non-bursting (n=8) and bursting (n=1) activity. This study does not support the notion that these drugs would produce enhancement of either periodic activity or bursting in cells recorded from medial or lateral somatosensory thalamic nuclei of naive rats; it is thus unlikely that they can account for this activity in recordings from thalamic neurons of chronic pain patients.

289.10

MORPHINE ANALGESIA IN THE FORMALIN TEST IN THALAMIC AND DECORTICATE RATS. B.K. Matthies* and K.B.J. Franklin, Dept. Psychol., McGill University, Montreal, PQ, Canada H3A 1B1.

Rats with partial decortication or transection of the brain just rostral to the thalamus, were tested for their response to nociceptive stimulation in the formalin (FN) and tail flick (TF) tests, and for the effect of morphine on these responses and motor activity. Undrugged rats showed vigorous responses to nociceptive stimulation in both tests. All thalamic, and some decorticate rats, exhibited continuous pain over 60 min in the FN test, rather than the typical biphasic response. In thalamic rats, doses of morphine (4 & 8 mg/kg) that were analgesic in sham operates produced catalepsy, and analgesia in the TF test, but not in the FN test. After morphine, rats with most of the neocortex removed were cataleptic and analgesic in both tests. Rats with lesions ventral to the rhinal fissure, extending into the piriform cortex and underlying structures showed analgesia in the TF but not in the FN test, and no catalepsy. On the basis of this and other evidence the amygdala may be critical for opioid analgesia in the FN test, and may play a role in the cataleptic effect of opioids.

290.1

HABENULA LESIONS ATTENUATE LATERAL HYPOTHALAMIC STIMULATION-PRODUCED ANALGESIA IN THE FORMALIN TEST. P.N. Fuchs and V.C. Cox*. Dept. of Psychology, University of Texas at Arlington, Arlington, TX 76019.

The purpose of the experiment was to determine whether the integrity of the habenula (Hb) complex was necessary for analgesia resulting from lateral hypothalamic (LH) stimulation for tonic pain. Female albino rats were divided equally into three groups (n=8). The LH group received electrical brain stimulation of the LH during formalin induced pain. The LH-Hb group received bilateral electrolytic lesions of the Hb complex. The LH-Caudate (LH-C) group received asymmetrical bilateral electrolytic lesions within the area of the caudate nucleus. Both LH-Hb and LH-C groups received LH stimulation during formalin induced pain. The results demonstrate that lesions of the Hb had no effect on nociceptive baseline response, but Hb lesions attenuated LH stimulation-produced analgesia in the formalin test. The modification of the analgesic effect of LH stimulation in the formalin test following Hb complex damage cannot be attributed to a non-specific brain damage effect since bilateral caudate nucleus lesions of similar extent as Hb complex damage failed to modify the analgesic effect of LH stimulation. It appears that the Hb is necessary for LH stimulation-produced analgesia for tonic pain. The critical damage was probably the lateral habenula nucleus since it receives a major input from the LH.

290.2

THE ANTINOCICEPTION PRODUCED BY STIMULATION OF THE DORSOLATERAL PONTINE TEGMENTUM IS MEDIATED IN PART BY NEURONS IN THE VENTROMEDIAL MEDULLA. J.A. Paice* and H.K. Proudfit. Department of Pharmacology, University of Illinois at Chicago, Chicago, IL 60680.

We have demonstrated that electrical stimulation of neurons near the A7 catecholamine cell group in the dorsolateral pontine tegmentum (DLPT) produces antinociception that is blocked by intrathecal injection of serotonergic antagonists. In addition, we have shown that neurons in this area of the pons project to the area of the ventromedial medulla (VMM) that contains spinally-projecting serotonin neurons. The present studies were designed to determine whether activation of these DLPT neurons by microinjection of glutamic acid could produce antinociception that is blocked by microinjection of the local anesthetic tetracaine into the VMM. The excitatory amino acid glutamate was microinjected into the DLPT, near the A7 cell group, of partially anesthetized female Sprague Dawley (Sasco, Inc.) rats and the latency to a paw-withdrawal response to noxious heat was determined before and after glutamate microinjection. Glutamate microinjection elevated response latencies for an average of 2.5 minutes (SEM=0.58). Forty minutes later, tetracaine was microinjected into the VMM and glutamate microinjection into the DLPT was repeated. The antinociception produced by glutamate microinjection was blocked by the microinjection of tetracaine into the VMM. These findings provide evidence that neurons in the DLPT project to the VMM, and are involved in modulating nociception. (Supported by USPHS grants DA03980 to HKP and predoctoral NRSA NR06310 to JAP.)

290.3

PARABRACHIAL AREA (PBA) AND NUCLEUS RAPHE MAGNUS (NRM)-INDUCED INHIBITORY EFFECTS ON THE ACTIVITY OF NOCICEPTIVE AND NON-NOCICEPTIVE TRIGEMINAL SUBNUCLEUS CAUDALIS (Vc) NEURONS ACTIVATED BY CUTANEOUS OR DEEP INPUTS. C.Y. Chiang*, J.W. Hu and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, Toronto, Ontario M5G 1G6, Canada.

Both PBA and NRM have been implicated in central nociceptive mechanisms, and the present study tested for and compared modulatory influences from PBA and NRM on nociceptive cutaneous and deep inputs to single Vc neurons. Extracellular recordings were made in Vc of adult rats anesthetized with urethane/α-chloralose, and conditioning stimulation (CS) applied to NRM and PBA (10 s repetitive stimuli, 0.2 ms, 400 Hz, 5-20 μA). Neurons were classified as low-threshold mechanosensitive rapidly adapting (RA, n=10) or slowly adapting (SA, n=6), wide dynamic range (WDR, n=16), nociceptive-specific (NS, n=5) or deep only (D, n=7). Spontaneous and mechanically evoked activity in WDR, NS, D and SA neurons was markedly inhibited by both NRM (15/15, 5/5, 7/7, 5/6) and PBA (15/16, 3/3, 6/7, 4/5), but fewer RA neurons were inhibited by PBA (1/8) than by NRM (5/9). PBA and NRM were equally effective in inducing potent inhibition (>50%) of cutaneous nociceptive inputs to WDR and NS neurons (PBA 17/19; NRM 19/20), although PBA may be less effective than NRM in inducing potent inhibition of deep nociceptive inputs to these neurons (2/4; 4/4) and to D neurons (3/7; 7/7). Both PBA and NRM-induced inhibition outlasted CS only in a few cases and its magnitude increased with increases in CS frequency (5-400 Hz). Glutamate (0.2M, 0.1-3 μl) injected into PBA however produced a long-lasting, potent inhibition in 6 of 7 neurons tested. Thus, PBA as well as NRM can powerfully inhibit both deep and cutaneous inputs to Vc neurons. Supported by Canadian MRC and NIH (grant DE09559).

290.4

EFFECTS OF ELECTRICAL AND CHEMICAL STIMULATION OF LATERAL RETICULAR NUCLEUS (LRN) ON DORSAL HORN NEURONS (DHN) IN THE CAT. U.T. Oh*, T.S. Moon, Y.K. Cho, J.Y. Kwak, K.H. Ko and H.C. Shin. Lab. of Physiology, College of Pharmacy, Seoul National University, Seoul, Korea 151-742.

Electrical stimulation of LRN prolongs latency of tail-flick reflex, and electrical stimulation of LRN and adjacent areas inhibits dorsal horn neurons. Since many ascending and descending axons are located close to the area, it is necessary to inject chemicals such as L-glutamate (L-glu) to LRN to see whether inhibition of DHNs is caused by stimulation of LRN or axons of passage. Thus, the purpose of the present study was to determine if L-glu injection to LRN inhibits DHNs in the cat. Along with this, neural responses of DHNs to electrical stimulation of LRN were also characterized.

Sixteen cats were anesthetized with alpha-chloralose (60mg/kg). Recordings were made from 65 DHNs located in the L3 to S1 segments of the spinal cord. Sixty-two DHNs were tested for responses to peripheral stimulation; 32 cells were WDR cells, 17 were HT, and 6 and 7 cells were Deep and LT cells, respectively. Responses of DHNs to electrical stimulation (100uA, 100Hz, 100us) of LRN and adjacent area were tested for 55 DHNs: 38 DHNs were inhibited, 15 failed to show any response and 2 were excited. The electrical stimulation inhibited not only spontaneous activities but responses of DHNs to noxious peripheral stimulation. Effect of L-glu injection (500nl, 100nmole) to LRN was tested to 7 DHNs. Among these cells, 3 were inhibited, 3 were unaffected, and one cell was initially excited and then inhibited by the injection. Five more injections were made to areas distant to LRN, and none of these injections inhibited DHNs, suggesting that inhibition resulting from L-glu injection was localized to LRN. In summary, electrical and chemical stimulation of LRN inhibited DHNs, and the inhibition was due to activation of neurons in LRN. Supported by RCNDD grant of Korea.

290.5

DIFFERENCES IN THE PROJECTIONS FROM THE NUCLEUS RAPHE MAGNUS (RMg) TO THE NUCLEUS LOCUS COERULEUS (LC) IN DIFFERENT SUBSTRAINS OF SPRAGUE-DAWLEY RATS: IMPLICATIONS FOR THE MODULATION OF NOCICEPTION. W.L. West and H.K. Proudfit.* Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60612.

We have recently provided anatomical evidence that LC neurons in Sprague-Dawley rats obtained from Harlan Sprague Dawley, Inc. innervate the spinal cord dorsal horn, but LC neurons in rats from Sasco, Inc. innervate the ventral horn. In addition, LC neurons modulate nociception in Harlan rats, but not in Sasco rats. We have also demonstrated that efferent projections from the RMg innervate the A5 and A7 catecholamine cell groups, but not the LC in Sprague-Dawley rats obtained from Sasco, Inc. We designed experiments to determine if the afferent projections from the RMg to the LC and other pontine catecholamine cell groups were the same or different in Sprague-Dawley rats obtained from Harlan, Inc. Fluoro-Ruby, an anterograde and retrograde tracer was injected iontophoretically into the RMg of animals from both vendors and brainstem sections were processed for tyrosine hydroxylase-immunoreactivity. In Sasco rats, there were moderate projections to the A5 and A7 cell groups, but only sparse projections to the LC. In contrast, Harlan rats had moderate projections to the LC, but only minor projections to the A5 and A7 catecholaminergic cell groups. These results provide evidence for a nociceptive modulatory pathway from RMg to LC to spinal cord dorsal horn in Harlan rats and from RMg to the A7 cell group to the dorsal horn in Sasco rats. This work was supported by USPHS Grant DA03980 from the National Institute on Drug Abuse.

290.6

THE PROJECTION OF NORADRENERGIC NEURONS IN THE A5 CELL GROUP TO THE SPINAL CORD IN THE RAT USING ANTEROGRADE TRACING: EVIDENCE FOR A ROLE OF A5 NEURONS IN MODULATING NOCICEPTION. F.M. Clark* and H.K. Proudfit, Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60680.

Noradrenergic neurons located in the A7 catecholamine cell group have been shown to innervate the spinal cord dorsal horn and stimulation of these neurons produces antinociception. Stimulation of noradrenergic neurons in the A5 cell group also produces antinociception, but the areas of the lumbar spinal cord innervated by these neurons have not been described. Experiments were designed to trace the projections from the A5 cell group to the spinal cord, using the anterograde tracer phaseolus vulgaris leucoagglutinin and immunocytochemistry. The results of these experiments indicated that A5 noradrenergic neurons project ipsilaterally through the dorsolateral funiculus and terminate primarily in the ipsilateral deep dorsal horn and intermediate zone (laminae IV-VI) in lumbar and cervical segments, and the thoracic intermediolateral cell column. These results provide anatomical support for a role of A5 neurons in modulating nociception. Supported by USPHS Grant DA03980 from the National Institute on Drug Abuse.

290.7

QUANTITATIVE BEHAVIORAL ANALYSIS OF RAT FLEXION HINDLIMB WITHDRAWALS TO GRADED NOXIOUS SKIN HEATING AND THEIR SUPPRESSION BY STIMULATION IN MIDBRAIN PERIAQUEDUCTAL GRAY (PAG) AND LATERAL RETICULAR FORMATION (LRF). R.L. Kitchell* and E. Carstens. Dept. of Animal Physiology, Univ. of Calif., Davis, CA 95616.

Most animal pain tests measure the latency of a nocifensive response, but provide no information on how suprathreshold responses are altered by analgesics. We therefore developed a method to measure the magnitude of rat hindlimb withdrawals to graded noxious heating of the paw, and have presently investigated effects of stimulation in midbrain analgesia areas.

One week following implantation of bipolar stimulating electrodes in PAG and LRF, rats were behaviorally tested. The rat rested comfortably in a restrainer with the protruding left hindpaw attached to a Peltier thermode. EMGs were recorded in biceps femoris during attempted limb withdrawals evoked by graded noxious heat stimuli (40-52° C, 5 s duration, 2 min intervals). Responses were quantified as area beneath the integrated EMG. Effects of PAG or LRF stimulation were tested by delivering electrical stimuli (100 ms, 100 Hz trains, 3/s, 10-800 uA) during the heat stimulus.

PAG and LRF stimulation suppressed withdrawals in an intensity-dependent manner. Thresholds (range: 10-400 uA) were lower, and recruitment of reflex suppression greater, for LRF vs. PAG stimulation. Withdrawal magnitude increased linearly from 40-52° C. PAG stimulation produced a significant reduction in mean slope of the stimulus-response function with no change in threshold, while LRF stimulation produced a parallel rightward shift with a significant increase (4° C) in mean threshold (N=11 to date).

Medial or lateral midbrain stimulation thus exerts parametrically distinct suppressive effects on limb withdrawals, identical to the effects observed previously on stimulus encoding by lumbar dorsal horn neurons.

290.8

QUANTITATIVE BEHAVIORAL ANALYSIS OF THE RAT TAIL FLICK REFLEX AND ITS SUPPRESSION BY STIMULATION IN MIDBRAIN PERIAQUEDUCTAL GRAY (PAG) OR LATERAL RETICULAR FORMATION (LRF). E. Carstens* Dept. of Animal Physiology, Univ. Calif. Davis, CA 95616.

The widely-used tail flick assay is a latency measure that gives an indication of pain threshold, but provides no information on suprathreshold responses or their modulation by analgesics. We therefore developed a method to measure the magnitude of tail flicks, and have investigated their modulation by stimulation in midbrain analgesia areas.

The same rats from the preceeding study (Kitchell & Carstens, 1992) were used. The rat rested comfortably in a restrainer, and the extended tail was attached to a radial array of force transducers to measure rostral, dorsal and horizontal isometric forces during attempted tail flicks elicited by graded noxious radiant heat pulses (38-58° C, 5 s duration, 2 min intervals) delivered unilaterally to the tail. Areas beneath force traces were measured to calculate integrated force vectors (N-sec). Brain stimulation was as described in the preceeding abstract.

Tail flicks were suppressed in an intensity-dependent manner, with LRF more effective than PAG stimulation. Hindlimb and tail flick reflexes were usually suppressed equally; in 4 rats tail flicks were suppressed at lower intensities compared to limb withdrawals. Tail flick magnitude increased linearly from threshold (40° C) to 50° C and then leveled off. PAG stimulation (N=12 rats to date) produced a mean stimulus-response function that was linear from 40-58° C, and whose slope was significantly lower (with no threshold change). LRF stimulation (N=11) produced a parallel rightward shift in the stimulus-response function with a significant increase (4° C) in threshold. Thus, the tail flick reflex is under the same two descending influences that operate on limb withdrawal reflexes and dorsal horn neurons.

290.9

RESPONSES OF SPINAL DORSAL HORN NEURONS TO NOXIOUS HEATING OF THE RAT'S TAIL: STIMULUS CODING, INHIBITION BY MIDBRAIN STIMULATION, AND BEHAVIORAL CORRELATES. D.K. Douglass* and E. Carstens. Dept. of Animal Physiology, Univ. of Calif., Davis, CA 95616.

Despite widespread use of the tail flick reflex as a pain assay, little is known about spinal interneurons in the reflex arc. We therefore investigated (a) whether spinal neurons respond to noxious tail heating, (b) if they are inhibited by midbrain stimulation, and (c) if the neuronal results correspond with behavioral data obtained from the same rats.

The same rats used in the preceeding behavioral study (Carstens, 1992) were anesthetized with sodium pentobarbital. The sacral-coccygeal spinal cord was exposed by laminectomy for single-unit recording from neurons with cutaneous tail input. Unit responses to the identical noxious radiant heat stimuli used in the preceeding study were quantified as the number of action potentials/10 s period beginning 2 s after heat onset.

In 10 rats to date, 29 units responded to noxious tail heating. Receptive fields ranged from unilateral areas of a few sq. mm to the entire tail. Mean responses to noxious heat increased linearly from a threshold near 38° C to 50° C and then levelled off. In 9/10 rats, neuronal responses were inhibited by stimulation in midbrain periaqueductal gray (PAG) or lateral reticular formation (LRF) at the same or slightly higher current intensities that had previously suppressed the tail flick. Neuronal responses were suppressed by PAG stimulation such that the slope of the mean stimulus-response function was significantly reduced with no threshold change. LRF stimulation produced a parallel rightward shift of the mean stimulus-response function with a significant increase in threshold. There was thus excellent correspondence between neuronal stimulus encoding and behavioral measures of tail flick magnitude, and their modulation.

290.10

ALTERED DESCENDING MODULATION FROM THE ROSTROVENTRAL MEDULLA IN CAPSAICIN-TREATED RATS. M. Zhuo* and G.F. Gebhart. Department of Pharmacology, College of Medicine, The University of Iowa, Iowa City, Iowa 52242

Descending biphasic modulation from the rostroventral medulla (RVM) on the spinal nociceptive tail-flick (TF) reflex and on lumbar spinal dorsal horn neuron responses to noxious cutaneous stimuli were studied in adult rats treated as neonates with capsaicin or vehicle. In vehicle-treated rats, descending facilitatory and inhibitory effects on the TF reflex were produced from the RVM. Electrical stimulation in the RVM produced biphasic (n=11), only inhibitory (n=13) or only facilitatory (n=1) effects on the TF reflex. In capsaicin-treated rats, the proportion of sites where electrical stimulation only inhibited the TF reflex was significantly less and the number of sites in RVM where electrical stimulation only facilitated the TF reflex was significantly greater than in vehicle-treated rats. In electrophysiological experiments, the number of sites where electrical stimulation produced only facilitatory effects on responses of spinal dorsal horn neurons to noxious stimulation (thermal or mechanical) of the skin were increased from 13.3% of the total sites in vehicle-treated rats to 40.0% in capsaicin-treated rats. The number of sites where stimulation only produced inhibitory effects on responses of spinal dorsal horn neurons to noxious stimulation was decreased from 46.7% of the total sites in vehicle-treated rats to 35% in capsaicin-treated rats. The present study suggests that neonatal capsaicin treatment has significant effects on bulbospinal systems important to the modulation of spinal nociceptive transmission.

290.11

DUAL LIDOCAINE INJECTIONS INTO VB THALAMUS ARE NOT ANALGESIC IN THE FORMALIN TEST. J.E.McKenna* and R.Melzack. Dept. of Psychology, McGill University, 1205 Dr. Penfield Ave. Montreal, Que. Canada H3A 1B1

The ventrobasal thalamus (VB) has long been proposed as a critical nociceptive sensory relay. Previous studies have indicated, however, that injection of the local anesthetic lidocaine into VB caused no observable changes in pain behavior expressed after peripheral formalin injection, while similar treatment of centromedial/posterior thalamus significantly reduced/enhanced pain behaviors. This experiment was undertaken to determine if pain behaviors would be affected by lidocaine treatment of a larger part of VB. Long-Evans rats received 2 x 1.0 µl unilateral intracranial infusions of 2% lidocaine or saline, before or after a 60 µl injection of 2.5% formalin acetate into the contralateral hindpaw. Dual lidocaine infusion into VB did not cause a reduction in pain behaviors, regardless of the time of central administration relative to the peripheral stimulus. These results indicate that VB may not be critically involved in formalin-induced nociception.

Supported by NSERC grant A7896.

291.1

NON-PEPTIDE NEUROKININ ANTAGONIST PREVENTS CAPSAICIN-INDUCED BLOOD PRESSURE REFLEXES IN RABBIT AND RAT. R. Amann*, M. Eder and J. Donnerer. Dept. Exp. & Clin. Pharmacology, Graz Univ., A-8010 Graz, Austria.

RP 67580 has been shown to be a potent neurokinin (NK)-1 receptor antagonist which exhibits analgesic properties (Garret et al., 1991). We have compared the effect of RP 67580 and that of the NK-1 antagonist CP-96345 (Snider et al., 1991) in anesthetized rats and rabbits on blood pressure, and on the reflex blood pressure fall produced by close injection of capsaicin into the rat femoral artery and the rabbit central ear artery, respectively.

In both species, RP 67580 (up to 7 µmol/kg i.v.) had no effect on blood pressure but attenuated the capsaicin-induced depressor reflex which recovered 50-80 min later. Inhibition could be overcome by increasing the dose of capsaicin. CP-96345 (0.7-7.0 µmol/kg i.v.) produced a long-lasting hypotension which prevented evaluation of a possible influence on the depressor reflex. CP-96344, the inactive enantiomer with no NK antagonistic properties, produced similar hypotension as CP-96345.

In rabbits, intracisternal injection of RP 67580 (100 nmol) as well of CP-96345 (30 nmol) had no effect on blood pressure but caused reversible inhibition (61% and 46%, respectively) of the capsaicin-induced depressor reflex.

The results suggest an analgesic effect of NK antagonists, which, in the case of CP-96345 seems to be masked by side-effects which are not related to NK receptor antagonism.

Garret et al., (1991) Proc. Natl. Acad. Sci. USA 88, 10208
Snider et al., (1991) Science 251, 435
(supported by FWF grant 7676)

291.2

TEMPERATURE AND PAIN THRESHOLDS ARE INFLUENCED BY DIFFERENT GLUCOCORTICOIDS IN MAN. G. Fehm-Wolfsdorf*, C. Körbächer, C. Maier, R. Arndt. Inst. of Psychology & Dept. of Anaesthesiology, University of Kiel, D-2300 Kiel, Germany.

Dexamethasone as compared to hydrocortisone differentially influence sensory thresholds of several modalities. The present study aimed to look for comparable effects on temperature and pain thresholds in male non-smoking volunteers. 26 subjects participated in three medication sessions, each, and were pretreated with either 30 mg hydrocortisone, 1 mg dexamethasone or the respective placebo pills according to a double-blind cross-over Latin-square design. Temperature and pain thresholds were obtained by a Path-Tester MPI 100 with a standard procedure starting at 32°C or 40°C, respectively. Temperature applied to the ball of the non-dominant thumb rose 0.7°C/ sec. Pain thresholds considerably vary between subjects: thus, in an additional untreated baseline session we studied the relationship between individual thresholds, cortisol response to the pain stimulation, and personality factors (anxiety and repression-sensitization). Salivary cortisol was determined at the start, middle and end of all sessions. As expected hydrocortisone treatment led to elevated temperature thresholds, but not pain thresholds. Dexamethasone had no effect other than placebo. High tendency to "situational control" in the repression-sensitization-questionnaire was positively correlated to high cortisol response within the untreated sessions, and negatively correlated to pain thresholds. Thus a combination of endocrine reactions to pain with personality variables may reliably predict interindividual differences in pain perception.

Research was supported by the Deutsche Forschungsgemeinschaft (DFG).

291.3

TEST-DEPENDENT ANALGESIC EFFECT OF THE SELECTIVE NON-PEPTIDE NK-1 RECEPTOR ANTAGONIST CP96,345. O.-G. Berge* and M. Ståhlberg. Astra Pain Control, Preclinical Research, S-151 85 Södertälje, Sweden.

The non-peptide NK-1 receptor antagonist CP96,345 provides advantages over the previously available peptidergic substance P antagonists with regard to selectivity and penetration of the blood-brain barrier. We have investigated the effects of intraperitoneally administered (±)-CP96,345 in four tests of nociception in mice.

(±)-CP96,345 (8 - 73 µmol/kg) had no effect when tested 15, 30, 45 and 60 min after injection in the radiant heat tail-flick test, the constant temperature hot plate test (58 °C) or the increasing temperature hot plate test (42 - 50 °C, rate 3 °C/min).

Doses from 2.4 to 73 µmol/kg reduced the response in the late, inflammation-related phase of the formalin test (licking induced by 20 µl, 1% formaldehyde in saline injected subcutaneously into one hind paw). Only 73 µmol/kg affected the early phase.

At 73 µmol/kg, some sedation was evident, possibly interfering with the response in the formalin test. Increasing the dose to 109 µmol/kg in two animals produced profound stupor, precluding any behavioral testing.

The data suggest that substance P plays a role in some but not all kinds of pain.

291.4

INFLUENCE OF SHOCK SEVERITY ON THE ACTIVATION OF ANALGESIC SYSTEMS IN PENTOBARBITAL TREATED RATS. J.W. Grau*, P.A. Illich, K.D. Burks and M.W. Meagher, Dept. of Psychology, Texas A&M Univ., College Station, TX 77843.

Terman et al. (Science, 226, 1270) showed that exposure to severe shock can elicit "analgesia" on the tail-flick test in pentobaribital anesthetized rats. Their least severe shock parameters generated a naltrexone reversible ("opioid") analgesia and the most severe elicited a naltrexone-insensitive ("nonopioid") analgesia. They proposed that a coulometric relation (shock intensity x duration) predicts whether the opioid or nonopioid system is engaged. The present experiments tested this hypothesis.

Experiment 1 looked at the minimum shock intensity and duration that generates analgesia on the tail-flick test in pentobarbital (40 mg/kg) anesthetized rats. After baseline testing, subjects were exposed to three tailshocks and pain reactivity was tested 5 more times. We found 1.5-s/1.0mA, 25-s/1.0mA, & 25-s/0.5mA shocks elicited analgesia, and 1.5-s/0.5mA, 3.0-s/0.5mA, & 25-s/0.3mA did not. The results fail to support the coulometric relation but can be accommodated by a relation derived from Steven's power law: shock duration x intensity**3.5.

Experiment 2 tested the impact of naltrexone (14 mg/kg) on the analgesia observed in pentobarbital anesthetized rats. In contrast to Terman et al., naltrexone did not attenuate the analgesia observed after the least severe shock schedules (e.g., 1.5-s/1.0 mA & 25-s/0.5 mA), but did attenuate the analgesia observed after more severe shocks (e.g., 2-s/3.0mA & 25-s/1.5mA). Very severe shocks (75-s/3.0mA) elicited a naltrexone-insensitive analgesia.

291.5

A GENETIC ANALYSIS OF SWIM STRESS-INDUCED ANALGESIA IN SELECTIVELY BRED MICE. J.S. Mogil*, P. Marek, W.F. Sternberg, M.A. Spence and J.C. Liebeskind. Dept. of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

We have previously demonstrated that 3 min forced swims in 32°C, 15°C and 20°C water produce swim stress-induced analgesia (SSIA) that is mediated by opioid, non-opioid, and mixed opioid/non-opioid mechanisms, respectively. The present study sought to examine the patterns of inheritance of these SSIA paradigms, and also of morphine analgesia, in mice selectively bred in Poland towards high (HA) and low (LA) 20°C SSIA for more than 20 generations. To this end, Mendelian test-cross populations were bred from 21st generation HA and LA parents, including: F_1 hybrids (HAxLA), backcrosses to both parents (F_1xLA, F_1xHA), and F_2 hybrids (F_1xF_1). All populations were tested on the hot plate (56°C) immediately prior to and 2 min after a 3 min forced swim in 15°C, 20°C or 32°C water, and also immediately prior to and 25 min following a systemic injection of 10 mg/kg morphine. Results indicate that the LA genotype is partially dominant over the HA genotype with respect to the SSIA paradigms, but the reverse is true with respect to morphine analgesia. As well, a non-parametric statistical analysis comparing observed phenotypic values with those predicted by Mendelian ratios indicates that a single-locus hypothesis for 32°C SSIA and morphine analgesia cannot be ruled out. This suggests that the magnitude of opioid SSIA and morphine analgesia in selectively bred mice may be determined by a single major gene. Supported by NIH Grant NS07628 and an Unrestricted Pain Research Grant from the Bristol-Myers Squibb Company.

291.6

NON-COMPETITIVE NMDA ANTAGONIST, MK-801, AND GLYCINE ANTAGONIST, ACEA-1011, PREVENT THE DEVELOPMENT OF TONIC PAIN FOLLOWING SUBCUTANEOUS FORMALIN. Anthony L. Vaccarino[1]*, Przemyslaw Marek[2], Benjamin Kest[2], Eckhard Weber[3] and John C. Liebeskind[2], [1]Department of Psychology, University of New Orleans, Lakefront, New Orleans, LA 70148, [2]Department of Psychology, University of California, Los Angeles, CA 90024 and [3]Department of Pharmacology, University of California Col. Med., Irvine, CA 92717.

Several lines of evidence indicate a critical role of *N*-methyl-D-aspartic acid (NMDA) receptor in the development of persistent pain following injury. Subcutaneous injection of formalin produces a biphasic pain response: an early, transient phase followed by a late tonic phase. It has recently been demonstrated that the late phase of the formalin test is a partial result of long-term changes in central nervous system function produced by neural activity generated during the early phase. The present study examined the involvement of the NMDA receptor in the development of the late pain produced following subcutaneous injection of formalin into the hind paw in mice. Blockade of the NMDA receptor by the ion channel blocker, MK-801, prior to formalin injection, but not after, reduced pain during the late phase. Similarily, the blockade of the NMDA allosteric site by the novel glycine site antagonist, ACEA-1011, also reduced the pain response in the late phase. Supported by NIH Grant NS07628 and an Unresricted Pain Research Grant from Bristol-Myers Squibb Company.

291.7

SEX DIFFERENCES IN THE ANTAGONISM OF NON-OPIOID STRESS-INDUCED ANALGESIA: EFFECTS OF GONADECTOMY AND ESTROGEN REPLACEMENT. W.F. Sternberg,* J.S. Mogil, P. Marek, B. Kest, and J.C. Liebeskind. Department of Psychology, University of California, Los Angeles, CA 90024.

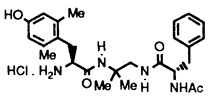
Sex differences in the neurochemical mediation of non-opioid swim stress-induced analgesia (SSIA) were examined in Swiss-Webster mice. Intact and gonadectomized mice of both sexes were tested for their analgesic response (hot-plate test; 56°C) to 3 min forced swimming in 15°C water. SSIA resulting from 15°C swim was previously shown to be naloxone-insensitive (i.e., non-opioid). Our laboratory has demonstrated that this form of non-opioid analgesia is attenuated by the *N*-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine (MK-801). We now report that this finding is specific to male mice. In female mice, neither naloxone (10 mg/kg; IP) nor dizocilpine (0.075 mg/kg; IP) significantly attenuated analgesia following 15°C swim. Thus, SSIA in intact females was neither opioid, nor NMDA- mediated, yet it was of similar magnitude to the SSIA displayed by intact males. After ovariectomy, females displayed a pattern of antagonism similar to males such that dizocilpine attenuated non-opioid SSIA. In a separate experiment, estrogen replacement (estrogen benzoate; 5.0µg/d) administered to ovariectomized mice over a 6-8 d period reinstated the dizocilpine-insensitivity of 15°C SSIA characteristic of intact female mice. These findings suggest the existence of a novel, female specific, estrogen dependent mechanism of SSIA.

291.8

TYROSYL DIAMIDES AS POTENT MU AND DELTA OPIOID RECEPTOR LIGANDS. D.W. Hansen Jr.*, N.S. Chandrakumar, R.K. Husa, K.B. Peterson, S.Tsymbalov, A.Stapelfeld, L.N. Williamson, M. Reichman, and P.M. Beardsley Depts. of Chemistry and Neurological Diseases, G.D. Searle & Co., 4901 Searle Parkway, Skokie, IL 60077.

In our effort to discover and develop δ opioid-selective agonists that possess potent analgesic properties without the negative side effects associated with µ opioid agonists, we evaluated a new class of antinociceptive amino acid diamides which are marginally selective for δ opioid receptors. A limited series of novel µ opioid-selective agents were recently disclosed by L.M. Sayre et al which consisted of a tyrosine and a variety of phenylalanine derivatives linked at their carboxyls by various diamine spacers. The tyrosine of the most potent analog was replaced by 2,6-dimethyltyrosine (DMT) to generate SC-50406. In contrast to its Tyr relative, this material not only had a preference for the δ opioid receptor but was significantly more potent in a variety of rodent analgesic assays by a number of routes of administration. In this paper we describe the design, synthesis, and biological properties of the structurally varied DMT diamides which we prepared to exploit this newly discovered lead.

SC-50406



291.9

Naltrexone - insensitive antinociception follows carbon dioxide anesthesia: a role for brain histamine H_2 receptors?

S.A. Mischler, L.B. Hough*, A.H. Battles and J.A. Raucci Jr., Dept. Pharmacology & Toxicology, and Animal Resources Facility, Albany Medical College, Albany, NY 12208.

Presently we have discovered and pharmacologically characterized the effects of CO_2 exposure on hot plate (52°) antinociception (ANC) in rats. Exposure (30 sec) to 100% CO_2 induced anesthesia with a duration of 2 - 4 min, followed by complete clinical recovery. This treatment resulted in significant ANC (25 - 30% of maximal effect) detected from 25 to 60 min later. Control animals, placed in the same chamber filled with room air, showed no such response. Carbon dioxide ANC was also additive with morphine ANC (5 mg/kg, s.c.). Pretreatment with a large dose of the opiate antagonist naltrexone (5 mg/kg, i.p., 7.5 min before exposure) did not significantly change CO_2 ANC, implying a non - obligatory role for endogenous opiates. In contrast, pretreatment with the brain - penetrating histamine H_2 antagonist zolantidine (5 mg/kg, s.c., 7.5 min, a treatment previously shown to inhibit naloxone - insensitive, footshock - induced analgesia) significantly attenuated the CO_2 - induced antinociceptive response. In the absence of CO_2 exposure, neither antagonist altered hot plate latencies. These findings suggest that: 1) brief exposure to anesthestic concentrations of CO_2 induces a long - lasting ANC that may represent a novel form of stress - induced analgesia, and 2) brain histamine may function as a mediator of this response (Supported by DA-03816).

291.10

THE EFFECTS OF SEROTONIN ANTAGONISTS ON STRESS INDUCED ANALGESIA FOR ACUTE AND CHRONIC NOCICEPTION. J. R. Prather, M. Shuck, E. Quinton* Psychobiology Lab., Univ. of Louisville, Louisville Ky. 40292.

Stress induced analgesia (SIA) is mediated through opiod (O)and non-opiod (NO)systems. This study investigated whether the two types of SIA were equally effective on acute and chronic nociceptive models, and whether $5HT_2$ and/or $5HT_3$ are involved. O-SIA was induced with a warm water swim (36°; 2.5 min.) and NO-SIA was induced with a cold water swim (2°; 1 min.) in C57/BL6j mice. Serotonin receptor antagonists (methysergide or metocloprimide) were administered one minute after stress and four minutes later the animals were tested on either the acute (hot-plate) or chronic (10µl, 10% formalin into rear paw; 60 min. observation) nociceptive test. Both SIA's produced analgesia on the acute test, but the NO-SIA was greater. Both SIA's were also effective in the chronic test, but the O-SIA was more effective in the early phase and the NO-SIA more effective in the late phase. The 5HT antagonists had inconsistent effects at the doses tested.

291.11

SWEET TASTE AND MORPHINE ANALGESIA DURATION: EFFECTS OF DRINKING SCHEDULE ON PAW LICK AND TAIL FLICK TESTS. K.F. Green*[1], K.Y. Hiyoshida[1], J.S. Jones[1], and J.J. Kim[2]. [1]Psych. Dept., Cal. State Univ., Long Beach, CA 90840-0901 and [2]NSF Young Scholar, Bellflower High School, Bellflower, CA.

Reduced morphine analgesia after flavor experience (RMAFE) has been found in adult rats given low doses of morphine after flavor exposures lasting one day to 5 weeks (e.g., Bergmann et al., Behav. Neural Biol.44, 347-353, 1985). Our laboratory has had inconsistent success in finding RMAFE, indicating a need to identify controlling variables. The present experiment used 4 groups of 8 rats each. It compared 3-h drinking periods that were continuous vs intermittent (nine 5-min periods equally spaced over the 3 h) in which rats were given a saccharin-dextrose solution or water. Analgesic responses to 4 mg/kg morphine (sc) were assessed with hind paw lick (51.5° C hot plate) and tail flick (4 sec baseline) tests. Analgesia was marked in paw lick and tail flick tests 30 min -- but not 60, 90, and 120 min -- after injection. At 30 min, intermittent flavor attenuated tail flick latencies but not paw lick latencies relative to intermittent water; continuous flavor vs water did not differ on either measure. However, the continuous flavor group showed strong negative correlations between amount consumed and paw lick ($r = -0.85$) and tail flick ($r = -.80$) latencies. Intermittent drinking thus provided experimental control over RMAFE in the tail flick test while continuous drinking provided statistical control in both tests.

291.12

VERAPAMIL POTENTIATES MORPHINE ANALGESIA AND REDUCES EUPHORIA IN HUMANS. D.B. Vaupel*, W.R. Lange and E.D. London. Neuropharmacology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224.

Verapamil (V) is one of several Ca^{2+} channel blockers which enhances morphine (M)-induced analgesia in rodents (G. Benedek and M. Szikszay, *Pharmacol. Res. Comm.* 16:1009, 1984). As this effect may have therapeutic utility, we assessed M-V interactions in human volunteers. The ability of V to potentiate M-induced analgesia and to modulate the subjective effects of M was evaluated in 12 experienced male heroin users. Five treatments were tested: saline (S)+S; S+M (10 mg); V (10 mg) + S; V (2.5 or 10 mg)+ M (10 mg). The first drug listed was infused i.v. over 2 min; i.v. infusion of the second drug was over 10 sec and was initiated half-way through the 2-min infusion. Analgesia was measured using a finger pressure test and hand immersion in ice water. The Addiction Research Center Inventory (ARCI) questionnaire was used to measure the positive subjective effects of M on mood.

Pain threshold, as determined with the finger pressure stimulus, was increased marginally by M ($p < .1$), but V was inactive. However V+M combinations, (V 2.5 mg + M 10 mg and V 10 mg + M 10 mg) significantly elevated pain threshold to produce analgesia. Similar nonsignificant trends were observed in the effect on pain threshold determined using the cold water stimulus. M produced euphorigenic effects based on the elevated scores ($p < .01$) of the MBG scale of the ARCI. V itself produced no subjective effects, but 10 mg of V antagonized elevation of MBG scores produced by M 1 h after treatment ($p < .01$). We concluded that the abilities of V to antagonize the euphorigenic effects of M and to enhance opiate analgesia are therapeutically useful.

291.13

ANTINOCICEPTIVE ACTION OF SANDOSTATIN® : IN VIVO AND IN VITRO STUDIES. A. Dray*, S. Bevan, R. Docherty, E. A. Campbell, I. F. James, M. Perkins, L. Urban and J. Wood Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6 BN, UK.

The distribution and actions of somatostatin in mammalian sensory systems suggests a functional role in nociception. Supporting this are clinical observations that the long acting analogue, Sandostatin® (octreotide) has analgesic activity (eg Pascal et al. 1991 Pain 47, 341). We have further investigated the analgesic activity of Sandostatin in various animal models and studied its effects on sensory neurons.

Sandostatin (up to 1mg/kg) showed little activity against an acute pain stimulus (heat, pressure, chemical) but was effective (10ng-100μg/kg, s.c.) against the inflammatory hyperalgesia induced by uric acid or Freunds adjuvant injection into the rat knee joint. In an in vitro spinal cord-tail preparation, spinal administration of Sandostatin (EC_{50}=50nM) attenuated heat and capsaicin-evoked responses. In cultured sensory neurons, Sandostatin (100nM) consistently inhibited voltage activated calcium currents but did not significantly affect K^+ efflux or calcium accumulation. Finally Sandostatin (1μM) inhibited K^+-evoked substance P release from afferent fibres in slices of guinea pig heart.

Systemically administered Sandostatin was antinociceptive only against inflammatory hyperalgesia or when administered directly to the spinal cord. Depression of calcium currents and inhibition of sensory neuropeptide release may account for these actions.

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291.14

COMPARISON OF THE ANALGESIC POTENCY OF LOCAL *VERSUS* SYSTEMIC ADMINISTRATION OF MORPHINE IN A RAT MODEL OF TONIC FACIAL PAIN. E.Eisenberg, B.P.Vos* and A.M. Strassman, Pain Physiology Lab, Dept. of Neurology, Massachusetts General Hospital, Charlestown, MA 02129.

Local administration of low doses of morphine can result in the blockade of mechanical or thermal hyperalgesia in inflamed tissue, presumably via activation of peripheral opioid receptors. The present study investigated the analgesic effect of local *versus* systemic morphine administration on tonic pain, using a model of formalin-induced facial pain in the rat.

Formalin (5%, 50μl) was injected subcutaneously into the vibrissal pad of adult rats (250-300g). Morphine sulphate at doses of 100, 250, 500 and 1000μg was subcutaneously injected either locally (same area) or systemically (in the neck), 30 min prior to or simultaneously with the formalin. Control animals received saline injections. The number of seconds of formalin-induced face grooming during a 42 min post-injection observation period was determined using computer-assisted video analysis.

Control animals displayed biphasic increases in face grooming directed to the vibrissal pad ipsilateral to the injection, consisting of an early "phasic" phase (0-6 min) and a delayed and prolonged "tonic" phase (12-42 min). Both local and systemic morphine administration produced a dose-dependent, naloxone-reversible decrease of both phases of formalin-induced face grooming. However, the suppression of face grooming was significantly greater when morphine was applied locally than systemically. Depending on the dosage, the time of injection and the response phase considered, local morphine administration produced 15%-58% more suppression of formalin-induced face grooming than systemic morphine. Local micro-injection of naloxone (10μg) completely reversed the analgesic effect of 1000μg local morphine (108%±22%), whereas the same dose of naloxone, applied systemically (i.p.), produced only a partial reversal (39%±4%).

These data indicate that locally administered morphine can exert an analgesic effect on tonic pain through an activation of peripheral opiate receptors.

291.15

PENTOBARBITAL ENHANCES ANTINOCICEPTION FROM β-ENDORPHIN WHILE SUPPRESSING THE ACTION OF MORPHINE. D.J. Smith*, B. Robertson and P.J. Monroe. Dept. of Anesth., WVU-HSC, Morgantown, WV 26506

Anesthesia modifies the antinociceptive potency of morphine (Banks et al., Pharm. Biochem. Behav. 31: 397, 88; Ding et al., Pain 43: 371, 90; Rosland and Hole, Anesth. Analg. 71: 242, 90). In the current study, the ability of pentobarbital to alter the action of β-endorphin or morphine on the tail-flick reflex was studied following administration of the opioids into the periaqueductal gray (PAG) of the rat brain. Anesthesia initiated with 35 mg/kg of pentobarbital (i.p.) was maintained using 20 % of the initial dose when animals exhibited a lid reflex. The anesthetic itself did not alter tail flick latency (TFL) (3.4 ± 0.13, prior to anesthesia; 3.25 ± 0.25, 40 min post anesthesia). Compared to values obtained in awake rats, pentobarbital caused a significant reduction in responses to morphine (3-30 nmol), while significantly elevating the responses to β-endorphin (0.1-10 nmol). There was a marked leftward shift (10 fold) of the β-endorphin dose-response curve with a 3 nmol dose yielding about 200 AUC units (area under the curve of the TF response for 60 min) in anesthetized, and 30 units in awake rats. Responses to morphine were absent at all but the highest dose where the average response was about 40 AUC units compared to 280 in awake animals. As they awakened, the anesthetized rats that had received morphine (10-30 nmol) exhibited rigidity in their limbs and tail ("catalepsy"), and the TFL rose to values expected in non-anesthetized, morphine treated rats. Rigidity was not observed in morphine-animals unless they were exposed to anesthesia, and was never seen in rats given β-endorphin. Since it appears that the actions of β-endorphin and morphine in the PAG may be mediated through separate neuronal pathways (Tseng and Tang, J.Pharm. Exp. Ther. 252: 546, 1990), these data would suggest that pentobarbital may differentially modify these systems.

291.16

THE SPECIFIC *N*-METHYL-D-ASPARTATE (NMDA) RECEPTOR ANTAGONIST MK-801 BLOCKS U-50,488, BUT NOT MORPHINE ANALGESIA. B. Kest*, P. Marek, and J. C. Liebeskind, Department of Psychology, University of California, Los Angeles, CA 90024.

The present study compared the effects of the specific NMDA receptor antagonist MK-801 (0.05 and 0.1 mg/kg, IP) on analgesia produced by the specific kappa opiate receptor agonist U-50,488 (7.5, 15 and 30 mg/kg, IP) and morphine (1 and 5 mg/kg, IP) using the tail-flick test in rats. Analgesia produced by all three doses of U-50,488 was completely blocked by the higher dose of MK-801, and was partially antagonized by the lower dose. In contrast, both doses of MK-801 significantly potentiated the analgesia produced by the low dose of morphine. MK-801 had no effect on analgesia following the higher morphine dose. Naloxone (1 mg/kg, IP) completely blocked analgesia following U-50,488 (7.5 mg/kg, IP), thereby confirming its opiate character. Since U-50,488 is a specific kappa receptor opiate, and morphine exerts its analgesic effect primarily through the mu opiate receptor subtype, the present results suggest a critical role for the NMDA receptor in analgesia involving the kappa, but not mu, opiate receptor and support more generally a distinction between the mechanisms by which the different opiate receptor subtypes mediate analgesia. (Supported by NIMH training grant 5T32MH17140 (BK) and by NIH grant NS07628 (JCL)).

291.17

OPIOID ANALGESIA AFTER SYSTEMIC ADMINISTRATION OF EIGHT OPIOID AGENTS IN AMPHIBIANS. C. W. Stevens* and Alan J. Klopp, Dept. of Pharmacology and Physiology, Oklahoma State University, College of Osteopathic Medicine, Tulsa OK 74107.

The relative analgesic potency of fentanyl, levorphanol, methadone, morphine, buprenorphine, meperidine, codeine, and nalorphine after systemic administration in Northern grass frogs, *Rana pipiens*, was done to compare opioid action in non-mammalian vertebrates to that observed in mammalian species. Opioid agents were diluted in saline to 4-6 log-spaced doses and administered subcutaneously into the dorsal lymph sac. Using the acetic acid test, nociceptive threshold (NT) was measured before (baseline) and at 60, 150, and 240 min after drug administration. Raw NT values were converted to maximum percent effect (MPE) values and dose-response curves generated by taking the maximal effect over the time course curve for each drug and dose. The ED50s (in nmol/g) and 95% confidence intervals (not shown here) were obtained by the PCS computer program. As shown in the Table, fentanyl was the most potent opioid and nalorphine the least potent, with morphine giving an ED50 of 86.3 nmol/g (=32.7 mg/kg). These data support the working hypothesis that the rank order of analgesic potency of systemic opioids in amphibians parallel that observed in mammalian models, with the caveat that greater doses may be needed due to the apparently tighter blood-brain barrier in amphibians. This research supported in part by OSU-COM and the Whitehall Foundation (A91-01).

Agent	ED50	Rel. Pot.
Fentanyl	1.4	61.6
Levorphanol	7.5	11.5
Methadone	19.9	4.3
Morphine	86.3	1.0
Buprenorphine	99.1	0.87
Meperidine	128.1	0.67
Codeine	140.3	0.62
Nalorphine	320.9	0.27

292.1

THE RELATIONSHIP BETWEEN THE APPEARANCE OF NEW ION CHANNELS AND ECTOPIC DISCHARGES FROM A REGION OF NERVE INJURY. Xie Y.K, Xiao W.H; Li H-Q and LaMotte R.H.* Dept. of Physiology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing 100005 CHINA

Peripheral nerve injury can produce abnormal sensory states such as pain, hyperalgesia and paresthesias. In the present study, we recorded electrophysiological discharges, from single A- and C-fiber afferent nerve fibers, that originated from a nerve injury produced by a chronic nerve constriction.

Four loose ligatures were tied around the sciatic nerve at the mid thigh region in 80 rats. In 24 of these rats, a sham operation was performed on the contralateral nerve. Eighty percent of the rats exhibited hyperalgesia on the nerve-injured foot, as tested by the method of Bennett and Xie (Pain 33:87,1989). Channel blocking agents were applied to the nerve at the site of prior surgery and discharges in single sciatic nerve fibers recorded proximally. For 40 A-beta fibers tested, Tetraethylamonium (TEA), a potassium channel blocker, greatly facilitated spontaneous ectopic discharges or evoked ectopic firing in previously silent axons. These discharges were inhibited by the calcium channel blockers Verapamil, La^{3+} and Mn^{2+}. Ectopic discharges were facilitated by high concentrations of K^+ (6 A-beta fibers) or noradrenalin (1 A-delta-, 3 C-fibers) and blocked by La^{3+} and Mn^{2+}. The tested agents had no effect on the evoked or spontaneous activities of normal axons. These results suggest that following nerve injury, the action potential generating part of the membrane expressed ion channels, in particular Ca^{2+} channels, that are not present in this region of normal sciatic nerves and that these channels are responsible for the generation of ectopic discharges. (Supported by Grants from National Natural Science of China and China Medical Board of New York).

292.2

KAINATE RECEPTORS ON C-FIBERS STIMULATE NOCICEPTIVE REFLEXES. B. Ault* and L. Hildebrand, Dept. Neurosciences, Sterling Winthrop Pharmaceuticals Research Division, Rensselaer, NY 12144.

C-fibers in dorsal roots, and small diameter DRG neurons, are depolarized by kainate (KA). The physiological role of C-fiber KA receptors is not known. Using the isolated spinal cord-tail preparation of the neonatal rat, the ability of peripherally-applied excitatory amino acids to stimulate nociceptive reflexes has been examined. Capsaicin (3 uM) superfused over exposed skin of the rat tail for 3 s evoked depolarization and spiking activity that was recorded in ventral roots. Domoate (0.1-10 uM) or kainate (10-300 uM) evoked nociceptive responses comparable to those evoked by capsaicin. L-glutamate (0.01-10 mM), quisqualate (0.1-1 mM) and AMPA (0.1-1 mM) were of reduced potency. L-aspartate (10 mM) and NMDA (1 mM) were inactive. KA responses were blocked by spinal application of 0.1 uM morphine, and were competitively antagonized by DNQX applied to the tail. The ability of KA receptors to stimulate nociceptive activity suggests that peripherally released glutamate may be involved in nociception.

292.3

ELECTROPHYSIOLOGICAL EVIDENCE THAT PERIPHERAL NK-1 RECEPTORS MEDIATE HYPERALGESIA IN AN ACUTELY INFLAMED JOINT MODEL OF ARTHRITIS. D.E. Kellstein* and M.M. Proffitt. Arthritis and Inflammation Section, Health Care Technology Division, Miami Valley Laboratories, The Procter and Gamble Company, Cincinnati, OH 45239.

Previous studies have implicated the release of substance P (SP) from peripheral C-fiber terminals in neurogenic inflammation, adjuvant-induced arthritis, and inflammation caused by intradermal or intra-articular injection of carrageenan. The present study used CP-96,345, a selective antagonist of SP at NK-1 receptors, to determine if peripheral NK-1 sites may be involved in inflammation-induced enhancement of the neuronal nociceptive response to ankle compression. Male Sprague-Dawley rats (400-550g) were anesthetized with urethane (1.5 g/kg). A laminectomy was performed from spinal segments L1-L4, and the dural membrane was retracted. Single unit extracellular recordings were made from wide dynamic range and nociceptive specific neurons of the spinal cord dorsal horn (laminae 2 to 7) during lateral ankle compression. Discretely graded stimuli (150-450 mmHg) were delivered for 10 sec at 2 min intervals with a pneumatic pressure controller, and the number of spikes during each stimulus was counted. For statistical comparisons, the total stimulus-response for each unit was converted to area under the curve; each neuron served as its own control. Compared to pretreatment values, injection of 4% kaolin (0.1 ml) and carrageenan (0.08 ml) (K+C) into the ankle joint significantly ($P<0.05$; $N=6$-10) increased compression-evoked firing (i.e., induced "hyperalgesia") 30, 60, and 90 min after injection. In a separate group of animals, intra-articular injection of CP-96,345 (50 nmol) significantly reduced firing compared to pretreatment controls; administration of drug before K+C also prevented the enhancement of neuronal activity normally induced by K+C at 30, 60, and 90 min. These findings suggest that activation of peripheral NK-1 receptors, presumably by SP, may mediate hyperalgesia in this acute model of inflammation.

292.4

CAPSAICIN STIMULATES cGMP VIA NITRIC OXIDE IN DORSAL ROOT GANGLION CELL CULTURES. M.B. Bauer*, S. Murphy and G.F. Gebhart. Dept. of Pharmacology, University of Iowa, Iowa City, IA 52242.

Dorsal root ganglia (DRG) harvested from embryonic 15-16 day old Sprague-Dawley rats were dispersed and grown in the presence of mitotic inhibitors to produce predominantly neuronal cell cultures. Such cultures are useful for investigating messengers involved in the transmission of peripheral stimulation, such as that produced by the putative algesic agents bradykinin (BK) and capsaicin. Previously we demonstrated that BK stimulates production of cGMP via nitric oxide (NO) in DRG neuronal cell cultures. Capsaicin (0.5 μM) stimulated cGMP production in DRG neuronal cell cultures but not in nonneuronal cells. This effect was inhibited by methylene blue (10 μM), an inhibitor of soluble guanlylate cyclase, and N-monomethyl arginine (10 μM), an inhibtor of NO synthase, suggesting the involvement of a nitrosyl compound. Atriopeptin II (1 μM), which activates particulate guanylate cyclase, also stimulated cGMP production in DRG neuronal cultures, but this was unaffected by methylene blue (10 μM). These results indicate that cGMP formed upon the release of NO may be involved in the excitation of first order neurons by nociceptive chemical stimuli.

292.5

THE RELATIONSHIP BETWEEN DORSAL ROOT CONDUCTION VELOCITY AND SUSCEPTIBILITY TO A LOCAL ANESTHETIC. R. A. Jaffe* and M. A. Rowe. Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5123

Persistant differential nerve block is difficult to explain. Supported by clinical observations, susceptibility to local anesthetics was believed to be inversely related to axon diameter. Recent studies on myelinated axons do not support this "size principle." We have examined the sensitivity of single unmyelinated (UM) and myelinated (M) DR axons to lidocaine.

Adult male rats were anesthetized with enflurane and lumbar dorsal roots were excised. The isolated roots were perfused at 37°C with artificial CSF. We studied 77 axons (CV = 0.53-25.3 m/s) using single-fiber techniques. After control measurements, each root was exposed to lidocaine HCl initially at 0.15 mM or 0.26 mM and subsequently advanced to a higher concentration (0.26 mM or 0.52 mM) if block failed to develop at the lower concentration.

Estimated lidocaine ED_{50} block concentrations were similar for M (0.24 mM) and UM (0.22 mM) axons. At sub-blocking concentrations, the occlusion of Na channels by lidocaine resulted in a proportional slowing of CV measured as an increasing latency. CV slowing at both 0.15 mM or 0.26 mM lidocaine was significantly greater for M (37%, 62%) than for UM (24%, 32%) axons. Regression analysis suggests a significant direct relationship between CV (axon diameter) and sensitivity to lidocaine.

292.6

EVALUATION OF iCGRP SECRETION FROM DENTAL PULP IN RESPONSE TO INFLAMMATORY MEDIATORS. D. Jackson*, M. Garry, M. Engelstad, H. Geier, K. Hargreaves, Dept of Restorative Sciences, Univ. of Minnesota School of Dentistry, Minneapolis, MN.

Although calcitonin gene-related peptide (CGRP) may participate in the development of neurogenically mediated inflammation, relatively little is known about the regulation and secretion of this neuropeptide from peripheral tissue. This study uses a previously validated dental pulp superfusion model (J. Dent. Res. 71:178,1992) to evaluate immunoreactive CGRP (iCGRP) secretion following exposure to prototypic inflammatory mediators. Bovine dental pulp tissue, collected shortly after slaughter, was sectioned into 200 µm cubes. The pulp was superfused with oxygenated Krebs buffer (300 µL/min @ pH 7.4 and 37°C). After baseline collections, prostaglandin E2, serotonin, bradykinin, and histamine (all at 1.0 µM) were added to the Krebs buffer for the remainder of the experiment. The collected superfusate fractions (2.1 ml) were assayed by validated radioimmunoassay. Data was analyzed using Student's-t tests and ANOVA and is expressed as mean ± s.e.m. Initial iCGRP secretion from pulp tissue following exposure to the buffer containing the inflammatory mediators was approximately four times greater than the basal release from untreated tissue (59.5 ± 10.3 fmol/G/7 min vs. 15.2 ± 2.6 fmol/G/7 min). The increase in iCGRP release was sustained under steady state exposure to the inflammatory mediators for more than 60 minutes (release at 60 min: 34.4 ± 6.0 fmol/G/7 min). Collectively, these results support the hypothesis that inflammatory mediators produced in response to tissue injury can elicit and maintain sustained release of iCGRP from a select population of primary afferent fibers.

292.7

PROSTAGLANDIN E2 IMMUNOREACTIVITY IS RELEASED DURING INFLAMMATION OF THE RABBIT TMJ. MT Roszkowski*, JO Swift, T Alton, and KM Hargreaves, Depts. of Restorative Sci, Oral Surgery,and Pharmacology, University of Minnesota, Minneapolis, MN

Selection of successful treatment modalities for management of the inflamed temporomandibular joint (TMJ) has been based largely on empirical findings. We have addressed this issue by using microdialysis probes implanted into the superior compartment of the TMJ of the anesthetized rabbit. Five male New Zealand rabbits (2-2.5 kg) were anesthetized with ketamine/xylazine and maintained with supplemental injection. A semilunar flap overlying both the left and right TMJ was elevated. The superior compartment of the TMJ was identified by palpation and confirmed by aspiration. Microdialysis probes (10,000 dalton cutoff) contained in a 27g needle were implanted into both right and left joint compartments and fixed by ligatures. Sterile Locke-Ringers buffer was pumped through the probes (8 µL/min) and 30 min fractions were collected. Carrageenan (4mg) or an equal volume of saline (0.1cc) were injected into the right and left TMJs 60 min after placement of the probes. The dialysate samples were measured for iPGE2 by a previously validated ELISA method. Data were analyzed by repeated measures ANOVA followed by Dundan's Test.

The results indicate that carrageenan injection produces a 10-fold increase in synovial levels of iPGE2 by 270 min after carrageenan injection (9.2±3.3 pg at baseline vs 104.4±40.1 pg at 270 minutes; $p<0.005$). The inflamed rabbit TMJ may prove to be an appropriate animal model to evaluate therapeutic interventions for the management of the inflamed TMJ in patients.

292.8

ADRENERGIC INHIBITION OF iCGRP RELEASE FROM CAPSAICIN-SENSITIVE FIBERS IN DENTAL PULP. M Engelstad, M Garry, D Jackson, H Geier, K Hargreaves*, University of Minnesota School of Dentistry. Minneapolis, MN 55455.

Several studies have suggested that the sympathetic nervous system modulates pain and inflammation, possibly through activation of adrenergic receptors located on peripheral nociceptive nerve terminals. Accordingly, bovine dental pulp was evaluated for modulation of the evoked release of the inflammatory neuropeptide, calcitonin gene-related peptide (CGRP), by pretreatment with norepinephrine. Pulp tissue from bovine mandibular incisors was extracted, chopped into 300 µm cubes, and placed in 2cc chambers for *in vitro* superfusion. Following a baseline recovery period, oxygenated Krebs buffer (pH 7.4 @ 37°C) containing norepinephrine (1mM) was superfused through the tissue before and after a stimulating pulse of capsaicin (10µM), a neurotoxin that selectively stimulates certain primary afferent neurons. Levels of immunoreactive CGRP (iCGRP) were analyzed by a validated radioimmunoassay. Data were analyzed by ANOVA and Duncan's test. Basal levels of iCGRP release were 68.4 ±8.2 fmol/g tissue/9 min superfusion. Following capsaicin stimulation, release of iCGRP doubled in control chambers that did not receive norepinephrine (132.0 ±16.1 fmol/g/9 min; $p<0.001$). In contrast, there was a near complete inhibition of iCGRP release in chambers that did receive norepinephrine (66.7 ±3.5 fmol/g/9 min; $p<0.001$). Similar results were obtained by pretreatment with the alpha agonist clonidine and the beta agonist salbutamol. Collectively, these results indicate that adrenergic agonists could inhibit the release of iCGRP from capsaicin sensitive pain fibers located in dental pulp.

292.9

PROSTAGLANDIN E_2 AND ELEVATION OF INTRACELLULAR CYCLIC AMP ENHANCE BRADYKININ-STIMULATED RELEASE OF PEPTIDES FROM RAT SENSORY NEURONS IN CULTURE. K. J. Waite and M. R. Vasko*, Dept. of Pharmacology and Toxicology, Indiana U. School of Medicine., Indianapolis, IN 46202.

Prostaglandins (PGs) increase the inflammatory and algesic actions of bradykinin (BK). One possible mechanism to explain this effect is that PGs increase the formation of intracellular cyclic AMP and this enhances the BK-induced release of transmitters from sensory neurons. To test this hypothesis, we studied the effects of both PGs and alteration of intracellular cAMP on resting and BK-evoked release of substance P (SP) and calcitonin gene-related peptide (CGRP) from rat sensory neurons grown in culture.

Neurons were dissociated from dorsal root ganglia of 16-18 day old rat embryos and grown in culture for 8-14 days. Release studies were performed by exposing neuronal cultures to HEPES buffer containing 3.5 mM KCl (basal release) or buffer containing PGE_2 and/or bradykinin (stimulated release). Buffer was then assayed for SP and CGRP using radioimmunoassay.

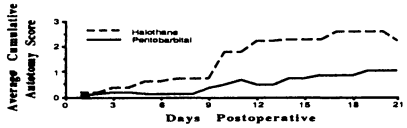
Exposure of cells to 1µM PGE_2 did not alter the release of either SP or CGRP. However, pretreatment of sensory neurons for 20 min with 1µM PGE_2 increased the release evoked by 10nM BK by 2-3 fold for both peptides. In a similar manner, pretreating sensory neurons with 100µM 8-bromo cyclic AMP, 1.5 µg cholera toxin, or 10 µM forskolin, significantly enhanced the BK-stimulated release of both SP and CGRP.

These results demonstrate that PGE_2 enhances the release of neurotransmitter from rat sensory neurons and suggest that this effect may contribute to PG-induced sensitization. Furthermore, one possible mechanism to explain the actions of PGE_2 on sensory neurons is an elevation of intracellular cyclic AMP. (Supported by PHS AR20582 and DA07176).

292.10

EFFECTS OF TWO DIFFERENT SURGICAL ANESTHETICS, SODIUM PENTOBARBITAL AND HALOTHANE, ON AUTOTOMY IN A RAT MODEL OF NEUROPATHIC PAIN. C. Fromm, J. A. DeLeo*, R. W. Colburn, D. W. Coombs, Anesthesia Research Lab, Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756.

Autotomy (self-mutilation) following peripheral somatic nerve damage is thought to be a behavioral marker for chronic neuropathic pain. The effects of 2 different surgical anesthetics on autotomy were studied in a rat model of sciatic cryoneurolysis (SCN). Under sodium pentobarbital (PB) anesthesia (40 mg/kg, i.p.), rats (n=21) underwent unilateral sciatic cryolesion with the contralateral side serving as an internal sham-operated control. The sciatic nerve was exposed by blunt dissection close to its emergence from the sciatic foramen and frozen with a cryoprobe (2 mm tip diameter, cooled to -60°C with NO_2 refrigerant) for a 30-5-30 sec freeze-thaw-freeze cycle. Under halothane (HAL) inhalation anesthesia (2% maintainence), a second group of rats (n=12) underwent identical surgery. Rats were observed every other day for about 5 weeks postoperatively, and the severity of autotomy was assessed using Wall's scale (1 point for bloody nail(s), 1 point for each distal or proximal phalange removed; max score = 11). Rats anesthetized with PB showed a decrease in severity of autotomy, or average cumulative Wall score, as compared with rats anesthetized with HAL.



These data suggest that use of a specific anesthetic during surgical procedures such as amputations may decrease the incidence of phantom-limb pain and other neuropathic pain syndromes. In addition, these results strongly support the use of autotomy and SCN as a behavioral marker and animal model for neuropathic pain.

292.11

OPIOID AGONISTS ENHANCE AND INHIBIT RELEASE OF PEPTIDES FROM RAT SENSORY NEURONS I. Dymshitz*, J. J. Chen, M.R.Vasko, Dept. Pharmacol. & Tox, Indiana U. Sch of Med, Indianapolis, IN 46202.

There is increasing evidence that different opioid agonists exert either inhibitory or facilitatory effects on primary afferent neurons; depending on the agonist concentration and receptor selectivity. To further study the actions of opioids on sensory neurons, we investigated the effects of the mu and delta selective opioids, (DAGO, and DPDPE, respectively) on the release of substance P (SP) and calcitonin gene-related peptide (CGRP) from cultures of rat dorsal root ganglia (DRG) and from rat spinal cord slices.

Neurons were dissociated from fetal DRG (E15-E17) and grown in culture for 9-12 days. After 10 min preincubation with opioids in a HEPES buffer, cells were exposed for 10 min to either 30 mM KCl or 100 nM capsaicin. Slices of spinal cord, obtained from adult rats, were superfused with Kreb's buffer with or without opioid agonists, and 250 nM capsaicin was used to stimulate peptide release. The levels of SP and CGRP in buffer were measured by RIA.

In DRG cell cultures, DAGO (10 nM-5 µM) inhibited KCl-induced release of SP and CGRP in a dose-dependent manner, with a maximal inhibition of 30-40% below control levels. In contrast, when cells were exposed to 100 nM capsaicin in the presence of the same concentrations of DAGO, a dose-dependent potentiation of the release of both peptides was observed. Application of DPDPE in the same concentration range did not significantly alter KCl-evoked release. In the slice preparation, DAGO (1 µM) had an inhibitory effect on SP and CGRP release elicited by capsaicin.

These results indicate that DAGO can either enhance or diminish the evoked release of SP and CGRP from sensory neurons. The opioid-induced potentiation or inhibition is dependent on the agent used to stimulate peptide release and on the neuronal preparation studied. (Supported by DA07176).

292.12

PROSTAGLANDIN E_2 ENHANCES THE SENSITIVITY TO BRADYKININ IN RAT SENSORY NEURONS GROWN IN CULTURE. G.D. Nicol* and M. Cui, Dept. of Pharmacology and Toxicology, Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Prostaglandins increase the sensitivity of sensory neurons to bradykinin (BK) presumably by lowering the response threshold to this pain producing agent. We sought to investigate the physiological mechanisms that produce this prostaglandin-induced hypersensitivity. Sensory neurons were dissociated from the dorsal root ganglions (DRG) of 15-17 day-old rat embryos, plated on small plastic cover slips coated with collagen, and grown for 5 to 7 days. The effects of prostaglandin E_2 (PGE_2) and BK were examined by utilizing the whole-cell patch clamp technique. PGE_2 (10 uM) had no direct effect on either the membrane potential or the action potential (AP) threshold as measured in current-clamp recordings from 6 of 8 neurons. Two of these neurons responded directly to the PGE_2 as indicated by an increase in the firing frequency of spontaneous APs. Exposure of the neurons to PGE_2 produced a small enhancement of the calcium current in voltage-clamp recordings. Application of 1 uM PGE_2 did not produce a significant increase above the control values. Both 10 and 30 uM PGE_2 caused increases of only 1.32- and 1.29-fold, respectively. However, a 10 min pre-treatment of the sensory neurons with 1 uM PGE_2 greatly enhanced the neuronal response to 100 nM BK. Under control conditions, the local application of 100 nM BK produced a small and slow increase in the inward current with relatively few large transient spikes of inward current. After exposure to PGE_2, BK again elicited a small and slow increase in inward current but the frequency of the transient inward currents was increased greatly. Similar results were obtained in current-clamp recordings wherein the frequency of BK-induced AP firing was enhanced by prior treatment with PGE_2. These findings suggest that PGE_2 does not have a direct excitatory action on the sensory neuron but rather a role in modulating the sensitivity to other stimulatory agents.

292.13

CARBA PROSTACYCLIN POTENTIATES CAPSAICIN-STIMULATED RELEASE OF NEUROPEPTIDES FROM RAT SENSORY NEURONS IN CULTURE. C.M. Hingtgen*, M.R. Vasko. Dept. of Pharmacology and Toxicology, Indiana U. School of Medicine, Indianapolis., IN 46202.

Because prostaglandin I_2 (PGI_2) injection into peripheral tissues produces an inflammatory response and sensitizes sensory neurons to noxious stimulation, it is possible that this prostanoid increases the release of transmitters from sensory neurons. We investigated whether the PGI_2 analog, carba prostacyclin ($CPGI_2$), enhances resting or capsaicin-induced release of substance P (SP) and calcitonin gene-related peptide (CGRP) from rat sensory neurons grown in culture.

Release experiments were performed by incubating sensory neurons grown in culture with a HEPES buffer containing 3.5 mM KCl (basal release) or buffer containing various concentrations of $CPGI_2$, capsaicin, or KCl. Buffer was assayed for SP and CGRP using radioimmunoassay.

Exposure of neurons to 1 μM $CPGI_2$ caused a 2 fold increase in the release of both SP and CGRP, compared to a 4-6 fold increase caused by either 30 mM KCl of 100 nM capsaicin. Pretreatment with 1 μM $CPGI_2$ potentiated the effects of 10 nM capsaicin (a nonstimulating concentration of capsaicin) on both SP and CGRP release. The release of SP increased significantly from a mean ± S.E.M. of 14 ± 3 fmol/well/10 min to 46 ± 4 fmol/well/10 min, whereas, the release of CGRP increased significantly from 47 ± 5 fmol/well/10 min to 198 ± 10 fmol/well/10 min.

These results demonstrate that $CPGI_2$ can sensitize sensory neurons to increase the release of transmitter. This sensitization may explain some of the inflammatory and hyperalgesic effects of PGI_2. (Supported by PHS AR20582).

292.14

PERSISTENT THERMAL AND MECHANICAL HYPERALGESIA INDUCED BY ULTRA-VIOLET RADIATION IN THE RAT. M.N.Perkins*, E.A.Campbell, D.Kelly, S.Patel, B.Sandells, L.Urban, and A.Dray. Sandoz Institute for Medical Research, Gower Place, London WC1E 6BN, UK.

Animal models of hyperalgesia and neuropathic pain are important for understanding chronic pain in man and for the evaluation of novel analgesic drugs. We describe here a model of thermal and mechanical hyperalgesia in the rat, lasting for up to 12 days, induced by ultra-violet (UV) radiation.

Female Sprague Dawley rats (100g) were exposed on one hindpaw to UVA light (intensity maximum 365 nm, 69mWatts/cm^2) for 90s and this was repeated 18hrs later. On each day following this the withdrawal threshold to a focussed beam of heat to the underside of both hind paws was measured. Mechanical hyperalgesia was assessed by a modified Randall-Selitto test. Drugs were given on various days following UV exposure.

On the first day after UV exposure (day 1) there was a 70% reduction in the latency of response to noxious heat. This was maintained for 7-8 days with recovery to control latencies occurring by day 12-13. The contralateral paw also exhibited thermal hyperalgesia reaching a maximum by day 5 but recovered by day 7. Mechanical hyperalgesia was maximal on day 5 with approximately 40% reduction in threshold returning to control levels by day 7. The thermal hyperalgesia was reversed by morphine on days 1 and 5 and this was naloxone sensitive. Ibuprofen, indomethacin and aspirin (10-500mg/kg, sc) were weakly active or non-effective when given at day 6. However, ibuprofen (200mg/kg, sc) reversed the hyperalgesia when given on day 1.

In this model of prolonged thermal and mechanical hyperalgesia there is an early inflammatory component and a later NSAID-resistant hyperalgesia.The contralateral hyperalgesia suggests the involvement of central mechanisms.

292.15

INTERLEUKIN FACILITATES Ca++ RELEASE IN ACUTELY DISSOCIATED DORSAL ROOT GANGLION (DRG) CELLS OF THE RAT. M. Kawatani* and L. Birder. Showa University, Dept. Physiology Tokyo Japan, 142.

It has been suggested that interleukin (IL) plays a key role in the inflammatory process. Using the Ca++ indicator FuraII-AM, we have visualized Ca++ transients in order to elucidate the mechanism of action of IL1α and IL1β . Addition of IL1α (0.1 - 1 ug) and IL1β (2 ug) in DMEM media resulted in an immediate, dose dependent increase in fluorescence due to increased Ca++ in acutely dissociated drg cells obtained from urethane anesthetized adult wistar rats (200-300 g). Blockade of Ca++ channels via addition of nifedipine (100-300 ug), or block of Ca++ activated K+ channels via use of Iberiotoxin (0.5-5ug) was ineffective in reducing the effect of IL1α . Indomethacin (100-300 ug), via prostaglandin blockade, inhibited the Ca++ increased by IL1α . These data may suggest that elevated Ca++ transients via IL1α may be due to release from intracellular storage sites mediated by increased prostaglandin levels.

292.16

POTENTIATION BY CAPSAICIN OF LIDOCAINE'S TONIC AND PHASIC IMPULSE BLOCK IN ISOLATED RAT SCIATIC NERVE. H.-C. Shin*, S.A. Raymond, U.T. Oh+ and G.R. Strichartz. Anesthesia Research Labs. Brigham & Women's Hospital, Harvard Medical School, Boston MA, 02115, +College of Pharmacy, Seoul National University, Seoul 151-742, Korea.

Compound action potentials (CAPs) of A- and C-fibers were recorded from isolated sciatic nerves of the rat to determine whether tonic and phasic block of impulse conduction induced by lidocaine was affected by low doses of capsaicin. Capsaicin alone (50 uM) did not change the CAPs of either A- or C-fibers. At a low frequency of stimulation (0.5 Hz), lidocaine (100 uM) for 30 min tonically reduced the amplitudes of A-fiber CAPs (by 20.6%, n=19) and C-fiber CAPs (by 28.4%, n=20). At higher stimulation frequencies (100 Hz for A-fibers, 20 Hz for C-fibers, 64 pulses), lidocaine (100 uM) phasically reduced both the A-fiber CAP (to a total steady state inhibition of 38.4%, n=7) and the C-fiber CAP (42.5%, n=7). Lidocaine's effects were 95-98% removed by 30 min wash. Although low concentrations of capsaicin (5-30 uM) caused no change of the tonic blocking action of lidocaine, 30 min of 50 uM capsaicin administration did induce a significant potentiation of tonic block (A-CAP: 35.7%, n=14; C-CAP: 38.2%, n=16). Lidocaine's phasic impulse block was similarly potentiated after 30 min of subsequent capsaicin administration (A-CAP: total inhibition=64%, n=7; C-CAP: 57.8%, n=7). Capsaicin's tonic and phasic potentiating effects were not reversed completely after 30 min of wash. These results suggest that capsaicin may be a useful agent for the reversible potentiation of impulse blockade by lidocaine, although the hoped for selective potentiation of C-fiber blockade was not observed in these rat peripheral nerves.

292.17

Gd^{3+} AS A PROBE FOR STRETCH ACTIVATED CHANNELS IN MAMMALIAN MECHANOSENSITIVE FREE NERVE ENDINGS. M.B. MacIver* and D.L. Tanelian. Pain Research Laboratory, Department of Anesthesia, Stanford University School of Medicine, Stanford, California 94305.

Gd^{3+}, at 20 to 100 μM, has been shown to block stretch activated channels in membrane patches from yeast, paramecium and invertebrate growth cones. It is possible that similar stretch activated channels could underlie mechanosensitivity in mammalian sensory neurons. The present study investigated this possibility using an *in vitro* preparation of mechanoreceptors innervating rabbit corneal epithelium. Extracellular recording electrodes were used to monitor multiple and single unit discharge from corneal nerves; response thresholds, discharge characteristics and receptive fields for mechanosensitive fibers were mapped. Corneal tissue was perfused with a PO_4 and CO_3 free HEPES buffer, since Gd^{3+} forms a precipitate with these anions. The HEPES buffer did not alter mechanoreceptor responses. Gadolinium chloride 10 to 250 μM in HEPES buffer did not alter mechanoreceptor responses following a 2 hr exposure time. Further studies will investigate longer exposure times (10 hr), higher concentrations (500 μM), and the membrane permeant compound gadopentetate dimeglumine to rule out the involvement of Gd^{3+}-sensitive stretch activated channels in mammalian mechanoreceptor sensory nerve endings.

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292.18

DILTIAZEM PRODUCES NON-Ca-DEPENDENT DEPRESSION OF CORNEAL C FIBERS. H. Lukatch*, M.B. MacIver and D.L. Tanelian. Pain Research Laboratory, Department of Anesthesia, Stanford University School of Medicine, Stanford, California 94305.

The calcium channel blocker diltiazem has been reported to specifically block corneal C fiber nociceptive responses, *in vivo*, although high concentrations were required (3 mM, 30 s, applied topically). The present study investigated concentration-dependent effects of diltiazem (10 to 250 μM) using an *in vitro* preparation of C fibers innervating rabbit corneal epithelium. Extracellular recording electrodes were used to monitor multiple and single unit discharge from corneal nerves. Low concentrations of diltiazem (10 μM) reduced C fiber tonic discharge frequencies to 83.6 +/- 9 (mean +/- SD; n=5) % of control, higher concentrations produced greater depression (100 μM - 71.5 +/- 27.3, n=6; 200 μM - 44.5 +/- 34, n=6) and total depression of tonic discharge occurred at 250 μM. The broad spectrum calcium channel blockers Ni^{2+} (200 μM) and Cd^{2+} (200 μM), in phosphate free solution, did not alter tonic discharge activity; however, lidocaine (20 to 80 μM) produced the same effect as diltiazem. Concentrations of diltiazem up to 250 μM did not alter Aδ fiber mechanoreceptors. These results suggest that the depressant effects of diltiazem on corneal C fiber discharge are not due to Ca^{2+} channel block, but may be due to sodium channel block.

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292.19

INHIBITION OF NEUROGENIC PLASMA EXTRAVASATION IN RATS BY A NON-PEPTIDE NK-1 ANTAGONIST RP67580. S.L. Shepheard, D.A. Cook, D.J. Williamson, R.G. Hill* & R.J. Hargreaves. M.S.D.Research laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex, U.K.

Plasma extravasation occurs within the dura and other receptive fields (lip, eyelid, conjunctiva) of trigeminal sensory afferent nerves upon electrical stimulation of the trigeminal ganglia. The non-peptide, NK_1 antagonist RP67580 is selective for rodent NK_1 receptors whilst its enantiomer RP68651 is inactive. Using these enantiomers (synthesized in our labs) we investigated the role of NK_1 receptors in mediating neurogenic plasma extravasation in the dura and extracranial tissues of rats.

Male S-D rats were anaesthetised with pentobarbitone sodium (60 $mg.kg^{-1}$, i.p.) and 10 min after i.v. injection of RP67580 (1-1000 $\mu g.kg^{-1}$), RP68651 (300 $\mu g.kg^{-1}$) or vehicle the right trigeminal ganglion or saphenous nerve was stimulated (5 Hz, 2 ms, 25 V, for 5 or 2 min). The left sides were prepared as sham controls. ^{125}I bovine serum albumin (70 $\mu Ci.kg^{-1}$, i.v.) was used to measure plasma extravasation. Dura, lip, eyelid or skin from the dorsal surface of the hindpaw was removed 5 min after stimulation, washed, weighed and counted for radioactivity. Results were calculated for each tissue as the ratio of extravasation in the stimulated/unstimulated sides. Data are given as the ID_{50} i.e. the dose producing half maximal inhibition of extravasation.

Following trigeminal ganglion stimulation RP67580 blocked dose-dependently plasma extravasation in all tissues. The ID_{50}'s in the facial tissues (lip and eyelid) were between 10 and 100 $\mu g.kg^{-1}$, whereas the ID_{50} in the dura was lower. RP67580 also reduced stimulation-evoked plasma extravasation in the hindlimb with an ID_{50} of 100 $\mu g.kg^{-1}$. RP68651 (inactive) given at a dose equivalent to the ID_{80} in the extracranial tissues (300 $\mu g.kg^{-1}$) had no effect on extravasation in any tissue.

The enantiomeric selectivity found in the present studies indicates that, in the rat, the plasma extravasation resulting from endogenous peptide release in a variety of tissues after electrical stimulation of cranial and extracranial sensory afferent nerve fibres is predominantly caused by activation of the NK_1 receptor.

BASAL GANGLIA AND THALAMUS III

293.1

NEURONAL AND BEHAVIORAL CORRELATES OF INTRASTRIATAL INFUSIONS OF AMPHETAMINE IN FREELY MOVING RATS. Z. R. Wang* and G. V. Rebec. Prog. Neural Science, Dept. Psychology, Indiana University, Bloomington, IN 47405.

Systemic amphetamine selectively increases and decreases the firing rate of motor- and nonmotor-related neurons, respectively, in the striatum of freely moving rats (Haracz et al., Brain Res., 489:365, 1989). To assess the role of striatal mechanisms in these differential neuronal responses, amphetamine (15-30 $\mu g/\mu l$) was infused (10 $\mu l/hr$) directly into the striatum of awake, behaving rats and single-unit activity was recorded simultaneously at the infusion site. Intrastriatal amphetamine typically activated motor-related, but suppressed nonmotor-related, neurons shortly after infusion onset. These changes in firing rate routinely preceded overt behavioral changes, supporting evidence that amphetamine-induced changes in striatal activity reflect a drug effect rather than behavioral feedback. Moreover, neurons in ventromedial and central striatum appeared more sensitive to amphetamine than other striatal neurons. Intrastriatal infusions of saline or extrastriatal infusion of amphetamine had no consistent effect. Our results indicate a direct role for both motor- and nonmotor-related striatal neurons in the behavioral effects of amphetamine and suggest regional differences in the sensitivity of striatal neurons to this drug.

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293.2

BEHAVIORAL AND NEUROPHYSIOLOGICAL CONSEQUENCES OF LARGE PUTAMEN LESIONS IN THE MONKEY. S.J. Mitchell*. VA Med. Ctr., Syracuse, NY 13210.

To understand the functional neural circuitry of the basal ganglia, we made unilateral excitotoxic lesions in the putamen of monkeys trained to perform a simple reaction-time reaching task, a finger coordination task, and a bimanual hand coordination task. The lesion aimed to damage the arm areas of putamen from 1 mm anterior to the anterior commissure through its caudalmost extent. Prior to the lesion, single cell studies were carried out during the reaction-time task to localize the arm areas of the putamen, and collect control data from arm-related cells in the globus pallidus. Ibotenic acid was injected at 12 sites in putamen, 6 sites per day, with one day intervening between the lesion days. For a period beginning with the first lesion day and lasting several days after the second lesion, animals were slow to react and move in the reaction-time task, were clumsy in the finger coordination task, and were impaired on the bimanual task. Deficits in the reaction-time task were bilateral although mild for the arm ipsilateral to the lesion. The contralateral arm was hypotonic. Long term behavioral impairments were variable and depended on the location and size of the lesion. Tonic discharge rates of neurons in both pallidal segments were not significantly altered by the lesions when damage was restricted to the putamen.

293.3

MICROEXCITABLE ZONES IN THE THALAMUS ARE SEPARATE FROM PALLIDAL-RECEIVING NEURONS. J.A. Buford*, M. Inase and M.E. Anderson. Depts. of Physiology and Biophysics and Rehabilitation Medicine and the Regional Primate Research Center, University of Washington, Seattle, WA 98185.

In the thalamus, most pallidal-receiving (PR) thalamic neurons are rostral to cerebellar-receiving (CR neurons). Ashe et al. (Soc. Neurosci., 1989) reported that movements were not elicited by microstimulation in rostral portions of the primate thalamus, whereas more caudal portions were microexcitable. He hypothesized that movements were elicited by microstimulation of CR, not PR, neurons.

We tested this hypothesis by comparing, in awake monkeys, the thalamic loci where pulse-train microstimulation produced muscle contraction (microexcitable zones) to the loci of thalamic cells (PR neurons) that exhibited fixed latency inhibition after single-pulse microstimulation in the internal globus pallidus (GPi). The GPi stimuli were delivered at locations where arm-related activity had been recorded.

Muscle contraction was elicited by stimulation at many thalamic locations. Microexcitable zones, however, were usually caudal and ventral to identified PR neurons. Our data support the hypothesis of Ashe et al. and emphasize the probability that excitation of PR thalamic neurons does not elicit movement. Supported by NIH NS15017 and RR00166.

293.4

CHANGES IN THALAMIC CELL DISCHARGE AFTER INJECTION OF MUSCIMOL INTO THE GLOBUS PALLIDUS. M. Inase, J.A. Buford, and M.E. Anderson*. Depts. of Physiology and Biophysics and Rehabilitation Medicine and the Regional Primate Research Center, University of Washington, Seattle, WA 98185.

Muscimol injected into the internal globus pallidus (GPi) causes flexor drift and slowed movement in the arm (Inase and Anderson, Soc. Neurosci. 1991). We tested whether this GABAA agonist disinhibited thalamic target neurons, presumably by inhibiting pallidal output.

The monkey held his hand at a central light (hold period) until a go-tone instructed him to move to a target light. In the *visible* condition, the target was continuously lit after the go-tone. In the *pre-cue* condition, the target was briefly lit 800 ms into the hold period and the monkey moved to the remembered target location. Thalamic neurons were studied before and after muscimol and were identified as pallidal-receiving (PR) if single-pulse stimuli at arm-related GPi loci produced fixed-latency inhibition of thalamic discharge.

After muscimol, the distribution of hold-period firing rates for PR cells included rates higher than those recorded before injection. A similar change in the distribution was noted in thalamic cells recorded near the PR cells. For PR cells recorded before and after injection, muscimol increased hold-period firing by about 50%. Movement-related PR-cell discharge increased by about 25% after muscimol. Because of the higher hold-period firing rates, the change in firing caused by movement (100*[MovementAverage - HoldAverage] / HoldAverage) was reduced by about one third after muscimol. The post-injection emergence of increased firing rates coincided with the appearance of flexor drift and decreased velocity. Supported by NIH NS15017 and RR00166.

293.5

ALTERATIONS IN THE PHYSIOLOGY OF INTRALAMINAR AND MOTOR THALAMIC NEURONS IN CATS WITH MPTP-INDUCED PARKINSONISM. D.S. Rothblat* and J.S. Schneider, Department. of Neurology, Hahnemann University, Philadelphia, PA. 19102.

Previously, we have shown that striatal sensory responsiveness and information processing is severely impaired in cats with MPTP-induced parkinsonism and profound striatal dopamine (DA) depletion. Following such extensive striatal DA depletion, the spontaneous activity of striatal and entopeduncular nucleus (ENTO) neurons increases while activity of globus pallidus neurons decreases. This data supports the hypothesis that thalamic neurons which receive inputs from the ENTO may be under abnormal inhibitory influences in the Parkinsonian condition. The present study examined the physiology of thalamic neurons which are afferent to the striatum (centromedianum (CM) and centralis lateralis (CL)) in effort to examine whether the loss of sensory responsiveness in the Parkinsonian striatum can at least in part be explained by a loss of sensory responsiveness in thalamic afferent structures. Spontaneous activity and responses to tactile stimuli were assessed in the CM and CL in awake, alert cats. Parkinson-like sensorimotor deficits were then produced by administering MPTP (7.5 mg/kg, i.p.) for 7-10 days. Spontaneous activity and sensory responsiveness was again assessed. Spontaneous activity of CM and CL neurons decreased by approximately 54% in cats with severe Parkinsonian motor deficits. Responsiveness to tactile stimuli also significantly decreased by approximately 85% when animals were Parkinsonian. Preliminary results obtained from the ventral anterior/ventral lateral (VA/VL) nuclei show similar trends. These results suggest that the previously observed increase in ENTO activity leads to decreased spontaneous activity and sensory responsiveness in intralaminar (IL) and motor thalamic regions. These changes in thalamic physiology, and particularly in the IL region, may contribute to the parkinsonian sensorimotor deficits observed in MPTP-treated cats at least in part by limiting the access of sensory information to the striatum and cortex. Supported by NIH grant NS23980.

293.6

CONTRIBUTIONS OF REUPTAKE INHIBITION AND DOPAMINE DENERVATION TO VOLUME TRANSMISSION OF DOPAMINE IN THE STRIATUM IN NORMAL AND MPTP-TREATED CATS. J.S. Schneider*, D.S. Rothblat and L. DiStefano, Dept. of Neurol., Hahnemann Univ., Phila., PA 19102.

Cats become parkinsonian after MPTP administration but typically recover motor function within 4-6 wks. Tissue dopamine (DA) levels in the dorsal lateral (i.e., sensorimotor) caudate nucleus (DL CD) do not differ between symptomatic and recovered MPTP-treated cats, but there is significant recovery of ventromedial caudate/nucleus accumbens (VM CD) DA levels in recovered cats. Despite the lack of tissue DA recovery in the DL CD, in vivo microdialysis shows an approx. 50% recovery of extracellular fluid (ECF) DA in this region. The present study was to determine the source of this ECF DA in the DL CD of previously parkinsonian cats. Cats were implanted with cannulae overlying the VM and DL CD on each side of the brain through which microdialysis probes (3-4 mm membrane) could be inserted. ECF DA levels were examined when cats were normal and after functional recovery from MPTP-induced parkinsonism. After basal levels of DA were measured in both striatal regions, KCl (60mM) was infused through one probe and the DA level was measured at both probes. In normal cats, KCl infusion stimulated a local 3-5 fold increase in ECF DA and no detectable increase in DA at the distant probe, approx. 5mm away. In normal cats treated with nomifensine (20mg/kg, i.p.), a DA reuptake inhibitor, DA released from KCl stimulation in one region was detected in the distant region by the second dialysis sample after infusion, suggesting enhanced diffusion after reuptake inhibition. DA released by KCl stimulation of the VM CD was, by the second sample after infusion, detected in the DL CD in cats functionally recovered from MPTP-induced parkinsonism. In these cats, DL CD DA increased more from diffusion than from local stimulated release. These results suggest that DA may exert its effects after extensive (but heterogenous) striatal presynaptic denervation significantly through volume transmission. We suggest that functional recovery following DA-depleting lesions may depend on the extent to which DA diffusion occurs and the tissue volume through which DA must diffuse. Supported by NIH grant NS-23980.

293.7

INACTIVATION OF THE SENSORIMOTOR TERRITORY IN THE INTERNAL PALLIDUM REVERSES PARKINSONIAN SIGNS IN MPTP-TREATED MONKEYS. M.S. Baron, T. Wichmann* and M.R. DeLong. Dept. of Neurology, Emory Univ. Sch. of Med., Atlanta, GA 30322

Recent studies suggest that increased neuronal activity in the internal pallidal segment (GPi) is a key element in the development of parkinsonian motor signs. This has led to renewed interest in neurosurgical lesions of GPi as treatment for Parkinson's disease. The goal of this study was to define the optimal target site for such lesions in parkinsonian monkeys.

A Rhesus monkey received bilateral intracarotid injections of MPTP (0.4 mg/kg/side), leading to stable parkinsonian motor signs (akinesia, bradykinesia, rigidity, flexed posture). In order to transiently inactivate small regions of GPi, injections of the $GABA_A$ receptor agonist muscimol (1 μg/1μl) were carried out in a 2 mm grid throughout the nucleus under electrophysiological guidance. The unrestrained animal was observed for 20 minute periods before and after the injection (20-40 min and 60-80 min post injection).

Injections in a small central area of GPi that contained neurons responsive to somatosensory stimulation led to marked contralateral improvement of akinesia, bradykinesia and posture, typically after 15 - 20 minutes and lasting several hours. Injections in other areas of the nucleus were less effective, and often induced atypical behavior (e.g. circling). Normal saline injections had no effect. Injections of muscimol into the external pallidal segment (GPe) failed to reverse parkinsonian signs.

These results indicate that lesions restricted to the "sensorimotor" portion of GPi can be an effective treatment for parkinsonian motor signs. Electrophysiological methods should help to localize the optimal target for lesioning in GPi and minimize the incidence of side effects.

293.8

BASAL GANGLIA MODULATION OF MOTOR CORTEX EXCITABILITY. Lawrence J. Ryan and David J. Sanders. Department of Psychology, Oregon State University, Corvallis, OR 97331-5303.

The basal ganglia are currently conceived to be part of parallel major and minor positive feedback loops originating in the cortex, traversing the basal ganglia and thalamus and returning to cortex. We test whether changes in basal ganglia function can directly alter motor cortex excitability via this loop.

Thresholds for evoking movements by electrical stimulation (50 ms trains, 333 Hz, 10-80uA, Pt-Ir electrodes) of primary motor cortex were determined in ketamine (100mg/kg, ip) anesthetized rats. Drugs were infused into the head of the neostriatum (0.4 uL over 90 seconds) and the motor cortex threshold was monitored for the next 60 minutes. The glutamate agonist kainic acid increased movement-eliciting thresholds to stimulation of the ipsilateral but not contralateral cortex. Thresholds returned near baseline by 60 minutes. In contrast GABA decreased thresholds of the ipsilateral motor cortex. These changes in excitability are likely due to modulation of the cortex rather than to direct competing descending influences on the muscles since cutting the loop with ibotenic acid lesions (7 days prior to testing) of the VL thalamus (n=6), but not the VM thalamus (n=5), eliminate the effect of kainic acid infusion.

Change in Motor Cortex Thresholds During First 10 Minutes After Infusion

Drug	N Infusions	Ipsilateral	Contralateral
0.25 ug/ul Kainic acid	7	+6.1%	-24.6%
0.75 ug/ul Kainic Acid	23	+185.5%	-11.1%
10^{-3}M GABA	8	-18.7%	-6.4%
0.75 KA - VL Lesion	6	-5.9%	-16.0%
0.75 KA - VM Lesion	5	+183.2%	-9.3%

Thus, the basal ganglia can directly modulate motor cortical excitability. These preliminary results suggests that increasing neostriatal activity inhibits the cortex, whereas decreasing neostriatal activity facilitates the cortex.

This research was supported by grant MH 45341 (to LJR) from the N.I.M.H.

293.9

SUBTHALAMOTOMY PRIOR TO MPTP ADMINISTRATION REDUCES SEVERITY OF PARKINSONISM IN MONKEYS. J. Guridi, MR Luquin, MT, Herrero, J. Laguna, J. Guillen, JA Obeso*. Movement Disorders Unit. Department of Neurology. Clinica Universitaria. Apdo. 192-31080 Pamplona-Spain.

A subthalamic lesion was made by Kainic acid injection into STN in two groups of macaques: 1). In 2 previously normal monkeys hemiballism become well established. MPTP (1mg/Kg, i.v.) was given up to 10 consecutive weekly doses. Animals showed a mild reduction in spontaneous activity, flexor posture and focal hypomimia but hemiballism persisted. This contrasted with the very severe and permanent parkinsonism induced in similar monkeys (n= 12) after a less agressive pattern of MPTP administration (0.5 mg/kg, 10 wks). 2) In 2 previously parkinsonian monkeys hemiballism developed in the contralateral limbs without clear cut motor improvement. These results support a pivotal role of the STN in parkinsonism but dyskinesias can coexist with parkinsonian sign following STN lesion.

293.10

EFFECTS OF DOPAMINERGIC AGENTS ON THE TONICALLY ACTIVE NEURONS OF THE STRIATUM IN HEMI-PARKINSONIAN MONKEYS. T. Aosaki, A. Ishida, K. Watanabe, H. Imai, A.M. Graybiel and M. Kimura*. Dept. of Physiology, Jichi Medical School, Tochigi 329-04, Japan, and Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Tonically active (type I) neurons in the striatum respond with inhibition to sensory stimuli that serve as cues for initiation of learned movements. In this study, we investigated the modulation of type I cell activity by the nigrostriatal dopamine system. After monkeys (2 Macaca fuscata) were well trained to lick a liquid reward after a click sound, the activity of type I cells was recorded during this learned behavior. About 70% of the type I cells (317/453 cells) showed significant inhibitory responses to the click stimuli. Then, the monkeys were rendered hemi-parkinsonian by local infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 4 mg) into the striatum by an osmotic minipump. The proportion of type I cells responsive to the click was dramatically decreased to about 15% (32/211 cells) in the depleted striatum, whereas the majority of Type I cells on the intact side (122/150 cells) remained responsive. When apomorphine (0.1-0.3 mg/kg) was injected systemically, 6 out of 9 non-responsive cells on the dopamine-depleted side acquired inhibitory responses to the click. After local application of 10 μM haloperidol on the intact side, using a concentric drug-applying recording electrode, responses to the click stimulus decreased significantly in 3 cells examined. Local application of dopamine (10 μM) immediately suppressed all firing of 14 type I cells examined. These results suggest that the behaviorally contingent activity of tonically active neurons in the striatum is strongly influenced by nigrostriatal dopaminergic inputs, and that these influences may be inhibitory. (Supported by Human Frontier Science Program)

293.11

LOCALIZATION OF FUNCTIONAL ACTIVITY IN RAT NEOSTRIATUM DURING TRAINED FORELIMB MOVEMENTS. *L. L. Brown*, M. F. Gonzalez† and F. R. Sharp†.* *Department of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461 and †UCSF, Department of Neurology, Veteran's Administration Hospital, San Francisco, CA 94121.

We have reported previously that electrical stimulation of forelimb motor cortex that induces movement in rats also causes activation in the ventral part of dorsolateral striatum. We here report that the same region is activated when a forelimb movement is made voluntarily. Rats were trained to reach through a hole and press a lever to receive pellets on a fixed ratio schedule of 16:1 or 32:1. Radiolabelled deoxyglucose was injected iv at the beginning of the final session; after 45 min the animals were killed with an overdose of pentobarbital and their brains were prepared for autoradiography. Experimental rats pressed the bar up to 5056 times during the deoxyglucose session and ate up to 158 pellets. After training, controls had been given pellets without a bar press for several weeks so that they would eat the same number of pellets as experimentals during the deoxyglucose session, but would not press the bar. The striatum was analyzed from 2.0 mm anterior to bregma to 1.0 mm posterior. Activation in the bar-pressing animals was different from controls in anterior striatum. Bar-pressing animals showed maximal activation in the region activated by forelimb motor cortex stimulation, while controls showed activation in the region activated by vibrissae motor cortex stimulation. The area of maximal activation was, however, smaller than in the motor cortex stimulation animals. The results confirm localization of forelimb motor neural activity in rat, during a natural movement, that is consistent with a topographic organization of activity in rat neostriatum.

293.12

GLUTAMATERGIC MEDIATION OF ORAL MOTOR CONTROL IN THE RAT'S STRIATUM: INTERACTIONS WITH DOPAMINERGIC AND CHOLINERGIC SYSTEMS. M. Pisa* D. Bosiljevac and D. Brain. Dept. Biomed. Sci., McMaster Univ., Hamilton, Ont., Canada, L8N 3Z5.

We previously showed that, in rats, bilateral injections of the wide-spectrum glutamate receptor antagonist kynurenic acid (12-72 nmol) in the lateral striatum induce an increase of nondirected (in vacuo) oral movements, suggesting a role of corticostriatal glutamatergic transmission in oral motor control. In the present study, we examined the hypothesis that this effect is mediated by increased activity of local dopaminergic and cholinergic systems. The oral hyperkinetic effect of kynurenate injections (36 nmoles in .4 ul) in the lateral striatum was confirmed. Co-injections of either SCH23390, sulpiride or cis-flupenthixol, which block D1 receptors, D2 receptors, and both D1 and D2 receptors, respectively, failed to reduce the kynurenate effect at doses known to abolish the oral stereotypies induced by the dopamine receptor agonist apomorphine. It appears, therefore, that the oral hyperkinesia induced by blockade of glutamatergic transmission in the lateral striatum is not mediated by a local increase of dopaminergic hyperactivity. The role of cholinergic mechanisms is being investigated and will be reported at the meeting. (Supported by the Scottish Rite Schizophrenia Research Program. M. Pisa is a Research Associate of the Ontario Mental Health Foundation).

293.13

DIFFERENTIAL ROLES OF THE CAUDATE AND PUTAMEN: BEHAVIORAL AND ELECTROPHYSIOLOGICAL ANALYSIS. K. Fujimoto*, M. Yoshida, H. Yamada, E. Nakamura. Dept. of Neurology, Jichi Medical School, Minamikawachi-machi, Tochigi, 329-04, JAPAN

In order to reveal a functional difference between the caudate (Cd) and the putamen (Put), we studied the effects of local injection of bicuculline (BIC) into these nuclei on the motor behavior in the cat. BIC is a reversible GABA antagonist and is assumed to activate efferent neurons of the Cd and the Put by removing the action of GABA-ergic inhibitory synapses on them.

Two weeks before the experiments, stainless-steel tubes for injections were inserted stereotaxically under the anesthesia with pentobarbital sodium. Recording electrodes made of insect pins were also inserted into the Cd, Put, entopeduncular nucleu (Ent), external pallidum (GP), subthalamic nucleus (STH), and substantia nigra (SN). Silver-ball electrodes were fixed on the motor cortex (Cx). In the experiments, BIC (3μg/1.5μl) was injected into the Cd and the Put respectively with the interval of at least 1 day, and the field potentials were recorded from the fixed electrodes. Behavior of the cat was recorded with a video camera and the movement of the neck was analysed with a X-Y recorder.

Shortly after the unilateral injection of BIC to the Cd, spikes were seen in the Cd and locomotor hyperactivity without any postural asymmetry or circling tendency was observed. Then, the spikes spread to the Cx, Put, GP, Ent, STH, and SN and these generalized spikes triggered the jerky movements of the neck toward the contralateral side. Shortly after the unilateral injection of BIC to the Put, spikes were seen in the Put and the jerky movements towered the contralateral side were observed. Next, the bursts of generalized spikes developed and the neck turned to the contralateral side dystonically. The dystonic posture lasted while the burst of spikes continued.

These results demonstrate that the Cd and the Put are differentially associated with locomotor and postural functions.

293.14

EFFECT OF DOPAMINE AGONISTS ON TURNING BEHAVIOR AND LOCOMOTOR ACTIVITY IN RATS WITH KAINIC ACID-INDUCED LESIONS OF THE SUBTHALAMIC NUCLEUS. **J.C. Pineda*, G. Flores, M. Rosales, J. Flores-Hernandez, A. Sierra, V. Martínez and J. Aceves. Dept. of Physiology. CINVESTAV-IPN. Mexico.**

Here we have explored the role of subthalamic nucleus (STN) on turning behavior and locomotory activity (LA) induced by dopamine (DA) activation. Kainic acid was injected either unilaterally or bilaterally into the rat STN. Shams animals received ascorbate in saline. Glutamic acid, glutamine and GABA contents were measured in substantia nigra reticulata (SNr) and entopeduncular nucleus (EPN). Unilaterally lesioned animals exhibited spontaneous contralateral turning, lasting 12 days. Systemic apomorphine (1 mg/Kg sc) or methamphetamine (4 mg/Kg sc) induced ipsilateral turning in these animals. In bilaterally lesioned animals, SKF 39383 (1 and 5 mg/Kg sc) increased, while quinpirole (0.5 and 2 mg/Kg sc) decreased LA when compared with controls. Glutamic acid levels were significantly decreased in the SNr and EPN of the experimental animals, but no decrease was observed in glutamine or GABA contents. This results show the relevance of the STN in the LA induced by activation of DA receptors.
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293.15

DOPAMINERGIC INPUT TO THE SUBTHALAMIC NUCLEUS REGULATES THE MUSCULAR TONE IN THE RAT. **G. Flores, J. Valencia, A. Sierra, M. Rosales, J. Hernández* and J. Aceves. Dept. of Physiology. CINVESTAV-IPN. Mexico.**

The subthalamic nucleus (STN) plays a crucial role in the control of motor functions. The STN receives a dopaminergic input from substantia nigra compacta (SNc). However, the effect of dopamine (DA) on subthalamic neurons is not clear. Here we studied the role of the DA innervation of the STN on muscular tone of extensor muscles (gastrocnemius-soleus). 6-hydroxydopamine (6-OHDA) was unilaterally injected into the STN. Shams received ascorbate in saline. Muscular tone was measured by electromyography. DA content was measured in STN, striatum (str) and accumbens (acc). The 6-OHDA lesion provoked an ipsilateral increase in muscular tone, DA levels were significantly decreased only in the lesioned STN. No change in either DA content or on muscular tone was observed in sham animals. These results suggest that the dopaminergic input to STN is inhibitory. The loss of this input, would disinhibit the subthalamic neurons, which would, in some way, cause the increased muscular tone.
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293.16

REPEATED APOMORPHINE-INDUCED ROTATIONS POSSESS A LEARNING COMPONENT. C.S. Fong, S.J. Boyson*, B.J. Hoffer, J.L. Hudson. Dept. of Pharmacology and Neuroscience Training Program, Univ. of CO HSC, Denver CO 80262.

Apomorphine-induced turning has been used, by many laboratories, to evaluate the extent of unilateral nigrostriatal denervation and functional striatal reinnervation by catecholaminergic grafts after 6-hydroxydopamine (6-OHDA) lesions. It has been noted that the pre-graft rotational pattern is usually double-peaked and that fetal ventral mesencephalic grafts, or dopaminergic drugs, will alter the second peak but leave the first relatively intact. We hypothesized that the first peak may possess a learning component and will therefore be unperturbed by the above treatments. We investigated this by injecting 6-OHDA, unilaterally, into the nigrostriatal pathway of several groups of Fischer 344 rats. One experimental group was repeatedly tested with 0.05mg/kg apomorphine. A second group received similar injections but were prevented from rotating. Vehicle control animals were also studied for both of the above experimental groups. Our results support the "learning" hypothesis in that the first peak is not present after the initial injection of apomorphine but emerges and is present upon the second and subsequent injections. Furthermore, the restrained but apomorphine-injected rats, as well as the control animals, manifest no first peak upon their first freely-moving apomorphine test; the second and subsequent rotations, however, do show a first peak. We therefore conclude that learned rotations are a significant component of the first peak of apomorphine-induced turning. These rotations are unlikely to be related to the cellular changes induced by 6-OHDA lesions.

293.17

TARGET SPECIFICITY AND NEUROTRANSMITTER TYPE OF FETAL C.N.S. TRANSPLANTS ARE IMPORTANT FOR FUNCTIONAL REINNERVATION OF 6-HYDROXYDOPAMINE TREATED RATS. J.L. Hudson, P. Bickford, B.J. Hoffer*, I. Strömberg. Departments of Pharmacology and Psychiatry, Univ. of Colorado Health Sci. Ctr., Denver, CO 80262 and Department of Histology and Neurobiology, Karolinska Institutet, Stockholm, Sweden.

The ability of grafts of fetal ventral mesencephalic dopaminergic (DA) neuroblasts to reinnervate the unilaterally DA-denervated rat striatum and improve motoric asymmetry has been well documented in several laboratories. The importance of host target specificity, and catecholamine (CA) neurotransmitter species, in the ability of grafts to ameliorate rotational responses to apomorphine and to affect electrophysiological characteristics of striatal neurons has not been systematically studied. We unilaterally lesioned male Sprague-Dawley rats with 6-hydroxydopamine and verified the lesions using 0.05mg/kg apomorphine s.c.. Some of the animals subsequently received, intrastriatally, either DA neuroblasts from ventral mesencephalon that normally innervate the striatum, or from arcuate nucleus that do not. Additionally, two control groups were included that received either a catecholaminergic graft from locus coeruleus or a graft of cerebral cortex. Only the fetal ventral mesencephalic grafts were able to reduce apomorphine-induced rotations and normalize striatal-cell firing rates; firing rates with substantia nigra grafts were 1.5 Hz $\pm$ 0.25, with arcuate nucleus grafts were 5.6 $\pm$ 0.8, with locus coeruleus grafts were 5.1 $\pm$ 1.1, and with cerebral cortex grafts were 4.4 $\pm$ 0.5. Moreover, only the ventral mesencephalic grafts produced a histochemically demonstrable CA reinnervation of striatum. We thus conclude that target specificity and neurotransmitter type are critically important in the ability of a graft to functionally reinnervate the 6-hydroxydopamine denervated striatum.

293.18

A SINGLE INTRAMESENCEPHALIC INJECTION OF BDNF INDUCES PERSISTENT ROTATIONAL ASYMMETRY IN RATS. CW Shults*ΔT, RT Matthews Δ, and PJ Langlais +ΔT ΔDept. of Neurosci., UCSD; +Psychol. Dept., SDSU, TNeurology Ser., VA Medical Ctr., San Diego, CA 92161

Brain-derived neurotrophic factor (BDNF) promotes survival of mesencephalic, dopaminergic neurons in vitro, and message for BDNF is transiently expressed in nigral neurons in rat pups and reexpressed in adult rats treated with intraventricular colchicine. To evaluate the effects of BDNF on the mesostriatal, dopaminergic system of the adult rat, we examined the effects of unilateral injection of BDNF into the mesencephalon. Rats were tested for amphetamine-induced (1 mg/kg) rotation before and at various times (2 d to 2 1/2 mon) after a single, unilateral injection of BDNF (1, 2, or 3 µg) or vehicle into the mesencephalon. Injections were made at the junction of the substantia nigra pars compacta and the ventral tegmental area . In a series of four experiments, we noted that after a single, unilateral injection of BDNF (2 or 3 µg) but not vehicle, animals exhibited a statistically significant increase in amphetamine-induced rotation contralateral to the side of injection. The pattern of rotation is consistent with increased activity of the mesostriatal dopaminergic system on the side of injection. The increase in amphetamine-induced rotation was greater at 10 days than at 2 days after injection of BDNF and persisted for up to 2 1/2 mon (the longest period studied). Levels of catecholamines and indolamines in the striatum and immunohistochemical studies of tyrosine hydroxylase-immunoreactive neurons in the mesencephalon will be presented. Our studies indicate that a single injection of BDNF into the mesencephalon can induce a persistent behavioral change in rats. Supported by United Parkinson Fndn. and VA Merit Review Program.

293.19

NEURONAL ACTIVITY IN RAT STRIATUM DURING A SIGNALED AVOIDANCE TASK: REGIONAL DIFFERENCES IN INFORMATION PROCESSING. I.M. White*, L.M. Bartolo, and G.V. Rebec, Prog. Neural Science, Dept. Psychology, Indiana University, Bloomington, IN 47405

The striatum, which receives substantial dopaminergic input, is known to play a critical role in active avoidance learning (Koob et al., Brain Res., 303:319, 1984). To begin to assess the neuronal mechanisms underlying this role, we recorded neuronal activity in the striatum during the performance of a signaled avoidance task in which rats were required to release a lever within 500 msec of the onset of an auditory signal to avoid footshock (White et al., Pharmacol. Biochem. Behav., 41:29, 1992). All animals were trained until performance reached or exceeded a 90%-avoidance rate for 5 consecutive sessions. Then they were surgically prepared for unit recording. Following a one week recovery, rats resumed training until performance was the same as before surgery. Striatal activity then was recorded during one or more sessions of 10-trials each. Data were obtained from more than 200 neurons, the majority of which responded in close temporal association with either the auditory signal or the lever-release response. Less than 20% of the cells responded to neither. Signal-responsive neurons tended to cluster in medial striatum, whereas response-specific neurons were found primarily in dorsolateral areas. Treatment with haloperidol (0.1 mg/kg, SC), a dopamine antagonist, 20 min before testing blocked performance as well as both signal- and response-related neuronal activity. Taken together, out results indicate a functional differentiation between medial and dorsolateral striatum in processing the signal that triggers a learned movement and the movement itself. Haloperidol, however, appears to exert uniform effects on both processing functions.

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293.20

NEUROTOXIC LESIONS OF THE NUCLEUS ACCUMBENS, BUT NOT NEOSTRIATUM, IMPAIRS PERFORMANCE ON A SIGNALED AVOIDANCE TASK IN RATS. G.V. Rebec*, M.P. Hrebin, H.M. Karsch, and I.M. White, Prog. Neural Science, Dept. Psychology, Indiana University, Bloomington, IN 47405.

Antipsychotic drugs block performance on a signaled avoidance response task in which rats are trained to release a lever within 500 msec of the onset of an auditory signal to avoid a footshock (White et al., Pharmacol. Biochem. Behav., 41:29, 1992). Although the mesotelencephalic dopaminergic projection is known to paly a key role in active avoidance learning (Koob et al., Brain Res., 303:319, 1984), relatively little is known about the involvement of specific target sites. To address this issue, rats were trained on our signaled avoidance task, and the nucleus accumbens or specific subregions of the neostriatum (dorsolateral, medial, or ventral) were damaged with ibotenic acid. One week after surgery, rats were run on the same task. To assess lesion effects, the average number of avoidances and the average avoidance latency were compared before and after lesion. Bilateral, not unilateral, damage to the nucleus accumbens significantly decreased the number of successful avoidances without disrupting the latency of either the avoidance or escape responses. In contrast, neither sham nor any of the neostriatal lesions altered performance on this task. Thus, the nucleus accumbens is a critical postsynaptic target of the ascending dopaminergic system involved in active avoidance learning.

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BASAL GANGLIA AND THALAMUS IV

294.1

SEROTONERGIC MODULATION OF NIGROSTRIATAL TERMINAL EXCITABILITY. L.D. Manley*, S.J. Young, M.S. Manley and P.M. Groves, Dept. of Psychiatry, University of California, San Diego, La Jolla, CA 92093-0603.

Studies suggest that striatal dopamine (DA) levels are affected by local application of serotonergic (5HT) agents. These effects may be due to the activation of presynaptic receptors on nigrostriatal DA terminals. Previous studies have also shown that changes in the activation of receptors on DA terminals are associated with systematic alterations in the electrical excitability of the axonal terminal field. We investigated whether local striatal application of 5HT drugs similarly altered DA terminal excitability. Antidromic responses elicited by stimulation of ventral striatum were recorded extracellularly from DA neurons of the substantia nigra pars compacta in urethane-anesthetized rats. Drugs were delivered (300 nl over 3-5min) to the stimulating site with an infusion cannula. Excitability changes were assessed by determining the threshold current required for antidromic activation before and after drug infusion. Administration of the 5HT2 agonist, DOI, produced a dose-dependent decrease in excitability (10 µM: -63.7±2.6%; 5 µM: -22.2±3.3%; 1 µM: -17.1±1.6%; 0.1µM: -7.4±0.4%, n=4/dose). Prior infusion of the 5HT2 antagonist, ketanserin (0.1 to 10µM, n=4), produced a decrease in excitability (3/4 rats) and, in all cases, completely blocked the action of a subsequent infusion of DOI (1 or 5µM). Studies are underway to determine whether these effects are dependent on dopamine or on the action of postsynaptic neurons. Additional experiments are being performed to investigate the actions of other 5HT receptor subtypes. This work is supported by DA 02854 and PHS 5T32 GM07752.

294.2

ELECTROCHEMICAL EXAMINATION OF THE CONNECTIONS BETWEEN THE PEDUNCULOPONTINE TEGMENTUM, SUBSTANTIA NIGRA AND CAUDATE-PUTAMEN IN THE RAT. C.D. Blaha and P. Winn* Depts. Psychol. & Psychiat., Univ. British Columbia, Vancouver B.C., V6T 1Z4, Canada (C.D.B.) and Dept. Psychol., Univ. St Andrews, Fife, Scotland KY16 9JU (P.W.)

The effects of cholinergic stimulation of substantia nigra pars compacta (SNPC) on DA efflux in the caudate-putamen (C-P) (measured by *in vivo* chronoamperometry: Blaha and Jung, 1991, J. Electroanal. Chem. 310: 317-334) was investigated in intact and pedunculopontine tegmental nucleus (PPTg) lesioned rats under urethane anesthesia. Using stearate-modified electrodes, a dose-dependent increase in oxidation current in the C-P corresponding to DA efflux was found following microinjection into the SNPC of nicotine (0.5 mM, 155$\pm$12% maximal increase as percentage of baseline; 5.0mM, 512$\pm$55%) or carbachol (0.5mM, 132$\pm$4%; 5.0mM, 331$\pm$18%). Increasing extracellular concentrations of ACh in the SNPC by infusion of the anticholinesterase neostigmine also increased C-P DA efflux (0.25mM, 144$\pm$10%; 0.5mM, 227$\pm$6%). The stimulatory effects of neostigmine on C-P DA efflux were confirmed using *in vivo* microdialysis (0.5mM, 159$\pm$11%). Compared to sham-operated controls, rats with quinolinic acid lesions of PPTg showed an attenuation ($p<0.01$) of the stimulatory effects on C-P DA of intranigral neostigmine (0.25mM, 120$\pm$4%), but PPTg lesions enhanced ($p<0.01$) C-P DA efflux in response to SNPC injection of the receptor agonist nicotine (0.5mM, 188$\pm$7%), suggesting that postsynaptic receptor supersensitivity had developed. Altogether, these data suggest (i) that there is a cholinergic innervation of the SNPC from the PPTg and (ii) that cholinergic neurons in the PPTg are indirectly involved in regulating the activity of the C-P by modulating the activity of DA-containing neurons in the SNPC.

294.3

AMPHETAMINE AND APOMORPHINE INDUCED LOCOMOTION AND ORAL STEREOTYPY IN PEDUNCULOPONTINE TEGMENTAL NUCLEUS LESIONED RATS. W.L. Inglis*, L.F. Allen, R.B. Whitelaw, M. Latimer, H. Brace and P. Winn Dept. Psychol., Univ. St Andrews, Fife, Scotland KY16 9JU

The effects of ibotenate lesions of the pedunculopontine tegmental nucleus (PPTg) on responses to systemic injection of d-amphetamine and apomorphine were examined in rats. The following groups were tested: bilateral IBO lesions of PPTg (0.12M: 2 X 0.2ul injections/hemisphere; n=7), bilateral IBO lesions of deep mesencephalic nucleus (DpMe) (0.12M, 0.4ul; n=8), sham lesions (phosphate buffer vehicle) of PPTg (n=6) and DpMe (n=5). For PPTg, 24h separated lesions in right and left hemispheres. All rats recovered from surgery: food and water intake was normal within 14 days of surgery (though DpMe lesioned rats increased water intake [never >5ml] compared to sham lesioned rats). All rats were then given injections of 1.5, 3.0 and 5.0 mg/kg AMPH (ip), and saline control injections, in a randomized order. Locomotion to 1.5 mg/kg was increased after AMPH in all groups compared to saline injections. At 3.0 and 5.0 mg/kg sham and DpMe lesioned rats showed normal AMPH stereotypies: rearing, sniffing and locomotion (3-4, Creese-Iversen). PPTg lesioned rats showed compulsive biting predominantly directed at their forepaws. Injections of 0.1, 1.0 and 3.0 mg/kg APO (sc; ascorbate-saline vehicle) showed similar effects: locomotion at 0.1 mg/kg but a significantly increased incidence of biting and gnawing at higher doses by PPTg lesioned rats. In this case biting was directed at cage bars rather than forepaws. Histological analysis (cresyl violet, NADPH-diaphorase) showed that lesions had been effective. These data demonstrate that (i) lesions in this area of the pons have discriminably different effects; (ii) locomotion stimulated by DA agonists is not affected by PPTg or DpMe lesions; (iii) the PPTg may play an important role in mediating dorsal striatal outflow.

294.4

CHOLINERGIC STIMULATION OF RAT SUBSTANTIA NIGRA: INTERACTIONS OF NICOTINIC AND MUSCARINIC RECEPTOR ACTIVATION ON BEHAVIOR. G.C. Parker* and P. Winn Dept. Psychol., Univ. St Andrews, Fife, Scotland KY16 9JU

Convergent lines of research show there is an excitatory cholinergic input to the substantia nigra pars compacta (SNPC) from the pedunculopontine tegmental nucleus (PPTg). Microinjection of muscarinic or nicotinic receptor agonists, or anticholinesterases, into SNPC increase DA efflux in the caudate-putamen and stimulate behavior for which there is a positive predisposition and a low baseline rate. We have now investigated the interaction between muscarinic and nicotinic receptor agonists in the SNPC. 55 rats were implanted with unilateral cannulae aimed at the SNPC and assigned to one of four drug groups: carbachol (CARB: 0, 0.039, 0.1, 0.257 ug), nicotine (NIC: 0, 0.055, 0.1, 0.25 ug), 0.055ug NIC + each dose of CARB, 0.1ug CARB + each dose of NIC. During each test rats received a 0.5ul/60sec microinjection through a stainless steel cannula into SNPC; doses were administered in an individually randomized order and successive injections were separated by 48h. Rats were placed in individual cages for 60min prior to microinjection and for 60min following and had free access to weighed amounts of tap water, lab chow, dry macaroni (1470 Kj/100g, 13.0g protein/100g) and polystyrene packing chips. NIC and CARB both stimulated significantly more feeding than SAL. Addition of a behaviourally activating dose of CARB to each dose of NIC caused a significant increase in feeding compared to NIC alone. However, addition of a behaviourally activating dose of NIC to each dose of CARB caused no significantly increased feeding compared to CARB alone. This suggests that cholinergic stimulation of SNPC can have behaviourally similar effects when acting through either muscarinic or nicotinic receptors but that the levels of stimulation at these receptors have different characteristics.

294.5

A TRANSIENT EXPRESSION OF SUBSTANCE P-LIKE NEURONS IN RAT SUBSTANTIA NIGRA (SN) AND VENTROTEGMENTAL AREA (VTA) PARTICIPATES IN DEVELOPMENT OF STRIATONIGRAL PROJECTION. N. Seno, T. Kono, Y. Kuga, S.T. Kitai, and E.J. Johnson*, Dept. of Anatomy and Neurobiology, University of Tennessee, Memphis, Memphis, TN.

Early appearance of monoamine and peptidergic neurotransmitters has been interpreted to be an indication of their role in development of the CNS. We have studied the development of SP like immunoreactive (SP-LIR) neurons in SN and VTA during embryonic (E14-E21) and postnatal periods (P0-P14). Immunohistochemical analysis were used to identify SP-LIR and tyrosine hydroxylase like immunoreactive (TH-LIR) neurons. Bromodeoxyuridine (BrdU) was used as a marker of cell birth date. In some cases, a retrograde tracer fluorogold (FG) was injected in the neostriatum to identify nigrostriatal neurons.

No SP-LIR immunoreactive SN and VTA neurons were seen at E16 while many neurons were already TH-LIR positive. At E17, SP-LIR or SP-TH-LIR double (d) labeled neurons and at E18 striatonigral projections identified by GABA/SP-LIR terminals were observed for the first time. The number of single or d-labeled SP-LIR neurons increased as striatonigral terminals density increased and was maximum at the postnatal day 0 (at birth). From P2, SP-LIR neurons decreased gradually in both nuclei, until their total disappearance at P14. At E18 and E19 more than 95% of SP-LIR neurons were d-labeled with TH-LIR. D-labeled neurons declined to about 10% to 20% by P6 and P7. BrdU immunocytochemistry demonstrated that most of SP-LIR positive neurons were labeled with BrdU injected at E12 and E13. FG study at E20 and P0 demonstrated that many SP-LIR neurons were nigrostriatal projection neurons. These observations indicate that a transient expression of SP-LIR in SN and VTA dopaminergic projection neurons may participate in promoting proper development of striatonigral (GABA/SP-LIR) connections with their target neurons in SN and VTA. *Supported by USPHS grant NS20702 and the Human Frontiers Program.*

294.6

MUSCARINIC MODULATION OF RHYTHMIC FIRING IN DOPAMINERGIC (DA) NEURONS OF RAT SUBSTANTIA NIGRA PARS COMPACTA (SNc) IN IN VITRO SLICE PREPARATION. Y. Kang*, T. Kono, Y. Kuga, and S.T. Kitai, Dept. of Anatomy and Neurobiology, The University of Tennessee, Memphis, Memphis, TN 38163.

Using a whole cell recording method, we have analyzed cholinergic modulation of rhythmic firing of DA neurons. Slices containing SNc were obtained from the brain of 10-21 day old rats and sectioned in 120-150 µm thickness. Biocytin was intracellularly injected into the recorded neurons and processed for tyrosine hydroxylase (TH) immunohistochemistry to identify their transmitter phenotype. During rhythmic firing in SNc DA neurons, a pacemaker like slow depolarization preceded each spike followed by a prominent afterhyperpolarization (M-AHP). A bath application of carbachol (1-100 µM) or muscarine (1-100 µM) increased the frequency of rhythmic firing with a decrease in the amplitude of M-AHP. The membrane potential was also depolarized. These phenomena were considered due to the blockade of K^+ currents. SNc DA neurons are also known to display membrane potential oscillation in the presence of TTX and TEA. With an increase in d.c. depolarization, the oscillation frequency increases while its amplitude decreases. A bath application of carbachol or muscarine in the presence of TTX and TEA suppressed spontaneous rhythmic membrane potential and current oscillation in a dose dependent manner. Atropine (20-50 µM) application in the media antagonized the carbachol and muscarine effects. These data indicate that rhythmic firing of SNc DA neurons could be modulated by extrinsic cholinergic inputs through activation of muscarinic receptors. *Supported by NIH grant NS 20702 and Human Frontiers Program.*

294.7

COUPLING BETWEEN DOPAMINE AND NON-DOPAMINE NEURONS IN RAT SUBSTANTIA NIGRA COMPACTA. S.T. Kitai*, T. Kono, and Y.Kang, Dept. of Anatomy and Neurobiology, The University of Tennessee, Memphis, Memphis, TN.

Anatomical and electrophysiological studies have indicated a coupling between substantia nigra compacta (SNc) neurons. We have further investigated a possible coupling among SNc dopamine (DA) neurons using intracellular recording and immunocytochemical techniques in in vitro slice preparation in the rat.

An atoxic binding fragment of tetanus toxin, Fragment C(TTC: 0.1~0.5% solution) with biocytin (2~3% in 0.05M Tris buffer and 0.5M KCl, pH 7.4) were intracellularly injected (300 msec duration, 1Hz, 15 to 30 min) to label the recorded and the coupled neurons. SNc neurons were identified as putative DA and non-DA cell on the basis of their electrophysiological characteristics (e.g., firing rate and action potential shape, etc.). Postinjection survival time varied from 5 to 120 min. Tyrosine hydroxylase (TH) immunocytochemistry was also used to identify the transmitter phenotype of the recorded and the coupled neurons. Serial sections (30 µm thick) were made through the slice (500 µm thick) for immunocytochemical analysis for Biocytin, TTC, and TH antibodies.

Intracellular analysis revealed that, in addition to typical SNc DA cell action potentials, small action potentials (SAP) (3-5 mV) followed by hyperpolarizing potential (3-5 mV in amplitude and 100-200 msec in duration) can be recorded. SAPs were abolished with TTX and their firing frequency could be manipulated by depolarizing and hyperpolarizing current injection. Rhythmic firing of DA neurons appeared to be modified by SAPs. Anatomical data demonstrated 2-3 TTC/ Biocytin labeled neurons with apparent contacts at varying distance among them. Immunocytochemical analysis indicated that a majority of coupling was between the DA neurons and TH immuno-negative neurons. These data indicate an existence of internuclear control of DA cell firing by non-DA neurons in SNc. *Supported by USPHS grant NS20702 and the Human Frontiers Program.*

294.8

DEVELOPMENT OF NEUROTRANSMITTER PHENOTYPES IN THE NEOSTRIATUM AND THE SUBSTANTIA NIGRA AND THEIR EFFERENT PROJECTIONS STUDIED BY A COMBINED DOUBLE IMMUNOCYTOCHEMICAL TECHNIQUE WITH A RETROGRADE TRACING METHOD. T.Kono*, M. Takada, and S.T. Kitai, Dept. of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, TN 38163

Using a double immunofluorescence histochemistry for the neurotransmitter or neurotransmitter-synthesizing enzyme, or the neuropeptide and bromodeoxyuridine (BrdU) thymidine analogue, as a cell birthday marker, we studied the birth date and neurotransmitter phenotypes and neuropeptide expression date of cells in the neostriatum (ST) and substantianigra (SN). BrdU was injected intraperitoneally (50mg/kg b.w.) to pregnant Wistar rats with gestation days of 11 through 20 (N=13 for each day). Litters were sacrificed on the various embryonic ages (E12 through E21, postnatal day 0 (date of birth), P1,P3,P4,P7 and P14) under a deep anesthesia and perfused with 20% formalin in 0.1M phosphate buffer (PB) (pH 7.4) or 4% paraformaldehyde in 0.1M PB(pH 7.4). The brain was removed and coronal or sagittal sections (30µm thick) were made through the ST and the SN for immunocytochemical procedures for glutamic acid decarboxylase, GABA, enkephalin, substance P, acetylcholine transferase, somatostatin, tyrosine hydroxylase and BrdU antibodies. In addition, fluorogold (0.02µl) was injected in the ST of the embryo at E14 and E15 and in the SN at E18, E19 and E20 in order to study the time of the establishment of efferent connections. The results indicate there is a difference in 1) the birth dates and the death dates of various neurotransmitter phenotypes, 2) the time of expression of various neurotransmitters, and 3) the time of the establishment of efferent projections for ST and SN neurons. *Supported by USPHS grant NS20702 and Human Frontiers Program.*

294.9

ELECTROPHYSIOLOGAL PROPERTIES OF ACCUMBENS CORE AND SHELL NEURONS. P. O'Donnell*and A. A. Grace. *Dept. Behavioral Neuroscience and Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

The electrophysiological properties of neurons in the core and shell regions of the nucleus accumbens were studied using intracellular recording techniques in rat brain slices maintained *in vitro*. The passive membrane properties of the 49 core cells and the 20 shell neurons studied were overall quite similar, although several significant differences were found. The time constant was faster in shell (6.8±1.5 msec; n=4) than in core neurons (11.2±3.1 msec; n=12). In most accumbens neurons the action potentials evoked by membrane depolarization showed firing-frequency accommodation, and were preceded by a slow Ca^{++}-dependent depolarization that was observed more commonly in core than in shell neurons. Following TTX administration, all-or-none spike-like events resembling high-threshold calcium spikes were observed in both regions, although TTX-insensitive events resembling low-threshold spikes were oberved in core, but not in shell neurons. In addition, synaptic responses (EPSPs) observed following amygdaloid or cortical afferent stimulation in shell neurons were smaller in amplitude (5.3±2.1; n=4 vs. 8.6±3.3 mV; n=12) and exhibited a lower probability of evoking spikes (1/13 vs. 11/28) in comparison to the responses of core neurons to cortical afferent stimulation.

In summary, many of the basic physiological properties of core and shell neurons are similar. However, in each case where differences were noted, such as the differences in time constant, low-threshold spikes, and synaptic responses, the results suggest that the shell neurons may be less excitable in nature. Supported by USPHS MH 45156, MH 42217, and NS 19608.

294.10

INFLUENCE OF VENTRAL PALLIDUM ON RAT DORSAL THALAMIC NUCLEUS NEURONS. A. Lavin* & A.A. Grace. Depts. Behavioral Neuroscience & Psychiatry, Univ. Pittsburgh, Pittsburgh, PA 15260.

The basal ganglia is thought to regulate motor behavior and affective states by its projections to the thalamus. In these experiments we used *in vivo* intracellular recording in rats to analyze the spontaneous activity and the response of dorsal thalamic nucleus neurons to electrical stimulation of the ventral pallidum (VP). A total of 20 neurons were recorded in the ventromedial (VM) and ventro-posterior medial (VPM) nuclei of the thalamus. Most of the neurons recorded (11/20) exhibited a spontaneous oscillatory burst firing pattern, with the oscillations ocurring at frequency of 6.2±0.6 Hz. Furthermore, four of the cells exhibited slow hyperpolarizing waves (0.9-1.5 Hz, 440-640 msec in duration) resembling spindle activity. In addition, 8 neurons had spontaneously ocurring ipsps which occured at 4-6 Hz and consisted of fast and slow components. In recordings made with KCl-filled electrodes, only the first component of the ipsp was reversed to a depolarization. VP stimulation evoked 3 responses in thalamic neurons: 1) 55% of the neurons (11/20) responded with an ipsp (latency 6.0±3.0 msec; amplitude 9.5±6.1 mV); 2) 25% (5/20) responded with an epsp (6.5±3.7 msec, amplitude 4.0±1.2 mV); and 3) 15% (3/20) showed no response. Furthermore, in 4/5 cells that respond to VP stimulation with epsps, continued stimulation caused the response to slowly change from an epsp to an ipsp (ipsp latency 8.3±2.1 msec; amplitude 5.6±1.1 mV). In addition, in 3 neurons located in the laterodorsal thalamic nucleus (LDVL), VP stimulation had no discernable effect. Given the anatomy of this system a likely source for the spontaneous and evoked potentials could be the reticular nucleus of the thalamus.
Support: USPHS MH 42217, MH 45156, NS19608.

294.11

EFFECTS OF DOPAMINE AGONISTS ON GABA-ACTIVATED INWARD CURRENTS IN MEDIUM SPINY NEURONS OF RAT STRIATUM: A WHOLE CELL VOLTAGE-CLAMP STUDY. J.Y. Chang*, M.F. Pacheco† and D.J. Woodward. Department of Physiology & Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157; and † CUIB-Univ. de Colima, Colima, Col 28045 Mexico.

The neuronal circuitry in neostriatum includes complex interactions between several neurotransmitters. GABAergic medium spiny neurons (MSN) send an output to other basal ganglia structures to regulate locomotor activities, and has collateral inhibitory connections to other MSNs. This neuron type also receives dense dopaminergic input from the substantia nigra pars compacta. The aim of the present study was to explore possible interactions between dopamine and GABA on MSNs using whole cell voltage-clamp methods. Acutely dissociated MSNs were obtained from the striatum of newborn rats (1-10 days old) after papain treatment (1 mg/ml, 1-2 hrs at 32°C) of the tissue. GABA-activated whole cell currents (I_{GABA}) were recorded in MSNs by means of an EPC-9 system under isomolar chloride concentration in the bath and pipette solutions, with V_h=-55mV. Drugs were applied by pressure ejection from independent pipettes (2 μm tip diameter) positioned 30-60 μm from the neuron being recorded. Brief application (4-5 sec, 1-3 psi, 0.1-1 mM in the pipette) of the dopamine D_1 agonist SKF-38393 inhibited I_{GABA} (20% suppression or more). The D_1 effect on I_{GABA} was attenuated by the D_1 antagonist SCH-23390 (1-10 μM) and by the protein kinase inhibitor H-7 (1-3 μM) in the bath solution (higher concentrations of H-7 inhibited both I_{GABA} and the D_1 agonist effect), and was mimicked by forskoline (3 mM in pipette). The dopamine D_2 agonist quinpirole inhibited I_{GABA} in only 3 out of 14 neurons tested and inconsistently evoked a rebound enhancement of I_{GABA}. The results indicate dopamine D_1 receptor activation produces a suppression of I_{GABA} in the isolated MSN preparation and suggests that modulation of ligand-gated inhibition by GABA may be one action of dopamine. *Supported by DA02338 and MH44339 (DJW); DGICSA-SEP and CONACYT (MFP).*

294.12

CHRONIC ENSEMBLE NEURON RECORDINGS IN RAT NEOSTRIATUM AFTER 6-OHDA: NETWORK PROPERTIES. S.F. Sawyer*, A.B. Kirillov, C.D. Myre and D.J. Woodward. Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157

The aim of this work was to employ extracellular recordings of multiple single neurons in the neostriatum to examine altered local activity and network interactions in normal rats and after unilateral destruction of the dopaminergic nigrostriatal projection from intracerebral injections of 6-OHDA. We report here indications of inhibitory, reciprocal synaptic interactions between neostriatal neurons in awake, behaving rats. As many as 24 neostriatal neurons were recorded concurrently from chronically implanted arrays of microwires (200-300 μm separation between adjacent microwires). Functional interactions between neurons were assessed with cross-correlation and ensemble analysis. Correlated spike activity of neuronal subpopulations were evaluated with graphical computer animations over short (seconds) and long (minutes) time scales. The spatial domain of cross-correlation interactions was restricted to neighboring microwires (200-500 μm range), and were most common between neurons recorded from the same microwire. These interactions were typically inhibitory and constant across experimental sessions. Administration of dopaminergic drugs to 6-OHDA-treated animals alterated spike activity that coincided with the time course of circling behaviors, and increased the number of cross-correlated neurons. Dopaminergic agonists often biased interactions such that one neuron in a pair of functionally connected neurons increased its spike activity whereas the other neuron was inhibited, the latter presumably due in part to increased inhibitory drive from recurrent inhibitory collateral connections from the active neuron. We interpret these reciprocal inhibitory events as due to the local circuit switching phenomena predicted by computer simulations of working memory properties of neostriatal circuits. *Supported by NIDA 2338 and NIMH 44337.*

294.13

A CADMIUM-SENSITIVE VOLTAGE-DEPENDENT CONDUCTANCE IS TRANSIENTLY EXPRESSED DURING DEVELOPMENT IN RAT NEOSTRIATAL NEURONS. F. Trent*[1], Z.C. Xu[2], C.J. Wilson[2], and J.M. Tepper[1]. Aidekman Research Center, Center for Molecular and Behavioral Neuroscience, Rutgers The State University of New Jersey, Newark, NJ, USA 07102[1] and Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis, TN, USA, 38163[2].

Intracellular recordings from neostriatal (NS) neurons were obtained in vivo from urethane-anesthetized Sprague-Dawley rat pups from birth (P1) to P40 and in adult males, and in vitro from neonatal brain slices. NS neurons in neonates ≤P15 both in vivo and in vitro displayed a transient voltage-sensitive depolarizing response (TDR) evoked by intracellularly applied depolarizing current pulses. The TDR appeared as a positive hump that arose near the onset of applied depolarizing transients. In vivo the TDR activated at potentials more negative than -37.2±1.6 mV (mean ± SEM; n=42). The mean maximal amplitude was 18.1±1.6 mV (n=40) and mean duration was 23±1 ms (n=44). The TDR was observed in 56% of NS neurons in P6-10 neonates (n=73), 47% at P11-15 (n=68), 6.1% at P16-29 (n=49) and was never seen in older animals. In >50% of neurons exhibiting the response, the TDR could be activated by depolarization from rest (-49.1±2 mV; n=45) although conditioning hyperpolarizing pulses (≥60 ms) increased both its frequency of occurrence and maximum amplitude. Inactivation of the TDR could be achieved by depolarizing prepulses that brought the cell to -40 mV or above. In vitro, the TDR was largely unaffected by TTX (1 μM) and almost completely abolished by cadmium (500 μM) suggesting that it is composed of an inward calcium current. In vivo, 59% of the neurons from P6-15 pups that displayed the TDR also exhibited a prominent T-current-like depolarizing response following the offset of hyperpolarizing current pulses that was never seen in older animals. Supported by MH45286 and NS26473.

294.14

POSTNATAL CHANGES IN NEOSTRIATAL SYNAPTIC INPUT. N.A. Sharpe*, F. Trent, and J.M. Tepper. Aidekman Research Center, Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ, USA 07102.

Anterograde labeling of corticostriatal fibers with biocytin was used to characterize corticostriatal afferents in adult and neonatal rats. In adult rats (>8 weeks old) the majority of corticostriatal boutons terminated on the heads of dendritic spines (88%). The remaining corticostriatal synapses were either axodendritic or axosomatic. Virtually all labeled boutons that formed axospinous synapses contained small (40 nm) round vesicles and formed asymmetric synapses. The mean synaptic cleft width was 224±25 nm (mean±S.D.).

In neonatal (P14) rats, many vesicle-containing labeled profiles were observed, but relatively few labeled synapses were found. These profiles appeared significantly less densely packed with vesicles than in adults. In addition, the total synaptic density of the neostriatum was less at P14, (4.4±1.5 synapses/100 μm^2) than in adults (9.7±2.9 synapses/100 μm^2), t=4.97, p<0.001. Although most of the labeled asymmetric synapses observed in adults terminated on dendritic spines, in the neonates only 21% of all asymmetric synapses ended on spines while 72% terminated on dendritic shafts. The absolute density of axodendritic synapses did not differ between neonates (3.2 synapses/100 μm^2) and adults (3.0 synapses/100 μm^2). These data indicate that by P14, the synaptic neuropil of neostriatum is not yet fully mature, consistent with previous electrophysiological studies.

Supported by MH 45286.

294.15

MUSCARINIC MODULATION OF CALCIUM CURRENTS IN ACUTELY DISSOCIATED RAT NEOSTRIATAL NEURONS. A. Howe* and D.J. Surmeier, Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis, TN 38163.

Although important clinically, the role of the cholinergic interneuron in regulating the excitability of other neurons in the neostriatum is unclear. Previous studies in other tissues have indicated that calcium currents are modulated by cholinergic agonists acting at muscarinic receptors. Using the whole-cell voltage-clamp recording technique we have shown that calcium currents in neostriatal neurons are modulated by muscarinic agonists.

Neurons from the neostriatum of young adult rats (3-5 wks postnatal) were dissociated using techniques previously described by our group. In brief, striata were dissected from 400 μm slices. Striata were then incubated in oxygenated saline containing pronase E (1.5 mg/ml) at 32 degrees C. After a 30 minute incubation, tissue was washed and mechanically dissociated in low calcium. (200 μM) Whole-cell recordings employed conventional techniques at room temperature.

Muscarine (500 nM) and/or carbachol (50 μM) application produced a significant inhibition (>20%) of calcium current in most neostriatal neurons studied (>95%, n>100). The modulation was antagonized by atropine (1 μM) as well as by pirenzipine (1 μM). Application of carbachol in the presence of GTPγS resulted in irreversible modulation while preincubation with pertussis toxin (PTX) blocked the modulation indicating the involvement of a PTX sensitive G-protein. The modulated component could be blocked with either dihydropyridines or ω-conotoxin. This work was supported by USPHS grant NS 28889.

294.16

DOPAMINE RECEPTOR AGONISTS ALTER RESPONSES EVOKED BY EXCITATORY AMINO ACIDS IN NEOSTRIATUM AND NEOCORTEX. C. Cepeda*, Z. Radisavljevic, N.A. Buchwald and M.S. Levine. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024

Our laboratory has demonstrated that dopamine (DA) potentiates responses evoked by N-methyl-D-aspartate (NMDA) but attenuates responses evoked by glutamate or Alpha-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid (AMPA) in neostriatum or neocortex. The present study was designed to assess the contribution of specific DA receptor subtypes (D_1 and D_2) to this differential modulation. In slices of rat neostriatum or neocortex, excitatory responses were evoked by iontophoretic application of NMDA (0.1M, pH 8) or AMPA (0.1M, pH 8) within 100-200 μm of the intracellularly recorded neuron. Selective DA receptor agonists SKF-38393 (D_1) or quinpirole (D_2) were bath (SKF-38393, 2-25 μM; quinpirole, 1-25 μM) or iontophoretically applied. SKF-38393 consistently potentiated excitatory responses induced by NMDA in neostriatum or neocortex in a dose dependent manner (11/11 cells). The amplitude of the NMDA-induced depolarization was larger, the frequency of occurrence of action potentials increased and the response had a longer duration. Excitatory responses induced by AMPA were also potentiated by the D_1 agonist (5/7 cells, 2 did not respond). The effective threshold concentration of the D_1 agonist was 5 μM. In contrast, quinpirole attenuated NMDA-induced responses in a dose-dependent manner (6/8 cells, 2 did not respond). The amplitude of the depolarization was reduced and the frequency of occurrence of action potentials decreased. The effective threshold concentration of the D_2 agonist was 1 μM. These findings indicate that activation of D_1 or D_2 receptors has opposing effects on responses evoked by excitatory amino acid receptor agonists. They indicate further that effects of DA release may depend upon relative proportions of D_1 and D_2 receptors activated. (Supported by USPHS HD 05958 and AG 7462).

294.17

VOLTAGE-CLAMP ANALYSIS OF BURST FIRING INDUCED BY N-METHYL-D-ASPARTATE IN RAT MESENCEPHALIC DOPAMINE NEURONS IN VITRO **Vincent Seutin, Steven W. Johnson and R. Alan North*. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.**

Intracellular recordings from dopamine neurons *in vitro* showed that N-methyl-D-aspartate (NMDA) induces bursts of action potentials separated by large hyperpolarizations (Johnson et al., Soc. Neurosci. Abstr. 1992). Single-electrode voltage-clamp experiments showed that NMDA (20 μM) caused an inward current (200-300 pA) at -60 mV. In NMDA, voltage steps from -60 to -40 mV for 1 s were followed by an outward tail current (100-300 pA) that declined over 1 - 3 s. This was blocked by APV (50 μM). The tail current was unaffected by K^+ channel blockers and was not blocked in Ca^{2+}-free solutions. It was blocked in solutions with no added Mg^{2+} and much reduced in a low (20% of normal) Na^+ concentration. The tail current was increased by a high K^+ concentration (10.5 mM) and blocked by a low K^+ concentration (0.5 mM). It was also blocked by ouabain (2 μM), DNP (0.1 mM) and glucose-free solutions. We suggest that this outward current corresponds to the hyperpolarization between bursts of action potentials, and that it is due to activation of an electrogenic sodium pump.

294.18

BURST FIRING INDUCED BY N-METHYL-D-ASPARTATE REQUIRES ACTIVATION OF AN ELECTROGENIC ION PUMP IN RAT DOPAMINE NEURONS. **S.W. Johnson*, V. Seutin and R. Alan North. Vollum Institute, Oregon Health Sciences University, Portland OR 97201.**

Dopamine cells fire in bursts *in vivo*, but with single regularly-spaced action potentials *in vitro*. Using intracellular recording from midbrain slice, N-methyl-D-aspartate (NMDA; 10 - 30 μM) converted single-spike firing to burst firing in about 90% of neurons. Burst firing consisted of 3 - 20 spikes followed by an inter-burst hyperpolarization of 5 - 30 mV. Tetrodotoxin blocked action potentials but did not block rhythmic hyperpolarizations which underlie burst firing. NMDA receptor activation was required because burst firing produced by NMDA, aspartate (500 μM), or focal electrical stimulation was blocked by APV (50 μM) but not by CNQX (10 μM), and because kainate (1 - 10 μM) and quisqualate (1 - 10 μM) did not cause bursts. Burst firing was blocked by solutions containing no-added Mg^{2+}, low Na^+ (20 mM Na^+, 126 mM choline substitution), 0.5 mM K^+, no-added glucose, ouabain (2 - 3 μM), or dinitrophenol (100 μM). It was not blocked by intracellular BAPTA or by solutions containing no-added Ca^{2+}. Apamin (0.1 - 1 μM) potentiated burst firing. *It* is concluded that NMDA-induced burst firing requires activation of an electrogenic energy-dependent Na^+/K^+ ion pump.

OCULOMOTOR SYSTEM: SACCADES

295.1

A MODEL FOR 3-DIMENSIONAL EYE-HEAD SACCADES. D Tweed*, B Glenn, T Vilis. Depts of Physiology and Ophthalmology, Univ of Western Ontario, London, Canada, N6A 5C1.

Eye and head together rotate with 6 degrees of freedom, while gaze direction has just 2. Thus there is a (6 - 2 =) 4-D set of eye-head configurations compatible with any given gaze direction. We present flow diagrams, and supporting data from 6 human subjects, for a feedback model of eye-head saccades with the following features: 1. Eye and head are not driven toward the nearest configuration compatible with the desired gaze direction, but to particular positions fitting additional constraints -- Donders' laws of eye in head and head in space -- i.e. eye and head are each restricted to 2 degrees of freedom. 2. There is in general no Donders' law of eye in space, but the eye and head adopt configurations that approximate such a constraint for most gaze shifts. 3. The head is driven not by gaze error but by head position error alone, with no contribution from eye position signals. 4. The eye is driven toward a desired orientation *in space*, not in the head. 5. However, the eye position error signal saturates so as not to drive the eye to its mechanical limits in the orbit. In 1-D, saturation simply involves clipping any oversized signals, but 3-D saturation can take many forms. A saturation mechanism that allows eye in space to approach its target value as closely as possible while keeping eye in head within limits predicts transient departures from Donders' law of eye in head and 2 distinct patterns of curvature that are observed in 3-D eye in space trajectories. 6. The VOR is shut off in the direction of current eye in space error; we show that this feature would allow even misguided head movements to carry the gaze line toward the target.

295.2

EFFECTS OF TEMPORAL CONTEXT (CONDITIONAL PROBABILITY) ON SACCADE LATENCY IN MACAQUE. D. Hanes, F. Tu and J. Schall*. Department of Psychology, Vanderbilt University, Nashville, TN 37240

The influence of temporal predictability on saccade latency (SL) was investigated in a visual tracking task in *Macaca mulatta*. Fixation of a yellow spot was followed by target presentation (4 positions, 10° ecc.). Following a foreperiod (FP), a trigger signal (TS) was given by a change in the color of the fixspot: green for go (87% of trials), red for nogo. The temporal predictability of the TS was manipulated. Blocks of trials were run with constant FPs (cFP condition, 0 to 1000 ms in 50 ms steps), with FPs sampled equally from a range (rectangular distribution, rFP condition, 0-500 or 0-1000 ms) or with decreasing *likelihood of longer times (exponential distribution* eFP condition).

Variation of SL could be accounted for by the conditional probability of TS. With the most predictable TS (cFP condition) many anticipatory saccades (SL < 100 ms) were observed; however, mean SL of nonanticipatory saccades was constant across FPs. With a less predictable TS (rFP condition) numerous anticipatory saccades were observed near the end of the FP. Mean SL of non-anticipatory saccades was greater than that observed in the cFP condition, but mean SL decreased with FP. The relation of SL to FP in the rFP condition scaled according to the range of FPs used. With the least predictable TS (eFP condition) the incidence of anticipatory saccades was lowest. Mean SL following short FPs was equal to that observed in the cFP condition, and following an initial rapid decline in SL, there was no further reduction in SL with FP.

SL following a particular FP depends on the context within which that FP occurs. Using this manipulation, neurons responsible for regulating saccade initiation can be identified by their sensitivity to the conditional probability of events. (EY08890, McDonnell-Pew and Sloan Foundation)

295.3

TWO CLASSES OF CELLS WITH SACCADE-RELATED ACTIVITY IN THE MONKEY SUPERIOR COLLICULUS. D. P. Munoz*[1,2] and R. H. Wurtz[1].
(1) Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD, USA 20892; (2) MRC Group in Sensory-Motor Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

We studied saccade-related cells in the deeper layers of the alert rhesus monkey superior colliculus (SC) to see if we could identify a "moving hill" of activity as reported in the cat (Munoz et al. *J. Neurophysiol.* 66: 1642). We identified two populations of saccade-related cells. *Burst cells,* located 1.2 - 2.0 mm below the dorsal surface of the SC, discharged high frequency bursts of action potentials immediately prior to the initiation of saccades of a specific amplitude and direction that defined a movement field and had almost no preparatory or long-lead activity before the saccade. *Preparatory cells,* found 1.5 - 2.5 mm below the dorsal surface of the SC, had anticipatory discharges in addition to saccade-related bursts. Preparatory cells had movement fields with no distal border; they were active for all saccades greater than their optimal amplitude. In the preparatory cell layer, prior to saccade initiation, activity built up at a specific locus on the motor map coding the amplitude and direction of the impending movement. Once the saccade began, there was a continuous change in the spatial distribution of activity in this population across the motor map. Preparatory cells located progressively more rostral to the initially active zone were activated sequentially until fixation cells, located in the rostral pole, were activated and the saccade terminated. In the burst cell population, activity also built up at a specific locus on the SC motor map immediately prior to saccade initiation and then diminished as the saccade was executed; there was no change in the spatial distribution of activity. In summary, of the two classes of saccade cells in the monkey SC, the preparatory cells have activity most similar to the "moving hill" cells seen in the cat SC while the burst cells show no such activity.

295.4

IMPENDING SACCADES SHIFT VISUAL RECEPTIVE FIELDS IN SELECTED INTERMEDIATE SUPERIOR COLLICULAR NEURONS. M. F. Walker† and M. E. Goldberg*. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892. †HHMI-NIH Research Scholar.

Visual neurons in the lateral intraparietal area (LIP) of the rhesus monkey use information about an impending saccade to shift transiently their retinal receptive fields to compensate for anticipated changes in the visual scene. When a saccade will bring a visual stimulus into their receptive fields, some LIP neurons begin to fire before the saccade is executed, while the stimulus still lies outside the receptive field. By this mechanism the parietal cortex updates its representation of visual space at the time of an eye movement, allowing spatially accurate processing of visual information without waiting for new retinal input. LIP projects strongly to the intermediate layers of the superior colliculus; therefore, we have sought to identify and characterize saccade-related receptive field shifts in visually responsive collicular cells.

We recorded from neurons in the superficial and intermediate layers of the superior colliculi of two rhesus monkeys. Approximately 25 percent of all intermediate layer visuomovement (VM) cells demonstrated a transient shift of their visual receptive fields at the time of a saccade. This was observed even when the stimulus was not behaviorally relevant, but in some cases the shifted activity was enhanced when the stimulus was the target of a subsequent saccade. The response of most cells began only after the saccade had been completed, when the stimulus was in the receptive field. A few of these cells also responded after the location of a previously flashed stimulus was brought into the receptive field; in LIP almost all cells have such memory trace responses. Cells with predictive shifts tended to be located more deeply within the intermediate layers and to have large visual receptive fields and movement fields. Several quasivisual cells were also identified; these showed predictive receptive field shifts, and the shifted responses were enhanced by a second saccade to the stimulus location. No superficial layer neurons were found to shift their receptive fields; their responses could be explained purely by retinal reafference.

These experiments demonstrate the presence of saccade-induced shifts of visual receptive fields in the intermediate layers of the superior colliculus.

295.5

INITIAL EYE POSITION DEPENDENCE OF SACCADES ELICITED BY MICROSTIMULATION IN CAUDAL NUCLEUS RETICULARIS TEGMENTI PONTIS. T. Yamada, D.A. Suzuki*, K.F. Betelak, and R.D. Yee. Dept. of Ophthalmology, Indiana Univ. School of Medicine, Indianapolis, IN.

Microstimulation and lidocaine "lesion" results complement single unit recording in implicating a role for caudal nucleus reticularis tegmenti pontis (cNRTP) in regulating saccadic eye movements. μstimulation was delivered, via the recording μelectrode, where saccade-related unit activity identified cNRTP. Visually guided saccades were elicited with a backprojected, 0.25° target jumped to various eccentric positions. Biphasic μstimulation pulses: 0.3 msec; 100-800 Hz. Train durations were 15-2000 msec and current intensities ranged from 10-60 μA.

The elicited eye movements (EMs) were ipsilateral and similar to spontaneous saccades with respect to the relationship between peak eye velocity and amplitude. Both the latencies and amplitudes of evoked EMs decreased with increases in μstimulus frequency. The amplitude of elicited EMs decreased for more ipsilateral, initial eye positions. These saccades were not, however, goal directed. For some NRTP sites, saccades to remembered target locations were not compensatory when preceded by evoked EMs. Injection of 1 μl, 4% lidocaine into the cNRTP resulted in hypometric ipsilateral, visually-guided saccades.

Our results resemble those obtained from cMRF more than SC.

Initial eye position dependence of evoked "pursuit-like" EMs has been observed with μstimulation in rostral NRTP (1991). Similar dependence of saccades evoked with μstimulation in cNRTP would implicate initial eye position dependence as a basic function of NRTP in regulating EMs.

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295.6

SACCADIC EYE MOVEMENTS TO BRIEFLY FLASHED TARGETS. C. S. Barnes and P.E.Hallett*. Dept. of Physiology, Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

An interesting feature of the study of Hallett & Lightstone (1976a) was the occurrence of normal latency, normal accuracy saccades to small targets that were briefly flashed in darkness. We have sought to replicate their findings, and those of Doma & Hallett (1988) who developed a model for the latency of saccades to variably intense continuously exposed targets. Our experiments have used yellow-green LED targets at 16 horizontal positions, in the range 2.0-5.5 deg to left and right of centre, in darkness. Up to 5 subjects and 5 luminous intensities have been examined for exposure durations mainly in the range 1 to 200 msec. Pupils have been fixed in control experiments. If the duration is made sufficiently brief, latency increases, accuracy decreases, or the saccade may fail to occur. This "critical duration" increases as the luminous intensity of the target is reduced towards the cone threshold. We discuss the plausibility of modelling saccadic latency in terms of a "waiting time for a threshold number of photons" and a "transit time".

295.7

TECTO-RETICULO-SPINAL NEURONS CONTRIBUTE TO THE GENERATION OF SLOW POST-SACCADIC EYE MOVEMENTS IN THE CAT. E. Olivier *, A. Grantyn, M. Chat and A. Berthoz, Laboratoire de Physiologie Neurosensorielle, C.N.R.S., Paris, France.

Motor correlates of post-saccadic activity of identified tecto-reticulo-spinal neurons (TRSN) were studied in alert, head-fixed cats. Recordings were made from tectal axons in the pons. Some axons were injected with HRP to reveal terminal patterns in the periabducens region.

We have observed that visually triggered saccades are often followed by slow eye movements (post-saccadic drifts, PSD) approximately in the direction of preceding saccades. PSD have average mean velocity of 15 ± 4.3 deg/s and mean duration of 104 ± 50 ms. Their amplitude (mean 1.2 ± 0.6 deg) can reach 4-8 deg and contribute substantially to the total gaze shift. The duration of PSD is positively correlated (r=.72-.99) with the duration of phasic neck EMG increments reflecting an intended head movement. When saccades are followed by PSD, especially in combination with neck muscle contractions, TRSN bursts have longer durations. This is due to a slower decay of the firing rate after the saccade-related part of the burst. In such cases, a low frequency discharge may persist even beyond the termination of PSD. TRSN bursts are sometimes associated with visually-evoked slow eye movements having the characteristics of PSD but produced in the absence of saccades. HRP material shows highest termination densities in reticular regions containing EBN, IBN, "neck bursters" and phasic-sustained "eye-neck" reticulo-spinal neurons (EN-RSN). In the abducens nucleus, densities of terminations given by TRSN collaterals correspond to only 60% of those reported for EN-RSN.

We suggest that prolonged TRSN discharges are at the origin of slow orienting eye movements of the cat. Most likely, net modulation of ocular motoneurons is induced by a cascade connection "TRSN - EN-RSN" to the abducens nucleus. This connection ensures also synergic activation of neck motoneurons, and apparently does not depend on the "pulse" signal of the saccadic generator.

295.8

PET ACTIVATIONS FOR VISUAL AND MEMORY SACCADES. F.M. Miezin*, M.Corbetta, G.L.Webb, M.E.Raichle and S.E.Petersen. Dept.Neurol. & Neurol.Surg., McDonnell Ctr. for Studies of Higher Brain Function, Wash. Univ. Sch. of Med., St.Louis, MO 63110.

Normal volunteers were scanned with PET activation methodology during the execution of visually or memory-guided saccadic eye movements. In the visually-guided task (VG), a saccade was triggered on each trial by the onset of a briefly flashed LED located 15° peripherally at six random locations. In the memory-guided tasks, saccades were triggered by the offset of a fixation point LED, and directed toward targets which had been presented 5 sec (long memory delay, LMD) or 500 ms (short memory delay, SMD) before. Two control tasks were also run in which fixation was maintained on the central LED with or without simultaneous peripheral stimulation. The VG task activated: 1. bilateral parietal-occipital cortex, including cuneus, precuneus, lateral posterior parietal; 2. bilateral FEF; 3. SEF & anterior cingulate; 4. bilateral thalamus. Also, an enhancement was observed in primary visual cortex and/or lingual-fusiform gyrus. The same regions were similarly activated by both memory tasks. When the memory component was isolated by subtracting the SMD from the LMD condition, significant responses were observed in: 1. left putamen/caudatus; 2. ventral anterior cingulate/caudatus; 3. left frontal cortex, near precentral sulcus. These findings are consistent with single unit recording in monkey in posterior parietal and frontal cortex related to saccade generation, and in frontal cortex and basal ganglia related to spatial delayed activity. However, the superior parietal localization for eye-movement and spatial attention (Miezin et al., ARVO 1991) suggest differences in the specific organization of the posterior parietal cortex between humans and monkeys.

295.9

A COMPUTATIONAL MODEL FOR MODIFIED SACCADE TRAJECTORIES. E.A. Henis[1], T.R. Stanford[*2], and D.L. Sparks[2]. 1. Inst. for Research in Cognitive Science/CIS, 2. Dept. of Psych., U. of Pennsylvania, Phila. PA 19104.

An earlier study examined the effects of available processing time on the metrics of saccades to a visual goal (Stanford et al., *Soc. Neurosci. Abstr.* 16:901, 1990). Briefly, subjects synchronized saccade onset to the fourth of a series of equally spaced tones. Targets were presented randomly at one of two equally probable locations, and the processing time was controlled by presenting the target at various times prior to movement onset. When the interval between target presentation and saccade onset was > 175 ms, the resulting saccades were directed toward the target. For shorter time intervals, saccades were typically directed to a point between the two possible locations, followed by a separate correction. Occasionally, however, saccade direction changed in mid-flight, resulting in a continuous trajectory that curved toward the target. In this study, an explicit model to account for these curved trajectories was quantitatively tested. The horizontal and vertical components of the curved trajectories were each simulated by the addition of the corresponding components of two time-shifted elemental trajectories: one from the initial eye position to an intermediate location, and a second one from that location to the final eye position. The components were then combined to simulate the entire modified trajectory. The kinematic details (paths and velocity profiles) of predicted motions were in good agreement with the measured ones for a broad spectrum of curved saccades.

The possibility that curved saccades are generated via the superposition of two simpler saccades has been previously proposed (Evinger & Fuchs, *J. Physiology* 285:209-229, 1978). Our results provide quantitative support for this hypothesis. Using this scheme the oculomotor system might generate prompt responses to novel stimuli. According to this scheme, each elemental movement is independently generated in a similar manner as a simple saccade to a single goal. An analogous mechanism has been shown to account for curved hand trajectories (Henis & Flash, In: Neural Information Processing Systems-4, Morgann-Kaufman, 1992). Thus, our results suggest similarities in the mechanisms underlying the generation of eye and hand motions. (Support: NIH EY01189 & F32-EY06320).

295.10

SACCADE-RELATED BURST CELLS OF THE CENTRAL MESENCEPHALIC RETICULAR FORMATION (cMRF) ASSOCIATED WITH DYNAMIC MOTOR ERROR. D. M. Waitzman*, University of Connecticut, 263 Farmington Ave, Farmington, CT, 06030, USA.

Recent experiments have shown a linear correlation between the decline in burst discharge and dynamic motor error in a subpopulation of saccade related burst neurons (SRBNs) in the superior colliculus (SC) (Waitzman et al., *J. Neurophysiol.*, 66:1716-37, 1991). Since reticulotectal neurons (Moschovakis et. al., *J. Neurophysiol.*, 60:263-302) in the cMRF represent a significant input into the SC, we have now examined the discharge properties of 17 SRBNs in the cMRF of one monkey during visually guided saccades. At least two groups are noted: one whose discharge is cut-off with the end of contralaterally directed visually guided saccades (clipped cells N=11) and another whose discharge continues for up to 50 msec following the end of the eye movement (partially clipped, N=6). Many neurons in the cMRF have a high spontaneous level of activity while the monkey is actively fixating. This discharge level is inhibited during saccades to the ipsilateral side and some of these neurons return to their background rate of activity *prior* to the end of the eye movement. Since cMRF neurons project to *both* ipsi- and contralateral SC, the difference burst was calculated by subtracting the discharge during ipsilateral movements from that generated during contralateral saccades. The difference burst is closely correlated with dynamic motor error for both clipped and partially clipped cells. These results are consistent with the hypothesis that the SRBNs of the SC receive a differential input from cells in the cMRF and this discharge encodes dynamic motor error.

295.11

SACCADIC BURSTS OF ABDUCENS NEURONS DO NOT FULLY COMPENSATE FOR ORBITAL NONLINEARITIES. C. Siebold, L. Ling, and A. F. Fuchs*, Regional Primate Research Center, and Departments of Psychology and Physiology and Biophysics, University of Washington, Seattle, WA 98195

Within the oculomotor range, the metrics of saccades less than twenty deg generally do not depend on where in the orbit the movement is initiated. Therefore, the burst of ocular motoneurons during saccades may be adjusted to compensate for orbital non-linearities. We examined this issue by recording from abducens neurons during saccades of constant amplitude initiated from different eye positions.

We recorded from over 40 neurons in the abducens nucleus of 3 monkeys. At orbital positions below the position threshold the peak firing frequency usually increased with initial position. For saccades beyond threshold the peak firing rate often saturated. The saccadic amplitude for which peak burst frequency saturated varied for different cells, but there was usually no saturation for saccades less than 5°. Even for saccades < 5° the slope of the relation between peak firing rate and eye starting position never exeeded the slope of the relation between steady firing rate and eye position during fixation. Therefore, the size of the motoneuron burst does not correct for orbital non-linearities.

For saccades of the same amplitude, the number of spikes in the burst increased with initial position even if peak burst rate was saturated. Similarly burst duration increased although saccade duration did not. The longer burst durations occurred because burst lead time increased as saccades were initiated more in the unit's on-direction even if the unit's threshold lay outside the oculomotor range.

For saccades with different amplitudes, the peak burst frequency is determined by the saccadic end position. Thus the burst is constant for all saccades reaching the same position in the orbit. This result is consistent with Collins' measurements of tension in the extra-ocular muscles during saccades.

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295.12

AUDITORY HEAD SACCADIC GAIN. James H. Fuller*
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Nine young adult humans executed auditory-evoked eye-head gaze shifts in a task identical that previously reported for visual stimuli (Soc Neurosci.: 861, 1991); the two tasks were interleaved. The non-aligned mode was a standard single-step gaze shift between fixation and target speakers; in the head-aligned mode the jump started with the head and fixation speaker aligned. Fixation/target speakers varied in position relative to the midline; amplitudes were 40, 60, and 80 deg.

The head velocity profiles, target/gain patterns, etc., showed gaze shifts were similarly executed in both tasks. Non-aligned gains in all subjects were elevated (vis, 0.27; aud, 0.42); like eye velocity, peak head velocity was slower. The head-aligned gains were lower in head-movers and higher in non-movers. Several instances of group reversal (head-mover exhibits non-mover traits, and vice-versa), and group trends showed a homogenization of head movement propensity; head-movers develop more midline attraction and non-movers more spatial head aiming. It is proposed that there is a wide continuum in head movement control: ideal head-movers use extrinsic (earth-fixed), and non-movers use intrinsic (e.g., body/head-oriented) coordinate reference frames; head-based auditory sensory acquisition and/or central mechanisms reduce the continuum.

295.13

DYNAMICS OF SUPERIOR COLLICULUS BURSTERS OBSERVED WITH THE USE OF A SHORT-LATENCY AVERAGING SACCADE PARADIGM J.A.Edelman, E.L.Keller*, and K.Arai. Smith-Kettlewell Eye Research Institute, San Francisco, CA 94115 and University of California, Berkeley, CA 94720 and Mitsubishi Kasei Corp, Yokohama 227, Japan

Van Opstal and Van Gisbergen (Exp. Brain Res.,1990) demonstrated that the superior colliculus (SC) participates in generating averaging saccades in monkey, but could not determine if the discharge of collicular burst cells unambiguously coded the metrics of these saccades. We wished to quantify how the SC codes averaging saccades in a short-reaction-time task that did not require target selection. In 1/3 of our trials, two targets separated by 45 deg were presented at locations neighboring the center of the cell's motor field. Single targets were presented in 2/3 of the trials to discourage formation of a strategy. Monkeys were rewarded for a short-latency saccade to a window surrounding the target configuration. All SC cells studied burst before and during both one-target and averaging saccades to the same location, corroborating the previous report. However, numerous spatio-temporal differences were apparent. Almost all cells fired less for averaging saccades in a 30-ms prelude to the main burst. In approximately half the cells peak firing rate was also smaller. Some cells showed a late burst following the main burst at a time when discharge during normal saccades was rapidly declining. These temporal differences could not be accounted for by averaging saccade velocity, which was similar to that of one-target saccades. Distributions of 1-target and averaging saccade activity within the motor field were fit separately by a neural network multivariate technique. Distributions computed for the averaging saccades were generally more flat with a smaller peak. These data suggest that programming of saccadic eye movements, as reflected in collicular discharge, is not complete before the SC motor burst, and that SC motor-related activity is dependent on target configuration.

295.14

IS THERE AN INFLUENCE OF SLEEP DEPRIVATION ON SACCADIC EYE MOVEMENTS?

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Sedating as well as stimulating drugs influence the peak velocity and duration of saccadic eye movements of a given amplitude, i. e. change the rise of the main sequence. In order to investigate the specificity of these findings the influence of sleep deprivation representing a rather unspecific precondition was examined.

Eye movements were measured in twelve healthy male volunteers using infrared reflection technique. Horizontal saccades from 5 to 20 degs. were elicited using a 0.3 deg. laser spot. Direction, frequency and amplitude changes occurred in pseudorandom order. Eye movements were registered every two hours between 8 am and 8 pm after a night of sleep deprivation as well as after a night's sleep.

Preliminary results reveal a substantial decrease in the slope of the main sequence after sleep deprivation. Thus, the reduced peak velocity of saccades after application of sedating drugs may be rather due to the reduced vigilance than to specific actions on the paramedian pontine reticular formation.

295.15

INCREASED VERGENCE SPEED DURING COMBINED SACCADIC AND VERGENCE EYE MOVEMENTS IN MONKEYS IS CENTRALLY PROGRAMMED. W. M. King*, University of Rochester Medical Center and Center for Visual Science, Rochester, NY 14642.

Previous reports have established that the speed of vergence eye movements in humans and monkeys is augmented when vergence and saccades occur in combination. To determine if the increase in vergence speed during saccades is centrally programmed, single unit activity from medial rectus motoneurons and premotor vergence cells was recorded in alert monkeys. Eye movements were measured binocularly using search coils. Monkeys were trained to make eye movements between near and far targets (2-18 deg vergence) that were offset horizontally or vertically (up to ±20 deg) from each other. Peak divergence or convergence speed was always greater during gaze shifts that included saccades (in any direction) compared to those that did not include saccades. Vergence eye movements with saccades appeared to be a combination of a fast component superimposed on a slow component with the temporal characteristics of classically described vergence. The fast component was always associated with saccades, and peak vergence speed occurred in synchrony with peak saccade speed.

Motoneurons and vergence neurons exhibited changes in discharge that correlated with saccade-related increases in vergence speed. Changes in vergence cell discharge led changes in vergence speed by at least 30 msec. Some vergence cells exhibited high sensitivity for vergence speed but not position. They discharged weakly with slow vergence changes in the absence of saccades but vigorously for fast vergence changes that were coupled with saccades. These data suggest that the augmented vergence speeds associated with gaze shifts that include saccades are centrally programmed in the brainstem.

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295.16

CHARACTERISTICS OF SACCADES TO SOMATOSENSORY TARGETS. J. M. Groh* and D. L. Sparks, Inst. of Neurological Sciences and Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Previous physiological studies have demonstrated that cells in an important oculomotor structure, the superior colliculus (SC), respond to visual, auditory and somatosensory stimuli in the anesthetized animal (Drager and Hubel, *Nature* **253**:203, Stein et al., *Science* **189**:224). Behavioral studies have compared saccades to auditory targets with those to visual targets (Jay and Sparks, *Comparative Perception Vol I*,ed.Stebbins and Berkeley,1990), but little attention has been given to the characteristics of saccades to somatosensory stimuli. Before the functional significance of the SC somatosensory neurons can be understood, the role of somatosensory stimuli in guiding saccadic eye movements must be examined. The purpose of this study was to compare saccades to visual and somatosensory targets in rhesus monkeys.

Monkeys were trained to perform saccades to the locations of visual and somatosensory targets. The animal simultaneously grasped two levers mounted out of view beneath a visual display. Vibration of either of the levers served as a silent somatosensory stimulus. Monkeys performed a variety of tasks requiring fixation of a visual target followed by a saccade to a visual, somatosensory, or combined target. On simple trials, the onset of the target was simultaneous with the offset of the fixation light. The monkey was rewarded for making a saccade to the target location within 500 ms. On delayed trials, target onset preceded fixation light offset by 600-800 ms. The offset of the fixation light served as the cue to initiate a saccade to the target within 500 ms.

For both simple and delayed trial types, saccades to somatosensory targets were of lower velocity and longer duration than equivalent amplitude saccades to visual targets. The latency from target onset (simple type) or fixation light offset (delayed type) to movement onset was longer for somatosensory than for visual saccades. Somatosensory saccades were significantly less accurate than saccades to briefly illuminated visual targets.

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295.17

The Responses of Quasi-Visual (QV) Cells of the Superior Colliclus (SC) During Target Presentation, Movement Specification and Movement Initiation. P. W. Glimcher* and D. L. Sparks, Department of Psychology, University of Pennsylvania, Philadelphia, PA 19104.

The responses of collicular QV cells (Mays & Sparks *J. Neurophysiol.* **43**: 207) were examined in movement selection tasks which spatially and temporally segregate movement selection from movement initiation and visual target presentation. Rhesus monkeys were trained to fixate a central yellow LED. While fixation was maintained, two eccentric yellow LEDs were illuminated, one inside of, and one outside of, the response field of an isolated QV cell. After 1000 to 1500 msec the two eccentric LEDs were extinguished. Four hundred to 600 msec later the fixation LED changed color to either red or green. Red signaled that the remembered location of the upper eccentric LED served as the target for a saccade and that the lower LED served as an unreinforced distractor. Green identified the lower LED location as the target. The fixation target was extinguished after a further 750 msec and animals were rewarded for making a saccade to the remembered location of the rewarded target. Variants of this task have been used to study the prelude bursters of the SC which encode the metrics of an impending saccade, after selection but before movement initiation (Glimcher & Sparks *Nature*, **355**: 542).

QV cells responded vigorously after the onset of an LED within their response field. The frequency of this discharge was reduced after 200 to 500 msec in most QV cells. Target offset did not abolish this responding. Finally, units maintained a more vigorous discharge if the LED location they responded to served as the target than if it served as the distractor. QV cells studied typically showed a lower target/distractor selectivity than has been measured in prelude bursters.

Converging lines of evidence suggest a role for the SC in the movement selection/specification process. Electrical stimulation of the SC, below threshold for movement initiation, has been shown to specify movement metrics (Glimcher & Sparks *Invest. Opth. & Vis. Sci.* **33**: 3320). Collicular prelude bursters encode movements selected for future initiation. QV cells are shown here to differentiate between targets and distractors.

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295.18

SACCADES TO REMEMBERED TARGETS EXHIBIT ENHANCED ORBITAL POSITION EFFECTS IN MACAQUES. E.J. Barton and D.L. Sparks*. Inst. of Neurological Sciences and Dept. of Psychology, Univ.of Pennsylvania, Philadelphia,PA. 19104

The amplitude and direction of visually guided saccades in the light is relatively independent of the initial orbital position within the range of +/- 20 deg. Saccades to remembered targets have been characterized as being less accurate and exhibiting an upward bias (Gnadt et al., *Vis. Res.* 31:693,1991). Contrary to visually guided saccades, remembered saccades are markedly influenced by the initial orbital position. The effect of orbital position was examined in rhesus monkeys trained to perform both visually guided and remembered saccades from different starting positions. For remembered trials, the monkeys waited 500 ms after the target was extinguished to look to the remembered location.

The amplitude and direction of saccades to remembered targets were modulated by the orbital position of the eye, with all orbital dependencies being exaggerated as the background illumination diminished. For horizontal targets, centrifugal saccades, especially those with an eccentric origin, have a smaller horizontal component than centripetal movements. For example, the amplitudes of saccades to the remembered locations of target displacements of 25 deg. could differ by as much as 10 deg. when initiated from orbital positions differing by 30 deg. The upward bias was also influenced by the initial orbital position, increasing monotonically with downward deviations (decreasing with upward). Vertical saccades to remembered targets exhibited a significant horizontal bias, which was greater for downward than upward saccades. There was a null point on the horizontal meridian at which this bias disappeared. As the starting position deviated from this null point, the direction of the saccades were altered in such a way as to introduce a horizontal component, which brought the eyes towards the null point. The greater the deviation from the null point, the greater the horizontal bias.

The null point of the horizontal bias and the vertical offset were modulated by patching one eye.

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295.19

DISCHARGE CHARACTERISTICS OF PUTATIVE INHIBITORY BURST NEURONS IN THE HEAD-FIXED AND HEAD-FREE CAT. K.E.Cullen*, D. Guitton, W. Jiang, M. Paré, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A-2B4.

In prior studies in the cat we have shown that brainstem omnipause neurons and collicular output neurons have discharges that are best correlated to the trajectory of gaze during an orienting gaze shift that is generated when the head is unrestrained. We now report on gaze-related activity of inhibitory burst neurons (IBNs). IBNs are located in a discrete area of the brainstem, just caudal to the abducens nucleus. They burst during ipsilateral saccadic eye movements and project to the contralateral abducens motor neurons, thereby suppressing the activity of the antagonist lateral rectus muscle during saccades.

We have recorded from putative IBNs in the cat during four experimental conditions: 1) spontaneous saccades made head-fixed; 2) vestibularly driven quick phases generated by whole body rotation; 3) passive movements of the head on the body; and 4) active orienting gaze shifts made head-free. IBN discharges were best correlated with the metrics of gaze motion in both the head-fixed and head-free conditions; in the following relationships. 1) Burst duration was proportional to gaze duration. 2) Peak firing frequency was proportional to peak gaze velocity. Also the trajectories of neuronal firing frequency and gaze velocity profiles were similar. 3) Total number of spikes (NOS) was proportional to gaze amplitude (G_a). The slope of NOS vs G_a was greater for saccades made head fixed than for gaze shifts made head-free. This would be expected if IBN discharge reflected the burst generator signal, and if the eye and the head shared a burst generator: a given burst in the head-free condition is linked to the summed movement of eye and head, and therefore would produce a larger gaze shift than would be seen in the head-fixed condidtion.

295.20

SACCADE-RELATED NEURONS IN THE MACAQUE'S ZONA INCERTA HAVE OMNIVECTORAL PAUSES. T. P. Ma* and N. K. Hunt, Department of Anatomy, University of Mississippi Medical Center, Jackson, MS 39216.

We have made extracellular recordings of single cells in the ventral thalamus of a rhesus monkey (*Macaca mulatta*) while it executed spontaneous (made in the light and dark without presented stimuli), visually-guided (made in the dark to a target on a tangent screen), and memory-guided (made in the dark to the location where a target was presented) saccades. Electrolytic microlesions (10 µamp negative current, 25 sec) were placed where cells were recorded before the electrode was advanced farther. Electrode tracks were reconstructed in cytochrome oxidase-reacted and Nissl-counterstained sections and the locations of microlesions were identified. Microlesions marking the location of 8 cells were in the zona incerta (ZI). These cells have moderate rates of activity (25-40 Hz) except during saccades. We were not able to elicit visual responses in these cells. When the monkey made a saccade, the activity rapidly declined at the beginning of the saccade and remained below background levels until after the end of the eye movement. The beginning of this pause in activity occurs near the start of the saccade. The neuron returned to its background level of activity at some point after the end of the saccade. Neither the duration of the pause nor the end of the pause could be related to the saccade. The pause in activity is present for saccades in every direction and amplitude, and for every behavioral paradigm tested. In light of the strong GABAergic projection from ZI to the deeper layers of the superior colliculus (SC), and the timing of the omnivectoral pause in incertal activity related to the beginning of saccades, we hypothesize that ZI functions within a feedback loop that controls the initiation of saccades by the ventral superior colliculus. *Supported in part by a Grant-in-Aid from Fight for Sight (TPM), NIH grant RR05386 (TPM), and a Dean's Research Fellowship (NKH).*

295.21

MORPHOLOGY OF INCERTOTECTAL AND INCERTOPRETECTAL NEURONS IN MACAQUES. J. C. Johnson*, G. A. Hoskins, G. A. Mihailoff, P. J. May, and T. P. Ma. Departments of Anatomy and Ophthalmology, University of Mississippi Medical Center, Jackson, MS 39216.

We injected WGA-HRP into the superior colliculus (SC) and/or pretectum of six cynomolgus monkeys (*Macaca fascicularis*) and examined the morphology of labeled neurons in the zona incerta (ZI). Incertotectal cells are primarily located in the ventral lamina whereas incertopretectal cells are primarily located in the dorsal lamina. Similarly, tectoincertal terminals are usually found in the ventral lamina and pretectoincertal terminals are usually found in the dorsal lamina. Both populations of labeled cells have somatic and dendritic morphologies similar to principal cells in Golgi-impregnated preparations (Ma et al., *J. Comp. Neurol.* 320:273, 1992). Moreover, measurements of the long axes of cells retrogradely labeled by either injection (15 to 44 μm) are comparable to those obtained from Nissl and Golgi preparations. All labeled cells examined with the electron microscope have similar morphologies and could not be distinguished from unlabeled neurons in the same section on the basis of organelle content or relationships with nearby structures. In addition, there was no difference between the size range and proportion of various terminal types that contacted dendrites labeled after injections into the SC or pretectum, nor were there any differences between labeled and unlabeled dendrites in the same section. We conclude that the LM and EM morphology of incertal neurons is relatively homogeneous supporting the hypothesis that the zona incerta functions by integrating multiple inputs into a single common efferent signal. Thus, ZI possesses the morphological substrate to subserve feedback control of saccadic eye movements in the primate. *Supported in part by a Grant from Fight for Sight (TPM) and NIH grants RR05386 (TPM), NS12644 (GAM), EY07166 (PJM).*

OCULOMOTOR III

296.1

TWO DIMENSIONAL SPATIOTOPIC MAPS OF PHORIA ADAPTATION. J.S. Maxwell* and C.S. Schor. University of California at Berkeley, Berkeley, CA 94720

If presented with diplopia (and the disparity is not too large), the visual axis of the eyes soon begin to realign in order to bring the two ocular images into registration with corresponding retinal points and eliminate the double image. Aftereffects of this change in alignment are present even in the absence of binocular visual feedback. The adaptation of phoria (the open loop difference in the position of the two eyes) to a stimulus presented in one location in space (ONE POINT paradigm) spreads to eye positions not specifically adapted. Some previous studies suggest that the adaptive response is largest at the site where the disparity was presented and falls off at more distant locations. Studies of Schor *et. al*, however, suggest that adaptation spreads uniformly across the field. Schor *et. al* also demonstrated differential phoria adaptation to two disparities of opposite sign, presented simultaneously at two different locations in the field (TWO POINT paradigm). Adaptation occurred whether the stimuli were separated vertically or horizontally, as long as the disparities were neither too large nor the two adaptation sites too close together. We have re-examined the spread of phoria adaptation to locations in the field not specifically adapted by measuring the change in phoria across a two dimensional surface using both one and two adapting sites. We found that the change in phoria was uniform across the field unless there was an opposing stimulus as in the TWO POINT paradigm, in which case, the phoria varied along the meridian in which the two disparities were given but constant along any particular orthogonal meridian. The time course of TWO POINT adaptation suggests that two mechanisms are involved in the early stages of phoria adaptation, one which immediately shifts the phoria uniformly across the field and another which then selectively adjusts the phoria to the demands of the stimulus. *Supported by EYO5532*

296.2

CORRESPONDENCE BETWEEN PERCEIVED TARGET VELOCITY AND SMOOTH PURSUIT GAIN. H.R. Piroozi and L.A. Abel*, Dep'ts of Ophthalmol. and Elect. Engr., IU Sch. of Med. and IUPUI, Indianapolis, IN 46202.

Visual tracking of moving objects and predicting their future position is a commonplace activity. We have studied the relationship between tracking performance and the perception of target velocity in 5 young normal subjects. Subjects pursued triangle wave targets for 2 or 3 half-cycles at 10, 20, 40 and 60°/s, with velocity, initial direction and stimulus duration randomized. Perceived velocity was estimated using the time between target disappearance and its predicted arrival at a prespecified position, as indicated by a button press. Simple reaction time (SRT) to the disappearance of the target was also obtained for the same stimuli. Eye velocity was calculated for the final half-cycle of tracking. Perceived target velocity was calculated both directly from the estimated target arrival time and after first subtracting SRT.

Pursuit gain was < 1 only at 60°/s for most subjects. SRT declined at the higher target velocities for all subjects. Perceptual gain (estimated/actual target velocity) was ≈ 1 for both methods at the 2 lower speeds. For the 2 higher speeds, subtracting SRT gave better perceived estimates of target velocity. Eye velocity paralleled the subtraction-based estimate in 3 subjects and the estimate which used the total time in the other 2. The corresponding declines in both perceptual and pursuit gain at 60°/s suggest that in normal subjects this limit on pursuit performance may derive from input processing rather than from any limit on motor command generation. Further determination of the relationship between SRT and estimate velocity is required, but this technique may serve as a means to evaluate both efferent and afferent processing in the pursuit system.

296.3

ASYMMETRICAL CYCLOFUSIONAL RESPONSES. Li Sun, I.P.Howard,and P.K.Kaiser*, The York Vision Group, York University, Toronto, Ontario, M3J 1P3, Canada

While investigating the dynamic properties of human cyclovergence, we noticed that several subjects had a severely asymmetrical response to sinusoidal cyclorotation of a dichoptic textured display. We used scleral search coils to record cyclovergence evoked by dichoptic textured patterns cyclorotating in antiphase through 6 deg at frequencies between 0 and 0.05 Hz. Cyclovergence was also measured by a psychophysical procedure in which subjects nulled the apparent misalignment of radial nonius lines superimposed on a small black disc at the centre of the cyclorotating textured pattern. A nonius procedure was also used to measure cyclophoria. We found that intorsional responses to top-inward stimulus rotation were much larger than extorsional responses to top-outward stimulus rotation. For 5 out of 6 subjects, the amplitude of intortional responses was in the range of 5--9 degs, while the amplitude of extorsional responses was in the range of 0.3--3 degs. Similar results were also obtained from the psychophysical nonius procedure. Cyclovergence is an involuntary response presumably designed to keep the eyes in orientational alignment and, in particular, to correct for misalignment due to cyclophoria. We hypothesized that people with a strong cyclophoria would show a corresponding asymmetry in cyclovergence. However, we found that the direction and magnitude of asymmetrical cyclovergence was not related to the direction and magnitude of a subjects' cyclophoria. A similar dissociation is known to exist between horizontal fixation disparity and horizontal phoria and between convergence/divergence asymmetries and phoria.

296.4

PRIMED TO FAIL. SELF INHIBITION OF THE BLINK. C. Evinger*[1,2], J.J. Pellegrini[1] and P.A. Sibony[2], Depts. [1]Neurobiology & Behavior and [2]Ophthalmology, SUNY Stony Brook, Stony Brook, NY 11794

A unique aspect of the blink is that the occurrence of a blink produces the stimuli to elicit a reflex blink. Rapidly closing the eyelid generates sensory stimuli that activate primary trigeminal afferents of the supraorbital branch of the trigeminal nerve (SO). Imposing these stimuli externally would elicit a blink. Thus, a blink should evoke a reflex blink. Paired electrical stimuli to the SO, however, demonstrate that the first reflex blink suppresses ensuing reflex blinks for 1-3 sec. Similarly, a voluntary blink suppresses subsequent SO evoked blinks for 400 msec. Thus, blinking initiates an inhibition of brainstem, trigeminal reflex blink circuits that decreases the probability of a blink launching a reflex blink. The present studies investigated the generality of blink self inhibition and tested whether reflex blinks modified the initiation of subsequent voluntary blinks.

We determined if the blinks evoked by saccadic eye and head movements suppressed subsequent SO evoked blinks. Measuring the orbicularis oculi EMG response to SO stimulation in normal humans revealed a clear reduction of SO evoked blink amplitude for at least 400 ms following a saccadic gaze shift. More importantly, we found that SO evoked blinks dramatically delayed the initiation of subsequent voluntary blinks. This result suggests that either inhibitory processes affect higher brain regions involved in the initiation of voluntary blinks or that the signal to make a voluntary blink remains active in the brainstem until the inhibitory processes subside.

Thus, the occurrence of a blink transiently prevents initiation of a reflex blink from the sensory stimuli produced by lid closure. The fact that this inhibitory process is reduced in patients with lid spasms suggests that disruption of this mechanism is a major cause of these spastic disorders.

Supported by EY07391 (CE).

296.5

STIMULATION OF THE SUPERIOR COLLICULUS (SC) SHIFTS THE FOCUS OF ATTENTION IN THE MACAQUE. R. Gattass* and R. Desimone. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

In a previous study of SC cells in animals performing a "spatial-attention" version of a matching to sample task with distractors, we reported that many SC cells respond better to an attended stimulus than to an irrelevant distractor. We have now examined the behavioral effects of electrically stimulating the SC in the same behavioral task, in one rhesus monkey. A sample stimulus was briefly presented at one location, followed, after a short delay, by a brief test stimulus at that (relevant) location. On some trials, a distractor was presented at another (irrelevant) location. The monkey, which maintained fixation throughout the trial, indicated whether the sample and test stimulus matched, ignoring the distractor. Electrical stimulation (200 Hz, 30-40 μA) was applied in the superficial layers of the SC, at the same topographic site as either the relevant or irrelevant stimuli. The animal was impaired when we stimulated at the site of the distractor stimulus, but not when we stimulated at the same site in the absence of the distractor, or when we stimulated at the site of the attended stimuli. These results are consistent with a model of attentional control based on target competition within the visual field.

296.6

SINGLE-UNIT ACTIVITY IN THE NUCLEUS RETICULARIS TEGMENTI PONTIS (NRTP) RELATED TO VERGENCE AND ACCOMMODATION. P.D.R. Gamlin* and R. J. Clarke. Dept. of Physiological Optics, School of Optometry, UAB, Birmingham, AL 35294.

Our recent physiological and anatomical studies indicate that the cerebellum is involved in the control of vergence and accommodation. Therefore single-unit activity in the NRTP, a precerebellar nucleus, has been investigated during these eye movements.

Cells in the NRTP of alert, behaving rhesus monkeys were encountered that exhibited large, transient increases in their firing-rate during near-to-far viewing. Many of these cells displayed some tonic activity with far viewing. This activity declined to zero with effective viewing distances of 30-100 cm. Cells were also encountered that showed a burst of activity during far-to-near viewing. Many of these cells had a tonic firing-rate that increased as a function of increases in convergence and accommodation. A few cells were encountered that had predominantly tonic firing-rates related to vergence and accommodation. Recorded cells were often close to neurons displaying saccade-related activity. Marking lesions confirmed that the recording sites were in the medial NRTP in the same area as that reported for saccade-related neurons.

The activity of some cells was tested during normal viewing, disparity vergence (blur open-loop), and accommodative vergence (disparity open-loop). In all cases, the firing-rate was modulated under all viewing conditions. Therefore, these cells would be expected to be located after the cross-links that couple the blur and disparity controllers to vergence and accommodation.

The NRTP is known to receive cortical afferents and to have reciprocal connections with the cerebellum. Therefore, this study suggests that some cells in the medial NRTP form part of a cerebro-cerebellar pathway modulating or controlling vergence and accommodation. (Supported by NEI Grants EY07558 and P30 EY03039).

296.7

COMPARISON OF SHIFTS OF ATTENTION IN PATIENTS WITH HUNTINGTON DISEASE (HD) AND NORMALS. T.Tsai, A.G. Lasker, D.S. Zee, S.E. Folstein, and C. Peyser*, The Johns Hopkins Hospital, Baltimore, MD 21205

We compared the ability of HD patients (n=25) and normals (n=22) to direct covert visual attention, using a thumb press reaction time task (TP) and a saccadic latency task (SL). For testing covert shifts of attention, the target appeared right or left 10°, 50-600ms after a central arrow cue that was valid (points in same direction), invalid (points in opposite direction), or neutral (points in both directions). There were 80 valid, 20 invalid and 50 neutral cues for each delay in TP task, and 80 valid, 20 invalid and 20 neutral cues for each delay in SL task.

HD patients showed abnormal eye movements; an inability to suppress reflexive saccades to a visual target (74-87% errors in HD vs < 25% errors in normals). In the TP task, the mean thumb reaction time (RT) for HD patients was 507 ± 97ms and for normals 327 ± 43ms, indicating a delay in motor response ($p < .0001$). In the SL task, the mean eye latency for HD patients was 301 ± 69ms and for normals, 283 ± 41ms (ns). For both experiments, the influence of attention (the difference between RT for invalid and valid cues) was the same in patients and normals. For a delay of 600ms, for the TP task, the difference in RT was 50 ± 75ms in HD and 39 ± 28ms in normals. For the SL task, the difference in saccade latency was 48 ± 40ms in HD and 40 ± 22ms in normals.

We conclude that HD patients are able to use cues to facilitate covert shifts of visual attention as well as normals, in spite of the delayed manual responses and the difficulty suppressing reflexive saccades. These results are compatible with the hypothesis that in HD, frontal-basal ganglia circuits are more affected (accounting for the eye abnormalites) than the parietal lobes (accounting for the sparing of attentional mechanisms).

296.8

3-DIMENSIONAL POSITION CODING OF EXTRA-OCULAR MOTONEURONS IN THE RHESUS MONKEY. Y. Suzuki, D. Straumann, V. Henn*. Neurology Department, University Hospital, CH-8091 Zürich, Switzerland.

We have investigated the relation between motoneuron activity and 3-dimensional eye position. Rhesus monkeys were chronically prepared for single unit recordings from the oculomotor, trochlear, and abducens nerve. 3-dimensional eye position was measured with the dual magnetic search coil technique. After recording spontaneous eye movements with the head upright, the monkey was put in a 20 and 40 deg right or left ear down position to induce counterrolling which increased the range of torsional positions by up to +/- 7 deg.

Data were sorted in bins of firing frequencies, and correlated to eye positions plotted in 3-dimensional space, i.e. with respect to the horizontal, vertical, and torsional components. Data points could be approximated by second order surfaces. This analysis extends the previously described iso-frequency curves to iso-frequency surfaces. Oblique and vertical recti motoneurons showed characteristic configurations of these surfaces with vertical and torsional components as deduced from geometrical analysis of muscle insertions on the globe.

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296.9

TORSIONAL AND VERTICAL EYE MOVEMENTS ELICITED BY ELECTRICAL MICROSTIMULATION OF THE ROSTRAL INTERSTITIAL NUCLEUS OF THE MLF. D. Straumann*, Y. Suzuki, V. Henn, B.J.M. Hess. Neurology Department, University Hospital, CH-8091 Zürich, Switzerland.

The riMLF contains premotor burst neurons with vertical-torsional on-directions for rapid eye movements. In the rhesus monkey, we have electrically stimulated this area and measured evoked eye rotations in three dimensions (search coil technique). The stimulation sites were selected by single unit recordings (short-lead burst neurons with vertical-torsional on-directions). Typical threshold currents to elicit eye movements were 40 μA (train duration 70 ms, pulse frequency 330 Hz). On/off-latencies ranged between 10 and 20 ms. Movements were always binocular with extorsion of the ipsilateral eye with or without a vertical-up or vertical-down component that was linked to the stimulation site. Movement directions deviated up to 35 deg from pure torsional. Amplitudes of eye rotations correlated linearly with the pulse interval and increased with current strength and train duration. Depending on stimulation parameters, mean eye velocities reached values up to 200 deg/s. After stimulation, the new torsional eye position was kept until the next spontaneous saccade reset the eye to zero torsion, i.e. into Listing's plane. Constant low level electrical stimulation produced nystagmus in the intorsional direction. These results complement findings from single neuron and lesion studies in the riMLF.

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296.10

EFFECTS OF FRONTAL EYE FIELD STIMULATION UPON OMNIPAUSE AND BURST NEURONS IN THE MONKEY PARAMEDIAN PONTINE RETICULAR FORMATION. M. A. Segraves*. Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208.

Frontal eye field (FEF) input to the monkey's paramedian pontine reticular formation (PPRF) conveys the amplitude and direction of an impending saccadic eye movement as well as the time to initiate that saccade (Segraves, 1991). The effects of this input upon omnipause neurons and burst neurons in the PPRF was examined in 1 rhesus monkey by electrically stimulating FEF sites where neurons could be antidromically excited from the pons.

High-intensity, short-duration stimuli silenced omnipause neurons with latencies of 5 ms or less. The omnipause neurons remained quiet for a time proportional to stimulus intensity and lasting up to 30 ms after a 6 ms train of 3 0.2 ms biphasic pulses at 330 Hz with a maximum intensity of 1000 μA. These stimuli sometimes evoked a saccade near the end of the break in pause neuron activity whose amplitude appeared to be curtailed by the resumption in firing of the neuron. Low-intensity, long-duration stimuli (75 μA, 70 ms train of 24 0.2 ms pulses at 330 Hz), traditionally used to evoke saccades from the FEF, also were followed by a cessation in omnipause neuron firing, but only after a delay of about 30 ms. For these stimuli, the neuron resumed firing when the stimulus was turned off. Presumably, FEF input to omnipause neurons is mediated by inhibitory neurons within the PPRF. High-intensity, short-duration stimuli excited burst neurons with latencies of 4.2 - 9.8 ms, suggesting that the circuit from FEF neuron to PPRF burst neuron is, at least, disynaptic.

FEF input to the PPRF is likely to play a role in triggering saccades by simultaneously inhibiting omnipause neurons and exciting burst neurons. In addition, this input could provide the PPRF with targeting information for the next saccade.

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296.11

A NEURAL NETWORK MODEL FOR AUDITORY-EVOKED ORIENTING IN PRIMATES. A.J. VAN OPSTAL, M.A. FRENS, R.F. VAN DER WILLIGEN AND C.C.A.M. GIELEN*. Dept. of Med. Physics and Biophysics, P.o. Box 6101, 6500 HB Nijmegen, The Netherlands.

In order to make an accurate eye-head orienting response towards an auditory target, an important localization cue for the auditory system is provided by Interaural Intensity Differences (*IIDs*). Because these differences are not available at the level of the cochlear nuclei, the brain has to reconstruct *IIDs* through neural computation. Monkey's are capable of making large excentric eye gaze shifts, which poses an interesting additional problem, since the auditory and visual sensory organs are generally not aligned. It has been found, that in monkey superior colliculus the auditory and visual representations take current eye position into account, in such a way that the neural map represents eye motor error. We present a simple neural network model that tries to account for these findings. A sound stimulus (random spectrum, azimuth and intensity) is transformed into intensity-related firing patterns in the tonotopic cochlear nuclei, taking relevant properties of head and pinnae into account. In the binaural stage, these cochlear patterns are combined. Eye position signals are added to the binaural output and transmitted to a topographic motor error map. The network is trained by error back-propagation. Two types of binaural units emerge, one that measures *IIDs*, the other relates to total sound energy. These units resemble broadly tuned *EI*- and *EE*-cells, respectively, amply demonstrated in the auditory system. We conjecture that both types are needed for coding the azimuthal position of sound relative to the head.

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296.12

EFFECTS OF PREDICTABILITY OF TARGET MOTION ON SMOOTH PURSUIT. R.S. Gellman* and W.A. Fletcher. Dept Clinical Neurosciences, Univ of Calgary, Calgary, AB, Canada T2N 4N1

When humans track simple ramp motions of unpredictable direction (left *vs* right) and speed, they initially produce a low acceleration smooth pursuit that is largely independent of target speed between 5-40°/s (Carl & Gellman *J. Neurophysiol* 57). We now report that making direction and speed predictable alters the acceleration, but not the latency, of the smooth pursuit response.

Eye movements of healthy volunteers were recorded with the search coil. The target appeared in the centre of the screen and, after an unpredictable delay, started moving. There were 3 conditions: (i) unpredictable direction and speed, (ii) predictable direction and unpredictable speed, and (iii) predictable direction and speed.

The stimulus-driven response started ≈130 ms after onset of target movement. This was true *for all conditions*, including those that tended to evoke anticipatory drifts: foreknowledge of motion did not modify response latency. The initial acceleration, in contrast, depended strongly on predictability: acceleration increased if the subject knew the direction in advance, and increased further if the subject knew the target speed. Acceleration became strongly dependent on target speed under both conditions. These findings show that (i) the initial component of pursuit, even when time-locked to the stimulus, is not invariant and cannot be described purely by parameters of image motion; and (ii) predictability of target motion allows the CNS to prepare a scaled response 50-100 ms earlier than usual, as shown for limb movements (Van Donkelaar et al., *Soc Neuro Abs 17*).

296.13

APPLICATION OF THE EQUAL INNERVATION PRINCIPLE TO THE ANALYSIS OF BINOCULAR EYE MOVEMENTS IN THREE DIMENSIONS. A.W.H. MINKEN, J.M.A. LOKATE and J.A.M. VAN GISBERGEN*
Department of Medical Physics and Biophysics, University of Nijmegen, Geert Grooteplein N21, 6521 EZ Nijmegen, The Netherlands.

The equal innervation principle (Hering) states that binocular eye positions can be decomposed into conjugate (CON) and disjunct (DIS) contributions. The CON signal denotes a common rotation (equal size and sign) in the two eyes while the DIS signal describes a rotation of equal size and opposite sign.

Binocular eye positions, represented as pairs of 3D rotation vectors, were measured with the 3D scleral search coil technique in four subjects. LED targets in two depth planes elicited a variety of gaze shifts in direction or in depth, or both.

The CON component, interpretable as the rotation of the cyclopean eye from a reference position, was always confined to a flat plane, close to the Listing planes of the two eyes measured in far vision. This was even the case in near vision, when the eyes showed clear deviations from the far vision Listing planes (Mok et al., 1992). Remarkably, we found that the DIS signal, present in near vision, had horizontal and torsional components with similar time courses. The torsional DIS component showed clear modulation by the elevation of the eyes (vertical CON component), by shifting from intorsion in up gaze towards extorsion in down gaze.

Based upon these data, we propose that the conjugate system operates independently from the disjunct system, but not vice versa. While their interaction causes cyclorotations in near fixations (violations of Listing's law), the combined operation of the two systems works in the right direction to maintain a binocular version of Donders' law and to prevent cumulation of torsion in the two eyes.

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296.14

INTRAOCULAR PRESSURE CHANGES DURING EDINGER-WESTPHAL STIMULATED ACCOMMODATION IN CHICKS. A. Glasser, D. Troilo, & H.C. Howland*, Neurobiol. & Behav., Cornell Univ., Ithaca, NY 14853

The role of intraocular pressure (IOP) in accommodation is controversial. An IOP mediated mechanism of accommodation has been suggested in mammalian (Coleman, 1970) and chicken eyes (Suburo & Marcentoni, 1983). We investigated IOP changes during accommodation in chicken eyes and their possible role in corneal and lenticular changes.

Accommodation was induced through stimulation of the Edinger-Westphal nucleus of 4 week old white leghorn Cornell-K strain chicks. We find a mean maximum total refractive change of 25.75 D (±.75 D SEM) as measured using infrared photoretinoscopy. A maximum change in corneal refracting power of 8.28 D (±1.01 D) was measured using keratometry. Significant changes in the axial dimensions of the eye were found during accommodation: lens thickness increased (0.20±0.01 mm) and there were decreases in anterior chamber (AC) depth (0.11±0.02 mm), vitreous chamber (VC) depth (0.13±0.02 mm), and axial length (AL) (0.05±0.02 mm). The anterior and vitreous chambers of the eye were cannulated and increases in pressure of ~2 mmHg were found in both chambers during accommodation.

An increase in AC pressure in chicken eyes is contrary to the results from mammalian studies and may be due to the corneal component of accommodation seen in chicks. The magnitude and direction of pressure changes in the AC are not only insufficient but also in the wrong direction to account for a steepening of the cornea based on in vitro studies (Glasser & Howland, 1991). While the causal effects of the modest vitreous pressure changes observed remain unclear, the pressure changes in the AC appear to be a consequence and not a cause of corneal accommodation.

296.15

ORGANOTYPIC NERVE/MUSCLE CO-CULTURE AS A POTENTIAL MEANS OF ANALYSIS OF DEVELOPMENTAL REGULATORY MECHANISMS FOR EXTRAOCULAR MUSCLE. J.D. Porter*, P.H. Bonner, and K.F. Hauser. Depts. of Anatomy/Neurobiology and Ophthalmology and Sch. of Biological Sciences, Univ. of Kentucky, Lexington, KY 40536.

The diversity and plasticity that is inherent in eye movement control systems is reflected in the unusual structure/function characteristics of extraocular muscle (EOM). Ontogenetic mechanisms that specify EOM fiber types are undetermined, although their unique myosin gene expression and morphology appear postnatally in the rat. We have used an organotypic explant culture system for the first time to test whether EOM primordia and motoneuron pools can be independently manipulated to assess myogenic versus neurogenic regulation of development of this system. Organotypic explants from 1-4 day-old rat pup EOMs were cultured with embryonic day 15 lumbar spinal cord for 10-17 days. Explant culture faithfully reproduces most of the features of *in vivo* development, while allowing daily visualization with Hoffman modulation and phase contrast microscopy. Muscle precursor cells migrated out of explants and subsequently fused to generate myotubes. Explants then supply progenitor cells; pre-existing fibers in explants degenerate. Spinal cord explants survive with cytoarchitectonic relationships essentially intact. Neurites made putative neuromuscular contacts with developing myofibers. Establishment of functional neuromuscular contacts was supported by observations of synchronous twitch activity after 8 days *in vitro*. Myosin heavy chain immunocytochemistry (monoclonals S58, F59, and MF20) was used to examine terminal differentiation of fiber types in explant cultures and indicated the presence of fibers containing routine fast and slow myosin isoforms. Electron microscopy documented myoblast fusion and formation of stereotypical contractile filament organization within developing myofibers. These studies demonstrate that precursor cells from EOM primordia can develop into functioning myotubes independent from their normal innervation from oculomotor motoneurons. Organotypic culture may prove useful in examining the mechanisms that regulate the development and maintenance of the unusual properties of EOM.

296.16

BLINK METRICS IN THE CHRONIC ALERT MONKEY: NORMATIVE DATA AND THE EFFECT OF BOTULINUM TOXIN. R.S. Baker and J.D. Porter. Depts. of Ophthalmology and Anatomy/Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0084.

Blink movement metrics were studied in three alert cynomolgous monkeys using electromagnetic search coils attached to the eyelids bilaterally. Monkey blinks were stereotypical, averaging approximately 20° in amplitude, with down-phase peak velocities approximately twice those of the corresponding up phases. Monkey blinks exhibited higher peak velocities and shorter durations than previously described for similar amplitude movements in man. Blink main sequence (amplitude-velocity) plots were linear and non-saturating within a 40° range for both up and down phases. Amplitude-duration behavior could be best described by a second-order polynomial.

Botulinum toxin is used in treatment of focal eyelid dystonia (blepharospasm). The toxin provides transient relief of continuous blinking through action as a presynaptic cholinergic blocker. Unilateral injection of botulinum toxin type A altered lid movement metrics in parallel with the morphologic changes that we have described previously (*Arch. Ophthalmol.* 109:396, '91). Blink gain (treated eye/untreated eye amplitude) decreased to <0.1 within 1-3 days and exhibited slow recovery starting 30-40 days post-injection. Initial overshoots in treated eye up phases were compensated for within 2 weeks. Recovery from toxin injection presumably is via preterminal sprouting of motoneuron axons that re-establishes functional neuromuscular junctions. During the recovery phase, while muscle fiber cross-sectional area was dramatically reduced, blink amplitudes were truncated, but their amplitude-velocity relationship still fell along the low end of the normal main sequence. Blink down-phase durations in the recovering eye exceeded those of normal blinks of the same amplitude. Downward lid saccades were not affected by toxin injections, thereby supporting observations by others that these movements are *not* mediated by the orbicularis oculi. Taken together, these data provide a quantitative data base for normal and toxin-compromised blinks that can be used to assess putative improvements in the treatment of focal dystonia of lid musculature.

296.17

DISCHARGE CHARACTERISTICS OF BRAINSTEM OMNIPAUSE NEURONS IN THE ALERT CAT. M. Paré* and D. Guitton, Montreal Neurological Institute and McGill Univ., Montreal H3A 2B4, Canada.

Omnipause neurons (OPNs), which cease firing for all saccades, have been implicated in controlling the duration of saccades by enabling or disabling the premotor burst generators. These neurons are regarded as one neuronal element linking the higher saccadic centers, which specify the movement metrics, to premotor neurons. In order to investigate the role of OPNs in saccade control the firing characteristics of these neurons, including the temporal relationships of the neuronal discharge relative to saccade parameters, were quantitatively analysed.

OPNs displayed a tonic activity during fixation periods. However, the regularity and the rate of that tonic activity varied amongst the units. OPN discharge was either regular or irregular. The average firing rate ranged from 55 to 150spikes/sec. Occasionally, OPNs had phasic excitatory visual responses. As shown by others, the duration of the cessation of OPNs firing was well correlated with the total duration of the concomitant saccade executed in head-restrained condition (mean r=0.93, mean slope=0.94). On average, OPNs ceased firing 20msec before the onset of a saccade and resumed their discharge closer to the saccade offset (10-15msec). Interestingly, the pause did not end at a fixed position error. Both the position error and the intantaneous velocity of the movement determined the time of the reactivation; for faster saccades, a larger error remained at the time of the pause ending. Similar results were obtained for gaze saccades executed in head-unrestrained condition.

The perisaccadic firing of OPNs varied considerably. Prior to the pause, the firing rate either abruptly dropped or declined slowly. Similarly, the resuming firing rate that followed the pause either rapidly reached its baseline or increased gradually. Moreover, OPNs were usually firing at a reduced rate during post-saccadic glissade suggesting that the gating action of OPNs is not an all or none process.

296.18

A BILATERAL MODEL FOR COORDINATED CONJUGATE AND VERGENCE EYE MOVEMENTS. A. C. Cova and H. L. Galiana* Department of Biomedical Engineering, Faculty of Medicine, McGill University, Montreal, Quebec, H3A - 2B4, Canada.

Traditionally, the Oculomotor Control System (OCS) has been considered as composed of several independent subsystems acting in parallel, where each dedicated subsystem controls a particular type of conjugate or disjunctive eye movement. Mathematical models built upon this assumption have mainly focused on input-output relationships, being difficult to relate to anatomy. This fact may limit the application of such models in the clinical environment. A new integrated approach for the modelling of the OCS is proposed, compatible with both known anatomy and physiology. This paper presents a single, bilateral model capable of predicting the general characteristics (dynamics) of vergence and the vestibulo-ocular reflex (VOR) during slow phase eye movements. It is motivated by growing evidence of interactions between conjugate and vergence premotor activities.

The model relies on the sharing of neural signals among several premotor structures located on both sides of the brainstem. A new possible role for the superior colliculi in the control of slow phase and vergence eye movements is suggested. The proposed model incorporates the presence of near response cells located in the midbrain [Mays et al. JNP'84; Judge and Cumming JNP'86; Mays et al. JNP'86] including modulation of their discharge rate with vergence angle. The model reproduces the reported activity pattern of abducens interneurons during vergence responses [Gamlin et al. JNP'89] and can provide tentative explanations for changes in VN modulation during VOR responses at different vergence levels. Additional extensions of the model can easily include burst cell populations for the simulation of fast vergence responses, and their interaction with conjugate saccades or vestibular quick phases. [Mays and Zee pers. comm.]

LEARNING AND MEMORY: PHYSIOLOGY III

297.1

COMPARISON OF NEURAL NETWORK SIMULATIONS OF BEHAVIOR AFTER VISUAL AND AUDITORY CORTEX LESIONS IN RATS. E.R. Delay*, D.Q. Nguyen, & N.J. Matsushima, Dept. of Psychology, Regis University, Denver, CO 80221.

To simulate postoperative relearning of a brightness discrimination, Delay et al. (Neurosci. Abst., 17: 484, 1991) modified Kehoe's layered network model of general transfer (Psychol. Rev., 95: 411-433, 1988) to treat hidden units as functional CNS structures. This study extended the model to relearning of an auditory intensity discrimination after auditory cortex lesions. While relearning of the visual discrimination by normal and visual decorticate rats was successfully simulated with two associative units and a response unit, three associative units were required to model discrimination behavior of intact and auditory decorticate rats. In each case, the unit representing the respective sensory cortex had the greatest impact on simulations of preoperative learning but showed little change in unit connection weights after cortical injury. However, the units representing intact neurological systems exhibited characteristics which compensated for the disruption of the network by lesions. This compensation was enhanced by general transfer effects of cross-modal training prior to relearning. The accurate simulation of post-operative relearning suggests this simplified model may be a useful tool for studying recovery of function. (Supported by NSF Grant BNS-8909803).

297.2

AREAL AND CELLULAR SEGREGATION OF SPATIAL AND OF FEATURE PROCESSING BY PREFRONTAL NEURONS
F.A.W. Wilson*, J.P. Skelly and P.S. Goldman-Rakic, Section of Neurobiology, Yale University School of Medicine.

Neurons in prefrontal cortex are known to be visually responsive. The question addressed in the present study is whether spatial and feature processing are segregated at the areal and/or cellular level. The sensory, mnemonic and motor activity of prefrontal neurons was examined in monkeys performing an oculomotor delayed response task with interleaved spatial (SDR) and pattern (PDR) stimuli. Fixation of a central point was followed sequentially by a cue (0.5 s), a delay (2.5 s) and a left or right saccadic eye movement. On SDR trials, spatial cues were presented 13 degs to the left or right; on PDR trials, pattern cues were presented behind the fixation point, each pattern associated with a left or right saccade. Electrode penetrations covering parts of the dorsolateral and inferior convexity cortex were regularly verified by x-ray. Regional differences were apparent. (1) Periarcuate neurons responded on both SDR+PDR trials generally with corresponding directional delay period activity. More rostrally, however, certain neurons responded specifically on SDR or PDR trials. (2) Cellular responses were also dissociable. Visual neurons located 15-20 mm lateral to the midline responded differentially to the two pattern cues. When this occurred, they were further tested on a picture task in which objects and other stimuli were presented for 1 s each. Stimulus-selective neurons responded in a graded fashion to the different stimuli; for 60 neurons, the ratio of responses to the most and least effective stimuli averaged 5.5. The most selective neurons responded exclusively to the pictures of faces. Neuronal activity sometimes continued after stimulus offset. No neurons responded on the basis of novelty or familiarity, indicating that stimulus selectivity in these cells was not related to visual recognition processes. These results suggest that neurons in the inferior convexity cortex process attributes of stimuli rather than their location in contradistinction to neurons situated more dorsally. Supported by MH44866 & MH 38546.

297.3

NEURONS IN THE PREFRONTAL CORTEX OF THE MACAQUE SELECTIVE FOR FACES. J.P Skelly*, F.A.W. Wilson, and P. S. Goldman-Rakic, Section of Neurobiology, Yale University School of Medicine, New Haven, CT, 06510.

It has been well established in other laboratories that a population of cells in inferior temporal cortex (IT) respond selectively to the presentation of pictures of faces. We have discovered that a similar population of neurons exist in a region of prefrontal cortex (PFC) which receives a direct projection from IT. We examined the responses of cells in PFC to the presentation of pictures of faces as well as to a variety of other stimuli. Using the scleral search coil technique animals were required to fixate a central fixation point for 0.5 sec which was then followed by presentation of a visual stimulus for 1.0 sec in the center of a video monitor.

We have found 9 out of 130 cells that responded selectively to visual stimuli responded specifically to the presentation of a subset of the face stimuli and showed little or no response to light spots, oriented bars, colored pattern stimuli, or a large variety of pictures of junk objects. The ratio of response magnitude to the best face vs. the best nonface ranged between 2.3 and 4.8 (mean = 3.7). Face selective cells were not randomly distributed throughout PFC, but were clustered on certain penetrations. On the basis both of sulcal patterns encountered on electrode penetrations compared with a stereotaxic atlas and comparison of X-rays of the electrodes with bony landmarks these face selective neurons appear to be within the IT recipient zone of PFC.

Given the lack of evidence for sensory or perceptual deficits in vision after prefrontal cortex damage it is unlikely that the face selective cells in PFC are involved in discriminating between faces. Rather, it seems probable that face discrimination is mediated by IT and that this information is accessed by PFC to enable cognitive processes such as working memory for faces. Preliminary results using faces as memoranda indicate that some PFC cells show face specific memory activity. (Supported by MH44866 and MH38546)

297.4

MONKEY ERPS DURING PASSIVE AND ACTIVE PROCESSING OF FACES. C. Nava, D. Swick* and J. A. Pineda. Department of Cognitive Science, University of California, San Diego, La Jolla, CA 92093.

Results from human studies indicate that a familiar face can activate "semantic" associations in terms of N400-like potentials elicited upon presentation of a face that is incompatible with a previously presented one. Face recognition plays a role in the ability of non-human primates to derive meaning from social communication and thus should result in N400-like potentials. To test this hypothesis, epidural ERPs were recorded in a priming paradigm in 1 juvenile and 2 adult macaque monkeys (Macaca fascicularis). Subjects viewed pairs of faces tachistoscopically-presented in four categories: human-human, monkey-monkey, monkey-human, human-monkey. One adult subject was trained to associate the occurrence of a monkey-monkey pair with a reward. In the passive condition, adult monkey ERPs exhibited a P1-N1a-N1b-P2 complex during the initial 400 ms poststimulus interval, and a long-duration negativity (LDN) during the last 400 ms of the 1400 ms epoch. Between these components, a prominent negativity occurred in response to monkey faces. This N400-like negativity was earlier in latency, larger in amplitude, and larger over left hemisphere sites to monkey than to human faces. It was also sensitive to the priming stimulus, exhibiting larger amplitudes when the prime category (S1) matched the target (S2) than when it did not. Thus, monkey-monkey responses exhibited larger amplitude potentials compared to human-monkey responses. Juvenile ERPs differed from adults in having no N1a peak while N400-like peaks were not as broadly distributed. Juvenile N400-like potentials were similar to the adult responses in their sensitivity to monkey faces and in their lateralization to left hemisphere sites. Active monkey ERPs differed primarily in the occurrence of an N2-P3 complex. P3 was larger in both amplitude and area, delayed in latency, and slightly larger over right hemisphere in response to monkey faces. P3 responses to matches were typically larger than to mismatches. These data suggest that monkey ERPs reflects both passive and active processing of faces and that N400-like negativities are sensitive to the processing of conspecifics, to priming, and primarily occur over the left hemisphere. Active processing of faces results in P3-like components that may reflect the novel quality of the stimulus. The different hemispheric origins of these active and passive potentials suggest that they do not share equivalent neural sources. (AFOSR 90-0325, McDonnell-Pew Center grant)

297.5

MONKEY P300 IN A MULTIMODAL "ODDBALL" PARADIGM: PHARMACOLOGICAL EVIDENCE FOR MULTIPLE NEURAL SOURCES. M. Westerfield, and J. A. Pineda*. Department of Cognitive Science, University of California, San Diego, La Jolla, CA 92093

Although considerable research has delimited the antecedent conditions necessary for P300 generation, it has not provided sufficient constraints to limit the number of functional theories. Some studies suggest that widely-distributed neurotransmitter systems, such as the noradrenergic-locus coeruleus (LC), could provide functional links among discrete neural sources (Pineda et al., 1989; Pineda et al., 1991). Two experiments examined the effects of the alpha-2 adrenergic antagonist L657,743 on free-running EEG activity and on ERPs to auditory and visual stimuli. In the first experiment, EEG from 4 squirrel monkeys (Saimiri sciureus) was recorded at the vertex before and after the administration of a saline placebo or two doses of the drug (0.01, 0.05 mg/kg IM). Only the 0.05 mg/kg dose produced a statistically reliable increase in beta-2 mean absolute power compared to placebo. In the second experiment, ERPs were recorded in the same subjects from an array of epidural electrodes during an 80-10-10 passive oddball paradigm. ERPs were collected before and after the administration of a placebo or three doses of the drug (0.01, 0.03, 0.05 mg/kg IM). The paradigm consisted of a repetitive background tone, a different pitch tone as the auditory oddball (AO), and a small yellow rectangle as the visual oddball (VO) event. Both oddball stimuli elicited large P300-like potentials distributed throughout the brain, with maxima at centro-parietal midline and lateral parietal sites. Modality-specific distributions were observed for the probability effect (the difference between responses to background and oddball) and for sequential dependencies. Drug effects on auditory and visual P3s were region- and modality-specific, suggesting the existence of three distinct sources located in midline, temporal, and parietal brain areas. The magnitudes of auditory and visual P300s were significantly reduced at parietal sites, while not changed or slightly enhanced at temporal electrodes. Midline sources appeared to be orthogonally modulated by these manipulations, with auditory P300s significantly reduced, while visual P300s were not affected, and in fact appeared to be enhanced. The results suggest that monkey auditory and visual P300s have shared and independent neural generators. These putative cortical sources differ in terms of their responsiveness to noradrenergic manipulations and in the direction of the modulation. (AFOSR 90-0325, McDonnell-Pew Center grant)

297.6

VISUAL AND MNEMONIC RESPONSES OF TE UNITS STIMULATED VIA TRANSCOMMISSURAL, FEEDBACK AND DIRECT PATHWAYS. S Sobotka* & JL Ringo, Physiol, U Rochester.

We recorded single units in inferotemporal cortex of monkeys working on a visual memory task. The optic chiasm and corpus callosum were cut, limiting interhemispheric (and interocular) information flow to the anterior commissure (AC). By recording in posterior IT (12 mm caudal to the anterior commissure) and by limiting input to the contralateral eye, stimulus driven activity was funneled across the AC and then caudally through a feedback step. By recording in anterior IT (in the regions innervated by the AC) activity was funneled across the AC, without a subsequent, requisite feedback. These responses were compared to those recorded when input was limited to the ipsilateral eye (direct input). For those units which were well driven through feedback and direct pathways (n=103), responses to various stimuli presented through the two pathways show significant correlation. Similarly, good correlation was found between responses from transcommissural (only) and direct pathways.

A trend toward a decrement-with-repetition (habituation) effect with repeated stimuli was seen in 71% of cells. Interestingly, when the eye of presentation (hence the pathway for the activity) was switched following initial presentations, much of the habituation disappeared. The responses recovered (on average) 54% of the reduction from the previous habituation.

297.7

UNIT DISCHARGE IN MONKEY'S PARIETAL CORTEX DURING PERCEPTION AND MNEMONIC RETENTION OF TACTILE FEATURES. Yong-Di Zhou and Joaquín M. Fuster*. Department of Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Parietal unit activity is recorded from monkeys trained to perform a haptic delayed matching-to-sample task with objects (rods) of identical dimensions but different surface features (direction of parallel ridges or texture). The task is performed in a fully automated apparatus, with all events electronically recorded, including hand contact with objects; the performing hand rests on a fixed pedal between trials. A trial consists of : (1) click signalling that the sample object, a vertical rod (diameter 19 mm), is in position for touch; (2) reaching and grasping the rod to feel its surface; (3) hand return to pedal; (4) delay of 14-20 sec (memorization period); (5) second sound signalling accessibility of two rods side by side (relative position changing randomly between trials); (6) choice (pull) of the rod matching the sample; and (7) fluid reward if match is correct. Data reported here are from 871 units in one monkey, about 1/3 of them in hand areas and adjacent associative cortex (area 5a). Almost all units showed changes of discharge in relation to some aspect of the task. A number of cells reacted to the click with short latency (in some cases <20 ms). Most click-activated cells were also activated by other task events (e.g., arm projection, sample touch). Cells activated by sample touch were common in hand areas; many had large receptive fields covering parts of more than one finger. Some cells differentiated sample rods by their features. Twenty-two per cent of all units showed sustained activation during retention (delay), while 14% showed sustained inhibition. In that period, 27 units, most of them in hand areas, fired differentially depending on the feature in active memory. The findings indicate participation of parietal neurons in haptic perception and temporary retention of tactile features. Hand areas are apparently not only involved in tactile discrimination but in active short-term tactile memory. Cells responding to task events of different modality may belong to distributed cortical networks accessible to the various associated inputs that the animal uses for integration of behavior.

297.8

ASSOCIATIVE CODING OF PLACE RELATED NEURONS *IN* MONKEY HIPPOCAMPAL FORMATION.
T. Ono*, S. Eifuku, H. Nishijo, K. Nakamura, and E. Tabuchi
Dept. Physiol., Fac. Med., Toyama Med. & Pharmaceu. Univ., Toyama 930-01, Japan.

We have previously recorded the place-related single unit activities from monkey hippocampal formation (HF) during the spatial moving task which increased firing specifically when the animals were in a specific place. In this study, we analyzed further the relation between place-related and task-related activities during the tasks. Of 238 HF neurons we recorded, 110 neurons had task-related activities and 79 had place-related, including 33 place-related neurons with task-related activity and 46 place-related neurons without task-related activity. Subsequently fourteen place-related neurons were also recorded in the condition the experimenter control the moving cab without imposing any operant task on the monkey. The place-related activities of 12 HF neurons turn to lose specificity to the place. These findings suggest associative coding in HF neurons between the specific behavior and the location of the animal.

297.9

WHOLE-BODY MOTION CELLS IN THE PRIMATE HIPPOCAMPUS
S.M.O'Mara', E.T.Rolls, A.Berthoz and V.Walsh Univ. Oxford, Dept. Exptl. Psychol., Oxford OX1 3UD, England.

In previous work on the role of the primate hippocampus in spatial processing and memory we have shown that some single hippocampal and parahippocampal gyrus neurons respond to views of the environment while a monkey is moved on a remote controlled robot-mounted platform in a cue-controlled test chamber (2m x 2m x 2m). Their activity did not occur in relation to the place where the monkey was located, but according to the place where the monkey looked (Rolls and O'Mara, 1992, Neurophysiological and theoretical analysis of how the hippocampus functions in memory. In Brain Mechanisms of Perception: From Neuron to Behavior, eds. T.Ono et al. Oxford University Press: New York). We describe here other hippocampal cells that respond to whole-body motion. Some of these cells responded to linear motion (n=9), and others to axial rotation (n=7). Some of these cells responded when the same motion occurred without a view of the visual field. These cells appeared to be driven by vestibular inputs. Other cells required a view of the visual field for their response. These cells appeared to be driven by optic flow. Further evidence that these latter cells were driven by optic flow is that some of them responded to rotation and linear motion of the test chamber while the monkey remained stationary.

These findings show that information about where the animal is looking, and about whole-body motion, is represented in the primate hippocampus. It is likely that this information is important in spatial memory and thus in spatial navigation (Rolls and O'Mara, 1992).

297.10

SPATIAL REFERENCE FRAMES OF HIPPOCAMPAL PLACE CELLS IN RATS. S.I. Wiener , R. Garcia and A. Berthoz. CNRS UPR 2, 75270 Paris & CNRS URA 339, Univ. Bordeaux I, Talence, FRANCE.

Hippocampal CA1 and CA3 pyramidal cells of the rat discharge when the rat occupies a small region (the "firing field") within a maze or an open field. Experimental displacements of salient environmental cues produce corresponding shifts in the location of the firing field. This supports the view that the spatially selective firing, and presumably the rat's knowledge of his position, is determined by the relations between an ensemble of distal cues or alternatively by the "local views" available at any given location. In contrast, internally generated information from the vestibular or kinesthetic sensory systems would not be useful in these formulations.

To test for the contributions of self-generated (and thus egocentrically referred) information on firing fields, hippocampal units were recorded extracellularly with fine-wire electrodes in 4 Long-Evans rats in a darkened open field (a 60 cm sided box). Each of the 4 corners and the center of the arena were alternately baited with water. One corner was distinguished as being the first corner to contain a reward and by having a cue card that was lit there during the remaining 3 corner visits. Periodically, the arena was rotated with the rat inside. However, the corner associated with the first reward and the lit cue card remained in its original position in the external (geomagnetic) reference frame.

In 19/19 analysed units, the firing field and, when present, directional selectivity rotated along with arena after at least one rotation. However 11/19 of these units also had changes in the reliability of spatial selective firing following other rotations. No firing fields maintained a constant position relative to the cue card; thus the external reference frame was not of principal importance. These results show that the hippocampus receives information regarding both egocentric and allocentric reference frames.

297.11

HIPPOCAMPAL COMPLEX SPIKE CELLS SYSTEMATICALLY SHIFT PHASE RELATIVE TO THE EEG THETA AS THE RAT RUNS THROUGH THE PLACE FIELD. J.O'Keefe* and M.Recce. Dept of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, England.

Complex spike cells in the hippocampus of the freely-moving rat signal the animal's location in an environment. We report the results of experiments on the phase relationship between place cells and the hippocampal theta waves as the rat ran in a straight line through the place field. Rats were trained to run back and forth on linear tracks for food rewards at each end or to run to the goal in a + maze. Each cell fires in a series of bursts in the place field. Typically the firing starts with one spike at the edge of the field, builds to a burst of several spikes in the middle, and tapers off during the exit. The first spike almost invariably occurs at the same fixed point in the theta cycle (e.g. the + peak of the CA1 theta for CA1 cells). On each successive cycle the phase correlate systematically shifts forward in time, the amount of precession varying from one cell to another but never exceeding 360° (or one full theta cycle). The phase correlate improves the spatial resolution of the place cells and suggests major roles for wave interference patterns and phase coding in the spatial functions of the hippocampus.

(Supported by the British Medical Research Council.)

297.12

AFTERDISCHARGE, BUT NOT BILATERAL PERFORANT PATH LTP OR KINDLING, DISRUPTS ACQUISITION OF THE WATER MAZE TASK. D.P. Cain*, E.L. Hargreaves, F. Boon, and Z. Dennison. Dept. Psychology, Univ. Western Ontario, London, N6A 5C2, CANADA.

To evaluate the effect of hippocampal LTP on acquisition of the water maze task, we induced LTP saturation by applying trains of high frequency pulses bilaterally through perforant path (PP) electrodes in rats (n=14) for 14 days. We bilaterally kindled Stage 5 seizures from the PP in other rats (n=5). Other rats (n=5) experienced a single PP-evoked afterdischarge (AD). Other rats (n=5) served as stimulated (1 Hz for 60 sec) or unstimulated (n=8) controls. All water maze training (12 1-min trials in 6 blocks of 2 trials each) began within 10 min after the last treatment. The bilateral LTP group acquired the task as quickly as both control groups. The kindled and single-AD groups were equally impaired on acquisition over the 12 trials. However, a second day of training in the maze in the absence of any treatments showed normal performance by all groups, indicating that it was the effect of the AD and not kindling per se that caused the impairment on Day 1. The severity of the behavioral impairment was related to the severity and duration of the last seizure in the kindled rats (regression coef.=+.91), but performance of the LTP rats was not related to the amount of LTP induced (regression coef.=-.26).

297.13

INACTIVATIONS OF ONE HIPPOCAMPUS (HPC) WITH TTX OR LIDOCAINE: LATERALIZATION AND INTERHEMISPHERIC TRANSFER (IHT) OF PLACE LEARNING IN RATS. A. Fenton and J. Bures. (SPONSORED). Inst. Physiol., Czechoslovak Ac. Sci., Prague 14220

Functional hemidecortication has been used to study IHT of visual discriminations. To use this approach in spatial memory studies, blockade of one HPC was produced by injecting 5 ng TTX into one dorsal HPC of chronically cannulated rats (n=20) before binocular training in the Morris water maze. Retrieval of the same task was then tested during block of either the ipsi- (I) or contralateral (C) HPC. Based on escape latencies (s) at the end of acquisition (A), and in I or C retrieval, lateralization absent after the first 4-trial block (A=49, I=40, C=44), and marginal after limited training (9 blocks) leading to an efficient search strategy (A=6, I=9, C=14) was complete in rats overtrained (15 blocks) to asymptotic target oriented navigation (A=4, I=4, C=12). Poor lateralization of place learning's early stages suggests their extrahippocampal basis. Shorter-acting lidocaine (1 ul, 4%) was similarly applied to investigate IHT of the place engram. 24 pretrained rats monocularly learned a new task during simultaneous lidocaine block of the HPC and visual cortex opposite the closed eye. Intact untrained eye retrieval, poor (30) on trial 1, improved to the trained eye performance (7) by trial 4. It is concluded that IHT is mediated by readout of the primary trace or by formation of the secondary engram in the untrained hemisphere.

297.14

THE EFFECTS OF HIPPOCAMPAL, GLOBUS PALLIDAL, AND BASAL NUCLEUS OF MEYNERT LESIONS ON SPATIAL MEMORY. K.E. Armstrong* and L.H. Hicks. Psychology Department. Howard University, Washington, D.C. 20059

An animal model was designed to compare the roles of the hippocampus (HP), globus pallidus (GP), and basal nucleus of Meynert (BNM) in spatial memory. The specific aim was to determine whether deficits due to HP, GP, or BNM lesions were dependent on the interaction between lesion location and task complexity. One-hundred and twenty male Sprague Dawley rats were selected for retention testing. First, they were randomly assigned to either sham operated control groups or one of three experimental groups (HP, GP, or BNM) and then tested on one of the following maze task: T-Maze task (TM), Complex Maze task (CM), or Both T-Maze and Complex Maze task (BTCM). The results showed that the ability to remember was dependent on the location of the lesion as well as the complexity of the task. The TM task was too simple to distinguish the groups. The CM task showed that the GP and BNM groups were more impaired than was the HP group. However, on the BTCM task, the HP group showed transient deficits in recall; whereas, both the GP and BNM groups showed persistent deficits in recall.

297.15

EFFECTS OF IBOTENATE ENTORHINAL CORTEX LESIONS ON A DELAYED NON-MATCHING TO PLACE TASK IN MICE. Y. H. Cho and R. Jaffard*. Laboratoire de Neurosciences Comportementales et Cognitives, CNRS URA n° 339, Univ. Bordeaux I, Avenues des Facultés, 33405 Talence, France.

The present study examined the effects of ibotenate lesions of the entorhinal cortex (EC) in mice on a delayed non-matching to place task (DNMTP), varying in the level of difficulty according to the number of interpolated arm visits between sample place presentation and subsequent recognition in a radial maze. *Preoperatively non-trained* mice were trained on the non-matching rule (P1: 1 interpolating visit: IV) until attaining the criterion of 85% correct responses. They were then submitted to memory testing at first, in a *progressive manner* ; P3 (3 IV) and then P5 (5 IV) including interspersed rule trials within each daily memory testing session, and then in a randomly *mixed fashion*.

Behavioral results indicated that naïve EC-lesioned mice require *three times as many sessions* as sham-operated controls to learn the rule. Subsequently, these experimental subjects performed significantly worse than controls in difficult (P3 and P5), but not simple rule problems during both the progressive and mixed procedures. Furthermore, when re-tested *1 month later* using the mixed procedure, EC-lesioned animals did not exibit *any signs of functional recovery*.

These results demonstrated that EC-lesions not only impair the acquisition of the DNMTP task, but also induce difficulty (occupied delay)-dependent memory deficits (accelerated rate of forgetting). This data will be compared to results obtained by subjects trained on the task prior to EC-lesions. Supported by CNRS URA 339.

297.16

SPATIAL, MOVEMENT- AND REWARD-SENSITIVE DISCHARGE BY MEDIAL NUCLEUS ACCUMBENS. A.M. Lavoie* & S.J.Y. Mizumori. Dept. of Psychology, Univ. of Utah, Salt Lake City, Utah 84112

It has been suggested that the nucleus accumbens (ACC) contributes to the integration of motivational aspects of a situation (i.e. reward) with appropriate motor outputs (Br. Res. Bull. 27:463-468,1991). Given that the ACC receives both hippocampal subicular and VTA afferents, we hypothesized that the ACC uses information from the hippocampus to direct future behavior. Specifically, ACC neurons may code information relevant to learned reward magnitude and/or motor responses as they relate to spatial/temporal aspects of behavior. This hypothesis was evaluated by recording single unit activity of rats (n=5) as they performed 8 trials daily on an 8 arm radial maze; alternate arms were baited with small or large reward.

Of the 36 cells recorded, 44% demonstrated firing which varied as a function of specific maze related behaviors and/or the location of the animal on the maze. Many cells fired in response to particular motor behaviors such as stopping, turning or directional locomotion. 30% of these cells were also modulated at the theta frequency (7-10 Hz). All of the spatial firing biases consisted of directional firing in one to three maze locations; these cells were usually quiescent if the animal remained still within the field.

Other behavioral correlates were associated with reinforcement. 14% of the cells fired in relation to reward encounter; two cells fired in anticipation of reward. Additionally, 8% of the cells fired at higher rates while approaching small versus large rewards.

Thus far, these data suggest that the accumbens is a candidate structure for integrating spatial information and reward significance with motor output.

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297.17

INTERDEPENDENCE OF HIPPOCAMPAL AND LATERAL DORSAL THALAMIC REPRESENTATIONS OF SPACE. S. J. Y. Mizumori* and J. D. Williams, Dept. Psychology, Univ. Utah, Salt Lake City, UT 84112

The lateral dorsal nucleus of the thalamus (LDN) may facilitate visuo-spatial learning by providing limbic cortex with information regarding the animal's current view (Mizumori & Williams, *Soc. Neurosci. Abstr.* 1991). The relationship between LDN and limbic cortex was evaluated by 1) comparing the dynamic properties of neural representations for place or head-direction in hippocampus or LDN respectively, and 2) observing the effects of LDN inactivation on hippocampal function.

Single unit activity was recorded in Exp. 1 as rats performed 15 spatial memory trials on a radial maze. LDN head-direction cells required at least 1 min of exposure to a lit room before stable directional firing was observed. In contrast, about 47% of hippocampal cells exhibited stable place fields after only 30 sec of such exposure. On other days, rats first performed trials 1-5 under lighted conditions. During trials 6-10 (performed in darkness), hippocampal place fields remained intact for about 5.5 min while LDN directional firing became disrupted after 3.5 min. In Exp. 2 (n=3 rats), tetracaine-induced LDN inactivation impaired spatial maze performance and disrupted hippocampal place field activity. This result is consistent with a previous finding that the specificity of LDN directional firing is correlated with improved choice accuracy. Together, these data suggest that hippocampal place cells depend on LDN head direction information for normal function. Specifically, it is hypothesized that feedback from hippocampus modulates visual information processing such that LDN directional representations are more highly resolved. This, in turn, could result in more precise spatial representations by hippocampus and improved spatial performance.
[Supported by NSF Grant 9120784]

297.18

HEAD-DIRECTION CELL ACTIVITY MONITORED IN A NOVEL ENVIRONMENT AND CONFLICT SITUATION. H.L. Burton* and J.S. Taube. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Neurons have been identified in the rat brain which discharge as a function of the animal's head direction. The nature of this spatial signal would suggest that the head direction cells are ideally suited for serving as part of an animal's navigational system. Two distinct navigational systems are known to be used by mammals: taking-a-positional fix and dead-reckoning. This study was designed to determine how animals use head direction cells for navigation. Head direction cell activity was monitored as an animal entered either 1) a novel environment or 2) an environment in which a conflict situation was set-up in relation to the established orientation cues.

Head direction cells were recorded in the postsubiculum and anterior thalamus of Long-Evans rats in a cylinder containing a single orientation cue. Following an 8 min session in the cylinder, a door was opened and the rat entered a U-shaped alleyway leading to a similarly cued rectangle. For most cells, the preferred firing direction remained constant between the cylinder and rectangle. In one case the preferred direction shifted 45-60° when the animal entered the alleyway, and remained shifted throughout the experiment, even when the animal returned to the cylinder. In the next session, with the animal out of view, the orientation cue in the cylinder was rotated 90° and the animal reintroduced into the cylinder. As expected, the cell's preferred firing direction shifted 90°. The rat was then permitted to walk back via the alleyway into the now-familiar rectangle. Immediately upon entering the alleyway, the preferred direction shifted back to its original orientation and remained the same in the rectangle. There did not appear to be any change in the cell's peak firing rate. When the rat was allowed to walk back and forth between the two environments, the cell's preferred firing direction was linked with the orientation of the cue for the corresponding environment. These results suggest that head direction cells may be involved in both the dead-reckoning and the taking-a-positional fix systems.

297.19

QUANTITATIVE ANALYSIS OF HEAD-DIRECTION CELLS RECORDED IN THE RAT ANTERIOR THALAMUS. J.S. Taube*. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Previous studies have identified neurons in the subicular complex which discharge as a function of the animal's head direction, independent of its behavior and location in the environment. This study was designed to determine how the head direction signal gets processed. Neurons in the subicular complex have reciprocal connections with the anterior thalamus. Although several studies have determined the behavioral correlates of neurons in the anterior thalamus, little is known about the possible spatial correlates of these neurons in the freely moving animal.

Long-Evans rats were trained to retrieve food pellets thrown randomly into a cylindrical apparatus containing a single orientation cue. Animals were then implanted with recording electrodes directed toward the anterior thalamus. Single cell recordings showed that 15 out of 35 cells discharged in relation to the animal's head direction in the horizontal plane, independent of the animal's behavior, trunk position, or location. For each head direction cell, there was only one head direction at which the cell discharged maximally. The preferred firing directions for these 15 cells were distributed equally over a 360° range. For 5 head direction cells, an automated video-computer system tracked the animal's head direction while simultaneously monitoring the neuron's firing rate. Quantitative analysis of these 5 cells showed that the mean peak firing rate was 42 spikes/sec (range: 15-118) and the mean directional firing range was 112° (range: 106-118°). These results indicate that the discharge properties of head direction cells in the thalamus are similar to the properties of postsubicular head direction cells.

297.20

PHOTOPERIODIC REGULATION OF TESTIS SIZE AND SPATIAL MEMORY FUNCTION IN THE ALBINO RAT: ORGANIZATIONAL AND ACTIVATIONAL EFFECTS. L. Davachi[1], C.L. Williams[1], S.C. Hinton[2] & W.H. Meck[2]*. Depts. of Psych., Barnard College[1] & Columbia Univ.[2], New York, NY 10027.

Male and female Sprague-Dawley rats were raised from conception in either short daylengths (SD = 8 h of light per day, 8L:16D) or long daylengths (LD = 16 h of light per day, 16L:8D). At 90 days all rats were gonadectomized and 2 weeks later training on a 12-arm radial maze with 8 baited and 4 unbaited arms began. During acquisition (Days 1-15) LD rats made significantly fewer working and reference memory errors than SD rats. Although significant differences in choice accuracy were not observed during steady-state performance (Days 16-30), LD and SD rats still differed in the response strategies used to solve the task. For example, during the last 15 days of training both sex and photoperiod influenced the magnitude and direction of rats' turning biases. LD rats were significantly more lateralized than SD rats, with LD females preferring to turn right and LD males preferring to turn left on exiting from a maze arm. Choice latency did not differ between LD and SD rats although it was predictive of choice accuracy for LD rats. Interestingly, for SD males testes size and body weight showed a significant positive correlation and both of these measures were negatively correlated with choice accuracy. In contrast, the correlation between testes size and body weight was non-significant for LD males, while testes size, but not body weight, was significantly correlated with choice accuracy. These data suggest that both spatial memory and reproductive function are subject to combined organizational and activational effects of seasonal fluctuations in daylength.

297.21

EFFECT OF DIABETES ON SPATIAL LEARNING AND MEMORY IN MICE. P.J. Riley* and N.E.Rowland. Dept Psychology, Univ of Florida, Gainesville, FL 32611.

Diabetes mellitus induces structural and functional changes in the central nervous system that may cause dysfunction in complex cognitive tasks. Simple shock-avoidance tasks have failed to show large impairments in either learning or retention in diabetic rodents. In the present study, we employ the harder task of spatial navigation to screen for impairments in diabetic mice.

Diabetes was induced by injection of streptozotocin in male CD-1 mice; controls received injection of vehicle. They were tested in a Morris water maze (with invisible underwater platform, requiring use of extra-maze cues) 6 and 14 weeks later. At each time, mice were given 10 trials per day for six consecutive days and videotaped measures of platform (escape) latency, distance swam, and time in the platform quadrant were determined. At 6 weeks, diabetic mice (mean blood glucose >4.0 g/l) showed no difference from nondiabetic controls in any of the measures of acquisition and retention of the task. At 14 weeks, the diabetic mice showed a slower escape latency, but no changes in either distance or quadrant time measures. Maze retention on week 14 was similar in the two groups. Diabetic mice are impaired only on time-dependent measures of neuropsychologic function.

298.1

Effects of excitotoxic lesions in ventral tegmental area, accumbens, ventral pallidum circuit on rewarding brain stimulation of the lateral hypothalamus in rats. P. Johnson*, P. Tehrany, and J.R. Stellar. Dept of Psychology, Northeastern University, Boston, MA 02115.

The ventral tegmental area [VTA], accumbens [Acc], and ventral pallidum [VP] form a circuit that is critical for the expression of dopamine associated behavioral activation and cocaine self-administration. Using the rate-frequency curve-shift method and testing at multiple currents, the reward threshold and motor/performance capacity following ipsilateral VTA and bilateral VP excitotoxin lesions were assessed. Surprisingly small reward degrading effects were observed two weeks post lesion when the region of cell death was restricted to VTA. Hypothalamic stimulation reward was diminished, however, in a few cases where the VTA lesion spread into the Substantia Nigra pars compacta. The bilateral VP excitotoxin lesions were also unsuccessful in producing large reward impairments, but did produce severe reductions in motor/performance capacity. Results are discussed in terms of a mediating vs. modulating role for dopamine in the expression of reward effects originating from sources outside the dopamine system.

298.2

COACTIVATION OF THE LATERAL HYPOTHALAMUS AND VENTRAL TEGMENTAL AREA YIELDS GREATER REWARD SUMMATION THAN COACTIVATION OF THE LATERAL HYPOTHALAMUS AND MEDIAL PRE-FRONTAL CORTEX. K.L. Conover*& P. Shizgal. CSBN, Dept. Psychol., Concordia U., Montreal, Quebec, Canada H3G 1M8.

Rats were offered a choice between a train of rewarding brain stimulation that was held constant within test sessions (the "standard") and a train that varied in current or frequency from trial to trial. The currents delivered through electrodes in the ventral tegmental area (VTA) and medial prefrontal cortex (MPFC) were varied to obtain trains that were equally preferred to a standard lateral hypothalamic (LH) train when all sites were stimulated at the same frequency. Summation of rewarding effects was measured by determining the frequency of LH stimulation that was equally preferred to a standard train consisting of concurrent (paired pulse) stimulation of either the VTA and LH or the MPFC and LH. Although paired pulse stimulation of either the VTA and LH or the MPFC and LH was more rewarding than stimulation of the LH alone, the rewarding effects of stimulating the VTA and LH summated more effectively than the rewarding effects of stimulating the MPFC and LH. This finding is consistent with the notion that the substrates for self-stimulation of the medial forebrain bundle and the MPFC are neither identical nor independent.

298.3

THRESHOLD FOR REWARDING VTA STIMULATION IS LOWERED TWENTY-FOUR HOURS AFTER INTRA-PREFRONTAL CORTEX MICROINJECTIONS OF THE D-1 ANTAGONIST SCH 23390. C.L. Duvauchelle* and C. Kornetsky. Boston Univ. School of Med., Boston, MA 02118.

A number of studies have shown that manipulation of dopamine systems in the medial prefrontal cortex (mPFC) can result in changes in the activity or function of subcortical dopamine neurons. In the present study, a rate-free method of determining threshold for rewarding brain stimulation was utilized to test for effects of an mPFC-applied D-1 antagonist. Animals were given bilateral microinjections of SCH 23390 (0.0, 0.125, 0.25, 0.375 and 0.5 ug/.5 ul) into the mPFC and brain stimulation thresholds were then tested immediately and 24 hours later. Although there was no effect on reward threshold immediately following the D1 antagonist, animals receiving SCH 23390 (.125 ug/.5 ul) 24 hours prior to testing demonstrated significantly lower thresholds. Since no trace of the DA antagonist is seen 3 hours after brain injection (Vezina et al, Soc. Neurosci. Mtg, 1990), this increase in the animal's sensitivity suggests the SCH 23390 treatment causes a change in the reward system that persists even when the drug is no longer present in the brain. (Supported in part by NIDA grant DA02326 and Research Scientist Award DA00099 to CK).

298.4

REWARD-RELEVANT NEURONS DIRECTLY LINK SELF-STIMULATION SITES IN THE ANTERIOR LATERAL HYPOTHALAMUS AND VENTRAL TEGMENTAL AREA. B. Murray* and P. Shizgal. CSBN, Dept. Psychol., Concordia U., Montreal, Quebec, Canada H3G 1M8.

Previous studies employing a behavioural version of the collision technique suggest that reward-relevant neurons directly link self-stimulation sites in the lateral hypothalamus (LH) and ventral tegmental area (VTA). In the present study, we asked whether this direct link extends into more anterior LH sites where electrolytic lesions degrade the rewarding effect of posterior LH and VTA stimulation. Six rats with electrodes in the anterior LH (ALH) and VTA served as subjects. Stimulation consisted of trains of paired conditioning (C) and test (T) pulses, with each electrode receiving one pulse from each pair. Collision was inferred when the rewarding effectiveness of the stimulation increased with C-T interval, and did so in the same manner regardless of whether the LH or VTA electrode delivered the C-pulses. In 4 of the 6 subjects, we found that rewarding effectiveness was lower at short C-T intervals (.4 msec) than at longer C-T intervals (6.4 or 12.8 msec), supporting the notion that some reward-relevant neurons directly link the ALH and VTA. The increase in rewarding effectiveness with C-T interval was smaller and more gradual than previously reported for more posterior LH and VTA self-stimulation sites.

298.5

BRAIN STIMULATION REWARD IN THE VENTRAL TEGMENTAL AREA INCREASES FOS EXPRESSION IN THE RAT BRAIN. D.F. Fiorino[1], G.S. Robertson[2], A.G. Phillips[1], H.C. Fibiger[2], and N. Swindale[3]*. [1]Dept. of Psychology, [2]Div. of Neurological Sciences, Dept. of Psychiatry, and [3]Dept. of Anatomy, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4.

Fos immunohistochemistry was used to map brain loci activated by rewarding electrical stimulation of the ventral tegmental area (VTA). Long Evans rats implanted with bipolar stimulating electrodes aimed at the VTA were tested for self-stimulation until stable rate-intensity curves were obtained. Rats displaying robust self-stimulation behavior were divided into three groups: 1) self-stimulators (SS), 2) yoked stimulators (YOKED), and 3) unstimulated controls. SS and YOKED animals received electrical stimulation (22uA, 60 Hz sine wave) for 30 minutes and were sacrificed 90 minutes later. Fos immunohistochemical procedures were conducted on fixed brain sections.

Electrical stimulation of the VTA increased Fos immunoreactivity in many areas which included the nucleus accumbens, cingulate cortex, portions of the amygdala, olfactory tubercle, lateral habenula, mediodorsal thalamus, and the VTA. The positive results in the YOKED group suggest that the pattern of activation observed here may be due in large part to activation of neural circuits underlying brain stimulation reward rather than motor systems mediating vigorous operant responding. This hypothesis will be tested further in experiments where the mesocorticolimbic reward pathway is lesioned.

298.6

REWARDING BRAIN STIMULATION INDUCTION OF FOS: PARALLEL BRAIN REGIONS ACTIVATED BY COCAINE. J. Buggy *, Z. Ying, A. Singha, and J. Valentine. Depts. of Physiology and Psychology, University of South Carolina, Columbia, SC 29208.

The *c-fos* immediate early gene is acutely induced in many brain regions by relevant physiological or pharmacological stimuli. Whereas the phosphoprotein Fos acts in the cell nucleus to regulate gene transcription, Fos immunoreactivity (Fos-IR) presents a useful mapping technique to identify activated neuronal systems. Fos-IR was determined in rats after rewarding brain stimulation self-administered to the ventral tegmentum area, origin of the dopaminergic mesolimbocortical neurons. After a 1 hr brain stimulation session, Fos-IR was observed in the ventral tegmental area and, as observed after cocaine administration, in numerous terminal fields of the mesolimbocortical dopaminergic system. Fos-IR was particularly dense in the medial prefrontal and cingulate cortex, olfactory tubercle, nucleus accumbens, lateral septum, bed nucleus of the stria terminalis, striatum, habenula, and amygdala. Fos-IR was usually present bilaterally although the number of labeled neurons was typically greater ipsilateral to the stimulation, especially in the prefrontal cortex, nucleus accumbens, striatum, and amygdala. Similar to reduced Fos-IR with repeated administration of cocaine, there was a reduction in Fos-IR in animals with rewarding brain stimulation sessions repeated daily for one week or longer.

298.7

DOPAMINE IN VENTROLATERAL STRIATUM MODULATES THE REWARD VALUE OF HYPOTHALAMIC STIMULATION.

S. E. Romer and D. B. Neill*. Dept. of Psychology, Emory University, Atlanta, GA 30322

It has often been observed that rats performing intracranial self stimulation (ICSS) of the lateral hypothalamus engage in oral behaviors such as intense licking and gnawing. The stimulation may be rewarding because it activates motor programs for feeding and other consummatory behaviors. We hypothesized that the ventrolateral striatum, which has been implicated in feeding, modulates these motor programs and thus modulates the reward of lateral hypothalamic stimulation.

We tested this hypothesis by manipulating dopaminergic transmission in ventrolateral striatum via intrastriatal microinjections of amphetamine or flupenthixol. The behavioral measure was 2-lever autotitration ICSS. The results were that amphetamine evoked resetting at lower current intensities (enhanced reward) and flupenthixol evoked resetting at higher intensities (reduced reward).

These results supported our hypothesis that dopaminergic synapses in ventrolateral striatum modulate the reward of hypothalamic stimulation.

298.8

PARABRACHIAL LESIONS HAVE LITTLE EFFECT ON MFB SELF-STIMULATION.

M. Waraczynski* and P. Shizgal. CSBN, Dept. Psychol., Concordia Univ., Montreal, Quebec H3G 1M8

The notion that parabrachial (PB) neurons may play a role in self-stimulation of the medial forebrain bundle (MFB) is consistent with the fact that the PB region supports self-stimulation and the fact that this region receives projections from and sends projections through the MFB. To test this hypothesis, a PB lesioning electrode and ipsilateral stimulation electrodes aimed at the LH and the ventral tegmental area (VTA) were implanted in male rats. At each stimulation site, rate-frequency curves were collected at each of 2-3 currents. In four rats, substantial damage to the PB region produced little or no increase in the frequency required to maintain half-maximal responding. The observed increases were similar in size to those seen following nearby lesions that spared the PB nucleus. In a fifth rat, a small lesion made through a PB electrode supporting self-stimulation also had minimal effects on self-stimulation of the LH and VTA. These results fail to implicate PB neurons in the rewarding effects of stimulating the LH and VTA.

298.9

EFFECTS OF AVERSIVE PAG STIMULATION ON SELF-STIMULATION AND SELF-ADMINISTRATION OF BRAIN STIMULATION.

M. Lepore* and K.B.J. Franklin. Dept. Psychology, McGill University, Montreal, PQ, Canada H3A 1B1.

Brain stimulation and drugs have both rewarding and aversive effects which may be simultaneous or sequential. Aversive effects are believed to limit the rate of responding and influence the rate of acquisition and extinction of responding reinforced by brain stimulation or drugs. We have used a computer to independently modulate the pulse frequency of brain stimulation delivered separately or simultaneously to a primarily rewarding site (MFB) and a primarily aversive site (PAG). By varying the parameters governing the increase and decrease of pulse frequency at the two sites, we explored the contributions of reward and aversion to the control of responding. It was found that rats subjected to continuous PAG stimulation would respond to self-administer reductions in stimulation pulse frequency, and their performance was similar to when they respond to increase the frequency of stimulation at a rewarding site. When aversive and rewarding stimulation were administered together, the rate of responding for rewarding stimulation was reduced as the pulse frequency of the aversive stimulation train increased. The properties of rewarding and aversive brain stimulation appear to be similar, except for sign.

298.10

Ch5 CHOLINERGIC NEURONS MEDIATE HYPOTHALAMIC REWARDING BRAIN STIMULATION VIA CONNECTIONS WITH A10 DOPAMINE CELLS.

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Microinjections of muscarinic blockers near A10 dopamine cells increase frequency thresholds for lateral hypothalamic rewarding brain stimulation (Kofman & Yeomans, 1989). Ch5 cholinergic neurons of the pedunculopontine nucleus (PPT) are believed to monosynaptically excite these dopamine cells (Bolam et al., 1991; Lacey et al., 1991). Since Ch5 cells are inhibited by muscarinic agonists (Leonard & Llinas, 1988), carbachol was injected unilaterally near medial PPT, where lesions block opiate place preference (Bechara & Van der Kooy, 1990). Carbachol (1-4 ug) raised frequency thresholds by over 400% bilaterally, similar to the effects of muscarinic blockers near A10 dopamine cells. In these same Ch5 sites, scopolamine (100 ug), a muscarinic antagonist, reduced thresholds by 20-80%. Preinjection of scopolamine blocked the carbachol-induced attenuation of reward, and preinjection of carbachol blocked scopolamine-induced hypersensitivity to reward, suggesting common receptors. Gallamine (20 ug), an M2 antagonist, reduced thresholds by 40%. Atropine (60 ug) injections near A10 dopamine cells blocked the facilitation of reward produced by scopolamine in medial PPT. Therefore, Ch5 neurons, via their linkage to A10 dopamine cells are critical for hypothalamic rewarding brain stimulation. (Supported by NSERCC grant to J.Y.)

298.11

INJECTION OF SELECTIVE DOPAMINE ANTAGONISTS INTO THE MEDIAL FOREBRAIN AND BASAL MIDBRAIN SITES OF RATS DURING SELF-STIMULATION.

S. Nakajima*, E. A. Rooney, and G. Tsouluhas. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada.

D1 and D2 dopamine agonists interact with each other in a synergistic manner to facilitate the reinforcing effect of hypothalamic stimulation (Nakajima & O'Regan, 1991). To locate the active sites of the D1 and D2 receptors involved in this function, selective antagonists were injected into the brain while rats were engaged in self-stimulation at a steady rate. SCH 23390, a D1 antagonist, suppressed self-stimulation when injected into the nucleus accumbens, the ventral tegmental area, or the substantia nigra. Raclopride, a D2 antagonist, suppressed responding when injected into the nucleus accumbens and its vicinity, but not all of the accumbens sites were affected by both antagonists. Injection of raclopride into the tegmental and nigral sites did not suppress responding except at the highest dosage tested, and no sign of facilitation was observed at lower dosage levels. Injection into the sites contralateral to the hypothalamic electrode had no effect. These results suggest that the synergistic interaction may take place between D1 and D2 receptors located some distance apart.

298.12

INJECTING THE κ-AGONIST U-50,488H INTO THE VENTRAL TEGMENTAL AREA, NUCLEUS ACCUMBENS OR MEDIAL PREOPTIC AREA DECREASES LOCOMOTOR ACTIVITY.

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Injections of *kappa (k)* agonists into either the cell body or terminal regions of dopamine neurons induce conditioned place aversions (Bals-Kubik et al., 1990 INRC: 11) and decrease male sexual behavior (Leyton & Stewart, 1991 Soc Neurosci Abs 17: 1539). To test whether stimulated central *k* receptors would decrease locomotor activity we injected the *k* agonist U-50,488H through bilateral cannulae aimed at the ventral tegmental area (VTA) (n=5), nucleus accumbens septi (NAS) (n=6) or medial preoptic area (mPOA) (n=5). During 60-min tests, given in counterbalanced order, U-50,488H (0.0, 0.0005, 0.05, 0.5, 5.0 nmol/0.5 µl/side) was co-administered with systemic injections of naloxone (0.0, 2.0 mg/kg, i.p.). At all three injection sites the highest dose of U-50,488H significantly decreased locomotor activity [$p<.05$]; differences between groups were not observed [$p=.8254$]. When the three groups were collapsed together, the two highest doses significantly decreased locomotion [$p<.01$]. U-50,488H-induced decreases in activity were not prevented by co-administered systemic injections of naloxone; naloxone itself significantly decreased locomotion [$p<.01$]. In contrast, we previously observed that intracranial injections of a selective *k* antagonist, nor-binaltorphimine, blocks systemic U-50,488H-induced decreases in locomotor activity (Leyton & Stewart, submitted). These findings suggest that stimulating *k* receptors in the VTA, NAS or mPOA decreases locomotor activity, possibly related to a decreased interest in environmental stimuli.

298.13

REWARD AND REINFORCEMENT DIFFERENTIATED. Estela Marroquin , and Anders Agmo*. Dept. of Psychology, Universidad Anahuac, Mexico City.

It has been proposed that reward (an affective state) and reinforcement (learning) may be experimentally distinguished. Saccharine may be rewarding but not reinforcing, while sucrose is known to have both of these properties. The two sweeteners produce release of dopamine in the nucleus accumbens. It is belived that release of dopamine is associated with reinforcement. If this would be the case, then saccharine and sucrose should be about equally reinforcing. The present studies evaluated this hypothesis. Conditioned place preference: A biased procedure is used (reinforcement in the non prefered compartment). The subjets (male rats) were allowed to drink a 0.1% saccharin solution or 18% sucrose for 15 min, and then transferred to the place preference box. No deprivation was used. Both sweeteners were consumed in similar amounts. Sucrose produced a reliable place preference whereas saccharine was ineffective. Discrimination learning: A straight runway ending in a choice box with two discriminative openings was used. 0.5 ml of the respective solution or plain water were available in the goal box. Ten sessions of five trials each were preformed. Sucrose produced reinforcement (learning) while saccharine was ineffective. These data suggest that reward and reinforcement are distinguishable and that the latter process does not depend exclusively in dopamine release.

298.14

EXAMINATION OF RELATIONS BETWEEN SELF-STIMULATION AND SNIFFING PATHWAYS. S. Ikemoto* and J. Panksepp. Dept. of Psychology, Bowling Green State Univ., Bowling Green, OH 43403.

Sniffing behavior appears to be a good indicator of exploratory and appetitive motivational states in rats. Since electrical self-stimulation of the brain (ESSB) may reflect the activation of such intrinsic processes, the relationship between ESSB and sniffing was examined. In order to identify neural sites involved in sniffing, Experiment 1 examined sites at which electrical stimulation can induce sniffing in the anterior telencephalic area (ATA) and the midbrain-pons [hence, the lower brain stem (LBS)] using anesthetized rats. Sniffing was elicited in many ATA and LBS sites. These sites appear to correspond to reported ESSB sites, but in the LBS, sniffing sites also included non-ESSB sites. Experiment 2 evaluated the relationships of ESSB and sniffing in the medial prefrontal cortex (MPC) and LBS of awake animals. All ESSB sites induced sniffing in MPC and LH-MFB sites. In the LBS, this was usually the case, but there were exceptions (one subject exhibited sniffing but no ESSB, and another exhibited ESSB and jaw movements without snffing). The third experiment examined the effects of lesions in the MPC or LBS on LH-MFB ESSB and sniffing. MPC lesions had no reliable effects on sniffing and ESSB from the LH-MFB. LBS lesions had no apparent effects on sniffing but disrupted ESSB (increasing thresholds and decreasing response rates). In summary, anatomical organizations of ESSB and stimulation-induced sniffing are very similar in the higher areas of the brain, but they can be particularly dissociated in the LBS. In general, sniffing substrates in brain appear to be more widely distributed than ESSB substrates.

298.15

EXAMINING MFB REWARD ANATOMY FROM ICSS PSYCHOPHYSICAL CHANGES IN MULTIPLE ELECTRODES AFTER ELECTROLYTIC LESIONS. D. Garity*, H. Albert, L. Jaehn, J. Nunnally, and J.R. Stellar. Dept. of Psychology, Northeastern University, Boston, MA, 02115.

Building on Gallistel's (JCCP, 92:997-98,1978) observation of the reciprocal relationship between intracranial self-stimulation (ICSS) current and rate-frequency half-maximal thresholds in a single electrode, Shizgal's laboratory (Forgie & Shizgal, in press) has developed a sophisticated technique for revealing the functional anatomy of normal reward-relevant medial forebrain bundle (MFB) fibers with a movable electrode. We extend this technique by using a fixed array of closely spaced electrodes to determine frequency-current (Hz-C) trade-offs before and after small electrolytic MFB lesions. Particular changes in the Hz-C curves after adjacent MFB lesions reveal aspects of alignment between lesion and electrode that may be efficacious in tracing brain stimulation reward-relevant MFB fibers. Also, a technique of implanting an IC-size rotary switch is presented to allow rapid movement between fixed electrodes with simple stimulation equipment.

(Supported by Whitehall Foundation)

298.16

EVIDENCE OF INHIBITORY G PROTEIN-MEDIATION OF STIMULANT AND OPIOID REINFORCEMENT. D.W. Self*, R.T. Mai and L. Stein. Dept. of Pharmacology, College of Medicine, Univ. of California, Irvine, CA 92717.

A substantial literature suggests that stimulant and opioid drugs exert their addictive or reinforcing actions in large part at dopamine D_2, and μ and δ opioid receptors. These receptors utilize pertussis toxin (PTX)-sensitive inhibitory G proteins for signal transduction. We tested whether PTX, injected into a reinforcement-relevant brain region (nucleus accumbens), could antagonize stimulant and opioid reinforcement using the self-administration paradigm. In control rats, bilateral intra-accumbens injections of heat-inactivated PTX (0.1 µg/ul/side) failed to alter the rate or pattern of cocaine (0.75 mg/kg/injection) and heroin (0.03 mg/kg/injection) self-administration. However, the same dose of active PTX produced significant compensatory increases in the self-administration rates of both drugs. The onset of the PTX-induced increases was delayed 5-6 days, consistent with the slow rate of G protein ribosylation and other in vivo measures of PTX effects. Increased self-administration in PTX-treated animals was initially characterized by highly regular, yet shortened interinjection intervals (mostly evident with cocaine), but progressed to burst-like responding over the next several days. Recovery of baseline performance was not observed even after three weeks. Compensatory increases in self-administration suggest that PTX antagonizes both cocaine and heroin reinforcement. Thus, these results support the hypothesis that inhibitory G proteins mediate both stimulant and opioid reinforcement (Self & Stein, Pharmacol. Toxicol. 70:87-94, 1992).

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298.17

DISSOCIATION OF REWARD AND PERFORMANCE CHANGES FOLLOWING CHRONIC TREATMENT WITH HALOPERIDOL.
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Previous studies have reported that chronic administration of the typical neuroleptic, haloperidol, increases rates of responding for rewarding electrical brain stimulation and this effect has been hypothesized to reflect an increase in sensitivity of the reward-relevant neural substrate(s). However, it is not clear whether the facilitation of responding is linked to an increase in the rewarding value of the stimulation and/or to a potentiation of performance following chronic drug treatment. In this experiment, we studied the effects of chronic haloperidol treatment on dorsal raphe self-stimulation using the curve-shift paradigm. Male rats were implanted with a stimulating electrode aimed at the dorsal raphe and trained to lever press for the delivery of 200 msec trains of 0.1 msec pulses of fixed intensity and variable frequency. Once reward thresholds were stable, rats received 20 daily injections of haloperidol (0.5 mg/kg, s.c.) or its vehicle. On day 21, all rats received a similar dose of haloperidol and were tested every day for 28 days, beginning 24 hrs after the last injection. Asymptotic rates of responding and reward thresholds were differentially affected by chronic drug treatment. Asymptotic rates of responding were increased by 30%, an effect that persisted for three weeks before returning to pre-treatment levels. Reward thresholds were elevated for both groups (23%) on the first day of drug withdrawal and returned to pre-treatment levels on day four. The magnitude and time-course of elevation in reward thresholds were the same for acutely- and chronically-treated rats, suggesting that the chronic treatment did not change the sensitivity of the reward-relevant neural substrate(s). These results show that performance only is potentiated following chronic haloperidol treatment, a phenomenon that could explain the increase in rates of responding for brain stimulation reward reported in previous studies.

Supported by a grant (P.P.R.) and a fellowship (S.M.B.) from MRC.

298.18

CHANGE IN ADENYLATE CYCLASE ACTIVITY FOLLOWING CHRONIC REWARDING BRAIN STIMULATION IN THE RAT. E.M. Bedwell*, C.J. Hillard, and E.A. Stein. Departments of Pharmacology and Psychiatry, Medical College of Wisconsin, Milwaukee, WI 53226.

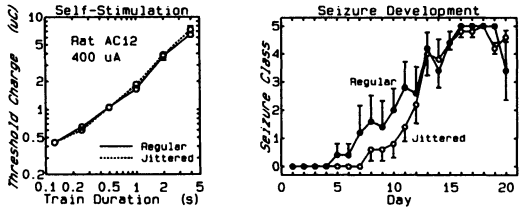
Recent reports of regional brain adenylate cyclase (AC) up-regulation in response to chronic cocaine and morphine raise the question of the role of this system in brain reward mechanisms. While dopamine (DA) is generally accepted as the principal transmitter involved in reinforcement, changes in second messenger systems involved in DA receptor activation are not well-understood. In this study, we report preliminary results of our investigation of the effects of chronic rewarding brain stimulation (RBS) on AC activity in the mesolimbic DA pathway. Rats were trained to respond for RBS from chronic ventral tegmental electrodes at a current set to that producing 80% maximal responding, and were allowed to respond twice/day in 45 min RBS sessions for 10 days. Rats were divided into 3 groups: active responders, yoked stimulation, and sedentary controls. Immediately following the 20th RBS session, rats were sacrificed and ipsilateral punches from 8 brain regions were isolated. Forskolin-stimulated (10µM) AC activity was determined in membrane preparations and was corrected for basal activity (ATP only). Results point towards an increase in forskolin-stimulated AC activity in yoked animals compared to controls in membranes from the nucleus accumbens and the ventral tegmentum. These results suggest that, in a manner similar to that seen in response to chronic cocaine and morphine, repeated exposure to RBS results in an increase in AC activity in some brain regions within the mesolimbic pathway. Supported in part by NIDA grant DA 06485 to EAS.

298.19

PULSE JITTER RETARDS THE DEVELOPMENT OF SEIZURES WITHOUT AFFECTING INTRACRANIAL SELF-STIMULATION CHARGE-DURATION FUNCTIONS.

G. Fouriezos* and J. Th. Rick. Sch. of Psychol., U of Ottawa, Ottawa Ontario, Canada K1N 6N5

Behaviourally derived charge-duration functions collected from three self-stimulating rats with lateral hypothalamic electrodes were not affected by a controlled, 50% jitter to the interpulse interval of rewarding trains. In a second experiment, we kindled two groups of 6 rats with once-daily, 1.0 s bursts of either regularly timed or jittered pulses to the amygdala until full motor seizures developed. Although both groups eventually attained Class 5 convulsions, the time to first seizure was about two days longer with jittered pulses. Thus, while the circuitry that accumulates input from self-stimulation is not affected by the irregularity of asynchronous pulses, the jitter does seem to retard the development of seizures.



INVERTEBRATE LEARNING AND BEHAVIOR III

299.1

CONTRIBUTION OF PERSISTENT PROTEIN KINASE A TO SEROTONIN-INDUCED LONG-TERM FACILITATION OF *APLYSIA* SENSORY-MOTOR SYNAPSES IN CULTURE. P.G. Montarolo, M. Ghirardi, and E. R. Kandel*. Ctr. Neurobiol. & Behav., Columbia P&S, HHMI, NY, NY 10032; Dip. Anat. & Fisiol. Umana, Univ. Torino, 10125 Italy.

The serotonin (5-HT)-induced long-term facilitation of *Aplysia* sensory-motor synapses leads to two protein synthesis-dependent sets of changes: (1) persistent activation of the cAMP-dependent protein kinase (kinase A) leading to persistent phosphorylation of the substrates phosphorylated during short-term facilitation (Bergold et al., 1990); and (2) growth of new presynaptic contacts. To determine the relative contribution of the persistently active PKA to the facilitation present throughout the first 24 hours of long-term facilitation, we used Rp-cAMPS (500 µM), a specific PKA inhibitor, to intercept the active kinase at various time points. In culture, five (5-min) pulses of 10 µM 5-HT induced an increase in EPSP amplitude of about + 156.32% (± 18.09, n = 11) when tested immediately after the end of the application of the five pulses of 5-HT. The amount of EPSP facilitation decreased progressively to + 126.21% (± 17.71, n = 7) at 12 h and to + 62.2% (± 7.2, n = 25) when tested at 24 h. Rp-cAMPS applied for 30 min at the end of the of the 5-HT application (1.5 h) or at 12 h after 5-HT dramatically reduced the synaptic facilitation to + 11.75% (± 7.17 n = 12) and + 33.5% (± 13.04, n = 11), respectively. By contrast, Rp-cAMPS applied at 24 h after the end of 5-HT training produced no statistically significant effect (+ 42.83% ± 12.29, n = 9). These results suggest that persistent PKA activity is particularly important during the first 12 hours after the induction of long-term facilitation by 5-HT, and that other mechanisms, perhaps the newly emerging morphological changes, carry the facilitation at later stages.

299.2

A POSSIBLE ROLE FOR A C/EBP FAMILY MEMBER IN APLYSIA LONG-TERM FACILITATION. C. Alberini, M. Ghirardi, R. Metz, A. Barzilai*, and E. R. Kandel. Ctr. Neurobiol. & Behav., Columbia P&S, HHMI, NY, NY 10032.

Long-term memory for sensitization in *Aplysia*, as well as for many forms of learning in vertebrates, requires RNA and protein synthesis. In *Aplysia* aspects of the transition from the short- to the long-term form can be studied on the cellular level in the synaptic connections between the sensory and motor neurons. In the sensory neurons injection of cAMP can induce both short-term and long-term changes. However, only the long-term form requires transcription. Thus, injection of the oligonucleotide encoding the cAMP recognition element (CRE) characteristic of the cAMP-inducible gene selectively blocks long-term facilitation, suggesting that one or more CREB-like proteins are involved. To explore the role of related transcription factors we are attempting to clone CREB related proteins. As a first step we have cloned the *Aplysia* homologue of NF-IL6, a transcription factor activated via a cAMP-dependent pathway in mammalian cells. Like its mammalian homologues, *Aplysia* NF-IL6 has a conserved b-zip domain. The remaining sequence, including the putative activation domain, appears to diverge. In vitro translation of the cDNA resulted in phosphorylated products of 38-42 kd that specifically bound to an oligonucleotide containing the NF-IL6 binding motiv (oligo NF-IL6), and also the C/EBP binding site of Rous Sarcoma Virus LTR (RSV-LTR). Injection of oligo NF-IL6 into the sensory neurons, but not of the two bases-mutated oligo NF-IL6, selectively blocked the 5-HT-induced long-term facilitation similar to the block produced by CRE. This suggests that NF-IL6 is involved in one of the activation steps of long-term facilitation. We are now studying transcription activation via NF-IL6 as well as its interactions with other cAMP-responsive transcription factors like CREB.

299.3

THE MAJORITY OF THE NEURONS IN THE APLYSIA ABDOMINAL GANGLION HAVE UNRELIABLE RESPONSES TO SIPHON STIMULATION. A.C. Tang*, C.X. Falk, L.B. Cohen, J.Y. Wu. Dept. of Physiology, Yale Univ. Sch. of Med., New Haven, CT 06510

Optical recording with a voltage sensitive dye was used to study the response of the neuronal population in the Aplysia abdominal ganglion. In the absence of learning, an identical stimulus (suction electrode;intensity=6v; duration=5ms; ISI=10-15min) was repetitively delivered to the siphon skin. We assessed the reliability of the neuronal population in responding to a constant stimulation, where the reliability is defined as the percentage of times a neuron responded. A neuron is considered to have responded when the chance of a background spike count being as large as the observed post-stimulus spike count is less than 5%. Our results show that (1) Stimulus response was widely distributed within the ganglion (about 75% of all recorded neurons responded to the siphon stimulation); (2) The reliabilities of these neurons had a broad spectrum, indicating that the neuronal population is made up of neurons with greatly varied response reliability; (3) The majority of neurons had unreliabe responses; (4) As a result, the response patterns across the neuronal pool triggered by an identical stimulus varied considerably. Studies from visual and computer sciences show that our visual system is built upon neurons of low reliability, and suggest that a reliable response can be synthesized from unreliable components. Given the existence of a majority of unreliable neurons in response to a siphon stimulation, their possible role in the production and plasticity of behavior requires future study.

299.4

OPTICAL RECORDING AND ANALYSIS OF *APLYSIA* LEARNING NETWORK IN ABDOMINAL GANGLIA. M. Nakashima*, S. Yamada, S. Shiono and K. Matsumoto# Central Research Laboratory, Mitsubishi Electric Corp., Amagasaki 661, Japan and #Department of Pharmacology, Hokkaido University, Sapporo 060, Japan.

We have been studying the neural network mechanism of the *Aplysia* gill-withdrawal reflex, using an optical apparatus with 448 detector elements (Nakashima et al, IEEE Trans. BME, 39, 26, 1992). Intracellular recordings were used simultaneously with the optical recording, to identify optically detected neurons as well as to detect neural signals from smaller cells which our optical apparatus could not detect. Siphon nerve was stimulated with a pulse train of electric shocks. The stimulation could elicit gill contraction in a similar manner to that caused by mechanical touch to the siphon. Repetitive touch to the siphon caused habituation, whereas 20 µM serotonin apparently enhanced the gill contraction when bath-applied to the abdominal ganglion (sensitization).

The spike trains of optically detected neurons were statistically analyzed by the technique based on the informational theory, calculating the quantity called "channel capacity" from one neuron to another (Yamada et al, submitted to Biol. Cybern., 1992). The calculation of channel capacity was useful to estimate neural connectivities.

The information theoretic analysis was applied to the spike trains detected in the sensitization and habituation experiments. The analysis technique was useful for detecting changes in the neural connectivities caused by the sensitization and habituation.

299.5

STRESS-EVOKED CHANGES OF CIRCULATING AMINO ACIDS IN APLYSIA CALIFORNICA. J. K. Krontiris-Litowitz*, E. T. Walters, and D. J. McAdoo. Dept. Biol. Sci., Youngstown State Univ., Youngstown, OH 44555, Dept. Physiol., U. Texas Med. Sch. at Houston, Houston, TX 77225, U. Texas Med. Branch, Galveston, TX 77550.

Previous studies have demonstrated that local mechanical and electrical stimuli can cause the release of amino acids from Aplysia body wall. In this study we investigate whether mechanical trauma can alter circulating amino acids levels sampled at a site distant from the trauma. Animals were stressed by cutting the right parapodium and pinching the tail for 1 minute. Glutamate and alanine levels were significantly increased at 15 minutes (+714%, +208% $p<.05$) and remained elevated for at least 6 hours. Conversely, asparagine and glutamine levels were significantly decreased at 60 minutes (35%, 40% $p<.05$) and remained reduced for at least 6 hours. These results suggest that selective alteration of amino acid content of hemolymph may reflect metabolic or physiological responses associated with stress.

299.6

FREQUENCY-DEPENDENT PLASTICITY OF LFS_B SIPHON MOTOR NEURON ACTIONS IN *APLYSIA*. C. Hickie* and E.T. Walters. Dept. of Physiology & Cell Biology, University of Texas Medical School at Houston, TX 77225.

The 4 LFS_B siphon motor neurons appear to account for flaring, tailward movements of the siphon in response to stimulation of the rear of the animal (Hickie & Walters, *Soc. Neurosci. Abstr.* 16:19, 1990). At least some of these motor neurons show frequency-dependent enhancement of the movements they produce, which may contribute to response-specific learning (*ibid.* 17:548, 1991). We have used detailed video analysis to further characterize peripheral plasticity and potential peripheral interactions of these motor neurons. Test stimuli were 1 s, 15-25 Hz trains of intracellular pulses just suprathreshold for producing a siphon movement. All 4 LFS_B neurons reliably showed enhancement of test responses by tonic background firing at 0.5-2Hz (n>15 cells)(see also Frost et al. *J. Neurobiol.* 19:297, 1988). This enhancement lasted <2 min after the offset of background firing. Brief, high frequency activation produced much longer-lasting, but more variable effects. A 2 s, 40Hz burst enhanced test responses for <2 to >35 min, with the average enhancement lasting >10 min (n=12 cells). Firing a burst in 1 LFS_B neuron had no effect on subsequent test responses of other LFS_B neurons. Similarly, tonic background activity in 1 LFS_B neuron did not affect the others. We are now using summation tests as an indication of convergence of different LFS_B neurons onto the same muscles. Convergent actions coupled with a lack of crosstalk of plasticity would imply a presynaptic locus of long-lasting, frequency-dependent facilitation in the periphery.

299.7

NEURAL MECHANISMS OF RESPONSE SPECIFICITY. II. CUTANEOUS TAIL AND MANTLE SHOCK PRODUCE DIFFERENTIAL EFFECTS ON THE SIPHON-WITHDRAWAL NEURONAL CIRCUIT IN *APLYSIA*. X. Fang* and G. A. Clark. Psychology Department, Program in Neuroscience, Princeton University, Princeton, NJ 08544.

Both sensitization and classical conditioning of the siphon-withdrawal response in *Aplysia* exhibit response specificity. To extend our previous physiological investigations using nerve stimulation, we have examined the effects of cutaneous shocks to the tail and mantle on behavioral and neuronal responses. We utilized a semi-intact preparation with the intact mantle organs, tail, and abdominal and ring ganglia. Cutaneous shocks were delivered to the tail or mantle through implanted silver wire electrodes, with shock intensity set at 400% of threshold for siphon movement. Behaviorally, tail shock and mantle shock elicited different reflexive siphon withdrawals (flaring and backward bending vs. constriction and forward bending, respectively); training with these two stimuli also differentially modified the response to a siphon tap presented subsequently. Physiologically, we found that LFS-B siphon motor neurons (which produce flaring and backward bending of the siphon) responded more vigorously to tail shock than did LFS-A siphon motor neurons (which produce constriction and forward bending of the siphon). In addition, LFS-B cells responded more strongly to tail shock than to mantle shock. In contrast, LBS siphon motor neurons fired more to mantle shock than to tail shock. These and related findings (Fang & Clark, *Soc. Neurosci. Abstr.*, 1990; Hickie & Walters, *Soc. Neurosci. Abstr.*, 1990, 1991) help identify the neuronal elements mediating the different forms of reflexive and learning-dependent siphon movements produced by tail and mantle shock.

299.8

A NEURAL NETWORK MODEL OF USE-DEPENDENT GAIN CONTROL IN THE SIPHON WITHDRAWAL REFLEX OF *APLYSIA*. D.E.J. Blazis*, T. M. Fischer, and T.J. Carew. Depts of Psychology and Biology, Yale University, New Haven, CT 06520.

In the siphon withdrawal reflex (SWR) of *Aplysia*, intracellular activation of a single member of a class of excitatory interneurons (the L29s) results in significant transient *inhibition* of reflex input to siphon motor neurons (MNs) [Fischer and Carew, 1991]. The mechanism underlying this inhibition involves L29 recruiting inhibition onto itself from inhibitory interneuron L30, which produces an IPSP in L29 that exhibits marked activity-dependent facilitation. Thus the L29/L30 interaction provides for use-dependent gain control in the SWR [Fischer and Carew, 1991].

We previously described a neural network model with a single L29 and a single L30 that generated important aspects of the interactions of these cells, as well as L29-induced inhibition of reflex input to MNs [Blazis et al, 1991]. In the SWR network there are at least 5 L29s and 2 L30s. Thus, our current simulations examined the effects of incorporating additional L29s and L30s into the model neural network. Our model was implemented using the network simulator GENESIS and consisted of single compartment cells. Biophysical and synaptic parameters were empirically derived or estimated from cellular studies. Initial results indicate that the expanded model shows improved descriptions of the firing patterns of L29 and of the magnitude of L29-induced inhibition of reflex input to MNs.

Since the behavioral implications of L29-induced reflex inhibition are unknown, we are currently using the model to examine the role of L29/L30 interactions in various forms of plasticity in the SWR, such as habituation, which is known to involve reduction in MN output with repeated sensory stimulation. Initial results show that diminution of reflex output occurs depending on stimulus duration and interstimulus interval. These results suggest that the inhibition created by L29/L30 interactions could augment other mechanisms, such as homosynaptic depression, thought to subserve habituation.

299.9

A SIMULATION APPROACH TO EVALUATING RELATIVE CONTRIBUTIONS OF MULTIPLE 5-HT-MODULATED K^+ CURRENTS TO SPIKE BROADENING IN *APLYSIA* SENSORY NEURONS. K.J. Belkin*, M.J. Goldstein, B.A. Goldsmith and T.W. Abrams Dept. of Biol. & Inst. of Neurological Sci., Univ. of Pennsylvania, Phila., PA 19104.

Prolongation of action potentials in *Aplysia* SNs contributes to facilitation of transmitter release during behavioral modulation of withdrawal responses. Spike broadening is due to a reduction in K^+ current produced by facilitatory transmitters, including 5-HT. Because 5-HT modulates multiple K^+ currents that are not pharmacologically dissociable, it is difficult to determine experimentally the contribution of each to broadening. To examine their contributions, we simulated a SN using the Nodus program (De Schutter, 1989). Data on voltage sensitivity and kinetics of activation and inactivation from published studies and our measurements were used in simulating K^+ currents. We simulated four K^+ currents that are modulated by 5-HT corresponding to: rapidly activating, V-insensitive and V-sensitive I_{K-S} channels (Schuster et al., 1991); the slowly activating, V-sensitive macroscopic I_{K-S} (Klein et al., 1982); and the rapidly activating, highly V-sensitive $I_{K-V,early}$ (Baxter and Byrne, 1989). 5-HT-like alteration in the properties of these currents resulted in both anti-accommodation and spike broadening. Modulation of the rapidly activating I_{K-S} substantially increased excitability, but had a minor contribution to broadening. Modulation of the slowly activating I_{K-S} decreased the SN's accommodation. Modulation of $I_{K-V,early}$ resulted in substantial spike broadening.

299.10

SPONTANEOUS BEHAVIOR OF THE NUDIBRANCH MOLLUSC *MELIBE LEONINA* IS A MARKOV PROCESS. A.E.Schivell*, S.S.-H.Wang, and S.H.Thompson. Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950.

We observed the spontaneous behavior of 20 individual *Melibe leonina* in the absence of overt stimulation and identified six canonical behavioral states: feeding (F), open hood (OH), roaming/open hood (R/OH), alert posture (AL), roaming/alert (R/AL), and resting (RST). We recorded behavioral states at 15-minute intervals in four six-hour sessions and calculated one- and two-step transition frequencies. The frequencies of one-step transitions quantitatively predicted the observed frequencies of two-step transitions through the Markovian relationship $P(A->B->C) = P(A)P(A->B)P(B->C)$.

The six behaviors fall into two behavior loops, feeding (F, OH, R/OH) and resting (AL, R/AL, RST), with 92% of one-step transitions occurring betweeen behaviors within the same loop. Total time spent by an individual animal in the feeding loop increases with body volume ($r = 0.87$). This tendency to remain in one behavior loop, in conjunction with the Markov model governing transitions, may be useful for putting whole-animal electrophysiological recordings in context. (Supported by BNS 9021217)

299.11

CHAOTIC DYNAMICS IN A MODEL NEURON SERVE AS PUTATIVE MEMORY MECHANISMS. C. C. Canavier*, D. A. Baxter, J. W. Clark, and J. H. Byrne. Dept. of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225 and Dept. of Electrical and Computer Engineering, Rice University, Houston, TX 77251.

We have extended our previous analysis of the bursting neuron R15 in *Aplysia* (Canavier et al., *J. Neurophysiol.* 1991) by examining the interaction of transient synaptic inputs with the nonlinear chaotic dynamics of the model.

In the absence of synaptic input, multiple modes of activity are associated with stable nested attractors that coexist in the eleven-variable phase space of the model at fixed parameter settings. The outermost attractor is associated with a periodic bursting mode. Inside that stable limit cycle is an attractor associated with a chaotic bursting mode (Type I). Nested within this chaotic attractor is yet another attractor associated with a distinct chaotic bursting mode (Type II). At the core of the nested attractors is found a limit cycle associated with a beating mode. Thus, we have found chaos to be transitional between bursting and beating in phase space, as well as in parameter space as reported previously (Canavier et al., *Biophys. J.* 1990).

A brief (one sec) train of synaptic input can switch the activity of the model from one mode to another "adjacent" mode. Moreover, the ability of the synaptic input to induce shifts between modes exhibits temporal specificity. For example, a depolarizing input just prior to a burst can shift the activity from Type I chaotic bursting to periodic bursting, whereas a similar input just after a burst can shift it in the opposite direction, to Type II chaotic bursting. No parameter changes are required for these shifts. Rather, these shifts occur because the state of the system is perturbed into the basin of another attractor. Thus, a transient input can dramatically alter the long-term activity of the system. These results suggest a role for chaotic dynamics in information processing and storage at the level of a single neuron. Specifically, brief "teaching" signals could induce persistent shifts in electrical activity, which could contribute to the expression and maintenance of persistent cellular alterations of a type necessary for learning and memory. Supported by a grant from the ONR.

299.12

SEROTONIN INHIBITS INHIBITORY INTERNEURONS IN THE NEURAL CIRCUITRY FOR THE TAIL-WITHDRAWAL REFLEX OF *APLYSIA*. Y. Xu*, J. P. Pieroni, L. J. Cleary and J. H. Byrne. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225.

Sensory neurons (SNs) in the pleural ganglion are hyperpolarized by mechanical stimulation of the tail outside their receptive fields. Previously, we identified two types of inhibitory interneurons (iINs) in the right pleural ganglion of *Aplysia* that may mediate this hyperpolarization (Xu *et al.*, 1991). These iINs are differentiated by the time course of the hyperpolarization that they evoke in SNs, and by the presence or absence of immunoreactivity to the inhibitory peptide FMRFamide. We also found that non-FMRFamide immunoreactive (NF-) iINs were distinctive in that they also hyperpolarized tail motor neurons (MNs) in the pedal ganglion.

The neuromodulator serotonin (5-HT) has well-established facilitatory actions on the connections between SNs and MNs, but little is known about its effects on other elements (*e.g.*, interneurons) in the neuronal circuitry underlying the tail-withdrawal reflex of *Aplysia*. We examined the effects of 5-HT on NF-iINs themselves, and on the associated hyperpolarization of SNs and MNs produced by NF-iINs. Identified NF-iINs were stimulated by 2-sec depolarizing current pulses at 5-min intervals. These successive test stimulations produced at least two effects: bursts of action potentials in the NF-iINs (with a consistent number of spikes in each test) and pronounced hyperpolarization of monitored MNs (and usually a small hyperpolarization of monitored SNs). Application of 5-HT (25 μM) after the second test stimulation hyperpolarized NF-iINs and suppressed spontaneous spike activity. 5-HT depolarized SNs and produced variable effects on MNs (*e.g.*, depolarization, hyperpolarization and triggering of spontaneous spikes). In NF-iINs, 5-HT decreased the number of spikes elicited by subsequent stimulations. In addition, the NF-iIN-induced hyperpolarization of the MNs and SNs was either decreased or absent after application of 5-HT, even in cases in which the number of spikes elicited by the stimulation was increased, via intensified current, back to the initial (pre-5-HT) value.

These results demonstrate that at least one subset of the iINs in the pleural ganglion is modulated by 5-HT. Thus, the sensitizing effect of 5-HT on the tail-withdrawal reflex may be accomplished, in part, via an inhibition of the activity of the NF-iINs.

299.13

QUANTITATIVE ANALYSIS OF THE MODULATION BY SEROTONIN OF THE VOLTAGE-DEPENDENT POTASSIUM CURRENT IN PLEURAL SENSORY NEURONS OF *APLYSIA*. J.A. White*, D.A. Baxter, and J.H. Byrne Dept. of Neurobiol. and Anat., Univ. of Texas Med. Sch., Houston, TX 77030

Baxter and Byrne (1989) found that serotonin (5-HT) modulates a TEA-sensitive, voltage-dependent potassium current ($I_{K,V}$) in pleural sensory neurons of *Aplysia*. The precise nature of the serotonergic modulation of $I_{K,V}$ was not analyzed, however. We isolated $I_{K,V}$ using TEA difference currents and examined its kinetics of activation and inactivation in control conditions and in the presence of 5-HT. The data were fit using a Hodgkin-Huxley type model. The kinetics and steady-state properties of this potassium current were sharply dependent on membrane potential for potentials above 0 mV and otherwise consistent with the criteria established by Rudy (1988) for delayed potassium currents. The effects of 5-HT on $I_{K,V}$ were complex. 5-HT significantly decreased the steady-state magnitude of $I_{K,V}$ (Iss). Iss in response to a voltage-clamp pulse from -50 mV to +20 mV was decreased by 50%. In addition, 5-HT significantly slowed both activation kinetics (the time constant of activation was increased by 29% at +20 mV) and inactivation kinetics (the time constant of inactivation was increased by 518% at +20 mV). No significant effect of 5-HT on steady-state values of inactivation was noted. Our mathematical descriptions of $I_{K,V}$ in control conditions and in the presence of 5-HT were used to estimate the contribution of serotonergic modulation of $I_{K,V}$ to the total 5-HT difference current. Effects of 5-HT on $I_{K,V}$ account for almost all of the 5-HT-induced reduction in outward current during the first 20 ms of a voltage-clamp pulse to +20 mV. This result implies that 5-HT may exert many of its effects on spike width in sensory neurons via modulation of $I_{K,V}$. Effects of 5-HT on $I_{K,V}$ are consistent with a model in which the maximal conductance underlying the current is decreased by 50% and the rate constants between open and closed states of both the activation and inactivation processes are diminished in magnitude across all membrane potentials. Supported by AFOSR Grant 91-0027 and NIMH Fellowship MH10215.

299.14

ACTIVATION OF PROTEIN KINASE C MIMICS SEROTONIN-INDUCED MODULATION OF A VOLTAGE-DEPENDENT POTASSIUM CURRENT IN PLEURAL SENSORY NEURONS OF *APLYSIA*. S. Sugita*, D.A. Baxter and J.H. Byrne. Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225.

Modulation of a voltage-dependent K^+ current ($I_{K,V}$) appears to play a major role in serotonin (5-HT) -induced spike broadening, which develops slowly in pleural sensory neurons (Baxter and Byrne, 1989, 1990). Little is known regarding which protein kinase mediates this modulation, however. Since activation of protein kinase C (PKC) by phorbol esters mimics a component of 5-HT-induced broadening (Sugita et al., 1991, 1992), we examined the effects of phorbol esters on $I_{K,V}$. Two-electrode voltage-clamp techniques were used to measure current responses to 200 ms pulses from -70 mV to -30, -20, 0, +20 mV in isolated clusters of pleural sensory neurons maintained at 15 ± 1 °C. Phorbol esters, phorbol-12,13-diacetate (3 μM) or 12-deoxyphorbol, 13-isobutyrate (2 μM), had little effect on membrane current at -30 and -20 mV. At more depolarized potentials, e.g. +20 mV, where $I_{K,V}$ predominates, phorbol esters produced complex modulation of the membrane current. Phorbol esters appeared to slow the kinetics of the current responses. This resulted in a decrease in outward current at the beginning of voltage-clamp pulse, which was followed by an increase in outward current at the end of pulse. This modulation is similar to 5-HT-induced modulation of $I_{K,V}$ (Baxter and Byrne, 1989; White et al., this volume). In addition, application of 5-HT after phorbol esters had little additional effect on membrane current. Inactive phorbols did not produce changes in membrane current or occlude the modulation by 5-HT. These results suggest that activation of PKC and 5-HT share some common mechanism in the modulation of $I_{K,V}$. Low concentration of TEA (2 mM), which blocked a Ca^{2+}-activated potassium current ($I_{K,Ca}$), blocked a portion of the phorbol ester-induced modulation at the end of voltage-clamp pulse, suggesting that an increase in outward current at the end of pulse is due to both an increase in $I_{K,Ca}$ (Critz and Byrne, 1990) and modulation of $I_{K,V}$. These results support the hypothesis that modulation of $I_{K,V}$ by PKC contributes to a component of 5-HT-induced spike broadening that develops slowly.

299.15

DIFFERENTIAL PHOSPHORYLATION WITH TREATMENTS THAT INDUCE SHORT-AND LONG-TERM FACILITATION IN *APLYSIA*. R. Homayouni *, J.H. Byrne, A. Eskin. Dept. Biochem., Univ. of Houston, Houston, TX 77204. Dept. Neurobiol. & Anat., Univ. of Texas Med. School, Houston, TX 77225.

Application of serotonin (5-HT) for 2 min produces short-term facilitation of tail sensory neurons (SNs) that lasts a few minutes, whereas repeated application of 5-HT for 1.5 hr produces long-term facilitation of SNs that lasts 24 hr. Both short- and long-term facilitation appear to be mediated at least in part by cAMP-dependent protein kinase. Activation of protein kinase C (PKC) may also play a role in mediating short-term facilitation produced by 5-HT (Sugita et al., this volume). Previously, we showed that different sets of proteins were phosphorylated immediately after 2 min, 25 min, and 1.5 hr durations of 5-HT (Homayouni et al., 1991). To investigate further the difference in events that may be responsible for inducing short-term and long-term facilitation we addressed two questions. Does the activation of PKC by phorbol diacetate (PDAc) alter the phosphorylation of the same proteins whose phosphorylation was affected by 5-HT? Are the same proteins whose phosphorylation was altered by 1.5 hr treatment of 5-HT also altered 1.5 hr after a 2 min treatment of 5-HT?

For each experiment, 6 pedal-pleural ganglia and their matched controls were incubated in ^{32}P for 20.5 hr. After 19 hr of labeling, experimental ganglia were treated with either PDAc for 1.5 hr or with 5-HT for 2 min or 1.5 hr. Clusters of sensory neurons were removed and the incorporation of ^{32}P into proteins was examined using 2D-PAGE. The application of PDAc for 1.5 hr increased the phosphorylation of 11 proteins and decreased the phosphorylation of 2 proteins. Four of these proteins were altered similarly immediately after 2 min 5-HT. The proteins whose phosphorylation was altered by 2 min 5-HT and PDAc are good candidates for proteins involved in mediating some of the aspects of short-term sensitizaiton.

The phosphorylation of 4 proteins increased and 1 protein decreased 1.5 hr after a 2 min 5-HT treatment. Among the 12 previously reported proteins whose phosphorylation was altered by continuous 1.5 hr 5-HT, 3 proteins were altered similarly 1.5 hr after a 2 min 5-HT and 9 proteins were altered only in the continuous presence of 5-HT. The 9 proteins that required continuous 1.5 hr 5-HT may play a role in the induction of long-term facilitation. The functional roles of these proteins are being studied by obtaining their amino acid sequences (Noel et al., this volume).

299.16

PHOSPHORYLATION AND SYNTHESIS OF INTERMEDIATE FILAMENT PROTEINS ARE AFFECTED BY TREATMENTS MIMICKING SENSITIZATION IN *APLYSIA*. F. Noel*, R. Homayouni, M. Nuñez-Regueiro, J.H. Byrne, A. Eskin. Dept. of Neurobiol. & Anat., Univ. of Texas Med. Sch., Houston, TX 77225 & Dept. of Biochem. & Biophys. Sciences, Univ. of Houston, TX 77204.

To investigate the involvement of individual proteins in learning and memory, we examined the effects of analogues of sensitization training on the synthesis and phosphorylation of proteins in pleural sensory neurons (SNs) of *Aplysia*. In one series of experiments we searched for proteins whose synthesis was altered by electrical stimulation of peripheral nerves of pleural-pedal ganglia over a 1.5 hr period. Incorporation of labeled amino acid was examined immediately after and 24 hr after electrical stimulation. In another series of experiments changes of phosphorylation of proteins were examined after a 2 min or 1.5 hr application of serotonin (5-HT). These studies focused our attention on two relatively abundant proteins. The incorporation of labeled amino acid into protein 17 (MW: 50; pI: 5.2) was increased 24 hr after electrical stimulation but was not affected immediately after stimulation. Although label incorporation into protein 6 (MW: 57; pI: 5.25) did not appear affected by electrical stimulation, its phosphorylation was increased after a 2 min application of 5-HT. Phosphorylation of protein 17 was increased after 1.5 hr application of 5-HT.

To determine the function of these proteins and their roles in learning and memory, amino acid sequences of peptides derived from the proteins by V8 protease digestion were obtained. Three partial sequences from protein 17 and 4 partial sequences from protein 6 were determined. The partial sequences from both proteins were highly similar to a previously sequenced intermediate filament protein (IFP) from *Aplysia* (Reimer et al., 1991). The peptides from protein 17 were 83 %, 96 % and 94 % identical to *Aplysia* IFP. The peptides from protein 6 were 100 %, 80 %, 100 % and 100 % identical to *Aplysia* IFP.

Intermediate filament proteins are one of the major components of the cytoskeleton. Phosphorylation of IFPs may regulate their assembly and interaction with other cellular components. The changes of synthesis and phosphorylation of proteins 17 and 6 may mediate the morphological changes that accompany long-term sensitization in *Aplysia*.

299.17

DELAYED APPLICATION OF ANISOMYCIN BLOCKS LONG-TERM (24 HR) cAMP-INDUCED MORPHOLOGICAL CHANGES IN PLEURAL SENSORY NEURONS OF *APLYSIA*. F.A. NAZIF*, J.H. BYRNE and L.J. CLEARY. Dept. Neurobiology and Anatomy, Univ. Texas Med. Sch., Houston, TX 77225

Intracellular injection of cAMP into sensory neurons elicits changes in morphology similar to those seen with long-term sensitization (Nazif *et al*, 1991a). The induction of these changes is dependent on protein synthesis during the injection period (Nazif *et al*, 1991b). Long-term facilitation of synaptic transmission by 5-HT is blocked by anisomycin applied concurrently with 5-HT, but not when application of anisomycin is delayed by 0.5 to 4 hr (Montarolo *et al*, 1986). Therefore, we examined the effects of delayed application of anisomycin on cAMP-induced morphological changes.

cAMP (200 μM) was iontophoresed into sensory neurons of paired pleural ganglia. Four hr later, one ganglion from each pair was incubated in culture medium containing anisomycin (10 μM), while the other was incubated in culture medium containing the inactive derivative deacetylanisomycin (10 μM). Three hours later, the ganglia were perfused with fresh culture medium. About 22 hr after nucleotide injection, the same neurons were pressure injected with HRP, incubated for another 2 hr, and processed. The number of varicosities was greater in neurons whose ganglia were bathed in deacetylanisomycin than in neurons from contralateral ganglia bathed in anisomycin (52 ± 13 SEM vs 17 ± 4 SEM; $P<0.02$; n=6). In control experiments, a 3 hr application of anisomycin alone had no significant effects on varicosity number 22 hr after application (31 ± 11 SEM vs 33 ± 10 SEM; $P=0.18$; n=9).

These data indicate that the morphological changes induced by cAMP are dependent on *de novo* protein synthesis not only during the injection period but also at least 4 to 7 hr afterwards. Although the time course of sensitivity to anisomycin is not identical to that for long-term facilitation, it is consistent with the time course for development of long-term morphological changes (Bailey and Chen, 1989). Moreover, changes in the synthesis of proteins have been observed as long as 24 hr after long-term training (Castellucci *et al*, 1988; Barzilai *et al*, 1989; Noel *et al* 1990). These data suggest that structural changes and transmitter release are regulated separately.

STRESS: GENERAL

300.1

ARGININE VASOPRESSIN POTENTIATES THE EFFECTS OF OF CORTICOTROPIN RELEASING FACTOR ON BEHAVIOR IN RATS. M.K. Salander, S.T. Ahlers, and P.A. Shea*. Naval Medical Research Institute, Bethesda, MD 20889-5055.

Research suggests that corticotropin releasing factor (CRF) and vasopressin interact in modulating the release of glucocorticoids in response to stress. The present study examined whether the facilitative effects of vasopressin on CRF would extend to a behavioral baseline which has been shown to be sensitive to CRF. Rats were administered intracerebroventricular (i.c.v.) CRF (0.1-1.0 μg) or saline 60 minutes prior to performing on a multiple fixed interval (FI) 60 sec/fixed ratio (FR) 20 schedule for food reinforcement. A session consisted of 10 components of each schedule which alternated, starting with the FI component. Thirty minutes prior to the test session rats were administered either 1.0μg arginine vasopressin (AVP) or saline (sub-Q) in the nape of the neck. All injections were given in a mixed sequence. Compared to saline control performance, administration of AVP slightly decreased the rate of responding in the FI component but did not effect FR responding. Administration of CRF produced dose-dependent suppression of FI and FR responding. Co-administration of AVP and CRF shifted the CRF dose-response curve to the left indicating that AVP potentiated the effects of CRF on behavior.

300.2

INDIVIDUAL CHARACTERISTICS AND BEHAVIORAL AND HORMONAL RESPONSES TO ACUTE STRESS IN RATS. V.M. Wiegant*, M. Broekhoven, A. Frankhuyzen-Sierevogel, M. Nijsen, I. Raats and G. Croiset. Rudolf Magnus Institute, Dept. Medical Pharmacology, University of Utrecht, The Netherlands.

Rats respond to a stressor with coordinated behavioral and neuro-endocrine reactions. It is thought that such reactions depend not only on quality and intensity of the stressor, but also on individual characteristics (innate and acquired) of the animal. The aim of the present study was to investigate the relationships between such characteristics, determined in water competition (ranking) and open field (innate activity) tests, and responses to acute stress in a shock-prod burying test.

For the water competition test, rats were housed five per cage with ad libitum food and water for at least two weeks. On four consecutive test days, water was withheld for five hours. Then drinking behavior was monitored for ten minutes, and rank numbers were assigned based on sequence and duration of access to the drinking nipple. Activity tests were performed on four consecutive days. Rats were placed in an open field for 10 min and total locomotor activity (travelled distance) was determined. Following assessment of the individual characteristics in these paradigms, the animals were surgically fitted with a jugular vein cannula for repeated blood sampling, and housed single-caged. After a one-week recovery period, they were confronted with an electrified prod that was inserted into the home cage. The behavioral response of each rat was analysed for time spend approaching and burying the prod. Blood samples for plasma corticosterone assay were collected 5 min prior, and 0, 5, 15 and 45 min after prod insertion.

High active rats (open field) displayed more burying behavior than low active rats, whereas low active rats spent more time approaching the prod. Both types of animals showed an increase in plasma corticosterone. High ranked animals (water competition test) spent significantly more time burying than low ranked rats, and showed more approaching behavior. In contrast to low ranked animals they did not show an increase in plasma corticosterone levels.

The results suggest that differences in behavioral and neuro-endocrine responses to a given stressor are determined by preexisting individual characteristics.

300.3

BEHAVIOR AND TELEMETERED AUTONOMIC RESPONSES TO ALCOHOL AND SOCIAL STRESS. W. Tornatzky* and K.A. Miczek. Dept. Psychology, Tufts University, Medford, MA 02155.

Animals react to social stress with defensive and submissive behavior as well as with increased cardiovascular activity and core temperature. To evaluate the involvement of alcohol and anxiolytics on telemetered heart-rate, core temperature and on behavioral reaction of a rat that is exposed for 1 hour to threats by an opponent, ethanol (0.1-3.0 g/kg, p.o.) and diazepam (1.0-10.0 mg/kg, ip.) were administered. The acute social stress situation consisted of brief physical agonistic interactions until the experimental rat was forced into a prolonged submissive supine posture, emitted ultrasounds and, subsequently, exposure to the opponent's threats, while being shielded from physical contact. Alcohol attenuates the stress induced hyperthermia, induces a dose-dependent decline in upright postures and a disruption of high-frequency ultrasonic calls. The protocol includes a time period prior to the physical interaction, where the defeat experienced intruder is confronted only with the olfactory cues of the resident homecage. In this period of conditioned anticipation of an upcoming defeat, alcohol in contrast to diazepam, decreases vocalization, defensive behavior and increases exploratory behavior. Components of the autonomic and behavioral responses to different grades of social stress can be pharmacologically differentiated.

300.4

TYROSINE REDUCES THE ADVERSE EFFECT OF HYPOBARIC HYPOXIA ON SPATIAL WORKING MEMORY OF THE RAT. H.R. Lieberman, B. Shukitt-Hale[1], S. Luo, J.A. Devine and J.F. Glenn*, Military Performance and Neuroscience Division, U.S.Army Research Institute of Environmental Medicine, Natick, MA 01760 and [1]GEO-CENTERS, INC., Newton Centre, MA 02159.

Exposure to hypobaric hypoxia rapidly produces profound decrements in various cognitive functions including learning and memory. The neurotransmitter precursor tyrosine has been reported to have beneficial behavioral effects when it is administered to experimental animals and humans who are exposed to various acute stressors. To determine whether tyrosine would protect animals from the adverse effects of hypobaric hypoxia on spatial memory, it was administered, in a divided dose, at 1.5 hours (400 mg/kg, i.p.) and again at 5.5 hours (400 mg/kg, i.p), after ascent to a simulated altitude of 5950 m (19,500 ft). Nine rats were treated with tyrosine and nine received vehicle. Working and reference memory were assessed using the Morris Water Maze task. Tyrosine treatment significantly reduced working memory escape time when compared to vehicle (53.4 sec ± 10.1 vs. 90.7 ± 7.3; mean ± SEM) ($p<.04$) but did not affect reference memory. There were no treatment related differences in performance when animals were tested the next day at sea level. The beneficial effects of tyrosine on working memory may be due to a direct effect of tyrosine on memory function or to other central or peripheral effects of this dietary catecholamine precursor. The absence of carry-over effects when animals were tested the next day supports the latter hypothesis.

300.5

EXPOSURE TO HEAT STRESS DISRUPTS THE ACQUISITION AND PERFORMANCE OF RESPONSE CHAINS IN RATS. J. Schrot* and J.R. Thomas. Naval Medical Research Institute, Bethesda, MD 20889-5055.

Performance of well-learned behavior is disrupted by exposure to heat. The extent to which heat interferes with the acquisition (learning) of behavior is less well understood. This study examined the effect of acute heat stress on both the acquisition and performance of four-member response sequences in rats. Four animals were trained on a four lever, four member repeated acquisition procedure in which each day of the week was associated with a different required sequence of responses. Each correct response advanced the sequence to the next member and the fourth correct response resulted in the delivery of a food pellet. Incorrect responses resulted in a three second period of timeout. This procedure generates a daily acquisition or learning curve. During the performance condition the sequence remained the same from day to day. The animals were exposed to a control air temperature of 22°C, or experimental temperatures of 31°C, 35°C, or 38°C for 30 minutes prior to the start of and during sessions. The results indicate that exposure to the two highest temperatures disrupted accuracy of responding during both the acquisition and performance of sequences. At the highest temperatures animals frequently did not complete sessions. With comparable temperatures, the animals tended to complete fewer sequences per session during the acquisition condition, indicating that acquisition was more sensitive to heat stress than was performance.

300.6

FURTHER EVIDENCE TYROSINE REVERSES BEHAVIORAL DEFICITS CAUSED BY COLD EXPOSURE. S. Luo, L. Villamil, C.J. Watkins* and H.R. Lieberman. Military Performance and Neuroscience Division, U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760.

Neurotransmitter-related behavioral deficits may occur when animals are under acute stress. The neurochemical alteration most consistently observed has been a reduction of brain norepinephrine (NE) levels. The objective of this crossover study was to determine if the NE precursor tyrosine could prevent or alleviate stress-induced adverse behavioral changes in male Fischer rats. On two separate trials nine animals were restrained and immersed in a water bath (17°C) for about 20 min. until a core body temperature (T_c) of 30°C was obtained. On one trial tyrosine (400 mg/kg, ip) was administered 30 min prior to hypothermia and on another saline was given. On a third trial animals were restrained and immersed in a 36°C water bath for 20 min, which did not alter normal T_c. Immediately following these treatments the animals were placed in a narrow cylinder filled with water maintained at 18°C and duration of immobility was assessed over a six minute period. A repeated measures ANOVA revealed a significant treatment effect ($p<0.0001$). Post hoc Duncan's comparisons showed that mean ($\pm$SEM) immobility following immersion in cold water was significantly greater than immersion in 36°C water (104 ± 15.0 vs. 31 ± 5.7 s. respectively, $p<.01$). In addition, when rats were treated with tyrosine mean immobility was 40 ± 5.1 s., a significant reduction compared to placebo treatment ($p<.01$) but not different from immersion in 36°C water. This study confirms that hypothermia was highly effective in producing behavioral depression which was reversed by tyrosine treatment.

300.7

AGED RATS: PLASMA CATECHOLAMINE RESPONSES TO ACUTE COLD STRESS. T. R. Mabry, H. Tong, R. McCarty, and P. E. Gold*. Department of Psychology, University of Virginia, Charlottesville, VA 22903.

One consequence of normal aging appears to be a diminished capacity to adapt to stressful stimulation. Specifically, aged animals and humans exhibit altered patterns of sympathetic-adrenal medullary activation when exposed to a variety of homeostatic challenges. In the present series of experiments, Fischer 344 male rats (3 and 22 months of age) were prepared with chronic tail artery catheters three days prior to exposure to acute stress (immersion in water at 20°, 25°, 30° or 35° C) for 15 minutes. Blood samples were collected under basal conditions and at 0, 15, 30 and 45 minutes post-immersion. Plasma samples were later assayed for content of norepinephrine (NE, pg/ml) and epinephrine (EPI, pg/ml) as a measure of sympathetic-adrenal medullary activity. Our findings indicate that aged rats have significantly greater plasma NE and EPI responses to immersion at 20° and 25° C and their return to baseline is more prolonged. In contrast, the plasma NE and EPI responses of aged rats more closely resembled those of young adult controls following immersion in 30° or 35° C water. These data are consistent with the view that aged rats display exaggerated plasma catecholamine responses to intensely stressful stimulation. These exaggerated physiological responses may underlie in part the inability of aged animals to adapt to or, in some cases, to survive significant environmental challenges.

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300.8

EFFECT OF STRESS ON THE DOMINANT/SUBMISSIVE RELATIONSHIP BETWEEN MALE RATS. A.B. Lucion and W.H. Vogel*, Dpt. of Pharmacology, Jefferson Medical College, Philadelphia, PA 19107 and Dpt. of Physiology, Federal University Rio Grande Sul, Porto Alegre, 90049, Brazil.

A dominant/submissive relationship between 2 rats based on frequency, total and mean duration of competitive water consumption was formed quickly and remained stable during all the experiment (control group). Nine dominant rats were exposed to a mild (2 hr restraint) and another group of 9 dominant rats to a severe (18 hr restraint) stressor. Restraint was repeated 3 times and the animals were always released 2 hr before the water competition test. During mild stress, there was a significant decrease of water intake by the stressed rats without an increase by the unstressed. After termination of the restraint, the dominant rats quickly regained their former positions. The severe stress caused a more profound decrease in water intake by the dominant rats with a concomitant increase by the submissive ones or an actual reversal of the dominant/submissive relationship. After termination of restraint, the dominant rats regained their former positions more slowly. Thus, acute stress of a dominant animal decreases aggression in the water competition test leading to a loss of social dominance. Cessation of stress restores the original social relationship. (A.B.L. fellowship CNPq Brazil).

300.9

THE EFFECTS OF STRESS ON SPATIAL LEARNING CAN BE ATTENUATED BY BLOCKING NMDA RECEPTORS (AND LTP). **T. J. Shors* Department of Psychology, Princeton University, Princeton, NJ 08540.**

A number of correlations between stress and LTP have led me to hypothesize that stress is inducing a form of synaptic potentiation similar to, if not the same as, LTP. For example, both stress and LTP increase classical conditioning (Shors et al., 1992; Berger, Science 224, 1984). If such a hypothesis were true, then stress should impair spatial learning, just as LTP does (McNaughton et al., J. Neurosci. 6, 1986).

Sprague-Dawley rats ($n=16$) were habituated to an 8-arm radial maze and half were restrained and exposed to 90, 1s, 1 mA tailshocks. 24 hours later, they were exposed to 5 booster shocks and tested 2 trials per day for 6 days. Relative to unstressed controls, stressed rats were impaired in acquisition of the maze [$F(5,70)=6.86$; $p<0.001$].

If stress impairs maze learning by inducing LTP, blocking NMDA receptors (and LTP) during the stressor should eliminate the impairment. 12 rats were injected with CGP (30 mg/kg), a reversible and competitive NMDA antagonist, and half were exposed to the stressor. Rats with CGP during the stressor were not impaired relative to stressed controls [$F(5,120)=3.08$; $p<0.01$]. Although CGP induced a notable motor impairment, rats with CGP alone learned as well as unstressed controls and CGP/stressed rats ($p>0.05$). These results suggest that the effects of stress on spatial learning are mediated through glutamate receptor activation and, potentially, LTP.

300.10

ADENOSINE ANALOGS MIMIC, METHYLXANTHINES REVERSE, AND GLUCOSE MITIGATES STRESS-INDUCED DEPRESSION IN RATS. Thomas R. Minor*, Wei-Chao Chang, & Jeff L. Winslow. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563.

The possibility was examined that the behavioral depression and escape deficits observed in rats exposed to inescapable electric shock (IS) 24 hr earlier result from compromised energy metabolism and a consequent increase in adenosine (ADO) mediated inhibition in the CNS. Caffeine (Caf: 1,7,14,30 mg/kg) and theophylline (3,10,17 mg/kg), ADO receptor antagonists, reversed (but did not prevent) escape deficits in IS rats in a dose-dependent manner. Amphetamine (AMP), another psychomotor stimulant, failed to improve the escape performance of IS rats and produced a progress deterioration of escape in restrained (no-shock) controls. The AMP-induced deficit was reversed by Caf. ADO analogs administered 24 hr after simple restraint (i.p. injection: .01-10 μmol/kg) mimicked the effect of earlier inescapable shock on test performance (NECA>R(-)PIA>S(+)PIA). A submaximal dose of NECA summated with earlier exposure to a submaximal number of inescapable shocks to maximize the test escape deficit. Finally, access to a 40% glucose solution over the 24 hr between inescapable shock pretreatment and escape testing markedly improved test performance. These data strongly implicate metabolic and purine-regulatory processes in behavioral depression following acute, traumatic stress. (Supported by R01 MH41170-03).

300.11

"NONOPIATE" COLD WATER SWIM-INDUCED ANALGESIA ATTENUATES MORPHINE ANALGESIA.

J.E. Grisel*, R.E. Grahn, L. Sutton, L.R. Watkins & S.F. Maier. Department of Psychology, University of Colorado, Boulder CO 80309

These experiments investigate the relationship between a "nonopiate" stress-induced analgesia (SIA) and the opiate analgesia produced by systemic morphine. Rats who were given morphine (1.5 mg/kg sc) prior to cold water swim (CWS; 4 min at 14°C) demonstrated less analgesia than those who received either morphine or CWS alone. These results are interpreted as evidence for a model of collateral inhibition (Kirschgessner et al., 1982) in which opiate and nonopiate systems can be mutually inhibitory. Since the specific mechanism(s) involved in this interaction are not yet understood, additional experiments manipulated variables known to differentially effect opiate and nonopiate analgesias. Rats given prior experience with escapable shock known to attenuate opioid analgesia (100, 1 mA, 5 sec tailshocks) did not show the attenuated levels of analgesia resulting from CWS + morphine. Inescapably shocked, restrained and home cage control rats did not differ from each other, and all showed decreased levels of analgesia in response to the dual manipulation. Further, adrenalectomy was shown to block the ability of CWS to attenuate morphine analgesia in inescapably shocked rats, but this effect was reinstated when rats were supplemented with corticosterone in their water.

300.12

IMMUNE STATUS ASSOCIATED WITH DYSTHYMIA, MAJOR DEPRESSION AND STRESSFUL LIFE EVENTS.

J. Griffiths*, A. Ravindran, C. Waddell and H. Anisman. Royal Ottawa Hospital and Carleton University. Ottawa, Ontario, Canada.

Depression and stressful life events have been associated with alterations of immune functioning. The present investigation sought to establish whether the ability to cope with major and minor stressors (daily hassles) would influence immune status in individuals suffering from either major depression or dysthymia (chronic mild depression). Circulating T cell subpopulations (activated T cells, CD4 helper, CD8 suppressor/cyctotoxic), B cells or NK cells were found not to differ among these patients relative to nondepressed controls. The depressed and dysthymic patients reported increased frequency of life event changes during the preceding 12 months, as well as the hassles encountered during the preceding month. The frequency and severity of these stressors were correlated with several immune measures. Moreover, immune status also varied as a function of the coping styles that patients had employed. The data are related to the notion that affective state per se may not be related to immune alterations; however, in depressed patients perceived stressors and coping styles are associated with immune status.

300.13

MODULATION OF IMMUNE RESPONSES IN NEONATALLY STRESSED RATS BY THE MATERNAL PRESENCE.

S.K.Sobrian*, M. Lazarevic, B.M. Markovic, V.J. Djuric and B.D. Jankovic. Dept. Pharmacology, Howard Univ. Col. of Med., Washington, DC 20059 and Immunology Research Institute, Belgrade, Yugoslavia.

Early experience can influence adult immune responses. Increases, decreases and no changes in tumor growth, disease incidence and survival time have been reported following neonatal manipulations. These discrepancies may reflect inadvertent stressing of control pups by maternal separation. To test this hypothesis, Wistar rat pups were sound stressed on postnatal days (PND) 15,18 and 21. Stress consisted of exposure to a 90 dB fire alarm bell that was programmed, on a variable interval schedule, to ring 60 times in 1 hr. Pups were stressed in groups of 3-4, either with (SPM), or without (SP) the dam present. Non-stressed controls were either isolated from (CP) or left with the dam (CPM) for the 1 hr period. All maternally isolated pups were maintained in an 80-85° F environment. At PND 46-49, rats were immunized with bovine serum albumin (BSA) in complete Freund's adjuvant; 14 days later, antibody production to BSA, and Arthus and delayed skin reactions to BSA and old tuberculin (OT) were measured. The severity of adjuvant-induced inflammation of the injected foot was also assessed. SP rats showed a decrease in the Arthus reaction to BSA in comparison to CP and CPM groups, which did not occur in SPM animals. The delayed reaction to OT was decreased in both SP and CP males; the response of SPM animals was unaltered. The inflammatory response was attenuated in both stressed groups. Antibody titers were reduced in CP, SPM and SP males. Therefore, the presence of the dam during neonatal stress can attenuate the stress-induced suppression of some *in vivo* humoral and cellular immune responses. The fact that some humoral responses were sensitive to maternal separation alone supports our hypothesis that maternally isolated non-stressed pups may not be adequate controls. (Supported by CIES and Republic of Serbia Research Fund).

300.14

STRESSOR EFFECTS ON IMMUNE AND CENTRAL NEUROTRANSMITTER ACTIVITY: STRAIN DIFFERENCES IN MICE.

L. Kerr, D. Francis & H. Anisman*. Dept. Psychology, Carleton Univ., Ottawa, Ontario K1S 5B6, Canada.

Stressors influence the splenic plaque forming cell (PFC) response and plasma antibody titers following administration of sheep red blood cells (SRBC). However, in CD-1 mice, this effect was dependent on the timing of the stressor relative to antigen treatment. A critical period existed, such that a stressor applied 72 hr after SRBC inoculation suppressed the peak immune response, but was without effect at other intervals. In the present report we demonstrate that the magnitude and time of the peak immune response to SRBC (10^6) varied across strains of mice (CD-1, C57BL/6ByJ, BALB/cByJ, DBA/2J). Moreover, a stressor (360 shocks, 2 sec, 300 uA) provoked strain-specific alterations of the immune response. Indeed, in some instances footshock elicited an immunoenhancement, rather than a suppression. Additionally, systemic SRBC administration provoked strain-dependent alterations of central norepinephrine and dopamine, but did not influence either plasma corticosterone or ACTH accumulation. The data are related to bidirectional communication between the immune and CNS.

300.15

PSYCHOBIOLOGIC CONSEQUENCES OF POSTNATAL REARING ON MEASURES OF SHOCK-INDUCED STRESS IN YOUNG RATS.

I. Goodman*, K. Vrana, S. Vrana, L. Beeler & A. Azzaro. Dept. of Behav. Med. & Psychiatry, Biochemistry, Neurology and Psychology, West Va Univ. Morgantown, WV 26506.

This study attempted to measure combined effects of weaning time (15 vs 30 day) and housing (single vs mult. caged) on future shock-induced (4 15-min sess. of brief footshock, .8 ma) stress in 45-d old male rats, using a factorial design. 8 treatment combinations were used. Fear assessment included locomotion in a novel open field, defecation, and plasma corticosterone (pCORT).

Among groups, 30d wean/single cage/shock rats, relative to their nonshock controls, showed most marked fear in tests initiated 48 hr after shock. They had elevated fecal bolus and pCORT and lower exploratory habituation (EH) mean scores. When 2 added groups of 30d wean/single cage/shock rats were given a single inj. of saline (SAL) or centrally-acting anxiolytic chlordiazepoxide (CDP, 5 mg/kg) prior to exploratory locomotion testing, SAL rats showed an EH score lower than CDP rats, a pattern like that found in shocked vs nonshocked rats in the prior expt. EH as a useful behavioral index of fear (Johnson et at, 1991) is supported. The findings raise questions regarding the role of time in relation to multiple life stressors and fear outcomes.

300.16

CORTICOSTEROID INDUCTION OF THREAT-INDUCED BEHAVIORAL INHIBITION IN THE PREWEANLING RAT.

L. K. Takahashi* and W. W. Rubin. Dept. of Psychiatry, Univ. of Wisconsin Med. Sch., Madison, WI 53792 and Middleton Veterans Hospital, Madison, WI 53705.

In rats, basal plasma corticosterone (CORT) increases after the first postnatal week and the hypothalamic-pituitary-adrenal system becomes responsive to stressors by the end of the second week. In addition, 14-day-old rats, but not 7-day-old pups, inhibit their emission of ultrasounds and freeze when socially isolated and exposed to an unfamiliar adult male rat, a naturalistic threat. This study examined whether CORT plays a major role in the developmental emergence of threat-induced behavioral inhibition.

Ten-day-old rats were adrenalectomized (ADX) and exposed to an adult male rat at 14 days of age. ADX rats emitted more ultrasounds ($p<.05$) and exhibited less freezing ($p<.01$) than intact rats. This noninhibited occurrence in vocalizations by ADX rats was not due to a potentiated increase in ultrasound production because when tested in social isolation without the adult male rat, vocal production did not differ significantly between groups. ADX rats treated daily with CORT (3mg/kg) from 10 to 13 days of age displayed significantly more freezing ($p<.05$) when threatened at 14 days of age than ADX rats treated with lower doses of CORT (0-0.3 mg/kg). Results indicate that during development one important action of CORT is to induce in rat pups the capability to adaptively inhibit behavioral activity when threatened. Supported by grant MH-43986.

300.17

CORTICOSTERONE MODULATES EXPLORATION VIA CENTRAL MINERALOCORTICOID RECEPTORS. M.S. Oitzl* and E.R. de Kloet. CBPS, Div. Med. Pharmacol., University of Leiden, NL-2300 RA Leiden, The Netherlands.

Corticosterone (B) exerts its central effects via mineralocorticoid (MRs) and glucocorticoid receptors (GRs). MRs display a high affinity for B and are occupied at low, basal levels of B; GRs are mainly occupied at stress levels of B. MRs are thought to be involved in the regulation of the threshold of the stress response, GRs in processes of information storage. To elucidate the role of MRs in the behavioral stress response we analyzed the exploration pattern of Wistar rats in a large open field with an object in its center. First, we demonstrated that B plays a role in this task: One day after adrenalectomy (ADX) rats explored significantly more the center area and its object compared to sham-ADX or intact rats. Treatment of ADX rats with a low dose of B (50 μg/kg sc; basal condition, and preferential occupation of MRs) normalized the exploration pattern. ADX and intact rats treated with a high dose of B (1000 μg/kg sc; stress condition) showed more exploration of the center. Blockade of central MRs by a selective MR antagonist (RU28318; 100 ng/μl icv) in intact rats prevented the B-induced increase in exploring the center area. - Occupation of MRs is necessary for the exploration, not only in basal but also during stress conditions.

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MONOAMINES AND BEHAVIOR: ACCUMBENS, STRIATUM AND FRONTAL CORTEX

301.1

AMPEROZIDE REDUCES PSYCHOSTIMULANT-INDUCED HYPERLOCOMOTION BUT NOT DOPAMINE RELEASE IN NUCLEUS ACCUMBENS. K.KIMURA, G.G.NOMIKOS*, T.H.SVENSSON. Dept. of Pharmacology, Karolinska Institute,Box 60400, S-10401, Stockholm, Sweden.

Amperozide (APZ) is a novel atypical neuroleptic that appears to selectively act on the limbic system. The present study investigated behavioral and biochemical effects of APZ on either d-amphetamine (AMPH) or cocaine (COC) treated rats. Behavior was assessed by locomotor activity measurements. Compared to saline controls, APZ (5 and 10mg/kg,SC) decreased locomotion. Hyperlocomotion induced by AMPH (1mg/kg, SC) or COC (10mg/kg,IP) was greatly reduced by APZ administered 20min earlier. Biochemical data was obtained by in vivo microdialysis in freely moving animals. APZ dose-dependently increased interstitial concentrations of dopamine (DA, +30%) in the nucleus accumbens (NAC). While AMPH or COC alone increased DA levels (450% and 270%, respectively), APZ had no effect on this increase. In contrast, APZ pretreatment dose-dependently attenuated the reduction of DA metabolites induced by AMPH and COC. These results indicate that APZ blocked hyperlocomotion induced by psychostimulants, without correlative changes in DA concentrations in NAC.

301.2

INVOLVEMENT OF NUCLEUS ACCUMBENS DOPAMINE IN INSTRUMENTAL RESPONDING FOR FOOD: A MICRO-DIALYSIS AND BEHAVIORAL INVESTIGATION. J.D. Salamone*, J.D. Sokolowski, P. Kurth and L.D. McCullough. Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020. Three experiments were conducted to investigate the role of nucleus accumbens dopamine (DA) in lever pressing for food on a continuous reinforcement schedule (CRF). In the first experiment, dialysis probes were implanted into the nucelus accumbens. Compared to food-deprived control rats, rats performing on a CRF schedule showed significant increases in extra-cellular DA, which were highly correlated (r=0.90) with the number of lever press responses. In the second experiment, rats were trained to press a lever on a CRF schedule, and a computer program recorded the interresponse time (IRT) for each response, which is an index of response latency. Accumbens DA depletion produced by 6-hydroxydopamine injection led to a minor and transient suppression of total number of lever pressing responses. However, DA-depleted rats showed a persistent slowing of responses as measured by their IRT distribution. The third experiment showed that, in contrast to the effects of accumbens DA depletion, extinction decreased the number of responses but increased the speed of responding. These results suggest that increased release of accumbens DA during operant performance may be acting to modulate the rate of responding.

301.3

INVOLVEMENT OF NUCLEUS ACCUMBENS DOPAMINE IN AVERSIVE MOTIVATION: A MICRODIALYSIS AND BEHAVIORAL INVESTIGATION. L.D. McCullough*, J. D. Sokolowski, M. Cousins and J. D. Salamone. Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020.

Four experiments were conducted to investigate the role of nucleus accumbens dopamine (DA) in aversive motivation. In the first experiment groups of rats were trained on a lever press avoidance procedure, in which 0.5 mA shock was presented for 5 sec every 30 sec, but the rat could escape shock or avoid shock for 30 sec by pressing a lever. Accumbens DA depletion produced by 6-hydroxydopamine injection led to a substantial decrease in lever pressing to avoid or escape shock. In the second experiment, dialysis probes were implanted into the nucleus accumbens. Compared to control rats and rats exposed to periodic shock, rats performing the avoidance task showed significant increases in extracellular DA, DOPAC and HVA. In the last two experiments, in vivo dialysis methods demonstrated that the anxiogenic drugs beta-CCE (1.25-2.5 mg/kg) and FG 7142 (10.0-30.0 mg/kg) both increased accumbens DA release. These results indicate that DA in nucleus accumbens is important for avoidance responding, and that the involvement of accumbens DA in motivation is not unique to positive reinforcement or drugs of abuse.

301.4

BEHAVIORAL RESPONSE TO SKF 38393 IN NUCLEUS ACCUMBENS SHOWS BOTH HEMISPHERIC ASYMMETRY AND REGIONAL NON-UNIFORMITY. N. A. Otmakhova, C.H. Woodworth and R.G. Robinson*

Dept. Psychiatry, Univ. of Iowa Coll. of Med., Iowa City, IA 52242.

Previous studies in our laboratory have shown that injections of the selective D1 agonist SKF 38393 into right nucleus accumbens (NA) in rat produced significantly more spontaneous activity in 1 hours than identical injections into left Na, suggested possible hemispheric asymmetry in the distribution of D1 receptors. The present study investigates whether this asymmetry is exhibited uniformly in both frontal and caudal regions of NA. Fifteen male SD rats were implanted with cannulae chronically and laterally in both frontal and caudal regions of the NA. Every other day animals were injected with either saline or 0.25, 0.5, or 1.0 ug SKF 38393 in 0.5 ul distilled water and monitored for two hours in Omnitech cages.

Results indicated that horizontal and vertical activity were significantly increased, but that circling and sterotypy were unchanged. For total horizontal distance, four-way ANOVA revealed significant effects for side of injection (right side advantage, $F(1,14)=5.09$, $p<0.05$), dose ($F(3,12)=12.55$, $p<0.001$) and time ($F(5,10)$-42.35, $p<0.001$). Although the effect of cannula position did not reach significance, post-hot t-tests revealed that horizontal activity was greater after caudal injections, and right vs. left asymmetry was more pronounced after frontal injections. For vertical time, significant effects were found for cannula position (caudal advantage, $F(1,14)=6.0$, $p<0.03$), time ($F(5,10)=25.68$, $p<0.001$) and position-dose interaction ($F(3,12)=4.67$, $p<0.03$), but not for laterality. These data support the conclusion that the D1 system of the NA differs not only between hemispheres, but also between frontal and caudal regions within the NA, and that the mechanisms involved in horizontal and vertical movement are different.

301.5

BASAL EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS DURING AMPHETAMINE WITHDRAWAL.
Donita L. Robinson*, Dianne M. Camp and Terry E. Robinson.
Dept. of Psychology, University of Michigan, Ann Arbor, MI, 48109.

The amphetamine (AMP) withdrawal syndrome is associated with changes in dopamine (DA) activity; and in rats, it is also associated with a transient depression in spontaneous nocturnal locomotor activity. The present experiment was designed to determine the relationship between behavioral depression and changes in DA neurotransmission by use of *in vivo* microdialysis. Although microdialysis is used extensively to sample DA in the extracellular fluid, conventional techniques do not allow strong inferences about the actual extracellular concentration of DA; however, recently developed *quantitative* dialysis techniques do. One such method is 'no net flux microdialysis,' in which perfusion solutions containing varying concentrations of DA are perfused through a dialysis probe implanted into the brain. The difference between the DA concentration in the perfusate entering the probe and the concentration in the perfusate exiting the probe is measured. The point at which this difference is zero, the point of 'no net flux,' provides a direct estimate of the extracellular concentration of DA. In the present experiment this method was used to assess the temporal relationship between the basal concentration of DA in the nucleus accumbens and behavior during AMP withdrawal. Rats were pretreated with escalating doses of AMP or saline and underwent 'no net flux microdialysis' 3, 7 or 28 days following the discontinuation of drug treatment. Spontaneous nocturnal locomotor activity was also monitored. Results will be reported at the meeting.

301.6

THE RELATIONSHIP BETWEEN MOTOR ACTIVITY AND DOPAMINE NEUROTRANSMISSION IN THE DORSAL AND VENTRAL STRIATUM ACROSS THE DAY-NIGHT CYCLE.
Pamela E. Paulson* and Terry E. Robinson. Department of Psychology, The University of Michigan, Ann Arbor, MI 48109

Although the effects of drugs on dopamine (DA) systems and behavior have been studied extensively, the relationship between DA neurotransmission and *spontaneous* changes in motor activity have received little attention. The present experiment was designed to do this by use of a fully automated *in vivo* microdialysis technique. Dialysate was sampled continuously across the day-night cycle (20 hrs) in both the dorsal and ventral striatum. Motor activity and feeding were also quantified over the same period of time. The results revealed both interesting relations, and *lack* of relations, between DA neurotransmission and behavior. For example, DA metabolites varied significantly across the day-night cycle, in both structures (night > day). However, there was no significant change in accumbens DA between day and night, despite a marked increase in nocturnal motor activity. Extensive correlational analyses on hundreds of data points did reveal a small, but statistically significant relationship between DA and behavior, but this accounted for only a tiny fraction of the total variance. For example, changes in DA neurotransmission in the dorsal striatum accounted for only 14.4% of the total variance in motor activity, and in the ventral striatum only 4.0%. These findings suggest that DA release plays only a minor role in regulating the large changes in motor activity that occur across the day-night cycle.

301.7

THE ACUTE EFFECTS OF AMPHETAMINE AND COCAINE ON BEHAVIOR AND EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS IN LEWIS AND FISCHER 344 RATS.
Dianne M. Camp*, Erin K. Wolfe and Terry E. Robinson.
Dept. of Psychology, University of Michigan, Ann Arbor, MI 48109.

Lewis (LEW) and Fischer (F344) rats, two inbred strains, differ in a variety of behaviors, including cocaine-induced locomotion and the propensity to self-administer several drugs of abuse. For example, LEW rats show greater self-administration of opiates, cocaine and alcohol than F344 rats. The mesolimbic dopamine (DA) system is thought to mediate the reinforcing effects of drugs of abuse, and therefore, strain differences in the mesolimbic DA system may underlie the strain differences in self-administration behavior. However, no strain differences have been reported in D1, D2 and mazindol binding. Therefore, the purpose of this experiment was to determine whether there are strain differences in the ability of amphetamine (AMP) or cocaine (COC) to elevate the extracellular concentration of DA in the nucleus accumbens. This was done by use of in vivo microdialysis in freely moving LEW and F344 rats. Animals received two dialysis tests with a single removeable microdialysis probe lowered into the right or left accumbens during one test and into the opposite hemisphere during a second test given one week later. Each rat received saline (i.p.) plus one of 4 doses of AMP (0.5, 1, 2, or 4 mg/kg, i.p.) or COC (5, 10, 20 or 40 mg/kg, i.p.) during each test, and the effect of the drug on both extracellular DA and behavior was quantified. Dose-response curves will be presented at the meeting.
Supported by NIDA grant #04294.

301.8

TIME COURSE OF CHANGES IN STRIATAL DOPAMINE NEUROTRANSMISSION AND BEHAVIOR FOLLOWING A UNILATERAL 6-OHDA LESION.
Z. Mocsary, E. Chan, M. Noordhoorn, D. M. Camp, I. Q. Whishaw & T. E. Robinson*.
Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI 48109.

The purpose of experiments reported here was to characterize time-dependent changes in DA neurotransmission following a unilateral 6-OHDA lesion of the substantia nigra in rats, using in vivo microdialysis. In the first experiment we examined very early changes in DA neurotransmission, because amphetamine (AMP)-induced rotation completely reverses direction between 4 and 8 days after a large *(>95%)* 6-OHDA lesion. All animals turn contraversive at 4 days and ipsiversive at 8 days. This change in the direction of rotational behavior is accompanied by changes in AMP-induced DA release on the lesion side. At 4 days the lesion side shows considerable DA release in response to AMP, but by 8 days the lesion side no longer responds to AMP. In a second experiment we examined changes in extracellular DA between 4 and 30 days following DA depletion of varying severity (20-99%). Preliminary data suggest that there is a gradual normalization of basal extracellular DA in the striatum between 4 and 30 days following a *partial* lesion (<95%). This is in contrast to reports of very rapid presynaptic neuroadaptations, based on postmortem tissue measures of DA synthesis and metabolism, and suggests that the normalization of extracellular DA may better account for the time course of recovery of function.
Supported by the National Parkinson Foundation & MRC (Canada).

301.9

A MICRODIALYSIS STUDY OF EXTRACELLULAR ACCUMBENS DOPAMINE AND SEXUAL BEHAVIOR IN FEMALE SYRIAN HAMSTERS.
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Release of dopamine in the nucleus accumbens has been associated both with response to incentive motivational stimuli and increased motor activity. Taking advantage of the immobility accompanying lordosis in female Syrian hamsters we sought to examine dopamine release in the nucleus accumbens using a motivated behavior unconfounded by high levels of motor activation.

Female Syrian hamsters were injected on consecutive days with either 10 µg estradiol benzoate or the oil vehicle. After the second injection, a removable dialysis probe was lowered into the nucleus accumbens, and the animal was left over night. The next day, four 15 min basal samples were taken after which estradiol-treated animals were injected with 500 µg progesterone and the remaining animals injected again with oil. Four additional samples were taken starting 3 hr after progesterone injection. Male hamsters were then placed in the cages with the females for 1 hr, with samples collected during that period. Preliminary results indicate that introduction of the male was followed by a significant increase in dopamine only in females pretreated with estradiol and progesterone. For these females, dopamine was elevated primarily in the first 15 min following introduction of the male, despite observations of high levels of lordosis throughout the 1 hr test session. These results indicate that release of dopamine in the nucleus accumbens is triggered by incentive stimuli involved in the initiation, but not necessarily the maintenance, of sexual behavior.

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301.10

MICRODIALYSIS DETERMINATION OF DOPAMINE IN FEMALE RAT STRIATUM AND ACCUMBENS DURING SEXUAL BEHAVIOR.
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Extracellular dopamine increases in the nucleus accumbens and striatum of male rats during copulation (Damsma et al., Behav. Neurosci., 106:181, 1992.). This present experiment examines the possible role of these brain regions in the female rat, with and without the opportunity for the female to pace the rate of the sexual encounter.

Ovariectomized rats with guide cannulae aimed at the left and right striatum and accumbens were hormonally primed with estradiol benzoate and progesterone. One group of subjects was acclimated to a testing arena containing a passable barrier for the females (adapted from Erskine and Baum, Pharm. Bio. Behav., 17:857, 1982.) to allow them to pace the rate of the sexual encounter. A second group was placed in the same arena, but without the divider, so they could not escape the male rat. A third group of animals were controls, injected with hormones but no male was introduced. The testing period was approximately one hour. After two practice sessions, animals underwent *in vivo* microdialysis. Concentrations of dopamine and its metabolites within the striatum and accumbens were measured before, during and after the sexual encounter or control interval. Following testing, animals were killed and their brains sectioned to verify probe placement.

Preliminary results indicate increased extracellular dopamine in both the accumbens and striatum during the first interval after the male was placed in the chamber, and again upon the removal of the male. Moreover, the dopamine metabolites DOPAC and HVA are elevated throughout the sexual encounter, suggesting these regions play a role in behavior involved in sexual activity.

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301.11

SEX DIFFERENCES IN DOPAMINE RECEPTOR BINDING IN STRIATUM AND ACCUMBENS: REGIONAL VARIATION AND RESPONSE TO ESTROGEN. **J.B. Becker*, T.J. Bazzett, M.D. Rice & S.H. Rybak** Psychology Dept, Neuroscience Prgm, & Reproductive Sciences Prgm, Univ. Michigan, Ann Arbor, MI 48104-1687.

Estrogen modulates striatal dopamine (DA) release, striatal DA receptor binding, and DA-mediated behaviors in rats. Last year we reported that estrogen treatment induces a rapid down-regulation in DA receptor binding in the striatum of females but not in males. This experiment was conducted to further investigate sex differences and estrogen effects on DA receptor binding in rat striatum and accumbens.

Castrated (CAST) or ovariectomized (OVX) adult male and female rats received s.c. injections of oil or 2 µg estradiol benzoate (EB)/100 g. In Exp. 1, animals received 4 injections of EB or oil at 24 hour intervals and were killed 4 hr, 24 hr, or 4 days after the last injection. In Exp. 2, animals received 1 injection of EB or oil and were killed 30 min or 4 hours later. Specific binding in striatum and accumbens to [^{3}H]SCH23390 and [^{3}H]spiperone were determined from fresh frozen coronal sections using quantitative autoradiography for D1 and D2 DA receptors.

Exp 1. Striatal D1 DA receptor binding was decreased by 23% 4 hr after repeated EB in striatum of OVX ($p<0.001$), but not in CAST rats. Interestingly, D2 DA receptor binding in rostral striatum and accumbens of oil treated OVX rats was significantly greater than that of oil treated CAST. Furthermore, 4 hours after EB treatment, D2 DA receptor binding was decreased in striatum of OVX, the same trend was seen for CAST. There was regional variation in the sex difference and in the effect of EB. In Exp. 2, one dose of EB in OVX produced a rapid down-regulation of striatal D1 and D2 DA receptors. We conclude that for striatal D1 and D2 DA receptor binding: 1) there are sex differences; 2) physiological doses of EB modulate binding; and 3) there is regional variation in these effects.

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301.12

SCHEDULE-INDUCED POLYDIPSIA AND THE NUCLEUS ACCUMBENS: MEASUREMENT OF DA EFFLUX AND EFFECTS OF NMDA LESIONS. R. Weissenborn*, C.D. Blaha, P. Winn and A.G. Phillips Dept. Psych., Univ. St Andrews, Fife, Scotland KY16 9JU (RW, PW); Dept. Psych. (AGP) and Psych. & Psychiat. (CDB), Univ. Brit. Columbia, Vancouver B.C., V6T 1Z4, Canada

The role of the nucleus accumbens (NAS) in the production of schedule-induced polydipsia in rats was investigated. In Expt. 1 the effects of acquisition of SIP on DA efflux in the NAS were measured using *in vivo* rapid-scan voltammetry (Blaha and Jung, J. Electroanal. Chem. 1991, 310:317-334). Recordings from chronically implanted stearate-modified electrodes (n=5) on 3 days preceding and 3 days following SIP acquisition showed that while DA efflux increased significantly within individual 60min sessions ($24\pm3\%$, $p<0.001$), absolute DA oxidation peak heights across days did not change. Thus, increased DA efflux in the NAS was not related to the acquisition of SIP. In Expt. 2 the effects of bilateral NMDA lesions of the NAS on acquisition of SIP were measured in rats. Lesions were made by 1.0ul 0.06M NMDA infused into medial NAS (n=10); sham-lesions were made by 1.0ul phosphate buffer (n=8). Although NMDA-lesioned rats had lower post-operative body weights than controls, recovery was not affected as food and water intake increased at the same rate. After exposure to 26 daily 60min SIP sessions, 7 out of 10 NMDA- and 4 out of 8 sham-lesioned rats had become polydipsic and were included in the statistical analysis. NMDA rats were found to drink 4.8 ± 0.3ml water, compared to 8.7 ± 0.7 by controls ($p<0.01$). The number of food hopper panel pushes was unaffected by NMDA lesions. NMDA lesions of the NAS therefore did not interfere with SIP acquisition but significantly attenuated water intake in polydipsic rats. Altogether, these data suggest involvement of other, non-DA components of the NAS or its projection sites in the acquisition of SIP.

301.13

DIFFERENTIAL INFLUENCE OF THE NUCLEUS ACCUMBENS ON PATTERNS OF RAT MOTOR ACTIVITY. M.P. Paulus*, C.W. Callaway, and M.A. Geyer. UCSD Dept Psychiatry, La Jolla, CA 92093.

Dopaminergic modulation in the nucleus accumbens (ACC) has been associated with varied locomotion as opposed to stereotypy elicited by dopaminergic stimulation in the caudate-putamen. The experiments were designed to test whether DA modulation exerts differential effects on the geometrical structure and the dynamical characteristics of rat movement sequences. Specifically, 32 animals were divided into two groups, 16 animals were lesioned by injections of 6-OHDA into the ACC, 16 animals received saline injections. 48 hr and 2 weeks after lesioning, the rats were tested for 2 hr in the Behavioral Pattern Monitor (BPM). 8 animals of each group were injected with saline or 5.0 mg/kg nomifensine, respectively. The following measures of rat motor behavior were obtained: amount of motor activity (*counts*, the temporal scaling exponent α), the average spatial scaling exponent (d), the fluctuation spectrum of local spatial scaling exponents ($f(d)$), the average dynamical entropy (h), and the fluctuation spectrum of local dynamical entropies ($S(h)$). 6-OHDA ACC lesions reduced the amount of activity in saline treated animals which was accompanied by a slight but non-significant increase in the average scaling exponent, d. Nomifensine reverted the reduced activity levels but increased strongly the average spatial scaling exponent, d. The fluctuation spectrum indicated that a reduced contribution of straight local movements and an increased contribution of local movements accounted for the shift of the average scaling exponent. The contribution of straight movements for 6-OHDA-lesioned animals was significantly reduced compared to the sham-lesioned animals. Moreover, nomifensine increased significantly the contribution of straight movements in sham-lesioned relative to 6-OHDA-lesioned rats. These results suggest that the contribution of straight and directed movement sequences is modulated sensitively in the ACC which might correspond to the initiation of goal directed motor behavior.

301.14

DISSOCIATION OF THE EFFECTS OF MK-801 ON BASELINE AND AMPHETAMINE STIMULATED LOCOMOTOR ACTIVITY AND DOPAMINE OVERFLOW IN THE NUCLEUS ACCUMBENS. J.P. Druhan*, H. Rajabi and J. Stewart, Center for Studies in Behavioral Neurobiology, Psychology Department, Concordia University, Montréal, Que, Canada, H3G 1M8.

We examined the effects of the NMDA antagonist, MK-801 on locomotor activity and on extracellular dopamine (DA) levels in the nucleus accumbens (NAS) both when given alone and when co-injected with d-amphetamine HCl (1.5 mg/kg, IP). Microdialysis probes (removable, concentric design) were inserted into the NAS through stationary unilateral guide cannulae 18 - 24 h before testing. On the morning of the test session the flow rate of the modified ringer solution was set at 1.75 µl/min and baseline samples were collected every 20 min for 2 to 3 hrs, with locomotor activity monitored continuously by photocells stationed in the test chambers. After this baseline period, rats in the first experiment were injected with MK-801 (0.125, 0.25 or 0.50 mg/kg, IP) or saline, and activity and DA overflow were measured for 160 min post-injection. In the second experiment, rats were given an injection of d-amphetamine 40 min after receiving these same doses of MK-801 and testing was continued for 120 min. The two highest doses of MK-801 (0.25 and 0.50 mg/kg) produced increases in locomotor activity when injected alone, and augmented the locomotor stimulant effects of d-amphetamine when co-injected with this drug. In contrast, MK-801 failed to increase DA overflow when given alone and it non-significantly elevated d-amphetamine stimulated DA overflow only at the highest dose (0.50 mg/kg). These results suggest that the locomotor activating effects of MK-801 are not due to actions of this drug on dopaminergic projections to the NAS.

301.15

PRIOR EXPOSURE TO INTRA-VTA AMPHETAMINE SENSITIZES THE NUCLEUS ACCUMBENS DOPAMINERGIC RESPONSE TO SYSTEMIC AMPHETAMINE AS MEASURED BY IN VIVO MICRODIALYSIS. P. Vezina* and B. Zurakowski. Neurosciences, Loeb Medical Research Institute, Ottawa Civic Hospital, Ottawa, Ontario K1Y 4E9 Canada.

Injections of amphetamine into the ventral tegmental area (VTA), but not the nucleus accumbens (N.Acc.), have been shown to sensitize rats to the locomotor activating effects of amphetamine, cocaine and morphine. These findings, together with others showing that this type of behavioral sensitization is paralleled by enhanced reactivity in mesolimbic dopamine (DA) neurons, suggest that the induction of sensitization in these neurons occurs in their somatodendritic region. The present experiment investigated this possibility. Rats received three injections of either d-amphetamine sulfate (2.5 ug/0.5 ul/side) or saline into the VTA, one injection every third day. Two weeks following the last injection, DA neurotransmission in the N.Acc. was assessed with in vivo microdialysis before and after a challenge with systemic amphetamine (1.0 mg/kg, i.p.). Prior to challenge, basal extracellular concentrations of DA in the N.Acc. did not differ in VTA amphetamine and saline preexposed animals. Following challenge, however, both groups showed an increase in N.Acc. DA but this was significantly greater (two fold) in VTA amphetamine preexposed animals. These latter animals also showed significantly higher extracellular concentrations of DOPAC and HVA in the N.Acc. before and after challenge. These findings demonstrate that amphetamine applied to the somatodendritic region of mesolimbic DA neurons sensitizes these neurons as evidenced by their enhanced N.Acc. DA response to a systemic amphetamine challenge.

301.16

STRESS-INDUCED SENSITIZATION OF DOPAMINE AND NOREPINEPHRINE RELEASE IN MEDIAL PREFRONTAL CORTEX. Paul J. Gresch*, Janet M. Finlay, Michael J. Zigmond and Alan F. Sved, Dept. of Cellular & Behavioral Neuroscience, Center for Neuroscience, University of Pittsburgh, Pgh, PA 15260

Prior exposure to chronic cold (5°C, 2-3 weeks) results in enhanced release of norepinephrine (NE) in hippocampus and medial prefrontal cortex (mPFC) in response to 30 min of low intensity intermittent tail-shock or tail-pressure (Nisenbaum et al., *J Neurosci*, 1991, Finlay et al., *Soc Neurosci Abs*, 1991). Whereas acute stress elicits an increase in dopamine (DA) release in mPFC, nucleus accumbens (NAS) and striatum (STR) (Abercrombie et al., *J Neurochem*, 1989), at present, it is unknown whether DA neurons also exhibit this sensitization following chronic cold. Therefore, we examined the effect of prior exposure to chronic cold on extracellular DA and NE in the mPFC and DA in the NAS and STR in response to tail shock using *in vivo* microdialysis. Basal DA and NE values did not differ between chronically cold-stressed and naive rats in any of the brain regions. In the mPFC, tail shock elicited a greater increase in DA and NE release in chronically cold-stressed than naive rats. However, the stress-induced release of DA in NAS or STR did not differ between cold-stressed and naive rats.

	mPFC-NE	mPFC-DA	NAS-DA	STR-DA
Chronic cold	128%* (5)	238%* (5)	46% (3)	41% (7)
Naive	56% (5)	78% (5)	32% (4)	41% (11)

Results are percent increase from baseline; (n) = # of animals,
* significantly different than naive, $p < 0.05$.

These data suggest that like cortical NE projections, cortically projecting DA neurons exhibit sensitization after chronic exposure to stress, whereas subcortical DA projections do not. Supported in part by a MRC fellowship (JMF) and USPHS grants MH43947 and MH45156.

301.17

EFFECTS OF REPEATED EXPOSURE TO STRESS ON EXTRACELLULAR DOPAMINE LEVELS IN PREFRONTAL CORTEX OF RAT. M.D. Doherty* and A. Gratton. Douglas Hosp. Res. Ctr, McGill Univ., Montréal, Canada, H4H 1R3.

We have recently reported that, with repeated daily exposure, increases in dopamine (DA)-related electrochemical signals recorded in nucleus accumbens (NAcc) during restraint stress become progressively larger (Doherty & Gratton, 1992). In this experiment, we examined the effects of five, once daily exposures to restraint (15 min) or tail pinch (10 min) stress on extracellular DA levels in prefrontal cortex (PFC) using high-speed chronoamperometry. Restraint caused a pronounced increase of the electrochemical signal in PFC that out-lasted the period of restraint but the magnitude of which did not change across test days. However, as previously reported with microdialysis (Imperato et al., 1991), releasing the animal from the restrainer further increased the signal. Interestingly, this second increase became progressively larger with repeated testing. In contrast to restraint, the amplitude of the electrochemical signal recorded during tail pinch increased with each daily test and no further increase in the signal occured upon termination of stress. These results and those of our previous studies suggest that differences between the stress responses of the meso-PFC and meso-NAcc DA pathways depend on the type of stress and the number of exposures to stress. Whereas a single exposure to restraint caused a pronounced increase in extracellular PFC DA levels, similar effects of restraint were observed in NAcc only after several daily exposures and while the effects of tail pinch on PFC DA levels increased with repeated testing, those of restraint did not; opposite to what was observed in NAcc. Unlike meso-NAcc DA neurons, meso-PFC DA neurons appear to respond to the cessation of a stressful event in addition to stress per se. Finally, pretreatment with apomorphine, at a dose (50µg/kg, s.c.) that inhibited restraint-elicited increases in NAcc, did not affect the electrochemical signal elicited by restraint in PFC. The differential effect of apomorphine could be due to the reported paucity of impulse-regulating receptors on meso-PFC DA neurons. Alternatively, it may suggest that stress-elicited electrochemical signals in PFC also reflect increased extracellular levels of an electroactive species other than DA. *Supported by FCAR and MRC.*

301.18

INVOLVEMENT OF PREFRONTAL CORTEX IN AMPHETAMINE-INDUCED SENSITIZATION OF MESOLIMBIC DOPAMINE NEURONS. K. E. Banks* and A. Gratton. Douglas Hosp. Res. Ctr, McGill Univ., Montréal, Canada, H4H 1R3.

The stimulant effect of d-amphetamine (d-AMP) on locomotor activity and on mesolimbic dopamine (DA) neurotransmission is potentiated--or sensitized--for some time following repeated, daily administration of this and other psychostimulants. We have previously reported that, with repeated daily presentation, increases in DA levels in nucleus accumbens (NAcc) elicited by a sex-related stimulus become progressively larger and more recently, we reported that this effect was potentiated in animals with 6-OHDA-induced lesions of DA terminals in prefrontal cortex (PFC). In the present study, we examined the role played by meso-PFC DA neurons in sensitization to the stimulant effects of d-AMP. Six weeks prior to testing, male rats received bilateral microinfusions of 6-OHDA or of vehicle (sham) into PFC. Following 3 days of habituation to the test chamber, the locomotor response of each animal to a test dose of d-AMP (0.5 mg/kg ip) was monitored. On each of the five following days, animals from both groups received either d-AMP (1.0 mg/kg ip) or vehicle. Five and then thirteen days later, the locomotor response of each animal to the test dose of d-AMP was again measured. Selective destruction of DA terminals in PFC was found to significantly facilitate development of sensitization to the locomotor stimulant effect of d-AMP. That is, while lesioned and sham-lesioned animals responded similarly to the first d-AMP test dose, repeated daily d-AMP injections sensitized lesioned animals to the effects of the two subsequent d-AMP test doses to a greater extent than sham-lesioned animals. Consistent with these results, d-AMP caused a 3-fold greater increase of DA-dependent electrochemical signals recorded in NAcc of lesioned than in NAcc of sham-lesioned animals. The results of this study are consistent with those of others indicating that the DA input to PFC exerts an indirect, inhibitory influence on mesolimbic DA neurotransmission. The present experiment also suggests that the long-lasting enhancement of mesolimbic DA neurotransmission that occurs with repeated exposure to a stimulus may reflect changes to a DA-sensitive mechanism in PFC. *Supported by FCAR, MRC and NSERC.*

301.19

NUCLEUS ACCUMBENS DOPAMINE DEPLETIONS: PROXIMATE STRIATAL AND BEHAVIORAL CHANGES. L.T.L. Tran* and E. Castañeda. Dep't. of Psychology, Arizona State University, Tempe, AZ 85284.

It has been suggested that some initial behavioral depression following brain damage might be due to secondary changes in other brain areas. To test this, rats were given bilateral 6-hydroxydopamine lesions of the nucleus accumbens and then tested for changes in extracellular levels of striatal monoamines using *in vivo* microdialysis. Rats were tested on days 2 and 3 post-lesion (Acute LX) or, after a recovery period, on days 14 and 15 (Chronic LX). Dopamine (DA), its metabolites DOPAC and HVA, and 5-HIAA were assayed by HPLC-EC from 20-min samples. After baseline measures, a Ringer's solution containing 60 mM potassium was infused through the microdialysis probe for 20 min to evoke depolarization-induced overflow. The next day after additional baseline measures, animals received d-amphetamine (1.5 mg/kg, s.c.). Extracellular levels of DOPAC, HVA and 5-HIAA were generally enhanced in the Acute LX group compared to Chronic LX and control animals. Also, the Acute LX group displayed an enhancement in amphetamine-stimulated DA release that was not observed *after* 2 weeks recovery (Chronic LX). Finally, these early changes in striatal DA release were related to a potentiation in AMPH-induced stereotypy. Results will be discussed in terms of how these changes may relate to recovery of function.

DRUGS OF ABUSE: PHARMACOLOGY OF COCAINE

302.1

COMPARISON OF THE REINFORCING EFFECTS OF THE NOVEL TROPANE ANALOG (PTT) WITH COCAINE. S.I. Dworkin*, H. Davies , E. Saikali , S.Childers and J.E. Smith. Center for the Neurobiology of Drug Abuse, Dept. of Phys/Pharm., Bowman Gray Sch of Med., Dept. Chemistry, Wake Forest Univ., Winston-Salem, NC, 27157.

Novel cocaine derivatives have been synthesized in a concerted effort to develop pharmacotherapies for the treatment of cocaine abuse. The reinforcing effects of one of these compounds 2(β)-propanoyl-3(β)-(p-tolyl)tropane (PTT) have been assessed in rats trained to self-administer cocaine under an FR 10 schedule and in drug naive subjects. Doses of PTT (1.0-90 µg/inj) were substituted for cocaine (0.33 mg/inj) during 3 and 6 hour self-administration sessions. All doses of PTT substituted for cocaine and resulted in an inverted "U" shaped function for injections self-administered. The pattern of self-administration consisted of alternating periods of long interinjection intervals (>20 min) followed by sustained periods of very rapid responding resulting in several very short interinjection intervals (<60 sec). This pattern was dose related, repeated throughout the 3 or 6 hour sessions, and was different from those maintained by either saline or the DA uptake inhibitor buproprion. PTT (10µg/inj) did not engender or maintain responding in drug naive rats, however, these subjects did self-administer cocaine following exposure to PTT. The novel cocaine derivative PTT maintains responding when acutely substituted for cocaine but does not appear to be reinforcing in drug naive subjects. Further evaluations of the pharmacologic, neurobiologic and behavioral actions of this compound should increase our understanding of the effects of cocaine and provide useful information for the development of pharmacotherapies for the treatment of cocaine abuse. (Research Supported by NIDA P50- DA06634)

302.2

EFFECTS OF THE NOVEL COCAINE ANALOG, PTT, ON EXTRACELLULAR DOPAMINE LEVELS IN THE NUCLEUS ACCUMBENS AND LOCOMOTOR ACTIVITY IN THE RAT. L.L. Devaud*, L.J. Porrino, S.R. Childers, H.W. Davies, E. Saikali, S.R. Dworkin and J.E. Smith, Dept. of Physiology & Pharmacology, Bowman Gray School of Medicine; Dept. of Chemistry, Wake Forest University, Winston-Salem, NC.

A series of novel cocaine analogs has recently been synthesized. The present experiments examined the effects of one of these, 2(ß)-propanoyl-3(ß)-(*p*-tolyl) tropane (PTT), on extracellular dopamine (DA) levels in the nucleus accumbens and on locomotor activity in the rat. These effects were compared to those of cocaine. PTT or cocaine was given iv to freely-moving rats and extracellular DA levels were measured by *in vivo* microdialysis. Extracellular DA levels were increased dose-dependently within 15 min of PTT treatment and remained elevated for up to 3 hrs. After cocaine DA levels returned to baseline within 1 hr of treatment. PTT, administered iv, also produced significant dose-dependent increases in locomotor activity as measured by photocell interruption. These increases persisted for 2.5 hrs after low doses and up to 4 hrs at the highest dose tested (2 mg/kg). Rearing and sniffing similar to that seen following cocaine were observed at lower doses (0.1-0.5 mg/kg) whereas intense stereotypic head bobbing was evident at higher doses (1-2 mg/kg). These data indicate that PTT is a potent cocaine analog with stimulant properties, but is different from cocaine in its duration of action and the form and intensity of its behavioral activating properties.
Supported by P50DA06634.

302.3

BINDING AND TRANSPORT PROPERTIES OF NOVEL 2-SUBSTITUTED TROPANE ANALOGS OF COCAINE. S.R.Childers*, T. Sexton, B. Bennett, C. Hyde, E. Saikali and H. Davies, Dept. Physiol/Pharmacol, Bowman Gray Sch. Med. and Dept. Chemistry, Wake Forest Univ., Winston-Salem, NC 27157.

A novel scheme utilizing vinylcarbenoid precursors has been developed for the synthesis of novel tropane analogs of cocaine. Using this method, 19 analogs have been tested for activity in binding to dopamine transporters in rat striatal membranes using [^{125}I]RTI-55, and inhibition of dopamine transport into dissociated striatal neurons from fetal rat brain. In all the analogs, the aryl group at the 3 position was directly bound to the tropane ring (as in WIN-35,428), and methyl or ethyl ketone moieties were present at the 2 position instead of the typical ester group. In general, all analogs displayed significant correlation (R=0.95) of IC_{50} values in displacing [^{125}I]RTI-55 binding and inhibiting dopamine uptake. Methyl and ethyl ketone groups in the 2 position did not affect potency compared to ester groups in the same position, but substituents on the benzene ring greatly affected potencies. Analogs which contained the 2-ketone in the β position were 10-80 times more potent than their α isomers. The two most potent analogs were the naphthyl and toluyl derivatives (IC_{50} values were 5-10 nM, vs. 170 nM for cocaine), while replacement of the aryl with either ethyl or cyclohexyl drastically reduced potency (to >100 μM and 5 μM, respectively). These novel compounds provide new understanding of SAR of cocaine analogs at dopamine transport sites.

Supported by PHS grant DA-06634 from NIDA.

302.4

POTENT PHENYLTROPANE COCAINE ANALOGS INHIBIT SEROTONIN (5-HT) DORSAL RAPHE CELL FIRING.

J.M. Lakoski*, H. Zheng, J.W. Boja[1], F.I.Carroll[1] and M.J. Kuhar[1], Dept. Pharmacol. & Tox., Univ. Texas Med. Br., Galveston, TX 77555 and [1]Addiction Research Center, NIDA, Baltimore, MD 21224.

Potent substituted-3β-phenyltropane analogs of cocaine have been developed with high affinity for cocaine and dopamine uptake sites in the striatum (Boja *et. al*, Eur.J. Pharm. 184:329-332, 1990). We have addressed whether 3β-(4-chlorophenyl)-(RTI-COC-31) and 3β-(4-methylphenyl)-tropane-2-carboxylic acid methyl esters (RTI-COC-32) interact with serotonergic function utilizing both *in vivo* and *in vitro* recording preparations of the dorsal raphe nucleus (DRN).

Electrophysiologic recordings were conducted in chloral hydrate anesthetized adult male Sprague-Dawley rats or in 400μm thick slices containing the DRN. Intravenous administration of RTI-COC-32 and RTI-COC-31 completely inhibited spontaneous cell firing with a 5X and 10X increased potency, respectively, as compared with cocaine (ID_{50} = 0.125 and 0.07 mg/kg versus 0.6 mg/kg). A 2X enhanced potency of RTI-COC-32 (ID_{50} = 4.2μm) in comparison to cocaine was evident *in vitro*. Indicative of a 5-HT reuptake inhibitor, maximal inhibition of cell firing produced by RTI-COC-32 was delayed beyond the period of drug perfusion and potentiated with addition of 10μm 5-HT. The data demonstrate a potent interaction of novel cocaine analogs with serotonergic neuronal systems.

Supported by PHS AG00450 (JML).

302.5

DIFFERENTIAL EFFECTS OF ISOTHIOCYANO-BTCP DERIVATIVES ON THE BINDING OF RADIOLABELED LIGANDS TO AND ON DOPAMINE UPTAKE IN RAT STRIATUM. L.P. RAYMON*, B. DE COSTA[1], C. DOMINGUEZ[1], M.E. ELDEFRAWI. Dept. Pharmacol. and Exp. Therap., UMAB, Sch. Med., Baltimore, MD 21201 and [1] Lab.Medicinal.Chem., NIH.

The potency of several isothiocyanate (NCS) derivatives of N-[1-(2-benzo(b)thienyl)cyclohexyl]piperidine (BTCP), differing in the position of the NCS group, on [^{3}H]BTCP, [^{3}H]cocaine binding, and [^{3}H]dopamine uptake in rat striatal membranes was studied *in vitro*. BTCP is a potent inhibitor of dopamine uptake, and [^{3}H]BTCP binds to high and low affinity sites. High affinity [^{3}H]BTCP and [^{3}H]cocaine binding have similar drug profiles. The isothiocyanate derivative in *meta* position to the sulphur of the thiophenyl was a poor inhibitor of [^{3}H]BTCP and [^{3}H]cocaine binding, as well as of [^{3}H]dopamine uptake. Preincubation with it, preferentially inhibited the low affinity [^{3}H]BTCP binding site. Furthermore, various isothiocyanate derivatives discriminated between cocaine and dopamine binding sites. When the isothiocyanate group was *para* to the sulphur of the thienyl moiety, the compound was as potent as BTCP or cocaine in inhibiting dopamine uptake but displaced cocaine binding very poorly.

These findings suggest that [^{3}H]BTCP and its NCS derivatives may be useful probes to investigate the dopamine transporters and in particular the cocaine receptor site.

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302.6

THE DOPAMINE TRANSPORTER: SELECTIVE INHIBITION BY NOVEL COCAINE ANALOGS. T.A. Kopajtic*, J.W. Boja, M. Milberger, F.I. Carroll, A.H. Lewin, P. Abraham, K. Parham and M.J. Kuhar. NIDA Addiction Research Center, P.O. Box 5180, Baltimore, MD 21224 and Research Triangle Institute, Research Triangle Park, NC 27709.

Previous work by our laboratories has determined that modifications of the cocaine molecule at the C-2 position resulted in increased selectivity for the dopamine transporter compared to the NE and 5-HT transporters. Modification of the highly potent cocaine analogs RTI-31 (3β-[4-chlorophenyl]tropane carboxylic acid methyl ester) and RTI-55 (3β-[4-iodophenyl]tropane-2β-carboxylic acid methyl ester) at the C-2 position also resulted in selective potency changes at the DA, NE and 5-HT transporters. Elimination of the methyl ester from the C-2 position decreased DA, NE and 5-HT potency 400-500 fold. Substitution of the 2 methyl ester with a carboxamide decreased DA potency 10 fold; however, NE and 5-HT potencies were reduced 50 and 75 fold respectively. Substitution of an isopropyl ester or a phenyl ester at the C-2 position had no or little effect upon DA potency, but NE and 5-HT potencies were reduced greater than 50 fold. This increase in selectivety for the DA transporter was reduced with halogen addition to the phenyl ring. The use of these cocaine analogs will allow for the selective study of the dopamine transporter.

302.7

INTRAVENTRICULAR PROCAINE ACTION ON RESPIRATORY PATTERNING. C.A. Richard*, R.K. Harper and R.M. Harper, Brain Research Institute and the Dept. of Anatomy & Cell Biology, UCLA School of Medicine, Los Angeles, CA 90024.

Intravenous or cerebral ventricular administration of cocaine results in a short-latency, substantial, and prolonged increase in respiratory rate, which is usually accompanied by a reduction in breath-to-breath variability. Cocaine has the potential to exert respiratory effects through anesthetic action, as well as through its traditional modes. We partitioned local anesthetic actions on respiratory patterning by recording diaphragmatic EMG responses to intraventricular cocaine and procaine administration and measuring total cycle time (Ttot), as well as the inspiratory and expiratory phase durations (Ti and Te, respectively). Adult cats were anesthetized and instrumented with EMG leads in the costal diaphragm and with ventricular cannulae. After 1 wk of recovery, baseline recordings were performed for 30-60 min in unanesthetized, unrestrained animals, following which an injection (0.625, 1.25, 2.5mg) of procaine or cocaine was delivered intraventricularly. Procaine significantly reduced Ttot at 10 min post-injection, with values rapidly returning to baseline after this time. Cocaine-induced respiratory effects persisted for 2-3 hr post-administration. No significant differences occurred between Ttot values for cocaine and procaine at 10 min. Breath-to-breath variability was reduced following some, but not all, procaine injections; such reductions occurred only at 10 min. We conclude that at least part of the early tachypnea that results from cocaine intoxication results from anesthetic effects. (Supported by DA04913)

302.8

COCAINE AND COCAETHYLENE: ACUTE AND SUBACUTE MOTILITY EFFECTS. Y. Wang and W.M. Davis*, Dept. of Pharmacology and Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677.

Concurrent use of cocaine (C) and ethanol (E) is a popular polydrug abuse pattern. A metabolite of C, cocaethylene (CE), is formed only in the presence of E. Motor activity after C, CE and a 50/50 combination (COM) was tested in CD-1 mice at doses equimolar to 0.3, 1, 3, 10 & 30 mg/kg of C. Both C and CE increased motor activity at the 2 highest doses, but not at lower ones. Motility after COM increased only at the highest dose. When mice received a 2nd exposure 1 wk. later to the same doses, the motor response to CE was elevated over the 1st exposure at the 2 highest doses, and to C at 30 mg/kg only. Daily dosing was then conducted in the 3 highest dose groups of each drug, and activity was tested on the 6th & 12th days of subacute dosing. Motility after the highest CE dose was lower on the 6th day than at the 2nd acute dose. Motility after doses of COM equimolar to 3 and 10 mg/kg of C increased so that 12th day activity was significantly greater than on 1st and 2nd acute exposures. Thus, the data are suggestive of both sensitization and tolerance development. (CE supplied by NIDA)

302.9

COCAINE DIFFERENTIALLY DECREASES SYMPATHETIC NERVE ACTIVITY IN ANESTHETIZED RATS. K.J. Varner*, T.P. Abrahams and M.C. Cuntapay. Dept. Pharmacology & Alcohol and Drug Abuse Center, LSUMC, New Orleans, LA 70112.

It is widely believed that an increase in sympathetic outflow is responsible for the increases in arterial pressure (AP), heart rate (HR) and circulating catecholamines after administration of cocaine. However, recent evidence suggests that cocaine decreases sympathetic nerve activity (SNA) in dogs and cats. The present study was performed to examine the sympathetic nerve responses elicited by the intravenous administration of cocaine in pentobarbital anesthetized rats. Platinum bipolar electrodes were used to record renal (RSNA, n=7), splanchnic (SSNA, n=6) or lumbar (LSNA, n=7) sympathetic nerve activity. Cocaine (0.005-5 mg/kg) elicited dose-dependent increases in mean AP (MAP) (max. 18±2 mmHg) and decreases in SNA on the 3 nerve types. The duration of the pressor responses (0.5-2.5 min.) was markedly shorter than the duration of the sympathoinhibitory responses (2-60 min.), indicating that the sympathoinhibitory responses were not baroreceptor reflex-mediated. The magnitude of the cocaine-mediated decreases in SNA between the 3 nerves was not uniform. For example, the maximum dose of cocaine (5 mg/kg) decreased SSNA 97±2%, RSNA 82±4% and LSNA 55±6%. For all doses, the rank order of sensitivity (most to least) to the inhibitory actions of cocaine was SSNA > RSNA > LSNA. As the dose response curves of MAP for the three experimental groups were virtually identical, the differential nerve responses were not due to differences in pressor-mediated baroreceptor reflex activity. We conclude that, in anesthetized rats, cocaine: 1) produces sympathoinhibition rather than sympathoexcitation, and 2) differentially inhibits activity in sympathetic nerves innervating different target organs.

302.10

CARDIOVASCULAR RESPONSES ELICITED BY COCAETHYLENE AND COCAINE IN INTACT AND ADRENAL DEMEDULLATED RATS. T.P. Abrahams*, W.L. Reed, Z. Zhang and K.J. Varner. Dept. Pharmacology & Alcohol and Drug Abuse Center, LSUMC, New Orleans, LA 70112.

Cocaethylene (benzoylecgonine ethyl ester, CE) has been implicated in the cardiovascular toxicity associated with concurrent use of cocaine (C) and ethanol. In the present study we compared the changes in arterial pressure (AP), heart rate (HR) and myocardial oxygen consumption (MOC) elicited by intravenous CE or C in conscious unrestrained rats. The involvement of adrenal catecholamine release in mediating these cardiovascular responses was assessed by administering CE or C 1 week after bilateral adrenal demedullation. In sham operated rats CE (n=6) and C (n=9) (0.005-5 mg/kg, iv) elicited similar dose-dependent increases in mean AP (MAP) up to 33±5 and 33±4 mmHg, respectively. The lower doses of CE (0.005-1 mg/kg) and C (0.005-0.5 mg/kg) elicited tachycardia (max. 37±6 and 20±6 bpm, respectively). The larger doses (CE=3-5 mg/kg; C=1-5 mg/kg) produced bradycardia (max. -38±3 and -34±22 bpm, respectively). Drug-induced changes in rate-pressure product (R-PP; HR x systolic AP) were used as an index of MOC. Doses of CE and C which elicited tachycardia also produced dose-dependent increases in R-PP (max, CE=27%; C=40%), while doses which produced bradycardia produced smaller increases in R-PP. Bilateral adrenal demedullation did not significantly change the magnitude or pattern of the MAP, HR or R-PP dose response relationships. We conclude: 1) CE and C produce a similar pattern of MAP and HR responses in conscious unrestrained rats, 2) CE, like C, increases MOC in conscious rats, 3) the pattern of cardiovascular responses elicited by CE and C does appear to be dependent on adrenal catecholamine release.

302.11

ACUTE COCAINE TOXICITY: THE EFFECT OF AGENTS IN NON-SEIZURE INDUCED DEATH. RW Derlet*, J Tseng, and TE Albertson. Emergency Dept., UC Davis, Davis, CA 95616

Death from cocaine intoxication results from one of multiple mechanisms including seizures, cardiovascular collapse or apnea. In the free-moving rat model, continuous seizures are the primary means of death. In order to study mechanisms of death unrelated to seizures, we supressed lethal seizures with diazepam and investigated the effect of several pharmacologic agents. **METHODS:** Male S.D. rats were pre-treated with vehicle, diazepam 5 mg/kg alone (Dzp) or in combination with and test agents; nifedipine 1 mg/kg (Nifed), propranolol 5 mg/kg (Prop) or prazocin 5 mg/kg (Praz). Five minutes after pre-treatment animals received cocaine 100 mg/kg. Each test group consisted of 15 animals and all agents were given i.p. Two animals in each group had cortical electrodes implanted and EEG's monitored. **RESULTS:** In Group 1, animals received vehicle followed by cocaine. The incidence of seizures was 100% and death 100%. Group 2 received Dzp in combination with Prop, Praz or Nifed resulting in no seizures and no deaths. Group 3 received Dzp followed by cocaine resulting in no seizures and 53% death. Group 4 received Dzp and Nifed followed by cocaine resulting in 0% seizures and 87% death. Group 5 received Dzp and Prop followed by cocaine resulting in 10% seizures and 87% death. Group 6 received Dzp and Praz resulting in 0% seizures and 13% death ($p \leq 0.02$). EEG recordings showed electrical spikes in all animals observed to have seizures and an absence in non-seizuring animals. **CONCLUSION:** Animals protected from seizures with Dzp have non-seizure deaths after high dose cocaine. Death in these animals is potentiated with Nifed and Prop, but protected with prazocin.

302.12

TIME COURSE OF COCAINE'S CNS ACTIVATION: AN AUTORADIOGRAPHIC BLOOD FLOW STUDY. E.A. Stein* and S.A. Fuller. Dept. of Psychiatry, Medical College of Wisconsin, Milwaukee, WI 53226.

Among the notable effects cocaine exerts upon the mammalian CNS is its potent reinforcing property. This action is extremely short lived and is thought to reflect cocaine's short plasma half life of 15-20 min. However, plasma levels alone apparently cannot predict these actions as, following a loading dose, rats self-administer additional cocaine prior to decreases in plasma levels. It thus appears that temporal alterations in regional brain activity may be critical in understanding cocaine's reinforcing ontogeny. With its 30 sec measurement window, the autoradiographic regional cerebral blood flow (rCBF) method of Sakurada et al permits a study of the time course of cocaine action simultaneously in large numbers of brain regions. As such, rats were prepared with acute femoral arterial and venous catheters. Four to five hours after surgery, rCBF was determined 1, 5, 15 and 45 min after a single 1.0 mg/kg IV cocaine injection. A heterogenous pattern of cerebral activation was seen. Of those regions which responded to cocaine, all did so with a time threshold of 1 min. Several groups of structures were evident: 1) those regions whose rCBF returned to baseline prior to the 5 min measurement group (including the claustrocortex, lateral and basomedial amygdala); 2) those returning to baseline between the 15 and 45 min measures (including the VTA, medial septum, ventral pallidum and cingulate); and 3) those demonstrating persistent rCBF alterations at least through the 45 min group (including accumbens, olfactory tubercle, hippocampus, lateral septum and caudate). It thus appears that cocaine's duration of action varies heterogenously across both time and brain structure. Supported in part by NIDA grant DA 05012 to EAS.

302.13

EFFECT OF REPEATED COCAINE ADMINISTRATION ON [^{3}H]WIN 35,428 BINDING *IN VIVO* IN DORSAL AND VENTRAL STRIATUM AND MIDBRAIN; REGIONAL DISSECTION AND QUANTITATIVE AUTORADIOGRAPHY Elizabeth J. Cline*, Nancy S. Pilotte, Wm. Mark Mitchell, Ursula Scheffel, and Michael J. Kuhar. NIDA Addiction Research Center, P. O. Box 5180, Baltimore, MD 21224, and The Johns Hopkins Medical Institutions, Baltimore, MD 21205

Studies examining neurochemical correlates of cocaine abuse have demonstrated regional differences in the distribution of DA uptake sites in mesolimbic and nigrostriatal pathways implicated in the behavioral effects of cocaine. Repeated cocaine administration can produce changes in stimulated DA release, DA uptake, and binding of various ligands to DA transporter sites in these pathways in rats. These effects are influenced by dose, dosing regimen, and route of administration

In these experiments, we examined the regulation of cocaine binding sites in rats given cocaine or saline for 10 days. Cocaine was given by either i.p. injection (10mg/kg) or i.v. infusion (total 10mg/kg in a 2 hr session). One or 10 days after the last cocaine exposure, *in vivo* binding of [^{3}H]WIN 35,428 was measured 1 hr after i.v. injection of either 10 or 20 μCi. Potential changes in *in vivo* binding as a result of cocaine administration were measured by dissolving and counting dissected tissue or by quantitative autoradiography. Preliminary results show the i.p. regimen did not change [^{3}H]WIN 35,428 binding in cocaine injected animals as compared to controls at either 1 or 10 days after the last injection. The results of these studies will provide further information as to the influence of regimen, route of administration, and time since cocaine exposure on regulation of transporter binding sites in vivo.

302.14

PRAZOCIN AND ICS 205-930 HAVE DIFFERENTIAL EFFECTS ON HIGH AND LOW RATES OF COCAINE- AND AMPHETAMINE INDUCED RESPONDING. B. J. Van Groll, R. B. Saunders and J. B. Appel*. Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, S. C. 29208.

Rats (n=8/group) were trained to press a lever for water under a schedule of continuous reinforcement and were treated with either cocaine or *d*-amphetamine (AMP), the a_1 antagonist prazocin (PRAZ) and the 5 HT_3 antagonist ICS 205-930 (ICS). When given alone, 1 and 3 mg/kg of cocaine had no reliable effects on response rates; 10 mg/kg decreased high rates. AMP had no effect on low and decreased high rates at a dose of 0.03 mg/kg, increased low and decreased high rates at 0.56 mg/kg, and had no effect on either rate at 3.0 mg/kg. When given alone, PRAZ (1 mg/kg) increased both low and high rates in animals previously treated with cocaine but not with AMP; ICS (10 mg/kg) decreased both low and high rates in animals previously treated with cocaine but not with AMP. When given in combination with cocaine (1 mg/kg), PRAZ (1 mg/kg) increased low and decreased high rates while ICS (10 mg/kg) increased both low and high rates. When given in combination with AMP, PRAZ (1 mg/kg) had no effects on low or high rates while ICS had no effect on low and decreased high rates. Combinations of cocaine (1 mg/kg), PRAZ (1 mg/kg) and ICS (10 mg/kg) lowered the rate-increasing effects and potentiated the rate-decreasing effects of the combination of cocaine and ICS. Combinations of AMP (0.03 mg/kg), PRAZ (1 mg/kg) and ICS (10 mg/kg) decreased low rates but did not affect high rates. These data point to the significance of drug history in determining rate dependencies and suggest that NE and 5-HT systems may be involved to a different extent in the actions of cocaine and AMP.

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302.15

EFFECTS OF TRAZODONE ON INTRAVENOUS COCAINE SELF-ADMINISTRATION IN RATS. G.F. Guerin*, C. Crow, K.A. Cunningham and N.E. Goeders. Departments of Pharmacology and Psychiatry, LSU Med. Center, Shreveport, LA 71130-3932

Although dopamine is involved in the stimulus effects and reinforcing properties of cocaine, recent data also suggest a role for serotonin in the mediation of these effects. Since anxiety and depression are often observed during cocaine withdrawal in humans, the following experiment was designed to investigate the effects of the serotonergic antidepressant drug, trazodone, on intravenous cocaine self-administration in rats. Adult male Wistar rats were trained to self-administer cocaine (0.25 mg/kg per 0.2 ml infusion delivered over 5.6 sec) on a fixed-ratio 4 schedule of reinforcement. When stable baselines of drug-intake were obtained, the rats were pretreated with trazodone (2.5 to 20 mg/kg, ip) 45 min before the start of the behavioral session. Acute pretreatment with relatively low doses of trazodone (e.g., 5 or 10 mg/kg, ip) completely blocked cocaine self-administration, suggesting a role for serotonin in the reinforcing effects of cocaine. Experiments are currently in progress to determine the effectiveness of chronic trazodone administration. This research was supported by USPHS grant DA06013.

302.16

COCAETHYLENE AND OTHER COCAINE ANALOGS: DIFFERENTIAL EFFECTS ON MONOAMINE UPTAKE AND BEHAVIOR. J.D. Elsworth*, J.R. Taylor, M.A. Medynski, P. Jatlow and R.H. Roth. Departments of Pharmacology, Psychiatry and Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06510.

The ethyl ester of cocaine (cocaethylene, CE) resembles cocaine in its affinity for the dopamine (DA) uptake site and in certain behavioral tests. The *in vivo* formation of CE following concurrent ingestion of cocaine and ethanol may explain the preference of cocaine abusers for this combination of drugs to cocaine alone. However, CE has been reported to be more toxic than cocaine. We tested the hypothesis that alteration of the ester group has a significant effect on potency and selectivity of cocaine as a monoamine uptake inhibitor and on its behavioral profile.

There was a 2-fold difference among ester analogs of cocaine in their Ki value for [^{3}H]DA uptake and [^{3}H]WIN 35428 binding; iso-propylcocaine and n-butylcocaine being the least potent and cocaine and CE the most potent. A more striking difference (25-fold) was observed in the Ki values for [^{3}H]5HT uptake and [^{3}H]paroxetine binding; CE and iso-propylcocaine being least potent and cocaine and n-butylcocaine the most potent. The analogs that were most selective for DA, compared to 5HT, were CE and iso-propylcocaine.

After injection of high doses (20 mg/kg i.p.) to rats, cocaine at first produced more motor activation than CE, but this initial difference between the drugs did not persist.

A different modification of the cocaine structure produces β-CIT (2β-carbomethoxy-3β-(4-iodophenyl)-tropane) which has been used in SPECT imaging studies. β-CIT showed no selectivity for the DA uptake site, yet was approximately 100 times more potent than cocaine at inhibiting [^{3}H]DA or [^{3}H]5HT uptake. Thus, a derivative of β-CIT with an altered ester group may be a high affinity ligand suitable for labelling the DA transporter selectively.

Supported by NIDA P50DA-04060 and DA-05119.

302.17

COCAINE-SENSITIVE AND -INSENSITIVE DOPAMINE UPTAKE IN PREFRONTAL CORTEX, NUCLEUS ACCUMBENS AND STRIATUM. J.R. Taylor*, J.D. Elsworth and R.H. Roth. Depts of Pharmacology and Psychiatry, Yale Univ. Sch. of Medicine, New Haven, CT 06510.

The reinforcing effects of cocaine are believed to be dependent on inhibition of dopamine (DA) uptake in nucleus accumbens and prefrontal cortex. As previous studies have suggested that DA uptake in nucleus accumbens, prefrontal cortex and striatum may differ, we have further characterized DA uptake in these regions.

A selective lesion of the prefrontal cortex DA terminals induced by simultaneous infusion of desipramine and 6-hydroxydopamine produced a corresponding decrease in DA concentration and *in vitro* [^{3}H]DA uptake. [^{3}H]DA uptake was resolved into two processes. One was very sensitive to cocaine, GBR 12909 or ouabain and was dependent on temperature and sodium ion concentration; this was responsible for most of the observed uptake in tissue from striatum and nucleus accumbens, but not from prefrontal cortex. There was no regional difference in the susceptibility of cocaine-sensitive [^{3}H]DA uptake to either cocaine or GBR 12909. The other type of [^{3}H]DA uptake, which represented a significant proportion of the total uptake in prefrontal cortex was relatively insensitive to cocaine, GBR 12909 and ouabain and was dependent on temperature, but not sodium ion concentration. Cocaine-insensitive [^{3}H]DA uptake was saturable and distinguished DA from 5HT, but not DA from norepinephrine. Cocaine-insensitive [^{3}H]DA uptake may be associated with glia and play an important role in regulating extracellular DA concentration when synaptic levels are elevated.

These results may account for some of the reported regional differences in sensitivity of DA uptake to inhibition by cocaine.

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302.18

ETHANOL ENHANCES THE BEHAVIORAL EFFECTS OF COCAINE AT VERY LOW DOSES. J. Peris*, J. Burry, S. Iyer & J. Scott. Dept. Pharmacodynamics, Univ. Florida, Gainesville FL 32610.

The stimulatory effects of cocaine are enhanced in rats consuming 8 g/kg ethanol daily (Pecins-Thompson & Peris, Psychopharmacol., in press) however ethanol drinking may not have occurred coincident with cocaine injection. In the present study, male rats were divided into 4 groups based on body weight and sucrose consumption in a 30-min period. For 14 consecutive days, rats received 30-min access to either sucrose (Groups S and C) or 10% ethanol in sucrose (Groups E and CE) followed immediately by a saline (Groups S and E) or cocaine (10 mg/kg, i.p.) injection (Groups C and CE). Locomotor activation and stereotypy were measured immediately after the first and fourteenth injection. On the first day, ethanol enhanced the stimulatory effect of cocaine ($U(12) = 27.5$; $p < 0.05$) but did not have an effect alone. After the fourteenth injection, both Groups C and CE exhibited cocaine sensitization but behavior of Group CE was still greatest ($U(12) = 23.5$; $p < 0.05$). Rats consumed an average of 0.8 ± 0.2 (Group E) and 0.9 ± 0.2 g/kg (Group CE) ethanol prior to the first injection but consumption decreased over days in Group CE ($F(13,224) = 3.5$; $p < 0.001$). Sugar consumption was similarly decreased over days in Group C ($F(13,546) = 3.1$; $p < 0.001$) indicating that a taste aversion to sucrose was formed in all cocaine-injected rats regardless of ethanol drinking. Although, this cocaine-induced decrease in ethanol consumption confounded interpretation of the behavioral data, very low ethanol consumption still increased the sensitized response to cocaine. Thus, even when ethanol consumption is too low to affect locomotion alone, it enhances both the initial and sensitized response to simultaneous cocaine. This work was supported by PHS grants AA08262 and AA00135.

302.19

IN VIVO CONCENTRATIONS OF COCAINE ARE ENHANCED FOLLOWING A SINGLE PREEXPOSURE TO COCAINE. **H.O. Pettit* and A.J. Eckstrom; Dept. of Neuroscience; SRI International; Menlo Park, California, 94025.**

Previous in vivo microdialysis work has revealed that cocaine concentrations in the blood and brain can be enhanced in animals that receive daily cocaine injections over a 10 day period (Pettit, et al., J. Neurochem. 55: 798-804, 1990). Microdialysis studies to be presented reveal that cocaine concentrations are also enhanced in the blood and brain of rats that have been given one injection of cocaine (20 mg/kg, s.c.) on the day before a challenge injection is given (30 mg/kg, i.p.). Effects of pretreatment on cocaine metabolism may explain how cocaine concentrations are increased. However, levels of the cocaine metabolite benzoylecgonine in both the i.p. cavity and the blood (and the cocaine\benzoylecgonine metabolism rate) were not significantly different between groups. Measures of cocaine levels in the peritoneal cavity following an i.p. injection revealed no significant differences between cocaine naive and experienced groups. Likewise, significant differences were not observed in the transfer rate of cocaine into the blood (i.v./i.p. cocaine concentration ratio) and into the brain (i.c./ i.v. cocaine concentration ratio). The results indicate that neither the rate of transfer of cocaine into the bloodstream or the metabolism of cocaine into benzoylecgonine can account for the increased cocaine concentrations produced by repeated cocaine administration. The results do not, however, discount possible effects of repeated administration on the metabolism of cocaine into ecgonine, another major cocaine metabolite. Although enhanced cocaine concentrations can be observed following an i.p. challenge, when the challenge injection of cocaine is given by an i.v. route of administration (5.0 and 7.5 mg/kg/0.1 ml) no differences in blood and brain cocaine levels occur between cocaine naive and experienced animals. Thus, the effects of repeated administration that enhance cocaine levels appear to occur prior to the entry of cocaine into the bloodstream. Present results indicate that a single preexposure to cocaine can produce effects that increase the concentration of cocaine in the blood and brain. These effects will play a major role in the development of reverse tolerance to the behavioral effects of cocaine.

302.20

DOSE EFFECTS OF ACUTE COCAINE ADMINISTRATION ON EEG AND AUDITORY EVENT-RELATED POTENTIALS IN RATS. W.M. Kaneko,*P. Robledo, and C.L. Ehlers. The Scripps Research Institute, La Jolla, CA 92037.

Cocaine administration appears to affect several CNS sites in the limbic forebrain. The nucleus accumbens has been implicated as an important site for the reinforcing aspects of the drug, whereas, the amygdala and hippocampus may be more sensitive to cocaine's "pre-kindling" actions. In the present study, EEG and auditory event related potentials (ERPs) were utilized to explore the actions of cocaine on several limbic sites. Eleven adult male Wistar rats were stereotaxically implanted with electrodes aimed at the dentate gyrus, dorsal hippocampus (CA1-CA2), amygdala, and nucleus accumbens. The rats received intraperitoneal injections of either saline, 2.5 mg/kg, 5.0 mg/kg, 10.0 mg/kg, or 20 mg/kg, in a latin square design. The ERPs were recorded in response to an auditory "oddball" paradigm consisting of frequent and infrequently presented tones. Cocaine was found to produce a significant dose-dependent main effect on both EEG and ERPs. This effect was to reduce the variance in the amplitude of several ERP components as well as the variance in power in several spectral bands. ERP effects of cocaine were most prominent following the frequent tone at the highest dose in the dentate, amygdala and nucleus accumbens, whereas lower dose effects were observed in the nucleus accumbens and dentate. EEG effects of cocaine were prominent at all doses primarily in the CA fields of dorsal hippocampus, although high dose effects were observed in the amygdala. These studies suggest that the sensory/cognitive effects of cocaine as quantified by ERPs may involve amygdala, nucleus accumbens as well as dentate gyrus. Whereas, state effects of cocaine which were quantified by EEG measures appear to be mediated through hippocampus CA fields. This reduction of variance of the EEG and ERPs suggests an increase in signal to noise ratio of these measures which may be related to a focussing of the animals attention and/or behavioral state, an effect previously ascribed to noradrenergic systems.

303.1

EFFECT OF CHRONIC LITHIUM TREATMENT ON PHOSPHOINOSITIDE-LINKED SIGNAL TRANSDUCTION PATHWAY IN RAT CORTEX. P.P. Li*, D. Sibony, M. Green and J.J. Warsh. Clarke Institute of Psychiatry, Toronto, Ontario, Canada, M5T 1R8.

Recent work indicates that the therapeutic action of lithium may be mediated through pertubation of postreceptor second messenger systems. To further elucidate the cellular mechanism of lithium, the effect of chronic lithium treatment on various components of the receptor-activated phosphoinositide (PPI) pathway was investigated. We found that chronic administration of lithium (0.2% LiCl, 21 days) to adult male rats did not significantly affect PPI hydrolysis in cerebral cortical slices induced by carbachol (1 mM) or NaF (10 mM). Neither did the same treatment alter the carbachol (1 mM)-potentiation of GTPγS (1-30 μM) stimulation of PPI hydrolysis (an index of receptor/G protein coupling) in cortical membranes. Immunoblotting studies revealed no changes in the levels of $G_{q/11}$ immunoreactivity in the cortex after chronic lithium. In contrast, the specific binding of [^{3}H]phorbol dibutyrate (PDBu) was significantly reduced in cytosol (-20%) and increased (27%) in the particulate fraction of rat cortex after chronic lithium, although the K_d of [^{3}H]PDBu binding remained relatively constant. A small and insignificant decrease (-13%) in the density of [^{3}H]InsP binding was also found in the cortex. The above data suggest that chronic lithium treatment affects neither the muscarinic cholinergic-linked PPI turnover, nor the putative G protein ($G_{q/11}$) responsible for phospholipase C activation. However, a possible translocation and activation of protein kinase C activity may be significant in the therapeutic effect of this mood stabilizing agent. Supported by the OMHF.

303.2

LITHIUM AND PILOCARPINE INDUCE IMMEDIATE EARLY GENE TRANSCRIPTION IN RAT BRAIN. M.B. Williams* and R.S. Jope. Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama, Birmingham, AL 35294.

Although lithium is used to treat bipolar affective disorder, its mechanism of action is unknown. Lithium generally inhibits second messenger production but in vivo lithium enhances cholinergic activity. For example, in rats administration of lithium (3 mmol/kg) or pilocarpine (30 mg/kg) alone has no effect on EEG recordings, but the combination causes generalized convulsive status epilepticus. In rat brain lithium potentiates pilocarpine-induced c-fos expression (Weiner et al. Br. Res. 553; 117, 1991).

We extended this finding in rat brain by measuring c-jun, jun B and jun D, as well as c-fos, mRNA and the drug dose and time-dependency of the increases. Pilocarpine (30 mg/kg) alone induced small increases in these mRNA levels and with lithium pretreatment large increases occurred concomitant with seizures. Lithium followed by a nonconvulsive dose of pilocarpine (5 mg/kg) also increased these mRNA levels more than pilocarpine alone. Work is in progress to determine the extent of the modulatory effect of lithium on the expression of each of these genes.

303.3

EFFECTS OF SEROTONERGIC ANXIOLYTICS ON BEHAVIOR MAINTAINED BY TIMEOUT FROM AVOIDANCE. M. Galizio*, K. L. Hale, M. Liborio, and M. Miller. Department of Psychology, University of North Carolina at Wilmington, Wilmington, NC 28403.

The effects of 5-HT1a ligands buspirone (.3-2.0 mg/kg), gepirone (3-20 mg/kg), and 8-hydroxy-2-(di-n-propyl-amino)tetralin (8-OH-DPAT) (.1-3.0 mg/kg) were studied, along with the benzodiazepine chlordiazepoxide (CDZ) (2.5-20 mg/kg), on rat behavior maintained by negative reinforcement. Concurrent performances were studied under conditions where responses on one lever postponed shock on a Sidman avoidance schedule and responses on a second lever produced brief periods of signaled timeout from avoidance. As in previous studies, CDZ increased responding on the timeout lever at doses that decreased avoidance. In contrast buspirone and gepirone decreased responding on both levers at all effective doses. Initially 8-OH-DPAT produced disruption of responding similar to buspirone and gepirone, but after repeated administrations, 8-OH-DPAT increased responding on both levers. The differences in the behavioral effects of these drugs were not expected on the basis of their known neuropharmacological or their anxiolytic properties.

303.4

PHARMACOLOGY OF CL 284,846, A NON-BENZODIAZEPINE SEDATIVE WITH REDUCED AMNESTIC LIABILITY. Ivana P. Day, Donald E. Clody, Michael M. Monaghan, Marc S. Abel‡, John P. Dusza and Bernard Beer*. American Cyanamid Company, Medical Research Division, Lederle Laboratories, Pearl River, NY 10965 and ‡The Chicago Medical School, Dept. of Cell Biology and Anatomy, North Chicago, IL 60064

The profile of effects for CL 284,846, N-[3-(-cyanopyrazolo[1,5-a]pyrimidin-7-yl)]-n-ethylacetamid, identifies this compound as a potential drug for the treatment of insomnia. It is orally active in rodents and primates and is pharmacologicaly similar to sedative benzodiazepine agents. It produces significant and dose-related anxiolytic-like effects on conflict procedures in rats and squirrel monkeys. In rats, this compound decreases motor activity, induces muscle relaxation, impairs coordinated movement, and promotes EEG sleep. In squirrel monkeys, it has a sedative-hypnotic profile of effects on vigilance behavior. CL 284,846 has anticonvulsant activity in rats similar to the benzodiazepine sedative-hypnotic drugs.

Similar to other sedative-hypnotic agents, CL 284,846 binds to brain benzodiazepine receptor sites (much more avidly to type I receptors), produces a significant enhancement of TBPS binding and has pharmacological effects that are antagonized by the benzodiazepine receptor antagonist Ro 15-1788.

In general, the potency of sedative-hypnotic effects appears to be slightly less than that of triazolam and greater than that of flurazepam. CL 284,846, at efficacious doses, has fewer side effects than standard BDZ sedative-hypnotics. It shows reduced tolerance, a lessened tendency to potentiate ethanol neurotoxicity, is devoid of next-day hangover effects and has a reduced amnestic liability.

303.5

SEDATIVE AND AMNESIC EFFECTS OF TRIAZOLAM AND ITS METABOLITES IN MICE, A.H.Tang* and C.S.Himes, The Upjohn Company, Kalamazoo, MI 49001.

The sedative and amnesic effects of triazolam and its metabolites were studied in mice using exploratory locomotor activity and the one-trial passive avoidance response, respectively. Drugs were administered on the first day, before measuring locomotor inhibition and training for passive avoidance. Memory recall was tested 24 hrs later with no further drug treatment. Triazolam administered either i.p. or p.o. 30 min before training had an amnesic effect at doses that also produced marked sedation. The same doses of triazolam given p.o. 4 hrs before training produced neither sedation nor amnesia. Pentobarbital sodium and ethanol at sedative doses also produced amnesia in mice. Both the sedative and amnesic effects of triazolam were antagonized by a simultaneous administration of the specific benzodiazepine antagonist, flumazenil. Five metabolites of triazolam identified in human urine were tested in the above paradigms using i.p. administrations. The major metabolite, α-hydroxy triazolam, was comparable in potency to triazolam for both sedative and amnesic effects. The other metabolites were all inactive in either measure. The results indicated that triazolam is similar to other benzodiazepines in producing anterograde amnesia at grossly sedative doses. The effects are mediated through the flumazenil-sensitive receptors and the metabolites of triazolam are either pharmacologically inactive, or have an identical sedative/amnesic effects as triazolam itself.

303.6

INITIAL SUB-SENSITIVITY TO BENZODIAZEPINE TREATMENT ON CONFLICT BEHAVIOR IN RATS: PARAMETRIC STUDIES ACROSS DRUG CLASSES. R. Kleinsorge* and R. Commissaris. Dept. of Pharmacy and Allied Health, Wayne State Univ., Detroit, MI 48202.

In rodent conflict paradigms, benzodiazepines (BZs) often exhibit sub-maximal anti-conflict (i. e. anxiolytic-like) effects initially, with maximal effects being observed only after 3-4 BZ exposures (Anxiolytic Initial Sub-sensitivity; AIS). The present experiments examined (1) whether AIS occurs with non-BZs and (2) whether prior exposure to non-BZs prevents the BZ-AIS. Female Sprague-Dawley rats were trained to stable responding levels on a repeated measures punished drinking paradigm. In Experiment-1, dose-response curves for pentobarbital (PB; 2.5, 5, & 10 mg/kg, ip, 15 min. pretreatment), chlordiazepoxide (CDP; 2.5, 5, & 10 mg/kg, ip, 30 min. pretreatment), and carbamazepine (CBZ; 5, 10, & 20 mg/kg, ip, 15 min. pretreatment) were determined in 3 groups (n=8 per group) of drug-naive rats using a 2 day balanced cross-over design, with each group receiving all doses of a single drug. Redetermination of the dose-response curves showed an AIS for the anti-conflict effects of CDP, but not for the effects of PB; the effects of CBZ were weak for both determinations. In Experiment-2, the rats from Experiment-1 were administered CDP (10 mg/kg, 30 min. pretreatment, ip) using a similar cross-over design. Compared to CDP-experienced rats, subjects with a history of repeated PB or CBZ treatments exhibited anti-conflict responses that were smaller in magnitude. These data suggest that (1) the AIS does not occur with non-BZ anxiolytics and (2) the BZ-AIS cannot be prevented by exposure to non-BZs. (Supported in part by USPHS MH#47181)

303.7

EFFECTS OF CHRONIC STIMULANT TREATMENT ON CEREBRAL METABOLISM IN ADULTS WITH HYPERACTIVITY. J. A. Matochik*, A. J. Zametkin, L. L. Liebenauer and R. M. Cohen. NIMH, Section on Clinical Brain Imaging, Bldg. 10/4N317, Bethesda, MD 20892.

The neural mechanisms of action of dextroamphetamine (DEX) and methylphenidate (MPH) in the treatment of Attention Deficit Hyperactivity Disorder (ADHD) are unknown. To measure cerebral glucose utilization, 34 adults diagnosed with ADHD received 4-5 mCi of 18-fluoro-2-deoxyglucose while performing an auditory attention task. Images were obtained with a Positron Emission Tomograph (PET) with 5-6 mm in plane resolution. Whole brain and 60 regional measures of glucose metabolic rate were extracted. Following the baseline PET scan, 16 patients received a daily dose of DEX individually titrated to achieve clinical effect and the remaining 18 patients received MPH. The 2nd PET scan was performed 6-8 weeks after the begining of drug treatment. Despite significant improvement in behavior (Connor's Rating Scale) and detectable blood levels for both stimulants, neither drug changed global or whole brain metabolism. Metabolism (normalized values) was significantly decreased in the right posterior frontal cortex (4% change) and right anterior putamen (5%) after chronic MPH administration, while DEX increased metabolism in the left anterior putamen (9%). Performance on the attention task was unaffected. Analysis of the responders versus nonresponders within each chronic drug treatment may reveal different cerebral metabolic patterns.

303.8

THE SEX AND AGE RELATED DIFFERENCES IN THE OPTIMAL DOSE OF L-DEPRENYL FOR INCREASING SOD AND CATALASE ACTIVITIES IN RAT STRIATUM. G.O. Ivy[1]*, M.-C. Carrillo[2], W. Switzer[3], N.W. Milgram[1] and K. Kitani[2]. [1]Dept.Anatomy and Cell Biology,Univ.Toronto,Scarborough,ON M1C 1A4, [2]Tokyo Met.Inst.Gerontology, Japan and [3]Neurosci.Assoc.,Knoxville,TN 37922.

We have shown that administration of the anti-Parkinsonian drug, l-deprenyl, increases activities of superoxide dismutase (SOD) and catalase (CAT) in striatum (ST) of young male (YM) rats. We now clarify sex and age related differences in the optimal dose of deprenyl for increasing SOD and CAT activities and demonstrate brain region selectivity. YM and Y and old (O) female (F) rats were infused s.c. with various doses of deprenyl or saline for 3 wks via osmotic mini-pump or by daily s.c. injection. YF rats required a 5-10-fold lower drug dose to optimally elevate SOD and CAT levels in ST than did YM rats while OF rats required intermediate doses. SOD and CAT levels were not increased in hippocampus (H) in any rats and glutathione peroxidase levels were not increased under any conditions. A study in YM rats showed that SOD and CAT activities increased in ST and substantia nigra (SN) but not in H, cerebellum or liver. Rat body weight, H and ST weights were unaltered by treatment, with the notable exception that ST weight was significantly lower in deprenyl treated Fs. We thus investigated possible ST degeneration in treated Fs and Ms using a variety of methods including the cupric-silver degeneration strain. No pathology was seen. Thus, l-deprenyl significantly increases activities of free-radical scavengers in ST of both M and F rats and in SN of M rats, but doses vary with sex and age. Further, no pathology was present in any brain region even with relatively high drug doses. Supported by Deprenyl Animal Health.

303.9

AN ANATOMICAL METHOD FOR ANALYZING PET DATA: VOLUMETRIC RECONSTRUCTION WITH MRI SURFACE SAMPLING VS TRADITIONAL SLICE ROI SAMPLING. E.A.Gastineau[1], H.L. Loats[2,3], C.A. Tamminga[1], H.H. Holcomb[1,3], T. Rippeon[2], D. Francis[2], M. McIver[2], D.Ross*[1]. [1]Maryland Psychiatric Research Center,Baltimore,MD 21247. [2]Loats Assoc.Inc, Westminster,MD. [3]Johns Hopkins Hospital,Balto.,MD.

Traditional Region of Interest (ROI) sampling of functional images such as FDG-PET have been on acquired axial slices. Reconstructing these slices into a volume allows for dynamic interaction with the data to render slices through any plane and generate surfaces. Here, an MRI and 3 FDG-PET scans were acquired from 8 patients enrolled in an on-off-off drug paradigm using Haloperidol. PET and MRI images were registered by overlaying the three orthogonal midplanes and adjusting the PET for X, Y, Z, translation and roll pitch and yawl, matching the functional image to the anatomy of the MRI. Once registered corresponding slices were constructed for all data sets and surfaces rendered. Surfaces were created using the MRI to describe a volumetric template. This template was then used to reconstruct surface views for the MRI and PET data sets. Atlases were described for the surfaces and midbrain area using the anatomy of the reconstructed MRI and applied directly to the associated PET studies. Measurements from the atlases were correlated with traditional slice ROI measurements and validation and reliability data is reported. Validation of both inter and intra-rater methods are also reported. Anatomical sampling based on reconstructed volumetric MRI data provides precise location for functional image sampling and shows the variability of activity within a perscribed area. Heretofore, this area was measured by a single ROI that was assumed to be representative. With the method described, volumetric as well as spatial relationships may be much more accurately reported.

303.10

MAGNETIC RESONANCE IMAGING REVEALS THAT BMS 181100 REDUCES EDEMA AFTER BILATERAL CAROTID OCCLUSION IN THE GERBIL. E. A. Marshall[1], S. L. Moon[2]*, K. E. Timko[2], M.A. Kapin[2], J.E. Rosenberg[1], and B.S. Krishnan[1]. Depts. of Analytical Research & Development.[1] and CNS Neuropharmacology[2], Bristol-Myers Squibb Co., Wallingford, CT 06492.

We have reported that BMS 181100 (Bristol-Myers Squibb, formerly coded BMY 14802) attenuated hippocampal histopathology and improved behavioral impairment resulting from 15-min bilateral carotid occlusion (BCO) followed by a 96 hr reperfusion/survival period in the Mongolian gerbil. We now report that proton NMR imaging after BCO shows that BMS 181100 decreased signal intensities in T_2-weighted images of the striatum relative to vehicle-treated controls. Standard Nissl stain confirmed the striatum as a site of severe damage in this model.

Gerbils received BMS 181100 (50 mg/kg) or vehicle 1 hr postsurgery, then once/day for 3 days. Once every 24 hrs after BCO, they were anesthetized and positioned in the imaging probe of a Bruker WM 360 NMR spectrometer fitted with a micro-imaging accessory. The radio-frequency coil was tuned to the proton Larmor frequency of 360.13 MHz. Sham-operated, vehicle and drug treated animals were imaged. At the end of the study, brains were fixed and stained with cresyl violet.

A consistent increase in signal intensity was noted in the striatum of occluded animals compared to that of sham controls. Evident from the first day of imaging, the striatal signal intensity in the BMS 181100-treated animals was smaller compared to the vehicle-treated animals. At 24 and 72 hrs, this signal attenuation was significant. Nissl stain indicated that the hippocampus showed cell death that is typical of this model, but also the striatum showed extreme cell loss.

303.11

DISCRIMINATIVE STIMULUS PROPERTIES OF THE COMBINED 5-HT1A PARTIAL AGONIST/5-HT2 ANTAGONIST WY-50324 IN RATS. K.L. Marquis, A.T. Shropshire, M.I. Piesla and J.A. Moyer*. Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

WY-50324, a combined 5-HT1A partial agonist/5-HT2 antagonist, is under development as an anxiolytic and antidepressant (Abou-Gharbia and Moyer, Drugs of the Future 15:1093-4, 1990; Barrett and Zhang, Drug Dev. Res. 24:179-188, 1991). The current experiment assesses the role of both mechanisms in the discriminative stimulus properties of WY-50324. Fifteen male Sprague-Dawley rats were trained to discriminate 2.5 mg/kg of WY-50324 from saline when administered i.p. 15 min prior to daily 10-minute two-lever food-reinforced operant sessions. Discriminative control was obtained in an average (± SD) of 17.5 ± 8.7 sessions after double alternation between drug and saline injection was initiated. Generalization of a range of doses of WY-50324 to the training dose occurred at an ED50 of 1.08 mg/kg ip. The full 5-HT1A agonist 8-OH-DPAT, given 15 minutes prior to test sessions, generalized to WY-50324 at an ED50 of 0.1 mg/kg i.p. The selective 5-HT2 antagonist ritanserin, when given at 1, 3 and 10 mg/kg sc 1 hr prior to test session, did not generalize to WY-50324. In addition, WY-50324 generalized to 8-OH-DPAT at an ED50 of 0.8 mg/kg ip in a separate group of rats trained to discriminate 8-OH-DPAT (0.4 mg/kg i.p.) from saline. Combinations of 3 mg/kg of ritanserin with various doses of 8-OH-DPAT did not significantly change the generalization of 8-OH-DPAT to WY-50324. These data indicate that the stimulus properties of WY-50324 are related to its agonist effects at 5-HT1A receptors at the training dose used. In addition, 5-HT2 antagonism does not block the 5-HT1A agonist effects of WY-50324. Given that ritanserin, by itself, does not function as a discriminative stimulus (Meert and Janssen, Drug Dev. Res. 18:119-44, 1989), the contribution of 5-HT2 antagonism to the discriminative stimulus effects of WY-50324 will need to be determined in antagonism experiments.

303.12

ACUTE EFFECTS OF ANTIDEPRESSANTS ON LOCUS COERULEUS (LC) DISCHARGE OF ANESTHETIZED AND UNANESTHETIZED RATS. A.L. Curtis* and R.J. Valentino. Dept. Mental Health Sci., Hahnemann University, Philadelphia, PA 19102, U.S.A.

Antidepressants are thought to produce their clinical effects by actions on central biogenic amine systems. This study characterized the acute effects of 4 antidepressants on discharge activity of the noradrenergic nucleus, LC. Because LC discharge is state dependent, antidepressant effects were compared in anesthetized and unanesthetized rats. In halothane-anesthetized rats desmethylimipramine (DMI) and phenelzine (PHE) decreased LC spontaneous discharge rate in a dose-dependent manner. In contrast, the atypical antidepressant, mianserin (MIA) increased LC spontaneous discharge rate, and the selective serotonin reuptake inhibitor, sertraline (SER) had no effect. In unanesthetized rats DMI had qualitatively similar effects but was 1/10th as potent. MIA and SER did not alter LC spontaneous discharge rates of unanesthetized rats. LC discharge was evoked by sciatic nerve stimulation in anesthetized rats and by auditory stimulation in unanesthetized rats. DMI decreased LC discharge evoked by sciatic nerve stimulation at doses greater than those that decreased spontaneous discharge. MIA and SER also decreased LC discharge evoked by sciatic nerve stimulation. In unanesthetized rats only DMI and MIA decreased auditory-evoked LC discharge and these drugs were 30-3000 times less potent in unanesthetized rats, respectively. These findings suggest that antidepressants have qualtitatively similar effects on LC spontaneous discharge but are less potent in unanesthetized vs anesthetized rats, perhaps because anesthesia potentiates the effects of antidepressants to suppress LC discharge. Potency and efficacy differences between antidepressants on auditory vs. sciatic nerve -evoked LC discharge may be due to the different neuronal circuitry underlying LC activation by these stimuli. Supported by MH42796 , MH 40008 and MH00840.

303.13

BEHAVIORAL EFFECTS OF FLUVOXAMINE ON SEVERAL PARADIGMS IN RODENTS. Y. Ichimaru*, T. Egawa, T. Imanishi and A. Sawa. Pharmaceutical Research Center, Meiji Seika Kaisha Ltd., Yokohama 222, Japan.

Fluvoxamine(FLV) is a serotonin(5-HT) reuptake inhibitor, which is marketed as an effective antidepressant in many countries throughout the world. A registration dossier for obsessive complusive disorder(OCD) has been submitted to the U.S. Food and Drug Administration. It is recently and widely accepted that 5-HT mediates various functions of the central nervous system, i.e., anxiety, mood, schizophrenia and memory, etc. In these experiments, the effects of FLV were investigated in several rodent depression and anxiety models. The antidepressant effects of FLV were tested in a male ICR mouse forced swim model. These data revealed the significant but weaker effect of FLV than that of desipramine. The anti-OCD effect of FLV was investigated in the following paradigms. The anxiolytic effect of FLV was not detected in Vogel-type conflict and elevated plus maze paradigms in male Fisher 344 rats. However, the anxiolytic effect of FLV via 5HT1A receptor was detected in the marble burying paradigm which is recently considered as a model of OCD. From these results, it is suggested that FLV has both antidepressant and anti-OCD effects.

303.14

ACUTE AND CHRONIC DESIPRAMINE INCREASES NOREPINEPHRINE CARRIER MRNA IN THE LOCUS COERULEUS. R. Veith*, P. Szot, A. Ashleigh, E. Petrie and D.M. Dorsa. GRECC, Seattle VAMC, WA 98108 and Dept. of Pharmacology, Univ. Washington, Seattle, WA 98195.

Desipramine (DMI), a tricyclic antidepressant, is known to have pronounced effects upon the postsynaptic and presynaptic noradrenergic (Nepi) system. Presynaptic effects include down regulation of α_2-adrenergic receptors, blockade of the transport protein responsible for the reuptake of Nepi after its release, and a decrease in tyrosine hydroxylase (the rate limiting enzyme responsible for synthesis of Nepi) mRNA in the locus coeruleus (LC). This study was performed to observe the effects of DMI on levels of the mRNA encoding the Nepi carrier protein in LC cells. In situ hybridization for the Nepi carrier mRNA was performed in animals receiving short term and chronic treatment with DMI. Three end-labeled oligonucleotides (108-150, 186-197 and 579-591) were used. Animals received either (a) 2 intraperitoneal injections of DMI (10mg/kg) or saline 24 hours apart (acute) or (b) 4 weeks of daily intraperitoneal injections of DMI (10mg/kg) or saline (chronic). Following the acute DMI treatment there was a tendency to increase ($p<0.10$) the Nepi carrier mRNA in the LC (OD control = 0.108 ± 0.01 (n=3) compared to OD DMI = 0.276 ± 0.07 (n=4)). However, chronic DMI treatment (4 weeks) resulted in a significant elevation (OD control = 0.207 ± 0.01 (n=4) compared to OD DMI = 0.316 ± 0.03 (n=4)) of Nepi carrier mRNA ($p<0.05$). This indicates that prolonged DMI treatment may increase the expression of the gene encoding the Nepi carrier. The mechanism of this increase in carrier protein mRNA or its involvement in the therapeutic effects of tricyclic antidepressants remains to be determined. (Supported by NS 20311 and the VA)

303.15

THE MECHANISM OF ANTIDEPRESSANT ACTION IN DEPRESSION. **P.L. Delgado*, H.L. Miller, R.M. Salomon, J. Licinio, L.H. Price, G.R. Heninger, D.S. Charney. West Haven VAMC and Dept. of Psychiatry, Yale University School of Medicine, 950 Cambell, West Haven, CT 06516**

Brain serotonin (5-HT) is reduced by depleting plasma tryptophan (TRP). Brain catecholamines are reduced by inhibiting their synthesis with the tyrosine hydroxylase inhibitor alpha-methyl-para-tyrosine (AMPT). If antidepressant drugs relieve depression by changing 5-HT or norepinephrine (NA), then reducing 5-HT or NA should alter mood in depressed patients having had therapeutic antidepressant responses to desipramine (DMI) or fluoxetine (FLU). **METHOD**: Thirty depressed (DSM-III-R) patients (5 AMPT, 25 TRP Depletion) having had therapeutic responses to DMI or FLU participated in 2 tests one week apart. Antidepressants are continued throughout testing. TRP depletion and AMPT tests were conducted in a double-blind, placebo-controlled, randomized crossover fashion. TRP depletion was as in our previous studies (Delgado et al., 1990). AMPT testing includes a baseline day, two days with AMPT 1 gm TID or diphenhydramine 50 mg TID and a follow-up day. Ratings (Hamilton Depression Scale (HDRS)) and plasma and urine (for TRP or MHPG and HVA levels) were obtained prior to, during and after testing. Depressive relapse was defined as a 50% increase in HDRS with total ≥20. **RESULTS**: 6/12 FLU responders but only 1/13 DMI responder relapsed with TRP depletion. Preliminary results show that 3/3 DMI-responders but 0/2 FLU-responders relapsed during AMPT testing. No patient relapsed during control (placebo) testing. **IMPLICATIONS**: Rapid depletion of plasma TRP transiently reverses antidepressant responses to FLU but not DMI. These results along with preliminary results from AMPT testing suggest that antidepressants may mediate their therapeutic effects through different mechanisms and highlight the importance of NA and 5-HT in antidepressant action.

303.16

CHRONIC TREATMENT WITH IMIPRAMINE AND CLOMIPRAMINE BUT NOT CLORGYLINE INCREASES m-CPP LEVELS IN THE BRAIN BUT NOT IN PLASMA. Teresa Tolliver, Charanjit S. Aulakh, James L. Hill, James M. Tolliver*, and Dennis L. Murphy. Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

We investigated the effects of chronic (21 days) imipramine (5 mg/kg/day), clomipramine (5 mg/kg/day), clorgyline (1 mg/kg/day) or saline treatment on m-chlorophenylpiperazine (m-CPP, a 5-HT_1 agonist) levels in the brain and plasma as well as hypothalamic levels of various neurotransmitters following intraperitoneal administration of 1.25, 2.5 and 5.0 mg/kg doses of m-CPP in male Wistar rats.

Chronic treatment with imipramine and clomipramine significantly enhanced m-CPP levels in brain but not in plasma by 74% and 33%, respectively, relative to saline treatment. Neither brain nor plasma levels of m-CPP were significantly affected by chronic clorgyline treatment. On the other hand, chronic imipramine and clomipramine treatment did not have any significant effect on hypothalamic levels of norepinephrine (NE), dopamine (DA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) while chronic clorgyline treatment produced significant increases in NE, DA and 5-HT levels and a significant decrease in 5-HIAA levels. These findings suggest that pharmacokinetic factors might partially contribute to modification of m-CPP's effects following chronic treatment with imipramine and clomipramine but not clorgyline.

303.17

EFFECT OF ANTIDEPRESSANTS ON 5-HT UPTAKE SITES AND 5HT1A RECEPTORS: HOMOGENATE BINDING AND AUTORADIOGRAPHIC STUDIES. E.K. Nénonéné[1]*, F. Radja[2], M. Ohayon[2], and T.A. Reader[2,3]. Centre de recherche en sciences neurologiques and Centre de recherche psychiatrique Fernand-Séguin (Départements de physiologie et de psychiatrie), Université de Montréal (Québec) CANADA.

The mechanism(s) of action of antidepressants has not yet been fully clarified, but all available evidence indicates that they affect monoamines, in particular serotonin (5-HT) nerve terminals and/or receptors. We compared *in vitro* the effects of the novel antidepressant Reboxetine (REB) with Imipramine (IMI), Trimipramine (TRI), Fluoxetine (FLU) and Buspirone (BUS). The ligands [^{3}H]citalopram and [^{3}H]paroxetine were used to examine 5-HT uptake sites, while [^{3}H]8-OH-DPAT was employed for 5-HT1A receptors. In homogenate studies, REB inhibited [^{3}H]paroxetine binding with an IC_{50} of 15.4 ± 5.8 µM; the rank order of potency was FLU > IMI > REB > TRI. For 5-HT1A receptors, competition of [^{3}H]8-OH-DPAT binding had the following orders of potency: BUS > TRI > IMI > REB > FLU for cortex, and BUS > TRI > FLU > IMI > REB for hippocampus. This survey thus shows that antidepressant drugs can compete with 5-HT1A receptors in the micromolar range. Quantitative autoradiography of 5-HT uptake sites showed that REB inhibited [^{3}H]citalopram binding with IC_{50} values of 4.1 ±1.3, 0.79 ± 0.2 and 2.5 ± 1.5 µM for dorsal Raphe nuclei, Substantia nigra and neostriatum, respectively. The main conclusions that can be drawn from these studies are that 1] REB has micromolar affinities for 5-HT uptake sites and 5-HT1A receptors, and 2] it exhibits a regional heterogeneity to compete with markers of 5-HT uptake sites. *(Supported by the [1] Savoy Foundation, the [2] FRSQ and [3] MRC Grant MT6967).*

303.18

EFFECTS OF CHRONIC ANTIDEPRESSANT TREATMENT ON CORTICAL [^{3}H]PAROXETINE BINDING AND SEROTONIN CONTENT. K.M. Dewar*[1,2], M. Ohayon[1], E. Nénonéné[3] and T.A. Reader[1,4]. Centre de recherche psychiatrique Fernand-Séguin and Centre de recherche en sciences neurologiques (Départements de physiologie et de psychiatrie), Université de Montréal (Québec) CANADA.

Serotonin (5-HT) appears to play a pivotal role in many central nervous system (CNS) functions such as sleep, feeding and motivation; many antidepressant drugs that influence these functions act via blockade of the 5-HT transporter. Serotonin uptake sites were quantified using high affinity binding of [^{3}H]paroxetine to cortical membranes of neonatal (7 days of age) or adult rats treated chronically (21 days) with tricyclic (Imipramine, Trimipramine) and monocyclic (Fluoxetine) antidepressants. The tissue concentrations of 5-HT and its metabolite 5-hydroxyindole-3-acetic acid (5-HIAA) were determined by HPLC with electrochemical detection, in the cingulate cortex, neostriatum, hippocampus and Raphe nuclei. Following treatment with the antidepressants, neither the maximum binding capacity (B_{MAX}; receptor number) nor the equilibrium dissociation constant (KD) of [^{3}H]paroxetine differed from control values in neonatal or adult treated animals. Furthermore, the concentration of both 5-HT and 5-HIAA were unchanged in the various brain regions examined. These data indicate that neither [^{3}H]paroxetine binding nor endogenous 5-HT content are altered by chronic treatment with drugs that block the 5-HT uptake site; suggesting that [^{3}H]paroxetine provides a reliable marker of 5-HT innervation density within the mammalian CNS, even following antidepressant drug treatments. *(Supported by the [1]FRSQ, the [2]Banting Research Foundation, the [3] Savoy Foundation and [4] MRC Grant MT-6967).*

303.19

JUVENILE DESMETHYLIMIPRAMINE TREATMENT REDUCES SENSITIVITY TO ADULT IMIPRAMINE ADMINISTRATION IN FORCED SWIMMING AND OPEN FIELD TESTS.
K.D.Dwyer* and E.J.Roy. Neuroscience Program, University of Illinois, Champaign, IL 61820.

Juvenile administration of antidepressants to rats has been proposed as an animal model of depression. In the present study we compared the effect of adult imipramine in female rats administered desmethylimipramine as juveniles (JDMI) with rats administered saline as juveniles (Jsal) and with rats that were untreated as juveniles (Junt).

Treated rats were injected with saline or 5mg/kg DMI daily between 1 and 3 wks old. Animals were tested in the swimming test at 10-12 wks and in the open field at 12-14 wks. In the swimming test imipramine increased activity among all juvenile groups when administered short-term after a pretest or when administered long-term prior to an initial exposure to the test. When administered with no prior pretest, short-term imipramine had different effects on Junt and Jsal rats and no effect on JDMI rats. In the open field short-term imipramine influenced all 3 juvenile groups, but long-term imipramine had no effect on JDMI rats. The results of this study indicate that JDMI treatment may have long-term effects on the mechanism underlying imipramine action.

GENETIC MODELS II

304.1

NEUROANATOMICALLY-SPECIFIC TRANSGENE EXPRESSION OF A NEURON-SPECIFIC HERPES VIRUS VECTOR STEREOTACTICALLY DELIVERED INTO THE RAT STRIATUM.
J.K. Andersen*, D.M. Frim, O. Isacson, and X.O. Breakefield. Neuroscience Center, Massachusetts General Hospital East, Charlestown, MA 02129 and Neuroregeneration Laboratory, McLean Hospital, Belmont, MA 02178.

Herpes virus infection with genetically engineered vectors is an effective way to deliver foreign genes to various cell populations in culture and *in vivo*. Selective delivery to neurons in the brain has been achieved using a replication-defective (TK-), recombinant herpes virus vector containing the bacterial lac Z gene under control of the rat neuron-specific enolase promoter. Direct stereotactic injection of 10^6 plaque forming units of this virus vector into the adult rat striatum resulted in transfer and stable expression of the transgene in a small number of neurons in the striatum, and in neurons ipsilateral to the injection site in the frontal cortex, substantia nigra, and lateral thalamus, as visualized by PCR, *in situ* histohybridization, and immunocytochemical and histochemical analyses. Neurons at these anatomical locations contain axons that project directly to the striatum. No other neurons within the brains of animals injected with the recombinant virus were found to express the transgene. As herpes virus can be transported in a retrograde fashion in neurons, these results indicate that gene transfer with this vector is constrained not only by the promoter used to drive gene expression and the type of cell that can harbor HSV in latency, but also by placement of viral particles at a specific anatomical location. This finding will help in designing strategies for gene replacement therapy for specific populations of neurons.

304.2

ALTERING CENTRAL NERVOUS SYSTEM PHYSIOLOGY WITH A DEFECTIVE HERPES SIMPLEX VIRUS VECTOR EXPRESSING THE GLUCOSE TRANSPORTER GENE.
D. Y. Ho[1], E. S. Mocarski[2] and R. Sapolsky*[1]. [1]Dept. of Biological Sciences, [2]Dept. of Microbiology and Immunology, Stanford Univ. Stanford, CA94305.

Because of their postmitotic nature, neurons are difficult subjects for gene therapy. To circumvent this, we have used a defective herpes simplex virus (HSV) vector to overexpress the rat brain glucose transporter gene under the control of the human cytomegalovirus *ie*1 promoter. This vector, designated as vIE1GT, was propagated using an HSV-1 temperature sensitive mutant *ts*756. Glucose transporter expressed from vIE1GT was readily immunoprecipitated from membrane fractions of vIE1GT-infected Vero cells. Using indirect double immunofluorescence techniques, vIE1GT was shown to be capable of enhancing expression of the glucose transporter in both cultured hippocampal neurons and glia. Glucose transport in such vIE1GT-infected cultures was increased approximately two-fold, and the increase was resistant to the effects of glucocorticoid treatment, which had been shown previously (Horner, et al., 1990) to inhibit glucose transport. The efficacy of this system *in vivo* was then tested by microinjection of vIE1GT into adult rat hippocampus and measurement with [^{14}C]2-deoxyglucose uptake autoradiography. vIE1GT significantly enhanced glucose transport in regions near the site of injection without causing adverse cytopathology; in particular, an approximately 10% increase was observed in a small sphere of tissue surrounding the injection site. Thus, this approach can be used to alter central nervous system physiology both *in vitro* and *in vivo*.

304.3

FOCAL ASTROCYTOSIS IN S100β TRANSGENIC MOUSE.
P.J. Yarowsky[1], B.K. Krueger[#], S. Buck[■], T. Michal[■], J.D.Gearhart[▲], R.H. Reeves[▲], and D.C. Hilt[■•]. Depts. of Pharmacology & Exp. Therap. [1], Physiology[#], and Neurology[■], Univ of Maryland Sch. of Med., Baltimore, MD 21201 and Dept. of Physiology[▲], Johns Hopkins University Sch. of Med., Baltimore, MD 21205.

Recent evidence suggests that astrocytes produce S100β, a Ca^{2+}-binding protein, glial mitogen and neurite extension factor. Levels of S100β have been found to be substantially elevated in Alzheimer's disease and in adults with Down Syndrome. Since S100β has been shown to act as a glial mitogen *in vitro*, it was of interest to examine the density and distribution of astrocytes in transgenic S100β mouse brain. S100β transgenic mice have been produced which overexpress mouse S100β mRNA (by 2.2 fold to 6.6 fold) in a tissue-appropriate manner as determined by northern blotting. Immunocytochemical staining of adult animals with an antibody against S100β demonstrates that there is overexpression of S100β in all brain regions of the transgenic. Immunostaining with antibody to the astrocyte specific protein, GFAP revealed focal astrocytosis in the olfactory bulb, hippocampus and cerebellum in S100β transgenic animals. This astrocytosis shows a dose-dependent relationship based upon the level of S100β expressed . These results suggest that S100β functions as a regulator of astrocyte proliferation in the CNS *in vivo* in a regionally-specific fashion. Supported by NIH and NSF.

304.4

• CHARACTERIZATION OF AN INHIBITORY FACTOR OF EPIDERMAL GROWTH FACTOR RECEPTOR PHOSPHORYLATION (EGF-R) IN CYST FLUIDS ASSOCIATED WITH GLIOMAS. •
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• 24 to 55% of gliomas have a cyst fluid (CF) component. Du to their closed contact with tumor cells they represent a good approach to study the biology of gliomas and to test the autocrine hypothesis. As EGF-R is the most frequently involved proto-oncogene in gliomas we searched for "EGF-like" factors in CFs.

We studied the effect of 30 different CFs on the EGF-R phosphorylation in A 431 cells membranes. CF effect was also analyzed in cell culture on A 431 and 3 human established glioma cell lines.

Unexpectedly, CFs, on the contrary of EGF, induced an inhibitory effect on EGF-R phosphorylation. Biochemical analysis suggested that it was an anti-tyrosine kinase effect. It persisted at high dilution and on cells in culture where an antitrophic effect was also observed for cell lines expressing EGF-R. This inhibitory effect was present in the 20-30 kDa fraction after HPLC superose 12 separation.

We conclude that an anti-tyrosine factor of 20-30 kDa is present in CFs from gliomas. Effect at high dilution and in vitro on whole cells suggest that this factors may play an anti-oncogenic role in the tumoral progression of gliomas. The recent report of EGF-responsive stem cells in the adult mice thalamus suggests that such factor may be important at the physiological level. Further purification and microsequencing are in process to clarify these issues.

304.5

SELECTIVE INDUCIBLE ABLATION OF HIV-TAT EXPRESSING GLIOMA CELLS BY RETROVIRUS GENE TRANSFER. D. Platika* and H. Rubinson. Depts. of Neurology and Neuroscience, Albert Einstein Coll. of Medicine, Bronx, NY 10461

We have previously demonstrated the killing of glioma cells in vitro and in vivo by retrovirus vector transfer of the herpes simplex thymidine kinase gene (TK) (Ezzeddine, et al. 1991). Unlike genes such as diphtheria toxin whose product is toxic to mammalian cells, TK is non-toxic except in the presence of specific nucleoside analogues such as acyclovir and ganciclovir. The report utilized a retrovirus based vector containing TK under the transcriptional control of the Moloney murine leukemia virus long terminal repeat. Selectivity was based on the fact that gliomas represent rapidly dividing cells in a setting of essentially non-dividing cells.

Selectivity and specificity can be further enhanced by utilizing tissue specific promoters or promoters that are induced in the cells targeted for ablation. We have designed a series of replication-deficient retrovirus vectors in which TK is under the transcriptional control of human immunodeficiency virus (HIV) promoter sequences. HIV demonstrates neurotropism and has been identified as the etiological agent in AIDS. AIDS has been associated with primary neoplasms at various sites including the CNS.

The vectors have been used to transduce C6 rat glioma-derived cells. Northern analysis revealed that the presence of HIV transactivator protein (tat) increases TK expression. Both in vitro and in vivo studies showed that in the presence of tat, these cells can be selectively killed with the use of acyclovir or ganciclovir.

304.6

DISTRIBUTION OF HUMAN LIGHT NEUROFILAMENT PROTEIN IN THE BRAIN OF TRANSGENIC MICE: AN IMMUNOCYTOCHEMICAL STUDY. J.-F. Mathieu, G. Doucet*, A. Vallée, L. Descarries and J.P. Julien. Centre de recherche en sciences neurologiques (Département de pathologie), Université de Montréal, and Montreal General Hospital Research Institute, McGill University, Montreal, Québec, Canada.

The distribution of human light neurofilament protein (hNF-L) immunoreactivity was studied in the brain of adult transgenic mice and compared to that of mouse NF-L in normal mice, using two monoclonal antibodies against NF-L, specific or not for the human protein (clones Bio/Can DP5-1-12 and Sigma NR4, respectively). In transgenic mice, numerous nerve cell bodies were labeled with both antibodies in several brain regions, such as cerebral cortex (laminae II/III and V), thalamus, amygdala, septum, substantia nigra and many brain stem nuclei, in addition to major fiber bundles. Other regions such as the striatum showed no cell body immunoreactivity whatsoever. The labeled cells in cerebral cortex were of the pyramidal type and unevenly distributed in large columns. Nerve cell body labeling was particularly striking in specific thalamic nuclei, where darkly stained, enlarged perikarya also displayed excentric and fragmented nuclei. At the electron microscopic level, these perikarya were mostly filled with disarrayed filaments to the extent that other organelles were repelled to a narrow rim underneath the plasma membrane. However, there were marked interindividual variations in the relative intensity of the cortical versus thalamic immunostaining. In normal mice, weaker immunoreactivity was present only after using clone NR4 antibodies, and confined to the major fiber bundles and pyramidal neurons of the cortex. These observations suggest that hNF-L expression and processing are regulated differentially in different regions of transgenic mouse brain, where these processes may even escape the regulatory capacities of some neurons, resulting in obvious neuropathological alterations. (Supported by FRSQ and MRC grants MT-10982, MA-9865 and MT-3544).

304.7

STUDIES OF CHROMOSOME DYNAMICS IN ALZHEIMER'S DISEASE CELLS: EVIDENCE FOR A DIFFERENTIAL RESPONSE TO MITOTIC ARREST AND THE OCCURRENCE OF LOW FREQUENCY CHROMOSOME 21 TRISOMIES. Lisa N. Geller*, Mark B. Benjamin and Huntington Potter. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

The overexpression of a gene or genes on chromosome 21 has been proposed as an explanation for the link between Down syndrome and Alzheimer's Disease (AD), since Down patients living into their 30s and 40s develop neuropathology essentially indistinguishable from classic AD. The amyloid precursor protein is now known to be encoded by a gene residing on chromosome 21, and mutations in this gene and at a proximal locus on this chromosome have been seen in some AD families. These mutations do not, however explain the vast majority of AD cases. We have been pursuing the hypothesis that the formation of trisomy 21 cell clones in the developing brain might explain sporadic and some familial cases of AD. We have examined AD cells growing in culture for the occurrence of spontaneous trisomy 21. Two methods are employed: chromosomal in situ hybridization to interphase cells using a chromosome 21-specific probe, and flow cytometry employing antibodies directed against the α2-interferon receptor which is expressed on lymphoblasts in proportion to the number of chromosomes 21 they possess. Secondly we have exposed lymphoblasts in culture to the antimicrotubule agent colchicine to examine the short-term effects such exposures have on chromosome dynamics. In situ hybridization has shown evidence for low but significant frequency spontaneous trisomy 21 in one AD cell line. Preliminary data from colchicine exposures, measured by premature centromere division, show that chromosomes in AD lymphoblasts are much more sensitive to colchicine exposure than control lines from similarly aged individuals. These data, taken together, suggest that chromosomes in AD cells respond differently to those in non-AD cells and may be more likely to undergo non-disjunction in response to transient microtubule disruption.

304.8

REDUCTION IN ANTIOXIDANT LEVELS IN TRISOMY 16 MOUSE BRAIN S.F.Chen*, J. Gafni, L.Reola, E. Carlson, C.J. Epstein and P.H.Chan. Depts of Neurology, Neurosurgery, Pediatrics, UCSF, San Francisco, CA 94143

Mouse trisomy 16 (Ts16), a model for human trisomy 21(Down syndrome), has been used as a tool to study the neurodegeneration involved in mental retardation and Alzheimer disease. Copper/zinc-superoxide dismutase (CuZn-SOD) is one of the genes mapped to the homologous regions of mouse chromosome 16 and human chromosome 21. Since Ts16 mice has one and a half times the regular gene dosage of CuZn-SOD, we studied the oxidative status of the Ts16 brain by measuring the oxygen radical scavenging system. Brains from Ts16 and euploid littermates at sixteen days of gestation were assayed for the activities of antioxidative enzymes [CuZn-SOD, glutathione peroxidase(GSH-Px), manganese-SOD(Mn-SOD), catalase(CAT)] and antioxidant levels [reduced glutathione(GSH), oxidized glutathione(GSSG) and ascorbate(AH_2)]. In Ts16 brain, CuZn-SOD activity is significantly increased over normal (3.14 vs 1.79 U/mg protein, $p<0.0001$), whereas activities of other antioxidative enzymes are not altered significantly [Mn-SOD (Ts/N:0.79/0.88U/mg protein), CAT (Ts/N:0.48/0.51 U/mg protein), GSH-Px (Ts/N:11.04/10.80 U/mg protein)]. However, both GSH and AH_2 levels are dramatically decreased in Ts16 brains (GSH,Ts/N: 20.2/9.1 nmol/mg protein, $p<0.001$; AH_2,Ts/N: 2.50/1.81 nmol/mg protein, $p<0.05$). Cell-specific changes were studied in cortical astrocyte and neuronal cultures prepared from fetal day 16 Ts16 and euploid brains. GSH levels are significantly decreased in both Ts16 astrocytes (Ts/N:42.68/77.06, $p<0.001$) and in neurons (Ts/N:10.30/14.30, $p<0.05$), whereas GSSG levels show a slight increase in Ts16 cultures. Furthermore, the uptake of glutamate is significantly reduced in Ts16 astrocytes. These observations suggest that, despite the overexpression of CuZn-SOD, there is a reduction in GSH and AH_2 levels in the Ts16 brains as compared to the normal ones. Whether these decreases in Ts16 mouse neurons and astrocytes contribute to neuropathological changes that are associated with oxidative stress needs further elucidation. Supported by AG-08938, NS-14543, and NS-25372.

304.9

REDUCED EXPRESSION OF NEURONAL PROTEINS IN PC12 CELLS TRANSFECTED WITH THE A4-C-TERMINAL PORTION OF AMYLOID PRECURSOR PROTEIN (APP)
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We previously reportd that PC12 cells transfected with A4-C-terminal APP DNA undergo morphological transformations, have incrased levels of immunologically detectable A4 in membranes, display reduced response to NGF, and secrete stimulatory factors which cause non-transfected cells to increae in size and extend neurites (Majocha, et al, J. Molec. Chem. Neuropathol., in press). Normal control PC12 cells contain immunocytochemically detectable levels of tyrosine hydroxylase, synaptophysin, and the 145 kD neurofilament protein (NFP). However transfectants that overexpress the A4 antigen do not appear to accumulate the proteins or do so at lower levels. AD brain also contains reduced levels of synaptohphysin and tyrosine hydroxylase, in addition to modified NFPs. Ongoing studies are aimed at determinig the mechanistic relationship between A4 overexpression and reductions in neuronal marker proteins. Supported by NIA P01 AG02126 and an award from the Metropolitan Life Foundation.

304.10

IN VITRO AND *IN VIVO* MODEL SYSTEMS FOR THE STUDY OF β-AMYLOIDOSIS. K. Fukuchi, S. S. Deeb[1], K. Kamino, C. E. Ogburn, A. C. Smith, D. Nochlin, S. M. Sumi, C. Furlong[1], P. A. Schwartzkroin[2], D. D. Kunkel, and G. M. Martin*. Depts. of Pathology, [1]Medicine (Div. of Medical Genetics), and [2]Neurosurgery, Univ. of Washington, Seattle, WA 98195.

We have previously demonstrated that overexpression of a C-terminal fragment of amyloid protein precursor (APP) brings about altered cleavage of APP, resulting in amyloidogenic and cytotoxic fragments (Fukuchi *et al., BBRC* 182:165). In further analyses, overexpression of the DNA construct in COS cells resulted in at least 6 C-terminal fragments (10-16.5 kDa by Tris-tricine SDS-PAGE) and their aggregates (23->200 kDa), suggesting the involvement of the C-terminal fragments in the intracellular formation of amyloid protein aggregates. P19 (embryonal carcinoma cell) transformants overexpressing the DNA construct degenerated to various extents corresponding to the degree of expression of the C-terminal fragments when the transformants were treated with retinoic acid for differentiation into neural cells. Transformants differentiated into muscle cells (DMSO treatment) survived as well as nontransformed P19 cells. When the DNA construct was introduced into SK-N-SH (a heterogeneous culture of neural and non-neural cells), the surviving cells overexpressing the C-terminal fragments lacked neuronal morphology. These results suggest selective neurotoxicity of the C-terminal APP fragments. Neuronally differentiating P19 transformants were transplanted into the hippocampus of C3H mice. APP immunoreactivity identified the transplanted P19 cells; adjacent cerebral vessels were also heavily stained. The construct was also used to develop transgenic mice. Strong expression of the transgene was confirmed in virtually all tissues by both Northern and Western blot analysis. Western blot analysis of the animals revealed tissue-specific processing of APP in the brain. Moreover, in contrast to the *in vitro* experiments, no significant neurotoxicity has so far been observed in the *in vivo* models. Both models, therefore, offer an opportunity to study suppressor mechanisms.

304.11

STRUCTURAL ALTERATIONS IN THE BRAINS OF MICE TRANSGENIC FOR THE CARBOXY TERMINAL PORTION OF THE AMYLOID PRECURSOR PROTEIN GENE (APP). M. L. Oster-Granite*[1], J. Greenan[1], and R. L. Neve[2].Div. Biomed Sci., Univ. CA, Riverside, CA[1] 92521-0121 and Dept. Psychiatry, Harvard University School of Medicine, Boston, MA[2] 02178.

Both individuals with Down Syndrome (DS) and patients with Alzheimer's disease (AD) develop characteristic pathologic changes in their brains, including granulovacular degeneration, neurofibrillary tangles, and neuritic plaques. Within the neuritic plaques of these individuals, a 42 amino acid peptide, β-amyloid or β/A4 accumulates. This peptide is derived from a much larger precursor molecule, encoded by the gene *amyloid precursor protein* (*APP*). *APP* is located on human chromosome 21 and is overexpressed in both DS and AD individuals. In DS individuals, overexpression results in large part because of triplication of the gene; in AD, the gene does not appear to be triplicated. Mice transgenic for but a portion of the APP molecule, the last 100 amino acids at the carboxy terminal (APP-C100) display altered brain morphology (Kammersheidt *et al.* 1992. Proc. Natl. Acad. Sci. USA, in press).

We have begun a systematic structural analysis of various lines of these APP-C100 transgenic mice, utilizing Golgi, light, and electron microscopic methods to provide finer structural correlates to the punctate vesicular immunoreactive material observed in hippocampus in lines of these transgenic mice with highest levels of transgene expression in the brain. We have extended systematic light and electron microscopic studies to other regions of the brain, including frontal cortex, entorhinal cortex, cerebellum, and spinal cord.

304.12

CONGENIC RECOMBINANT ANIMAL MODEL SYSTEMS: A TOP DOWN GENETIC STRATEGY TO ANALYZE QUANTITATIVE NEUROBEHAVIORAL TRAITS. C. Vadasz*. Laboratory of Neurobehavioral Genetics, Nathan Kline Institute for Psychiatric Research, Orangeburg, NY 10962 and New York University Medical Center, 550 First Avenue, New York, NY. 10016

Genetically standardized inbred mouse and rat strains are highly variable in neural and behavioral traits. However, in spite of concentrated efforts, genes responsible for polygenic strain differences have not been mapped and genetic correlation between neural and behavioral traits have not been established because inbred strains and recombinant inbred strains differ at a large number of loci. We suggest, that these difficulties can be circumvented by developing Congenic Recombinant Animal Model (CRAM) systems. CRAM systems are constructed by transferring a set of genes responsible for a phenotypic strain difference to the same genetic background by a series of backcrosses to one of the parental strains with concomitant selection for the desired trait. Subsequent inbreeding results in recombinant fixation of the transferred genes. In a CRAM system the genetic background of the lines is similar, however, they carry elements of the gene set which was responsible for the original strain difference. A CRAM system, focusing on the mesotelencephalic dopamine system, is being developed in our laboratory.

304.13

LATERAL PAW PREFERENCE IN INBRED MICE N.S. Waters* and V.H. Denenberg Biobeh. Sci. Grad. Deg. Prog., U. Conn., Storrs, CT 06269.

We have developed a novel measure of behavioral laterality in inbred mice, the lateral paw preference (LPP) test (Waters and Denenberg, *Phys. Behav.* **50,** 853). In this test a small unit, with preferred food available through two holes, is placed in the animals' cages. Each hole is most easily accessed by one paw, and the amount of food consumed from each hole is measured. We now report the results from 653 mice of 27 genotypes. Most mice were tested twice on LPP and once on the Collins' paw preference test, in which subjects reach for food from a centrally located tube, and the number of right-pawed reaches (RPE) out of 50 reaches is measured (Collins, *J. Hered.* **59,** 9). A weak but significant bias towards the left was found on the Collins' test (54.6% sinistral; χ^2=4.83, p<.05; N=581), similar to what has been previously reported. The population bias in the LPP test was stronger and in the opposite direction, with 60.6% dextral (χ^2=29.59, p<.0001; N=653). A Spearman correlation of 0.147 (p<.01, N=588) was found between LPP and RPE direction measures, and of 0.108 (p<.02, N=588) for magnitude of asymmetry, regardless of direction. Though significant, these correlations are small enough to be negligable. There were significant differences among the strains for magnitude of asymmetry on both measures. Using strain means, a Spearman correlation of 0.577 (p<.01, N=26) was found for the magnitude measure, while the correlation for direction was non-significant (r=0.196, p>.1). Collins has reported that magnitude of asymmetry has a genetic basis, and the strain correlation implies that this basis may be partially shared with the LPP absolute asymmetry measure, while direction of asymmetry on the two tasks is clearly under the control of different, probably epigenetic, factors.

Supported, in part, by NIH grant HD20806. We wish to thank Drs. E. Eicher, L. Mobraaten, C. and R. Wimer, who generously supplied mice.

304.14

A GENETIC APPROACH TO THE NEURODEVELOPMENTAL HYPOTHESIS FOR SCHIZOPHRENIA

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The lack of a clear genetic etiology in schizophrenia and the large number of possible causative genes make it helpful to design strategies that select candidate genes for the disorder. Several monoclonal antibodies showing different binding affinities to schizophrenic versus control brains have been identified. One antibody, called EP10, was used to screen two cDNA expression libraries, one derived from a neuroblastoma cell line and another from human temporal cortex, in an attempt to isolate candidate genes.

Clone WH4 was isolated from the temporal cortex library. Its 381 base pairs were sequenced and WH4 was assigned to chromosome 17, close (lod score > 11 by genetic linkage) to two loci involved in the development of the nervous system, the nerve growth factor receptor (NGFR) and the homeobox-2 gene cluster (HOX2@). Preliminary linkage analysis using a two gene hypothesis was conducted in an Italian schizophrenic family and has given a slight positive result (Lod score = +1.1 at theta=0.0).

Several lines of evidence indicate that a major etiological factor in schizophrenia is a disturbance in the development of the brain. The candidate gene WH4 is located near genes implicated in brain development and displays homology with an integrin molecule. Integrins have been implicated in brain development, as well as in cell adhesion. These factors prompt us to determine the role of this gene in neurodevelopment and its possible involvement in schizophrenia.

304.15

RECOMBINANT DROSOPHILA CYSTATIN AND ITS "ICELANDIC" MUTANT. M.P.Brown and L.E.Kelly* Dept Genetics, Melbourne University, Parkville, Victoria 3052, Australia.

We aim to develop a model system in Drosophila melanogaster to investigate amyloidogenesis.

A Drosophila complementary DNA (cDNA), cloned and sequenced in our laboratory, has been expressed as a fusion protein which inhibits cysteine protease activity.

The genetic lesion of Hereditary Cystatin-C Amyloid Angiopathy (HCCAA, formerly Hereditary Cerebral Hemorrhage with Amyloid of Icelandic type), which results in substitution of glutamine for leucine in a region conserved in Drosophila cystatin, has been reproduced in the recombinant fusion protein. This mutant fusion protein is a three-fold less potent inhibitor of cysteine protease activity than wild type fusion protein.

We postulate that defective regulation of cysteine protease activity is responsible, directly or indirectly, for cerebral hemorrhage in HCCAA.

304.16

MYOBLAST TRANSFER THERAPY(MTT) STRENGTHENS LEG MUSCLES OF DUCHENNE MUSCULAR DYSTROPHY (DMD) BOYS. P.K. Law*, T. Goodwin, Q. Fang, V. Duggirala, C. Larkin, J. Florendo, D. Kirby, M. Deering, H. Li, M. Chen, T. Yoo, J. Cornett, L. Li, A. Shirzad, T. Quinley, and R. Holcomb. Cell Therapy Research Foundation, Memphis, TN 38117.

Five billion normal myoblasts were injected into each of 32 DMD boys aged 6-14 to assess the feasibility, safety and efficacy of Phase II MTT. Forty-eight i.m. injections into 22 muscles took 10 minutes under general anesthesia. Eleven boys had received 8 million myoblasts each 1 year ago in Phase I MTT. In Phase II eight of them had their myoblasts subcultured from reserves frozen 1 year ago. The remaining boys received myoblasts cultured from 1-g muscle biopsies of normal males who might or might not be histocompatible with the recipient. Cyclosporine (Cy) is administered for 6 months after MTT to facilitate donor cell survival. There has been no evidence of an adverse reaction to MTT or Cy as determined by serial laboratory evaluations including electrolytes, creatinine and urea. Objective functional tests using the KinCom Robotic Dynamometer were conducted on 13 subjects aged 6 to 13 before MTT and at 3 months after MTT. Of the 69 knee extensors, knee flexors,and plantar flexors tested, 43% showed mean increase of 41.3% in isometric force, 38% showed no change, and 19% showed continuous force reduction of 23.4%. The remaining subjects await the 3-month post-MTT evaluation. The results indicate that 1) MTT is safe; 2) MTT increases muscle strength in DMD: 81% of the muscles tested showed either increase in strength or did not show continuous loss of strength; 3) more than 5 billion myoblasts can be cultured from 1 g normal muscle biopsy, providing unprecedented numbers of cells for MTT; 4) myoblasts, frozen over one year, retain the ability to proliferate from 10 million to 5 billion, and to form normal myofibers; 5) injections of 5 billion myoblasts have not provoked any immunologic rejection symptoms in the Phase II subjects, eleven of whom received 8 million myoblasts in Phase I MTT a year ago; 6) it is safe to perform multiple injections of myoblasts into lower limb muscles without formation of emboli; and 7) donor cell rejection by the recipient can be prevented with Cy when properly managed. (Supported by public donations)

304.17

INTRAPAIR-DISCREPANCY IN RIDGE-COUNT OF MZ TWINS AS A MARKER OF SECOND-TRIMESTER DISTURBANCES IN FETAL-DEVELOPMENT, H. Stefan Bracha, M.D*, Edward Taylor, M.S.W., Ph.D., Beverly Linington, M.A., Beata Przybyla, Ph.D., Child and Adolescent Psychiatry Division, Univ. of Arkansas for Med. Sciences and Psychiatry Service, VAMC, 116A1/NLR, North Little Rock, Arkansas 72114-1706.

The second-prenatal-trimester is a critical period of neural cell migration. Fetal injuries in second-trimester are difficult to study because of a lack of obstetrical markers. The dermal anatomical feature called ridge count is a stable anthropometric measure (Bracha et al 1991, 1992) and MZ twins have nearly identical ridge count. We took advantage of the fact that prenatal insults, especially ischemias, often do not affect both MZ twins equally. Since dermal cells migrate to form ridges during the second-trimester, we hypothesized that any discrepancies in the ridge count between MZ twins may be a marker of the second-trimester injuries to one twin. METHODS: 30 mothers of MZ twins completed a questionnaire about deleterious pregnancy events. We also measured intra-pair differences in ridge count within pairs. RESULTS: The second-trimester subscale of deleterious events was the best predictor of increased intrapair differences in ridge count and accounted for 36% of variance. The third and first trimesters accounted for less than 2%. Conclusion: MZ discrepancy in ridge count may serve as a marker for prenatal events that might affect only one MZ fetus in the second-trimester.
Sup. by grant MH-43537 and the U.S.-DVA

DEGENERATIVE DISEASE: ALZHEIMER'S—β-AMYLOID III

305.1

AMYLOID ß PROTEIN AND AMYLOID P COMPONENT IN ALZHEIMER'S DISEASE. T. Duong* and P. J. Acton. Terre Haute Center for Medical Education, Indiana Univ. Sch. of Med., Terre Haute, IN 47809.

The distribution of amyloid ß protein (AßP) was compared to that of amyloid P component (APC) in lesions characteristic of Alzheimer's disease (AD): the amyloidotic blood vessels (ABV), senile plaques (SP) and neurofibrillary tangles (NFT). Blocks from the hippocampus and neocortical regions of AD patients and normal aged controls were collected postmortem and fixed in 4% phosphate-buffered paraformaldehyde. Cryostat sections (30 μm thick) were processed by single- and double-antigen immunohistochemistry. AßP and APC were localized to ABV, which were mostly penetrating pial arterioles. Although the 2 proteins could be co-localized on the same vessels, their distribution did not always coincide: some portions of ABV were more immunoreactive to either AßP or APC. ABV immunoreactive only to one or the other protein were also seen. SP were labeled more often by AßP than APC antiserum. In neocortical areas, APC-immunoreactive SP were mostly localized in superficial layers of the cortex whereas AßP-labeled SP were seen throughout the gray matter. Both AßP and APC labeled the corona and the core of SP, as well as diffuse SP in layer I. In SP double-labeled for AßP and APC, APC also labeled dystrophic neurites whereas AßP did not. APC was localized to the majority of NFT in all regions studied whereas AßP was only observed in occasional NFT in the hippocampal CA 1 region and in the layer II of the entorhinal cortex. Although occasional NFT double-labeled for AßP and APC were seen, the NFT populations labeled by AßP and APC were mostly segregated. **Thus, the distribution of AßP and APC in AD lesions is not similar and this may reflect differences in their origin and /or the process leading to their incorporation in AD lesions.** This work was supported by the Turken Scholarship from the Alzheimer's Disease and Related Disorders Association, Inc.

305.2

AMYLOID PRECURSOR PROTEIN(APP) IMMUNOREACTIVITY IN ALZHEIMER'S DISEASE BRAIN WITH APP 717 MUTATION. N.Nukina, K.Hashimoto, H.Mizusawa, I.Nakano, I.Kanazawa, K.Murayama*,Department of Neurology,University of Tokyo, Japan

The amyloid core in senile plaques of Alzheimer's disease is composed of beta protein with a molecular weight of 4kD. Whether beta protein accumulation in AD is a byproduct of Alzheimer's pathology or a primary pathology has not been determined. Recent discovery of familial Alzheimer's disease which is linked to APP codon 717 mutation suggests that the beta protein accumulation is a primary pathology. It is important to clarify whether the processing of APP in those cases with the mutation is different from the processing in other cases. In this study we investigated the distribution of APP immunoreactivity in AD brains with Val-Ile mutation.

Antibodies used in this study were the following:monoclonal antibody against synthetic peptide residues 45-62 of APP, polyclonal antibodies against synthetic peptide residues 666-695 of APP,beta protein, human tau protein, and ubiquitin. Tissue sections from 2 cases with the mutation and 8 cases without the mutation were examined.

APP antibodies stained neurites around senile plaques and somtimes neuronal cell bodies. Antibodies against beta protein immunostained amyloid and diffuse plaques. Antibodies against tau and antibodies against ubiquitin immunolabeled tangles and neurites around senile plaques. Those immunostainings were observed in cases with and without the mutation. We could not find any qualitative differences in those cases, suggesting that those immunoreactivities observed in this study appear in the later phase of Alzheimer's pathological cascade.

305.3

THE EXPRESSION OF CELL ADHESION MOLECULES IN ALZHEIMER'S DISEASE

K.C. Breen & A.M. Gillian. Dept. of Pharmacology, University College, Belfield, Dublin 4, Ireland. (SPON: Brain Research Association).

The ß-Amyloid Precursor Protein (ßAPP) is a membrane bound glycoprotein which has been implicated in the mediation of neural cell adhesion. This protein is abnormally degraded by protoelysis in Alzheimer's disease (AD) to the 42 amino acid ßA4 polypeptide which is a major component of the characteristic amyloid plaques. It was of interest, therefore, to examine the expression of other cell adhesion molecules in AD to investigate any parallel changes.

The nerve cell adhesion molecule (NCAM) is a cell surface glycoprotein whose sialic acid content is developmentally regulated. Western blot analysis has demonstrated that although total brain levels of NCAM appear unaltered in AD, there is the appearance of an extra 170kd band, suggestive of altered proteolytic processing of the protein. Furthermore, there is an increase in the soluble form of NCAM in both serum and CSF.

There is also evidence of altered glycosylation in AD. While there is no reappearance of the embryonic (polysialylated) form of NCAM in AD, there is a significant decrease in brain membrane sialyltransferase levels, and also the levels of the carbohydrate adhesion epitope L2/HNK-1.

This may result in an altered neural adhesive state in AD which may alter cell-cell contact and thus contribute to the neurodegenerative process.

305.4

THE ROLE OF AMYLOID PRECURSOR PROTEIN (APP) IN NEURITIC OUTGROWTH AND ABERRANT SPROUTING IN ALZHEIMER'S DISEASE. E. Masliah*, V. Lev-Ram, M. Mallory, N. Ge, M. Ellisman, T. Saitoh,
San Diego Microscopy & Imaging Resource, Dept. of Neurosciences, University of California, San Diego, La Jolla CA 92093

Previous studies have shown that APP is localized in GAP43-positive aberrant sprouting neurites in the plaque (Masliah et al, Brain Res 574:312,1992). Thus APP might by involved in neurodegeneration in Alzheimer's disease (AD) by inducing an abnormal sprouting response. In order to study the role of APP in neuritic outgrowth, we examined organotypic cultures of neonatal rat (P1) hippocampus labeled with DiI and treated with N-terminal APP fragments and amyloid ß using time-lapse microscopy. In control preparations, DiI-labeled neuritic processes were imaged for 35 min and perfused with vehicle. Processes of neurons in control preps extended at rates of approximately 20 µm per hr. Experimental cultures perfused with a 100 µM amyloid ß solution (1-28, Sigma) and imaged for 35 min displayed a relative increase in fluorescence and motility. Process outgrowth was further enhanced with time. Further analysis of the presence of APP in growth cones was performed in brain sections of P1 rats, as well as organotypic culture slices. Laser-scanning confocal imaging of double-immunolabeled sections showed that a subpopulation of the anti-GAP43-immunoreactive growth cones contained APP immunoreactivity in the neocortex. These fine long neuritic processes were also positive with antibodies against phosphorylated neurofilaments (SMI 312) and were GFAP negative. APP strongly immunolabeled neurons in the inner cortical layers, while GAP-43 strongly immunolabeled the neuropil surrounding them. These observations suggest a possible role for APP in neuritic outgrowth and might explain the extensive neuritic response found in plaques of AD.

305.5

LOCALIZATION OF AMYLOID PRECURSOR PROTEIN IN SELECTIVE POSTSYNAPTIC DENSITIES OF RAT CORTICAL NEURONS. K. Shigematsu*, P. L. McGeer and E. G. McGeer. Kinsmen Lab. of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, B.C. Canada V6T 1Z3

One of the hallmarks of Alzheimer pathology is extracellular deposition of amyloid which is derived from a larger glycoprotein called amyloid precursor protein (APP). Although APP is thought to be a membrane associated protein, widely distributed in various cells including neurons, its ultrastructural localization in neurons is still unclear. We now report the results of immunoelectron microscopy using three specific antibodies against different synthetic fragments of APP. All three antibodies demonstrated a localization to organelles such as the Golgi apparatus, endoplasmic reticulum and vesicular-like structures. A minor proportion of staining with all three was on selective postsynaptic membranes, whereas staining of presynaptic membranes was not observed. The morphological evidence suggests that one role of APP may be in association with the function of selective synapses.
(Supported by a grant from the Alzheimer Society of B.C.)

305.6

STELLATE CELLS EXPRESSING APP, AMYLOID P AND LACTOFERRIN IN THE CEREBELLAR CORTEX IN ALZHEIMER AND ELDERLY CONTROL BRAINS. T. Kawamata, P. L. McGeer and A. Jakubovic* Kinsmen Lab. of Neurol. Res., U. Brit. Columbia, Vancouver, Canada, V6T1Z3

Amyloid deposits are frequent in the cerebellar cortex in Alzheimer disease (AD), but not in aged controls. Diffuse plaques are the most common type of amyloid deposits in the molecular layer, and emerging evidence suggests that such amorphous, largely nonfibrillary deposits may precede the appearance of altered neuritc and glial elements during development of consolidated senile plaques. We studied the distributions of APP (APP_{18-38}, $APP_{274-286}$, $APP_{528-540}$, $APP_{597-620}$, and $APP_{681-695}$), complement protein C4d, amyloid P component (AP) and lactoferrin (LF) in the molecular layer of the cerebellum in AD and aged controls. The antibodies to $APP_{597-620}$, C4d, AP and LF stained diffuse plaques and all the antigens, except C4d, were expressed in stellate and basket cells in both AD and controls, with the staining being upregulated in AD cases. In AD some of the positive dendrites and axons were closely associated with diffuse plaques. These data suggest that diffuse plaques may derive from the neurites of stellate neurons which show upregulated expression of APP, AP and LF in the molecular layer of the cerebellum in AD.

305.7

LYSOSOMAL HYDROLASES COLOCALIZE WITH β-AMYLOID IN DIFFUSE PLAQUES IN ALZHEIMER AND DOWN SYNDROME BRAIN. A.M. Cataldo* D.Hamilton, P.A. Paskevich, D.A. Mann, R.A. Nixon. McLean Hosp., Harv. Med. Sch., Belmont, MA, and Dept. of Path., Univ. of Manchester, U.K.

Our previous studies in Alzheimer Disease (AD) and Down Syndrome (DS) brains support a relationship between the presence of β-amyloid protein and elevated levels of lysosomal hydrolase activity. Hydrolases accumulate abnormally in neurons at risk to degenerate and are abundant in classical senile plaques (SP) of the neocortex and hippocampus. Intensely-stained neurons were often associated with classical SP. To further investigate this relationship, we studied the distribution of lysosomal hydrolases in diffuse type SP of the neocortex and cerebellum. Tissue sections from 20 AD and DS brains were analyzed immunocytochemically with antisera to cathepsin D, β-hexosaminidse A, and β-amyloid (residues 1-24). SP were identified histologically with a modified Bielschowsky stain and thioflavin S. In the cortex, diffuse SP were immunoreactive to hydrolase and β-amyloid antisera. These antisera colocalized in many SP. In the cerebellum, elevated amounts of hydrolase immunoreactivity were present extracellularly in most diffuse SP of the molecular layer in a similar topography to that of β-amyloid detected immunocytochemically. A subset of Purkinje cells in DS and AD brains were intensely hydrolase positive. In many cases, hydrolase positive diffuse SP were located in the vicinity of intensely stained dendritic arborizations of these Purkinje cells. Hydrolase-containing SP were also seen in the Purkinje cell layer. Perikarya of dying neurons are a principal source of hydrolases in many classical SP; however, in diffuse SP the degeneration of different structures, such as dendrites and other cell types, may account for the association of lysosomal hydrolases with β-amyloid deposits.

305.8

NEUROPATHOLOGICAL STUDY OF THE BRAINS OF THREE AGED CHIMPANZEES. M. Gearing*, J. Tigges and S. S. Mirra. VA Medical Center, and School of Medicine and Yerkes Regional Primate Research Center of Emory University, Atlanta, GA 30322.

While vascular amyloid and senile plaques have been described in the brains of aged mammals such as dogs, bears, and monkeys, neuropathological studies of aged great apes have been limited. We had the opportunity to examine the brains of three elderly chimpanzees: two females aged 59 and 56 and a male aged 45 years. External abnormalities were not observed in any of the brains. A battery of stains and immunohistochemical procedures revealed ßA4 amyloid deposition in meningeal and cortical vessels in both females. This was particularly striking in the 59 year old. The cortex and hippocampus in this older female also displayed diffuse plaques, predominantly in a perivascular distribution, as well as rare neuritic plaques. Preliminary ultrastructural study revealed fibrillar amyloid in vessel walls, but extravascular fibrillar amyloid could not be detected in plaque-rich regions. No amyloid was found in the brain of the male. Alz-50-positive neurons were seen in all three brains in the absence of any detectable neurofibrillary tangles or immunoreactivity with Tau-1 antibody. These Alz-50 labelled neurons were rare in the male, but were more readily observed in the two females, particularly in the lateral putamen. Ubiquitin immunohistochemistry revealed scattered neurons with cytoplasmic label and "dot-like structures" in the neuropil. In summary, these findings recapitulate those described in normal elderly people and emphasize the value of aged great apes as animal models for the study of changes related to aging and Alzheimer's disease. RR-00165, AG00001, AG10130, and VA Merit Award.

305.9

SPECIFIC METALS AND SALTS PROMOTE OR INHIBIT ß–AMYLOID DEPOSITION ONTO EXISTING PLAQUES IN ALZHEIMER DISEASE BRAIN

J.R. Ghilardi, C.J. Allen, E.R. Stimson, H.V. Vinters, J.E. Maggio*, P.W. Mantyh. Mol. Neurobio. Lab (151) VA Med. Cntr., Mpls., MN 55417; Dept. Pathol., UCLA, LA, CA,90024; Dept. of Biol. Chem. and Mol. Pharm., Harvard Med. School, Boston, MA 02115

ß-Amyloid peptide (ßA4), a 39–43 amino acid peptide, is the primary constituent of senile plaques and cerebrovascular deposits in Alzheimer disease (AD). Recently it has been reported that ßA4 neurotoxicity is related to its aggregation state. To explore the factors which influence the deposition of ßA4 onto amyloid plaques and cerebrovascular amyloid, an *in vitro* assay was developed using radioiodinated ßA4 in conjunction with homogenates or thin sections of AD cortex. This system was used to address the effect various metals, salts, pH and temperature have on the deposition of radioiodinated ßA4 onto pre–existing amyloid deposits in AD brain. The results from these experiments suggest that the presence of specific metals and salts can have pronounced effects on the ability of ßA4 to deposit onto preformed amyloid deposits. These factors may be important in regulating the growth and toxicity of amyloid deposits in AD. Supported by the NIH and the VA.

305.10

SPECIFICITY, SENSITIVITY AND ABILITY TO QUANTIFY AMYLOID DEPOSITS IN ALZHEIMER DISEASE BRAIN USING EITHER RADIOIODINATED ßA4, THIOFLAVIN S, CONGO RED OR ANTI–A4 ANTIBODIES

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The salient neuropathological feature of Alzheimer disease (AD) is the presence of a high density of amyloid plaques in the brain tissue of victims. Recent data has suggested that there is a correlation between ß-amyloid peptide (ßA4) deposition and the clinical severity of the dementia and that the amyloid plaques themselves are neurotoxic both *in vivo* and *in vitro*. A key tool in diagnosis of AD is the ability to visualize, characterize and quantify the amyloid deposition in AD brain. We have compared the sensitivity and specificity of 4 methods, radioiodinated ßA4, thioflavin S, Congo red and anti–A4 antibodies, for their ability to label amyloid deposits in both fixed and unfixed AD brain. While each of these techniques of labeling amyloid deposits has its strengths, radioiodinated ßA4 is unique in that it is highly sensitive, specific and easy to quantify. Supported by the NIH and the VA.

305.11

SERUM, ASSOCIATION CORTEX, AND CEREBELLAR C1q CONCENTRATIONS IN ALZHEIMER'S DISEASE AND NONDEMENTED ELDERLY PATIENTS. L. Brachova*, L.-F. Lue, J. Schultz, T. El-Rashidy, and J. Rogers. Sun Health Research Institute, Sun City, AZ 85372.

Concentrations of C1q were assayed by Western blot analysis of sera, superior frontal gyrus, and cerebellar homogenates from 5 Alzheimer's disease (AD) and 5 nondemented elderly (ND) control patients. Immunoreactive serum C1q concentrations did not differ in the two groups, whereas in the same patients AD superior frontal gyrus exhibited five-fold more immunoreactive C1q (0.460 ± 0.052 μg C1q/mg total soluble protein) than ND superior frontal gyrus (0.088 ± 0.011 μg C1q/mg total soluble protein). Cerebellar concentrations from these patients averaged 0.084 ± 0.007 μg C1q/mg total soluble protein in AD samples and 0.053 ± 0.001 μg C1q/mg total soluble protein in ND samples. The significant elevation of C1q in the superior frontal gyrus of AD patients and the relative paucity of C1q in the cerebellum correlates well with the extensive AD pathology of the former structure and the relative lack of degenerative pathology or clinical symptomatology of the latter in AD. Because β-amyloid is present in the AD cerebellum (albeit in a diffuse rather than compacted form), these data suggest that other factors such as complement activation may be necessary for the full expression of β-AP mediated neuritic damage.

305.12

COMPLEMENT ACTIVATION FAILS IN DIFFUSE PLAQUES OF THE ALZHEIMER'S DISEASE CEREBELLUM. L.-F. Lue*, and J. Rogers. Sun Health Research Institute, Sun City, Arizona 85372.

Immunohistochemistry of β-amyloid peptide (β-AP), complement proteins C4d, C3d, and C5b-9, and the HLA-DR marker LN-3 was performed on 40 μm cerebellar sections from Alzheimer's disease (AD) and nondemented elderly (ND) patients. Morphologically, two major types of β-AP deposit were identified: a diffuse type and a compacted type. Diffuse plaques were rare and compacted plaques were absent in ND cerebella. In AD cerebella, both diffuse and compacted plaques were observed, although the former were much more abundant. C4d immunoreactivity co-localized with diffuse and compacted plaques. C3d immunoreactivity co-localized predominately with compacted plaques, but could occasionally be observed in diffuse plaques. Unequivocal C5b-9 immunoreactivity was present only in compacted plaques. Activated microglia in the AD brain are known to express the LN-3 antigen and to possess complement receptors. In AD cerebella, LN-3 positive microglia were consistently clustered around compacted plaques, whereas no such association was found with diffuse plaques. In the classical complement cascade, formation of C4d is a relatively early step and formation of C5b-9 is the final step. These findings are therefore consistent with the hypothesis that complement activation runs to completion in the vicinity of compacted β-AP containing plaques, but fails to do so in the context of diffuse plaques. Since diffuse plaques greatly predominate in the AD cerebellum, these data may also help explain the relative paucity of cerebellar pathology and clinical symptomatology in AD.

305.13

ALZ-50 G1-IMMUNOREACTIVITY IN THE BABOON BRAIN: ENHANCED EXPRESSION IN AGING. **Y. Ma, C. Anderson*[1], E.J. Mufson, and J.H. Kordower. Dept. Neurological Sciences, Rush Presbyterian Med. Ctr. and [1]Dept. of Anatomy and Cell Biology, Univ. Illinois. Sch. Med., Chicago Illinois 60612**

Initial studies suggested that the protein Alz-50 may be an indicator of early degenerative changes in Alzheimer's disease. Recently, Alz-50-immunoreactivity has been observed in the normal human brain as well as in select nonprimate species. Therefore, we examined the expression of the Ab42, a class switch clone of the Alz-50 IgM antibody in young (age 4, 7 years old) and aged (21 and 33 year old) baboons. Baboons were perfused transcardially with 4% paraformaldehyde, their brains hemisected, and cut in the coronal plane (40 μm) on a sliding knife microtome. Only scattered Ab42-immunoreactivity was observed within thalamic and limbic structures in young baboons. In contrast, aged baboons displayed extensive Ab42-immunoreactive profiles within the neo and limbic cortices as well as subcortical structures which have been shown previously to degenerate in the aged and diseased human brain. These include the cingulate gyrus, entorhinal, and temporal neocortices, septum, the nucleus basalis, paraventricular nucleus, amygdala, and subiculum. Immunoreactivity was granular and principally localized within the peripheral portions of the cytoplasm. This age-related difference in Ab42 expression was specific since staining patterns of numerous other cytoskeletal proteins and neurotransmitter markers were similar in young and aged baboons. This study suggests that the Ab42 protein may be a marker for age-related changes in the primate central nervous system. (Supported by AG09466, AG10161, IDPH).

305.14

COLOCALIZATION OF CHOLINESTERASES WITH AMYLOID IN SENILE PLAQUES OF AGED AND ALZHEIMER'S DISEASE PATIENTS. P. Gómez-Ramos* and A. Morán. Dept.Morfologia. School of Medicine. U.A.M. Madrid Spain.

A sensitive modified Karnovsky-Roots protocol for Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) (Mesulam and Morán, 1987) showed positivity for either of both enzymes in all types of senile plaques (SP) of aged and Alzheimer's brains: diffuse, classical with or without a central core and burned out SP. In addition, AChE has been ultrastructurally localized in diffuse plaques as well as in both the core and the crown of classical plaques (Gómez-Ramos et al, 1992).

To study the colocalization of cholinesterases with amyloid in SP the following procedures were used: a) Quantitative comparison of cholinesterase positive SP (either AChE or BChE) and these SP stained with either Thioflavine-S or A4 protein immunocytochemistry, in adjacent or closely related sections. b) Double-procedures conbining cholinesterase histochemistry, (AChE or BChE) with either Thioflavine-S staining or A4 protein immunocytochemistry, in the same section. The results obtained suggest that all Thioflavin-S positive SP are also positive for at least one of both cholinesterases, whereas the Thioflavine-S negative preamyloid diffuse SP, only in some occasions were cholinesterase positives. Thus, it seems that AChE and/or BChE colocalize with amyloid even before it acquires the ß pleated configuration which leds to Thioflavin-S staining. Supported by FISS nº 90/0259.

305.15

SOLUBLE DERIVATIVES OF APP: A POTENTIAL RELATIONSHIP TO ABNORMAL CALPAIN I ACTIVATION IN ALZHEIMER BRAIN. T. Honda, J. Hamos* and R. A. Nixon. McLean Hospital, Harvard Medical School, Belmont, MA. 02178; University of Massachusetts, Worcester, MA

Alzheimer amyloid precursor protein (APP) is considered to be a membrane glycoprotein. To examine the processing of APP we determined the subcellular localization of APP and quantified the proportions of APP forms in control and Alzheimer disease (AD) brain. Homogenates of prefrontal cortex were separated into four fractions: soluble, microsomal, Triton X-100 soluble and insoluble membrane fractions. Protein was analyzed by SDS-PAGE and Western Blot using a monoclonal antibody to the N-terminal domain (Boehringer-Mannheim), polyclonal antisera to APP 578-606, or the amyloid ß region (1-28 and 12-28). In both control and AD samples, APP forms (100-130 kDa) were present in soluble fractions that were immunoreactive only with the antibodies which recognize the secreted domain of APP. In the course of other studies demonstrating an abnormal activation of calpain I (μCANP) in AD brain (Saito et al., these proceedings), we examined whether calpain activation was associated with alterations in the content of membrane and soluble APP forms. We observed that the concentration of soluble APP forms in brain displayed a very high inverse correlation with the degree of calpain activation (i.e., the percentage of the 76 kDa autolytically activated isoform of calpain) in AD brain ($r=0.885$, $n=13$, $p < 0.0001$). The content of membrane-associated APP was comparable in AD and control brains and did not correlate with calpain I activation. The significance of the soluble APP correlation is unclear at present, although a relationship between calpain activity and membrane protein trafficking and processing is reasonable in view of the localization, substrate specificity and suspected biological roles of calpain I.

305.16

BETA AMYLOID DEPOSITION IN SQUIRREL MONKEYS. G. Murphy, L.S. Forno*, L.E. DeLanney, B. Greenberg, J.W. Langston. DVA Medical Center, Palo Alto, CA 94304, California Parkinson's Foundation, San Jose, CA 95128, and The Upjohn Co., Kalamazoo, MI 49001.

Amyloid deposition in plaques and cerebral vessels was studied in two groups of squirrel monkeys of various ages. The first group consisted of control monkeys aged 3, 10, and 15-20 years, with 4 monkeys in each subgroup. The second group included 32 monkeys with a minimum age of 15 (14 controls, 18 MPTP treated). For the first group, methacarn-fixed sections from the frontal and occipital poles were reacted with antibodies to the ß-amyloid peptide (ßAP) and to the ß-amyloid precursor protein (ßAPP), and stained with Congo Red (CR). ßAP and CR positive plaques and vessels were seen in three of the oldest animals, and diffuse ßAP plaques were seen in one 10 year old, but the other animals were negative. ßAPP was observed in plaque neurites in the same three old animals, but the diffuse plaques in the 10 year old had no ßAPP. In the second group, formalin-fixed sections containing cerebral cortex and forebrain subcortical structures were examined with CR and Bielschowsky stains, and with the ßAP antibody. 27 of 32 animals (84%) had congophilic angiopathy, and ßAP in plaques and vessels. These results suggest that as in humans, amyloid deposition is rare at younger ages, and although most older monkeys show marked Alzheimer-like amyloid pathology, considerable variation exists, and some older animals escape such changes.

305.17

PLAQUES, CYTOSKELETAL CHANGES AND CELL DEATH IN THE ALZHEIMER HYPOTHALAMUS. J.A.P. Van de Nes[1,2]*, W. Kamphorst[3], R. Ravid[1], D.F. Swaab[1]. [1]Netherlands Institute for Brain Research; [2]Department of Psychiatry, Free University; [3]Department of Neuropathology, Free University, all in Amsterdam, The Netherlands. (Spon: ENA)

It is generally assumed that in the brains of patients with Alzheimer's disease the cytoskeletal changes are locally induced by plaques, followed by cell death. To test this hypothesis, sections from the suprachiasmatic, sexually dimorphic, paraventricular and supraoptic nuclei were stained with Alz-50 and anti-A4.

The results showed that 1) the supraoptic nucleus and the paraventricular nucleus, which do not undergo cell loss during aging or in Alzheimer's disease, did not stain for Alz-50-labelled cytoskeletal changes or anti-A4 positive plaques, thus supporting the cascade hypothesis. However, 2) cytoskeletal changes were observed in the sexually dimorphic nucleus of all Alzheimer patients but not in controls, while in this nucleus a similar decline in cell number was found during aging and in Alzheimer's disease. 3) The suprachiasmatic nucleus in Alzheimer's disease showed some cytoskeletal changes while this was not the case in the suprachiasmatic nucleus of normal subjects. In none of the patients studied was any plaque observed in the suprachiasmatic nucleus.

In conclusion, the occurrence of amorphic plaques, cytoskeletal alterations and cell death seem to be independent phenomena in the hypothalamic nuclei, rather than a cascade.

Brain tissue was obtained from the Netherlands Brain Bank in Amsterdam (Coordinator Dr. R. Ravid)

305.18

BETA AMYLOID PROTEIN IMMUNOREACTIVE SENILE PLAQUES IN INFANTILE DOWN'S SYNDROME. Ter-Minassian M, Kowall NW, McKee AC*. Massachusetts General Hospital, Boston, MA 02114

Virtually all patients with Down's syndrome develop Alzheimer's disease with aging, usually in their 4th to 6th decades. Using immunohistochemical methods and antibodies specific for beta/A4 protein (Angela and 1280,courtesy of D. Selkoe), tau 1 (courtesy of L.Binder), MAP 2 (5E2, courtesy of K.Kosik), and Alz 50 (courtesy of H.Ghanbari), in addition to modified Bielschowsky silver, Congo red and thioflavine S stains, we examined the brains of 3 infants with Down's syndrome (ages 6, 18, and 30 months) and 5 age-matched controls (6-30 months, mean 23 months). We found occasional silver positive, Angela and 1280 immunoreactive (imr) primitive plaques in 2 Down's infant brains (ages 18 and 30 months). The plaques were distributed in the superior frontal and transentorhinal temporal cortices, primarily in the 5th and 6th cortical layers. Congo red and thioflavine S stains were negative. Occasional beta amyloid imr neurons and imr astroglia were also found. Beta amyloid positive plaques were not identified in the control brains. No tau positive or argyrophilic neurofibrillary tangles, neuritic threads, or abnormalities of MAP2 were found in either the Down's or control brains. Diffusely Alz 50 imr neurons and beaded fibers were scattered throughout the CA fields and subiculum of the hippocampus and in the frontal and temporal cortices of all brains. Intensely Alz 50 positive neurons were most abundant in the subcortical white matter of the hippocampus, frontal and temporal lobes. The number of Alz 50 imr neurons did not differ appreciably with age. These findings show that abnormal deposition of beta amyloid protein may begin as early as 18 months in Down's syndrome and prior to any evidence of cytoskeletal pathology.

DEGENERATIVE DISEASE: ALZHEIMER'S—COGNITION

306.1

A SPECIFICALLY CONCEPTUAL PRIMING DEFICIT IN PATIENTS WITH ALZHEIMER'S DISEASE. L.A. Monti*, J.D.E. Gabrieli, S. L. Reminger, D. A. Grosse, and R. S. Wilson. Rush Alzheimer's Disease Center, Chicago, IL 60612 and Department of Psychology, Northwestern University, Evanston, IL, 60208.

Patients with Alzheimer's disease (AD) have a deficit on all explicit memory tasks (recall and recognition). In implicit memory or repetition priming (the influence of prior processing upon later reprocessing of a stimulus), AD patients have shown impaired learning on some tasks and intact learning on others. Characterization of what implicit memory processes are intact and impaired in AD has been difficult because dissociations have come from between-task comparisons, and various repetition priming tasks differ in many ways. We sought to determine whether AD patients have a specific deficit in conceptual priming by varying conceptual processing (with a depth-of-processing manipulation) in a single priming task (category-exemplar production). Subjects were 11 mildy-to-moderately demented AD patients and 10 age-matched normal control (NC) subjects. The experiment had two identical study phases followed by tests of either implicit or explicit memory. In both study phases, subjects judged whether exemplars of semantic categories referred to man-made or natural objects (deep processing) or were displayed in upper or lower case (shallow processing). For the implicit test, subjects were shown the category names and asked to generate 8 exemplars of each; the measure of priming was how often study-list exemplars were provided relative to baseline. The explicit test was identical except that subjects used the category name as a cue to recall the studied exemplars. Subjects recalled more exemplars after deep than shallow processing, and the AD group recalled fewer exemplars than the NC group. On the implicit test, both groups showed repetition priming, but only the NC group showed greater priming after deep processing. The AD patients showed the normal small magnitude of priming after shallow processing. The failure of patients to show greater repetition priming after enhanced conceptual analysis in the deep processing condition indicates a specific impairment of conceptual implicit memory in AD. Supported by grants from the NIA #P01 AG09466-02 and the Illinois Department of Public Health.

306.2

INTACT CONCEPTUAL PRIMING IN A PICTURE-NAMING TASK IN PATIENTS WITH ALZHEIMER'S DISEASE. S.M. Park, J.D.E. Gabrieli*, J.R.Tinklenberg, and J.A. Yesavage. Department of Psychology, Stanford University, Stanford, CA 94305 and VA Medical Center, Palo Alto, CA 94304.

In prior studies we showed that patients with Alzheimer's disease (AD) learned normally on picture-naming priming tasks despite impaired recognition for pictures and despite impaired repetition priming on conceptually demanding tasks such as word-stem completion. One interpretation of these results is that AD patients can not demonstrate conceptual priming. To test this interpretation, we used a picture-naming task in which subjects were exposed initially to pictures or to words (labels of pictures). Word-to-picture priming is conceptual because a word and the picture that it labels are conceptually (but not perceptually) related. The subjects were 7 mildly-to-moderately demented AD patients and 4 age-matched normal control (NC) subjects. In a study phase, subjects named 20 pictures and read 20 words of common objects and animals. For the priming test, subjects named 80 pictures (20 pictures named in the study phase, 20 pictures that corresponded to the words read in the study phase, and 40 new pictures). Naming latencies were recorded. In a second study phase, subjects named another 20 pictures and 20 words. They then performed an 80-item yes/no recognition test (20 study phase pictures, 20 pictures that corresponded to the words in the study phase, and 40 new pictures). AD patients were impaired on the recognition test and named pictures more slowly and less accurately than NC subjects. AD patients, however, showed a normal pattern of picture-naming priming: 1) repeated pictures were named most quickly; 2) new pictures were named most slowly; and 3) pictures corresponding to study phase words were named faster than new pictures but slower than repeated pictures. These results suggest that AD patients can show intact conceptual priming when identifying pictures.

306.3

INTACT PRIMING PERFORMANCE IN ALZHEIMER PATIENTS ON A WORD-FRAGMENT COMPLETION TASK. D. Cahn, D. Salmon*, W. Heindel, and N. Butters. Psychiatry and Neuroscience Depts., U.C.S.D. School of Medicine and VAMC, San Diego, CA 92161

Previous studies have found impaired stem-completion priming in Alzheimer's disease (AD) patients. Although this impairment has been attributed to a breakdown in the AD patient's semantic processing ability, impaired presemantic processes may also be mediating performance on this task. The present study investigated this issue by examining the performance of AD patients on another word completion task that is thought to be more dependent on presemantic than semantic processes. Two conditions were created so that subjects were asked to complete word fragments (i.e. A_ _A_ _IN for *assassin*) following both immediately and fifteen minutes after the presentation of the study list. A prime score was calculated by dividing the number of target fragments completed by the sum of target and distractor fragments completed. In addition, a recognition version of the task was given for both conditions. As expected, AD patients performed significantly worse than elderly normal control (NC) subjects on recognition memory in both the immediate and delay conditions. Although AD patients appeared to have more rapid forgetting than the NC subjects, the interaction did not reach significance, due possibly to floor performance in the AD patients. Despite impaired explicit performance, the AD patients did not differ significantly from the NC subjects on priming in either delay condition. These results suggest that AD patients can demonstrate normal priming performance when the task is dependent on presemantic rather than semantic processes. The impairment that AD patients show on the stem-completion task may indeed be attributable to an impaired ability to process the semantic component of the task.

306.4

SEMANTIC AND PRESEMANTIC CONTRIBUTIONS TO PRIMING IN ALZHEIMER'S DISEASE. C. Fennema-Notestine*, W. Heindel, D. Salmon, and N. Butters. Psychology, Psychiatry and Neurosciences Depts., UCSD, La Jolla, CA 92093 and VAMC, San Diego, CA 92161.

Alzheimer's Disease (AD) patients have been found to demonstrate both impaired and preserved priming performance across a variety of tasks. We and others have suggested that the priming deficit of AD patients is due primarily to impaired semantic processing ability and that the apparent inconsistency in AD patients' priming performance is due to differences in the degree to which different priming tasks are mediated by presemantic versus semantic processes. This issue was examined in the present study by comparing AD patients' priming performance to that of amnesic patients and normal controls on both a semantic and a presemantic version of a homophone spelling bias task. In the semantic priming condition, subjects were biased against previously preferred homophone spellings by presenting the homophones orally in a semantically related context. In the perceptual priming condition, subjects' spellings were biased by presenting the homophones visually in a semantically unrelated context. As expected, amnesic patients primed normally in both the semantic and presemantic priming conditions. Interestingly, AD patients primed normally in the semantic condition, but were significantly impaired relative to controls in the presemantic priming condition. These results suggest that AD patients' impairment on the spelling bias task may be mediated by presemantic processing deficits and that, under certain conditions, AD patients can demonstrate normal semantically-based priming. Thus, the previously reported variability in AD patients' priming performance across tasks may not be attributable solely to differences in the degree to which these tasks are dependent upon semantic processes.

306.5

CHARACTERISTICS OF GAZING EYE MOVEMENT FOR DIFFERENT DEPTH TARGETS IN PATIENTS WITH ALZHEIMER'S DISEASE. K. UOMORI++, M. YAMADA++, H. YOSHIMATSU++, S. MURAKAMI+, M. FUJII+, R. FUKATSU+, N. TAKAHATA+, M. WATANABE+*. ++ATR A&VP Res. Labs. (Kyoto, JAPAN), +Sapporo Medical College (Sapporo, JAPAN).

To clarify the stereopsis disturbance in patients with Alzheimer's disease(AD), we quantitatively analyze changes in binocular eye movement when subjects shift their gaze to different depths. The new eye movement analyzing system developed by ATR makes this analysis possible.

Subjects are patients in the early and moderate stages of AD, OND and similar-aged control subjects. Their binocular eye movement is recorded when they shift their gaze from targets faraway to targets close at hand, and vice versa. The targets are symmetrically arranged along the median plane, or asymmetrically, on opposite sides; 30 and 100 cm away from the eye. They are switched by a personal computer with a beep. The experiment is conducted in a dimly lit room with a structure capable of providing good binocular cues for depth.

In AD subjects, especially those in the moderate stage, vergence is limited and the amount of change in convergence angle decreases. They also try to focus on a target using saccadic eye movement instead of vergence. Furthermore, asymmetrical saccades are observed(where the saccadic movement amplitudes differ for each eye) even when targets are symmetrically along the median plane.

The new analyzing system will be useful in understanding visuo-spatial cognitive disturbances which are the most outstanding clinical features in early AD.

306.6

A COMPUTATIONAL MODEL OF NAMING IN ALZHEIMER'S DISEASE: SEMANTIC, VISUAL AND LEXICAL FACTORS. L.J. Tippett & M.J. Farah*. Carnegie Mellon University, Pittsburgh PA 15213.

An early and consistent sign associated with Alzheimer's Disease (AD) is difficulty naming objects. The predominant explanation of the naming deficit in AD is that it results from impaired semantic memory. However, two classes of findings seem to challenge this. First, the lower the name frequency, the less likely AD patients are to name an object, suggesting a deficit of lexical access (as opposed to semantic memory). Second, the lower the visual quality of the stimulus to be named (e.g. line drawings v. objects) the less likely AD patients are to name it, suggesting a deficit of visual perception.

The sensitivity of the AD naming deficit to lexical and visual factors is not necessarily incompatible with the semantic memory hypothesis. In parallel distributed processing systems, the different components underlying task performance are highly interactive and damage in one part of the system might therefore render the system as a whole more vulnerable to manipulations affecting the non-damaged components. We trained an interactive network to associate "visual" patterns with "semantic" and "lexical" patterns. When semantic units were lesioned, the network made disproportionate errors both to patterns trained with lower frequency, and when visual input patterns were degraded. This result shows that the hypothesis of semantic memory impairment is able to account for a variety of data on naming in AD, previously thought to challenge it.

306.7

NAMING PERFORMANCE IN ALZHEIMER'S DISEASE IS MORE IMPAIRED IN WOMEN THAN MEN. V.W. Henderson* and J.G. Buckwalter. Depts. of Neurology and Psychology, and the Andrus Gerontology Center, Univ. of Southern California, Los Angeles,CA 90033

Based on our preliminary observations (Buckwalter et al, *J Clin Exp Neuropsychol* 1992;14:23), we hypothesized that women with Alzheimer's disease (AD), when compared to men, would be more impaired on language tasks but would not necessarily differ in other cognitive domains. Data were available for 259 men and 358 women enrolled in the multicenter Consortium to Establish a Registry for Alzheimer's Disease (CERAD) and who met standard criteria for "probable" AD. Dependent variables, taken from the CERAD battery, were measures of language (oral naming), visuospatial skills (line drawings), and dementia severity (the short Blessed test of orientation, memory, and concentration). Hierarchical regression analyses were done to control for potentially confounding variables, forcing in subject age, age at onset of dementia symptoms, and years of education before a dummy-coded gender variable was entered. Women with AD performed significantly worse than demented men on the language task (mean naming scores of 10.1 vs. 11.4, semipartial $R = .12$, $p < 0.005$), but there were no significant differences on the drawing task (semipartial $R = 0.06$) or in terms of severity (semipartial $R = 0.01$). Among 348 nondemented elderly control subjects, women scored better than men on the Blessed test after controlling for age and education (semipartial $R = 0.09$, $p = 0.04$), but there were no significant gender-associated differences on CERAD language or visuospatial measures. Although gender accounts for only a small proportion of the total variance, we conclude that naming for women with AD is more impaired than for men with this disorder.

306.8

DENIAL OF ILLNESS IS RELATED TO DIMINISHED FRONTAL CORTEX GLUCOSE METABOLISM IN ALZHEIMER'S DISEASE. R.P.Friedland *, E.A. Weinstein and C.L. Grady. Section on Brain Aging and Dementia, NIA, NIH, Bethesda, MD 20892 and Alzheimer Center, University Hospitals, Cleveland, OH, 44120.

The mechanisms of denial/unawareness of illness in Alzheimer's disease (AD) are not well understood. We have found that denial/unawareness of impairment in AD is associated with confabulation, reduplicative delusions and disorientation, but is not associated with severity of illness or disease duration. Resting regional cerebral glucose metabolism was determined by positron emission tomography using (F-18) 2-fluoro-2-deoxy-D-glucose and a Scanditronix PC 1024-7B tomograph in 16 AD patients without denial/unawareness with 18 AD patients with denial/unawareness. Patients with denial/unawareness had lower rCMRglc in right ($p < .01$) and left ($p < 0.5$) prefrontal and right premotor cortices ($p < .01$), when compared to patients without denial/unawareness. Principal components analysis showed that the denial/unawareness group had relatively fewer patients belonging to the parietotemporal hypometabolism group which is dominant in AD. These data support the view that denial of illness is a reflection of frontal cortex impairment in AD.

306.9

THE NEUROLOGICAL CORRELATES OF MULTIPLE MEMORY SYSTEMS: [^{18}F] FDG AND PET STUDY IN EARLY ALZHEIMER'S DISEASE. D. Perani*, S. Bressi, S.F. Cappa, G. Vallar, M. Alberoni, F. Fazio, INB-CNR, Universities of Milano and Brescia, Scientific Institute H S Raffaele, 20132 Milano, Italy.

Patients with Alzheimer's disease (AD) are impaired in several aspects of memory function. Severe episodic memory impairment is one of the hallmark of the disease: there are however also defects of immediate memory, semantic memory and some aspects of procedural learning. In order to define the metabolic correlates of the memory disorders in AD, we measured local cerebral metabolic rates for glucose (LCMRglc) with [^{18}F]-FDG and PET in 18 early AD patients (mean age 64.72 +/- 8.74, disease duration 16.44 +/- 8.36 months, Mini-mental state 20 +/- 4.29). AD patients were given a neuropsychological test battery which explored short and long-term explicit memory, semantic memory, language comprehension and production, visuo-spatial processing, conceptual and abstract reasoning and implicit learning. Analysis of regions of interest, including the cortex and the main subcortical grey nuclei, was performed with ANALYZE software package (BRU/Mayo Clinic) on SUN (SPARC) workstation. The relationship between impairment of different aspects of memory function and LCMRglc was investigated with a stepwise multivariate regression analysis. The "best predictors" of cognitive variables, on the basis of their contribution to Hotelling T^2 value (F-to-enter $p<0.05$) were: for immediate memory, right parietal, left superior temporal and bilateral prefrontal areas; for episodic memory, bilateral hippocampus and cingulate cortex; for semantic memory, left cingulate, prefrontal and superior temporal cortex; for procedural memory, bilateral basal ganglia, prefrontal cortex and cerebellum.

This study indicates that, while the episodic memory impairment found in AD is associated, as in pure amnesia, with metabolic dysfunction of the mesial temporal cortex and its connections along Papez circuit, semantic and procedural memory defects are related to metabolic dysfunction of other neuronal networks, which include higher-order association cortices, cerebellum and basal ganglia.

306.10

CELLULAR SPECIFIC PATHOLOGY ALTERS PROBABLE CORTICO-AUTONOMIC EFFERENTS IN ALZHEIMER'S DISEASE. C.C.Chu*, G.W.Van Hoesen, A.R.Damasio, Dept. of Neurology, Univ. Iowa Coll. Med., Iowa City, IA 52242.

Brodmann's area 25 is the major component of the ventromedial frontal cortex whose damage in humans causes defects in social behavior and decision making. Because patients with Alzheimer's disease (AD) have such defects, we investigated the integrity of area 25 in 10 brains from AD patients and in 8 brains from age-compatible controls.

50 um sections stained with Thioflavin S, Alz 50, and thionin were charted using a computer coordinated microscope. The salient findings are: (1) In area 25, there is a selective laminar distribution of neurofibrillary tangles (NFTs) involving mostly cells of layer V. (2) Layer III contains none or only a few NFTs. (3) There are more NFTs in area 25 than in adjoining areas 11 and anterior insular cortex (AI). (4) In area 11 and AI, NFTs are evenly distributed in layers III and V. Layer V pyramids in area 25 thus appears highly vulnerable in AD.

In higher mammals layer V pyramidal cells in area 25 are the primary projection neurons to autonomic regulatory centers (amygdala, hypothalamus, and brainstem nuclei). Loss of pyramidal cells in layer V of area 25 would disrupt autonomic regulation, and compromise reactivation of somatic states. Elsewhere we have proposed that when this reactivation is triggered by socially significant stimuli, it plays a role in social behavior.

306.11

LONGITUDINAL ANATOMICAL AND BEHAVIORAL CHANGES IN ALZHEIMER'S DISEASE. J.P. Kesslak*, S.F. Nagata, C.W. Cotman and O. Nalcioglu. Depts Neurol, Psychobio and Radiol Sci, Univ Calif, Irvine, CA 92717. Alzheimer's disease (AD) is characterized by a progressive dementia and increased density of plaques, tangles and neural loss. Magnetic resonance imaging (MRI), a noninvasive, high-resolution method for quantifying longitudinal volumetric changes antemortem, has shown ventriculomegaly, cerebral, parahippocampal and hippocampal atrophy in AD. This atrophy correlates with behavioral deficits.

Twelve AD patients received annual MR scans and neuropsychological evaluations. Coronal inversion recovery images were quantification from the frontal pole of the temporal lobe to the posterior hippocampus. Neuropsychological tests included the Mini Mental State Exam (MMSE), word recall and recognition. Matched pair t-tests for brain areas and neuropsychological tests were computed to determine between year differences (Table 1). Correlations between anatomical and psychometric measures suggest the progressive degeneration in AD impacts on behavioral function. Atrophy in the hippocampus and parahippocampal gyrus may be severe and occur early in the disease, with subsequent sequential degeneration of other brain areas contributing to increased cognitive deficits.

TABLE 1. LONGITUDINAL CHANGES IN AD BRAIN

	Anatomical	Behavioral Correlations		
	% Change	MMSE	Recall	Recognition
VENTRICLE	26.1*	.192	.167	.028
HPC	.5	.085	.377	.240
PHPC	9.5*	.324	.420	.196
FRONTAL	6.7*	.514	.414	.072
PARIETAL	6.7*	.336	.113	.167
TEMPORAL^	9.1*	.291	.284	.596
STRIATUM	3.1	.019	.551	.104

*Significant change between assessment years ($p > .05$).
^Does not include hippocampus (HPC) or parahippocampus (PHPC).

306.12

DIRECT FUNCTIONAL STATUS ASSESSMENT IN THE HOME ENVIRONMENT: PATIENT PERFORMANCE VERSUS CAREGIVER APPRAISAL IN AD R.A. Mulnard*, and C.W. Cotman, Irvine Research Unit in Brain Aging, *University of California, Irvine, CA 92717 USA.*

While Alzheimer's Disease (AD) causes a protracted cognitive decline, the impact is also significant on the individual's activities of daily living (functional capabilities) within their home environment. These functional capabilities represent important links to self esteem and functional independence of the AD victim. Historically, measurement of these daily living skills in the demented elderly has relied upon caregiver report. Data from caregivers is used as an important means to evaluate the patient in the context of clinical activities. However, the accuracy of the caregiver's appraisal of these important functions has not been systematically tested.

The advent of new instrumentation which allows a direct measurement of functional status in the demented elderly, supported this investigation. Fifteen patients diagnosed with probable or possible AD and their caregivers were visited in their home environments for the purpose of simultaneously measuring the patient's actual performance and the caregiver's appraisal of the patient's functional capabilities. The DAFS (Direct Assessment of Functional Status, Loewenstein, et al, 1989) instrument was utilized for the measurements.

Significant differences were found between the patient's actual performance and the caregiver's appraisal of performance potential in the subscales of transportation, financial skills, shopping skills and the total DAFS scores. AD patients were indeed able to perform significantly better on three of the subscales than was predicted by the caregivers. However,the caregivers were highly accurate in appraising the patients' performance on the subscales of eating and grooming.

306.13

DELAY CLASSICAL CONDITIONING HAS A HIGHER SENSITIVITY FOR ALZHEIMER'S DISEASE THAN TRACE. J.M. Coffin,* L.S. Ferrante, & D.S. Woodruff-Pak. Psych. Dept., Temple Univ. & Phil. Geriatric Cntr., Philadelphia, PA 19141.

We demonstrated the sensitivity of the 400 msec CS-US delay eyeblink classical conditioning in differentiating probable Alzheimer's disease (AD) patients from non-demented elderly control subjects. We suspected that the 750 msec CS-US trace paradigm might be even more sensitive. Two groups of patients who met NINCDS-ADRDA criteria for probable AD were conditioned in either the trace or delay paradigm, and their performance was compared to elderly control subjects. Although AD patients in each group conditioned more poorly, sensitivity in the trace paradigm was lower (54% sensitivity) than it was in the delay paradigm (95% sensitivity). Here we explored whether AD patients initially conditioned in the trace paradigm would still perform more poorly in a subsequent delay conditioning task. Following an average 4-month interval, AD patients ($N=11$; mean age=86.8 yrs.) were retrained in the delay paradigm (500 msec, 1 KHz, 80 dB tone CS followed after 400 msec by a 100 msec, 5 psi corneal airpuff US). Total percentage of CRs was significantly poorer in the delay paradigm (19.6) than in the initially tested trace paradigm (31.1; $t=2.47; p<.05$). There was no evidence of retention. The 400 msec delay paradigm is more sensitive in discriminating probable AD patients from elderly control subjects. *Supported by grant IIRG-91-059 from the Alzheimer's Disease and Related Disorders Association.*

306.14

A COMPUTER-INTERACTIVE TASK DETECTS IMPAIRED MOTOR LEARNING IN MILDLY-DEMENTED ALZHEIMER'S DISEASE PATIENTS M.G. Baker*, W.J. Jagust, A.L. Leiman and B.R. Reed. University of California, Davis, Northern California Alzheimer's Disease Center, Berkeley, CA 94704.

Recent evidence suggests that early in Alzheimer's disease (AD), patients exhibit a loss of declarative knowledge but retain procedural knowledge and the capacity for its acquisition. The present study sought to determine whether motor learning tasks with a strong declarative component may be abnormal in AD patients. Subjects included ten mildly-demented AD patients (MMSE $\geq$ 18) and ten age- and education-matched controls. On a computer, subjects made finger responses to visual stimuli presented on-screen. After training for one of seven response patterns, subjects completed trials with an escalating number of stimuli, per the Digit Span Test. This was repeated for each of the seven randomly-presented tasks of varying complexity. AD patients showed diminished maximum number of responses correct for each task, increased response times, difficulty or inability to acquire response skills on complex tasks, and deficits in the ability to shift response patterns. These results suggest that a complex motor task is sensitive in detecting abnormalities that may reflect both declarative and procedural systems.

306.15

VISION IN ALZHEIMER'S DISEASE: PREVALENCE OF DEFICIT. I.D. Mendola*, A. Cronin-Golomb[1], S. Corkin, & J.H. Growdon Dept. of Brain and Cognitive Sciences & Clinical Research Center, MIT, Cambridge, MA 02139; [1]Dept. of Psychology, Boston University, Boston MA 02215.

We undertook a retrospective analysis of 12 vision tests given to 82 patients with Alzheimer's disease (AD) and 109 healthy control subjects matched for age and education. The healthy sample was used to establish a quantitative criteria for defining deficits. For each test, the criterion corresponded to the mean plus the standard deviation multiplied by a constant. The constant was obtained from the z-distribution such that $p = 0.01$. The cut-off score obtained would be expected from only 1 control subject out of 100. When we documented the number of AD patients who performed at or worse than this cut-off score, the percentage of patients impaired varied across tests as follows: backward pattern and flash masking, 58% and 44%; low spatial frequency contrast sensitivity, 40%; mental rotation, 38%; spatial orientation, 31%; incomplete-picture recognition, 30%; color vision, 28%; stereopsis, 21%; high spatial frequency contrast sensitivity, 4%; local and global motion perception, 0%; critical flicker fusion, 0%. The distribution of impairment across tests suggests hypotheses about differential vulnerability of object, motion, and spatial vision in AD, and about the relation between visual deficits and the density of neuropathological changes in visual cortices.

306.16

BOSTON NAMING TEST ERROR ANALYSIS IN ALZHEIMER'S DISEASE. C.G. Logan*, J.G. Buckwalter, & V.W. Henderson, Dept. of Neurol. and School of Gerontol., University of Southern California, Los Angeles, CA.

We analyzed oral responses of 30 "probable" Alzheimer's disease (AD) patients for the first 30 items of the Boston Naming Test. Errors were classified as non-responsive, perceptual, phonological, neologistic, perseverative, semantic, mixed perceptual-semantic, vague, or unrelated-other. The semantic errors were further classified as contrast coordinate, superordinate, subordinate, or circumlocutory errors. Neuropsychological measures of disease severity, attention, language, verbal fluency, memory, and constructional skills were also analyzed. Hierarchical regression analysis showed that after partialling out effects of age, duration of symptoms, education, and gender only language performance on the Token test and verbal fluency were significantly correlated with total number of errors and verbal fluency with total number missed. Language impairment on the Token test was significantly correlated with number of perseverative, neologistic and phonological errors. Constructional skills were predictive of combined perceptual and mixed semantic-perceptual errors. Impairment on an attentional task was related to number of neologisms. Measures of severity, memory, and verbal fluency were not significantly related to any error type. These results indicate that certain domains of cognitive dysfunction are related to different patterns of errors, suggesting that anomia in AD may be multiply determined.

As reported previously, gender differences were also found in total number correct, with women scoring significantly lower than men after partialling out control variables. In particular, women gave significantly more semantic and vague errors than men, and men tended to give more mixed semantic-perceptual errors than women. [Supported by P50 AG05142 and T32 NS07149.]

306.17

Temporal Lobe Regions on Magnetic Resonance Imaging Identify Patients with Early Alzheimer's Disease. M.B. Moss[1]*, R. Killiany[1], M.S. Albert[2], T. Sandor[3], J. Tieman[3] & F. Jolesz[3]. [1]Boston Univ. Sch. Of Med., [2]Mass. Gen. Hosp. & Harvard Med. Sch., [3]Brigham & Womens Hosp. & Harvard Med. Sch.

Neuropsycholgical and neuropathological evidence from recent studies of Alzheimer's disease (AD) has accumulated to suggest that structures within the temporal lobe may be involved early in the disease process. To assess this possibility, we examined on MRI, the volume of selective brain regions, including the hippocampus, amygdala, temporal lobe, inferior horn of the lateral ventricle and the basal forebrain, in a group of mildly impaired AD patients and a group of control subjects. Volumetric measurement of the regions were taken from consecutive, serial 1.5mm thick, 3-D, T-1 weighted magnetic resonance images. An operator, blinded to group classification, manually traced on a computer screen the borders of each region. Boundaries were operationally defined based upon neuroanatomical landmarks. The total volume for each region was then reconstructed by the computer and standardized as a percentage of the total intracranial volume for each individual case. Analysis of the data revealed that the hippocampus, the inferior horn of the lateral ventricles and the temporal lobe, but not the amygdala or basal forebrain, differed significantly in volume between the two groups. In addition, a step-wise discriminant function analysis demonstrated that a linear combination of the volumes of the hippocampus and the inferior horn of the lateral ventricles differentiated 100% of the patients from the age-matched normal controls. The finding of volumetric changes in the hippocampus are consistent with findings from behavioral, neuropathological and recent MRI studies that this limbic structure is involved in the early stages of AD and, when combined with measures of the inferior horn, may ultimately provide a clinically useful tool. "Supported by NIA grant P01-AG04953, Alzheimer's Association grant RG-89-116 and NINDS training grant 5-T32-NS07152-12."

306.18

Visuo-Construcyional Disturbance in Patients with Alzheimer's Disease.
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Disorganized eye movements in the patients with Alzheimer's disease(AD) are observed in making trials of constructional behaviors.Constructional behavior is regarded as two functions.The one is visuo-spatial-language and the other visuo-spatial motor function.So we examined visuo-spatial language function, visual concept,visual memory and visual cognition to detect eye movements by the vision analyzer(TKK939) when drawing and copying figures.

Subjects were 10 pre-senile onset AD and 5 multi infarct dementia as disease control and 20 age matched controls.All of cases were right handed. AD showed focal low IMP perfusion in bilateral parietal lobes,but left parietal lobe hypoperfusion were more accentrated. Clinical staging was in early stage(Sjogren's I-II).AD could understand our instruction but showed some language difficulties,constructional apraxia and some gnostic disturbances.

Gazing point,trajectory,gazing time and eye velocity were analyzed during drawing figures by means of verbal instruction,memory and during copying a cube.

Our results showed visual concept and memory of figures and the output neuronal systems were well preserved in the early stage in AD even if they showed constructional apraxia and other gnostic disturbances and that eye movements became normalized rather by verbal instruction and memory than in copyingfigures.

These results indicate that visuo-spatial motor function are relatively well spared and visuo-spatial-language function might became disturbed first, especially visual cognition or vertification process of visual cognition with language which seemed to be related to left parietal pathophysiological impairment.

306.19

Eye and Head Coordination System Disturbance in Patients with Alzheimer's Disease.
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*School of Medicine,Hokkaido University,Sapporo JAPAN

Visuo-spatial cognitive disfunction in one of the most prominent symptome in an early stage of Alzheimer's disease(AD) in addition to memory disturbance. The characteristic eye movements were demonstrated objectively,for the first time,using newly developed technology,the vision analyzer,during tracking the figures,following the line and copying the models.The gazing points showed disorganized and looked similar to those of Balint's syndrome.We assumeed to that normal function of various coordination system in the body are essential in order to catch an objectin fovea.We examined therefore eye and head coordination system,which seemed most important among these coordination system.

To detect the eye movement,vision analyzer(TKK939) was used.Head movement is detected using a magnetic coil attached to the subject who is surrounded by an AC magnetic field.Gazing points in space,which is the summention of both head and eye moovement,is calculated and superimposed on the video.We choose 10 right handed AD,5multi infarct dementia,10 age matched healthy controls. First,we observed the eye movements un copying a cube when head was fixed and also was free.In the next,we instructed the subjects to catch the targets located at 25 deg. and 50 deg. in right and left direction with eye and head movements.

The result showed that a tendency of gazing point to be localized away from the model or figure became more exaggerated when head was free than that when head was fixed.And in the second experiment,all of AD looked at the targets mainly with eye movements and only limited head movements were noted compared with other dementias and controls.Our deta suggest that eye head coordination is disturbed from the early stage in AD.This may play important role in pathophysiology of visuo-spatial dysfunction in AD.

306.20

P300 POWER LOSS IN AGING AND ALZHEIMER'S DISEASE. J.W. Ashford*, K.L.Coburn and K. Yamashita. Univ. Calif., Davis, VA, Martinez, CA 94553; Mercer Univ., Macon, GA.

The amplitude of the P300 component of the auditory event related potential, recorded using an "oddball" paradigm is decreased during aging and further diminished in Alzheimer's disease (AD). Using a brain mapping approach (BioLogic Brain Atlas, with 21 electrodes, averaged reference), the P300 was recorded from 13 young normals (YN:21-41), 11 elderly normals (EN:61-80) and 23 probable AD patients (AD:63-93; NINCDS-ADRDA criteria). Peak calculations were made on the smoothed values summed across all electrodes. Average P300 peak latency - YN: 315 ms (sd±19); EN: 364 (±46); AD: 361 (±51) - was prolonged in the elderly, but not further prolonged in the demented patients. Average P300 peak amplitude - YN: 13 uv (±5.2); EN: 8.3 (±2.6); AD: 4.9 (±3.3) - showed the expected pattern of deterioration. Total power (measured by summing the squares of each wave, smoothing and integrating ±2sd) revealed values - YN: 55 (±sd 38); EN: 23 (±15); AD: 8 (±7) - which showed this expected pattern more strongly. For AD with Mini-mental States >20, power = 9.0 (±11; 0-28); for MMS 10-20, power = 10 (±5; 5-19); and for MMS<10, power = 2.7 (±2; 0-5), indicating that the p300 is not obliterated early in AD, but is barely discernable in late AD.

306.21

LONGITUDINAL INVESTIGATION OF EYEBLINK CONDITIONING AND NEUROPSYCHOLOGICAL TESTS IN DOWN'S SYNDROME ADULTS. M.Papka,*[1] D.A.Rappaport,[2] E.W.Simon,[2] & D.S.Woodruff-Pak[1] Dept. of Psych.[1], Temple Univ., Phil., PA 19122 & Phil. Geriatric Cntr.[1], Elwyn, Inc.[2], Elwyn, PA.

To date, we have applied the eyeblink conditioning paradigm to a total of 43 Down's Syndrome (DS) adults, 22 below age 35 and 21 age 35 and older. Older DS adults, who presumably have developed Alzheimer-like neuropathology, emit fewer CRs than younger DS adults ($t(41)=3.29$; $p<.01$). Eyeblink classical conditioning in Down's Syndrome adults of a mean age of 47.8 years was similar to conditioning in Alzheimer's disease patients aged in the 80s. No differences between the two age groups of DS adults were found using the Slosson Intelligence Test, the Down's Syndrome Mental Status Examination, or the Vineland Behavioral Scales. About 14 months after initial testing, longitudinal data were collected on 6 of the younger and 12 of the older DS adults. Conditioning performance was improved for both age groups ($t(5)=3.16$; $p<.05$ for young, $t(11)=3.63$; $p<.05$ for old). However, conditioning performance of older DS adults was still poorer than that of younger DS adults, ($t(16)=3.05$; $p<.01$). There were no changes in neuropsychological test performance for either age group. DS adults show evidence of retention for periods of a year, but eyeblink classical conditioning is still sensitive to the presumed Alzheimer-like neuropathology.

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307.1

PROPERTIES OF CHOLINESTERASES IN BRAIN WITH ALZHEIMER'S DISEASE AND COMPONENTS OF WHOLE BLOOD. C. Wright, C. Geula*, M-M. Mesulam. Harvard Medical School, Boston, MA 02215.

Previous studies in our laboratory have shown that histochemically identified acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) exist in the tangles and plaques of Alzheimer's disease (AD). These cholinesterases (ChEs) are best visualized at pH 6.8 and are more resistant to several ChE inhibitors than fiber and perikarya AChE, which is best seen at pH 8.0. We now show that AChE and BuChE is visualized in glial nuclei, tangles, plaques and neuropil threads; that visualization of these ChEs is susceptible to inhibition by 5-hydroxytryptophan (5-HTP, 500uM); and that visualization of fiber and perikarya AChE occurs even in the presence of 5mM 5-HTP. Glycine, tyrosine, and tyramine at 1mM have no effect on ChE visualization. 5-hydroxytryptamine (5-HT) has similar effects as 5-HTP, tryptamine has a weak effect. Inhibition of AChE visualization by 5-HTP is dose-dependent. 50uM 5-HTP is required for inhibition of glial nuclei and neuropil thread AChE, while that of tangles and plaques requires 100uM and 500uM, respectively. Histochemical analysis for ChEs in whole blood smears identifies AChE and BuChE in formed elements like that found in glial nuclei and the histopathologic structures of AD. These ChEs are best visualized at pH 6.8, in comparison to pH 8.0; and their visualization is inhibitable by 5-HTP. Plasma BuChE measured by biochemical assay is also inhibitable by 5-HT and 5-HTP.

These studies indicate that the histopathologic structures of AD have ChEs with properties like those of whole blood and glial cells in contrast to perikaryal and fiber AChE. This suggests that potential sources of the ChEs in the histopathological structures of AD could be glial cells or whole blood components. Alternatively, these ChEs may be produced by neural elements in altered form; or produced normally and subsequently altered by interactions with the histopathologic structures of AD. Further experiments are necessary to investigate a possible role for these ChEs in AD.

307.2

MEMBRANE METABOLITES IN RAT FRONTAL CORTEX ARE DECREASED FOLLOWING NUCLEUS BASALIS LESIONS. T.C. Holmes*, R.M. Nitsch, and R.J. Wurtman. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139

Membrane phospholipid metabolism is abnormal in Alzheimer's disease. Phosphatidylcholine and phosphatidylethanolamine levels per cell are decreased as are brain choline (Ch) and ethanolamine, while glycerophosphocholine (GPC) and glycerophosphoethanolamine are increased. To develop a rat model for these changes, we examined the effects on frontocortical Ch and GPC of lesioning one cholinergic nucleus basalis (nBM) with ibotenic acid (10 mg/ml in PBS, 0.5 μl). After one week, frontal cortex choline acetyltransferase (ChAT, nmol acetylcholine/μg protein/hr) activity was decreased (26%, $p<0.01$, n=9) on the lesioned side. Tissue Ch and GPC levels also were decreased relative to those in control tissues (Ch:16%, $p<0.05$,n=9; GPC: 12%, $p<0.05$, n=9). These data suggest that impaired cholinergic neurotransmission alters phospholipid metabolism in cholinergic target regions. Supported by NIH, NIA and CBSMCT.

307.3

ALTERATIONS IN THE PHYSICAL PROPERTIES OF LEUKOCYTE MEMBRANES IN ALZHEIMER' DISEASE (AD). P. G. Antuono*, K. R. Nicholson and A. S. Bloom. Department of Neurology and Department of Pharmacology & Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

Several investigators have reported changes in the fluidity of a variety of biological membranes as a result of aging and Alzheimer's Disease. We have examined leukocytes from 13 patients with probable Alzheimer Disease (NINCDS/ADRDA criteria) and 9 age matched controls. Membrane fluidity was determined by fluorescence polarization. Three fluorescent probes were used; diphenylhexatriene (DPH), which primarily monitors lipid order at the hydrophobic acyl chain tail region of the membrane; TMA-DPH, which monitors lipid order at the surface head group region; and 12-anthroyloxystearic acid (12-AS), which monitors phospholipid rotations and viscosity near the membrane core. With 12-AS, significant increases in polarization were observed in leukocytes prepared from AD patients when compared to controls. Polarization values increased from 0.106 to 0.119 ($p< 0.05$). On the other hand, there were no differences between the two groups in the polarization of either DPH or TMA-DPH fluorescence. In contrast to these results, we have previously reported that the fluidity of brain membranes was increased in AD when measured using either DPH or TMA-DPH. These findings suggest that: a) physical properties of membranes in AD may be affected in both neuronal and non-neuronal tissues; b) in leukocytes, the changes appear to be due to an alteration in membrane viscosity rather than in lipid acyl chain ordering as was observed in brain. It is yet to be determined if these alterations are due to changes in membrane lipid or protein composition.

307.4

α_1-ADRENERGIC RECEPTOR DENSITY AND NOREPINEPHRINE LEVELS IN AGING AND ALZHEIMER'S DISEASE

Z.W. Li, M. Romagnano*, E.K. Richfield, L.M. Cousins and R.W. Hamill. Dept. of Neurology & Neurobiology and Anatomy, University of Rochester Medical Center, Monroe Community Hospital, Rochester, NY 14620.

The distribution and laminar density of superior temporal gyrus (STG) noradrenergic α_1 receptor was studied in postmortem human brain. Nine brains were obtained from Alzheimer's disease (AD) patients and six from aged controls. α_1 receptors were labeled with ^{3}H-prazosin (1 nM) and nonspecific binding was determined using phentolamine (10 μM) as a blank. The laminar distribution and average density of α_1 receptors were determined using *in vitro* quantitative autoradiography and video densitometry based on cortical cytoarchitecture of adjacent and nearly adjacent Nissl-stained sections. The α_1 receptor binding site was present in all laminae. However, a heterogeneous pattern was observed. The highest densities were found in lamina I and II, while moderate densities were present in lamina III and V/VI. Lamina IV contained the lowest level of receptor binding sites. In comparison to aged control STG, the AD group showed a significantly lower density of α_1 receptor binding in lamina IV and V/VI, but not in the other laminae. Furthermore, norepinephrine (NE) levels in STG were measured in 21 AD cases and 12 control subjects using HPLC. NE levels were significantly reduced in AD brain without significant alteration in protein levels. The decrease in the NE level and α_1 receptor density implies that the noradrenergic system is involved in the pathophysiology of AD, and may be related to dementia symptoms. The variable decrease in laminar density of α_1 receptors in STG in cases of AD suggests that α_1 receptor loss may be heterogeneous with certain laminae showing disturbances. The exact relationship between these alterations and specific cortical function or dysfunction remains to be determined. (Supported by NIH grant AG03644).

307.5

Neurotoxicity of Aluminum and Hydroxy-Aluminum Polymer on Cultured Neurons of Rat Cerebral Cortex: Tau Accumulation and Loss of Synapse. Masahiro KAWAHARA*, Kazuyo MURAMOTO, Kazuo KOBAYASHI and Yoichiro KURODA. Dept. of Molecular & Cellular Neurobiology, Tokyo Metropolitan Institute for Neuroscience, Fuchu-city, Tokyo 183, Japan.

Aluminum in drinking water has been indicated as a risk factor for Alzheimer's disease (AD) by many epidemiological surveies (*Lancet*, 339,713(1992)). ^{27}Al-NMR spectrometry has confirmed that mononuclear aluminum (Al^{3+}) polymerizes to form one type of hydroxy polymer ($[Al_{13}O_4(OH)_{24}(H_2O)_{12}]^{7+}$; Al_{13}) at pH 5.0. Although Al^{3+} and other Al-complexes including Al_{13} co-exist in water, their relative contributions to neurotoxicity are unknown.We applied 100μM Al^{3+} or Al_{13} to the cultured neurons of rat cerebral cortex at 37°C for 1h.

After 48 days, cultured neurons were stained by antibodies for tau and phosphorylated neurofilament. Al^{3+}-intoxicated neurons were stained more densely by antibody for tau than the control. Al_{13}-intoxicated cells were aggregated to form clusters, and a distinctive accumulation of tau was observed. The accumulation of tau was also observed in the case of continuous presence of Al^{3+} (100μM for 48 days). The clustering cells were connected by thick processes which were stained by antibodies for phosphorylated neurofilament and tau.

The effects on *in vitro* synapse formation between cortical neurons were investigated using multi-site fluorometry of $[Ca^{2+}]_i$ (Kuroda et al.*Neurosci.Lett.*135,255(1992)). After 7 days, Al_{13}-intoxicated neurons showed remarkable decrease in the frequency of $[Ca^{2+}]_i$ oscillation, which is correlated to the number of synapses, compared to Al^{3+}-intoxicated neurons.

These results support the idea that at least a part of AD is caused by long-term accumulation of aluminum, especially aluminum polymers which are more permeable than Al^{3+} through the cell membrane.

This work was supported in part by the Grant-in-Aid for Encouragement of Young Scientists, No.03857339, from the Ministry of Education, Science and Culture of Japan.

307.6

Altered pattern of nitric oxide synthase (NADPH diaphorase) staining in the hippocampus of Alzheimer brains. G. W. Rebeck* and B. T. Hyman Dept. of Neurology, Mass. General Hospital, Boston, MA, 02114.

Nitric oxide (NO), a free radical neuromodulator, has been implicated in NMDA mediated excitotoxicity and in long term potentiation. We have recently shown that the number of NO synthase immunoreactive neurons is unchanged in the hippocampal formation in Alzheimer disease (AD) despite substantial overall neuronal loss. Because NO synthase containing neurons and their processes are stained by NADPH diaphorase histochemistry, we used this stain to examine whether the pattern of NO hippocampal innervation is affected in AD. Control individuals (n=6) showed strong terminal staining throughout the molecular layer of the dentate gyrus, with a striking band occupying the middle 1/3. In AD (n=7), the pattern of diaphorase staining in the dentate gyrus was more homogenous, with a marked loss of staining in the middle 1/3. Densitometric analysis revealed the reduction in the middle portion to be from 18 to 31% ($p<0.001$), whereas staining in the inner 1/3 was not changed compared to CA4. The alteration in diaphorase was most striking in the most severely affected cases (Down's syndrome and familial AD). Altered innervation of neural structures by NO could contribute to neuronal vulnerability and loss in AD. Supported by NIHAG08487, and the Brookdale Foundation.

307.7

PARVALBUMIN IMMUNOREACTIVE NEURONS IN HUMAN AMYGDALA: DISTRIBUTION IN NORMAL AGING AND REDUCTION IN ALZHEIMER'S DISEASE. D.R. Brady* and E.J. Mufson, Lab. of Neurosci., NIA/NIH, Bethesda, MD 20892; Div. of Neurosci., Rush Med. Ctr., Chicago, IL 60612.

Antibodies to the calcium-binding protein, parvalbumin (PV), have been used previously to identify a subset of fast-firing GABAergic interneurons in the human hippocampal formation (HPF) and neocortex in normal and Alzheimer's diseased (AD) brains. Initial analyses indicated a selective, topographic loss of PV interneurons in the HPF and we have extended our analysis of limbic structures to include the amygdala. Post-mortem, paraformaldehyde fixed amygdala from normal ($\bar{X}$=62.7, n=7) and AD ($\bar{X}$=80.1, n=9) brains were obtained at autopsy. In normal amygdala, PV interneurons were aspiny, pleomorphic with extensive dendritic arbors and ranged from 6 to 25μm in size. PV neurons were observed almost exclusively within the corticobasal amygdaloid nuclei. The majority of PV neurons were distributed within the lateral (LT; $\bar{X}$=321.6) and basolateral (BL; $\bar{X}$=46.5) nuclei, and much fewer in the accessory basal, medial basal and cortical nuclei. An occasional PV neuron occupied the capsule of the central nucleus, but none were observed within the central nucleus. In AD, PV neurons displayed similar morphologies as controls. There was, however, a 49% decrease in PV neurons in the LT nucleus and a 20% reduction in the BL nucleus. The decrease in the LT nucleus is consistent with previous reports of areal decreases of greater than 50% in LT. The 20% decrease in BL nucleus in light of the 50% decrease in area, suggests a selective vulnerability of PV neurons in LT and BL nuclei in AD that may relate to their functional circuitry. Supported by NS 26146.

307.8

THE PERIRHINAL CORTEX (AREA 35) IN MAN AND ITS PATHOLOGY IN ALZHEIMER'S DISEASE. A. Solodkin *[1,2], G.F. Wu[1], R.O. Kuljis[2] and G.W. Van Hoesen[1,2]. Departments of Anatomy[1] and Neurology[2], The University of Iowa, Iowa City, IA 52242.

Brodmann's area 35 in man is a long strip of proisocortex positioned between the entorhinal periallocortex and temporal association isocortex. It lies lateral to the rhinal sulcus anteriorly, as in non-human primates, but continues posteriorly as the cortex on the medial bank of the anterior one half of the collateral sulcus. It is an important area from a neural systems viewpoint handling two-way traffic between the hippocampal formation and association cortices, and a functional role in memory has been documented recently in monkey behavioral investigations.

We have analized area 35 in AD and in aged compatible controls (N=45; mean autolysis time = 4.6 hrs) using cyto- and myeloarchitectonic stains (Nissl, Gallyas-myelin), histochemistry for oxidative enzymes (AChE) and for neuropathological lesions (thioflavine S) as well as immunolabeling for several neurochemicals such as glutamic acid decarboxilase (GAD), Parvalbumin (PAR), A-68 (Alz-50) and certain peptides (SP, NPY, SOM, CGRP).

AChE and GAD staining reveals a striking vertically organized distribution of neurites and cell bodies in area 35. Similar patterns are also found with PAR immunostaining but not with the peptides. Neurofibrillary tangles and dystrophic neurites as assessed with thioflavine S and Alz-50 stainings in Alzheimer's disease also showed a similar pattern of discrete vertical organization. The latter are seen most commonly in cases when the clinical signs of dementia were present for less than 5 years and proved to be a valuable tool for appreciating the extent and organization of the perirhinal cortex.

Evidence of anatomical compartmentalization outside primary sensory areas is unusual. Its presence in area 35 organization may be viewed as consistent with its seemingly complex role of directing hippocampo-cortical interactions and its early destruction in Alzheimer's disease may play a prominent role in the memory impairment that characterizes this disorder. Supported by NS 14944 and PONS 19632.

307.9

ALTERATIONS OF WHITE MATTER NEURONS IN AGING AND ALZHEIMER'S DISEASE. X. Tang* and R.E. Powers, UAB Brain Resource Program, University of Alabama at Birmingham, Birmingham, Alabama 35294

Neurons are present in white matter beneath the neocortex and many contain neuropeptides. The function of these small fusiform neurons is unclear. This study employs immunocytochemical methods with acrolien fixed human autopsy tissues (n = 20) from temporal lobe and antisera to somatostatin (SS), substance P (SP), neuropeptide Y (NPY), neurotensin (NT) and cholecystokinin (CCK). Formalin fixed human tissues were stained with antisera to Tau, the ALZ 50 antibody, as well as with silver preparations. Temporal lobe tissue from 16 young controls, 16 aged controls and 18 patients with AD were examined.

Large numbers of small fusiform neurons were stained with antisera to NPY, smaller numbers stained with antisera to SS, and SP and few were stained with antibodies to CCK or NT. In sections from superior temporal gyrus, middle temporal gyrus and mesial temporal lobe white matter, antibodies to cytoskeletal epitopes did not stain white matter neurons in young controls, but normal appearing neurons were immunostained with antisera to Tau and the ALZ 50 antibody in aged controls and AD cases. Small circular and flame shaped neurofibrillary tangles were stained with silver preparations or immunostained with Tau and Alz 50 antisesra in white matter neurons that resembled peptidergic neurons.

This study documents an elaborate network of small subcortical peptidergic neurons that are damaged in aging and AD.

307.10

AUTORADIOGRAPHIC INVESTIGATION OF MUSCARINIC RECEPTOR SUBTYPES IN HUMAN HIPPOCAMPUS W.P. Hoss*, R.W. Hamill, A. El-Assadi, L. Cousins and W.S. Messer, Jr. Department of Medicinal and Biological Chemistry, College of Pharmacy, The University of Toledo, Toledo, OH 43606 and Department of Neurology, University of Rochester, Rochester, NY 14620

Developing new therapies for Alzheimer's disease depends on localizing the subtypes of muscarinic receptor in brain and their presence or absence in different disease states. The binding properties of muscarinic receptors in sections of human hippocampus were examined using 0.2 and 2.0 nM [^{3}H]-(R)-quinuclidinyl benzilate ([^{3}H]-(R)-QNB) with and without 1.0 μM pirenzepine, AF-DX 116 and p-F-hexahydrosiladifenidol (p-F-HHSiD). Nonspecific binding was assessed using excess atropine. Tritium sensitive film was apposed to the sections for 35 days prior to development. Images were analyzed using optical density and tritium standards for calibration. Muscarinic receptor binding was compared with choline acetyltransferase activity in the human hippocampus to determine relationships between presynaptic cholinergic markers and muscarinic receptor subtypes.

Specific [^{3}H]-(R)-QNB binding was high in the dentate gyrus, CA1-4, subiculum and entorhinal cortex. Competition studies revealed high levels of inhibition for 1 μM pirenzepine and p-F-HHSiD, and lower levels for AF-DX 116. The data indicate that muscarinic receptors present in the hippocampus are predominately M_1 and M_3 receptors. Lower levels of M_2 receptors were identified using AF-DX 116. The relatively high affinity of pirenzepine for muscarinic receptors in human hippocampus supports the hypothesis that M_1-selective agonists might be of therapeutic value in the treatment of Alzheimer's disease. Determination of the levels of each subtype in Alzheimer's patients and in age-matched controls will be an important goal for future research. In addition, the relationship between choline acetyltransferase activity and M_2 receptors will be compared in various subregions of hippocampus. Supported by AG03644, NS01493 and the Ohio Department of Aging.

307.11

COMPARABLE DISTRIBUTION OF SOMATOSTATIN mRNA CONTAINING NEURONS IN THE HIPPOCAMPUS IN NORMAL AGING AND ALZHEIMER'S DISEASE, J. Epelbaum *, P. Dournaud, A. Slama, C. Videau, P. Cervera°, E. Hirsch°, F. Javoy-Agid°. U159 and °U289 INSERM, Paris, France.

Decrease in cortical and hippocampal Somatostatin (SS) concentrations are one of the neurochemichal hallmarks of Alzheimer disease (AD). Recent immunocytochemical studies concluded that the number of SS-ir neurons is maintained in AD hippocampus; thus, suggesting a metabolic defect rather than a specific cellular loss of SS-containing neurons. In this study, we used a ^{35}S-labelled-45-mer-oligonucleotide complementary to the SS coding sequence to visualize by in situ hybridization SS ARNm containing neurons in the hippocampus of age- and post mortem delay-matched AD (n=6) and controls (n=7). Labelled cells are located in all Ammonian fields. They represent 1 to 4% of the total cell number. Their distribution is similar in AD and control brains. Cells are more concentrated in the Hilus of the dentate gyrus as compared to CA1, CA2, and CA3 in which their number is equivalent. SS m RNA quantification by image analysis (Biocom RAG200) demonstrate an identical diminution (-38±3%) in all hippocampal fields in AD. In summary, neurons synthetizing somatostatin are still present in AD hippocampus with an identical distribution as compared to controls but the rate of SS mRNA/cell is diminished. This implies that the minor somatostatinergic decrease observed in AD hippocampus is likely due to a metabolic defect rather than to a specific cell loss.

310

SYMPOSIUM. STRATEGIES FOR THE STUDY OF ALZHEIMER AMYLOIDOSIS USING ANIMAL MODELS. I.D. Buxbaum, Rockefeller Univ. & A. Goate, Washington Univ. Sch. Med. (Chairpersons); C.J. Epstein, UCSF; R.L. Neve, McLean Hosp.; D.L. Price, Johns Hopkins Univ. Sch. Med.

Abnormalities of the Alzheimer amyloid precursor protein (APP) have been implicated in the development of Alzheimer disease (AD). In this symposium, the use of animal models for understanding the relationship between APP and the underlying mechanisms of AD will be discussed.

Alison Goate will introduce APP and summarize the recently discovered mutations in this protein which co-segregate with forms of familial AD; she will also describe strategies to target mutations to the APP gene. Trisomic and transgenic mice will be discussed as potential models for Down syndrome and AD by **Charles J. Epstein.** Regulation of the catabolism of APP by first and second messengers will be described by **Joseph D. Buxbaum** in the context of generating transgenic animals with altered APP catabolism. **Rachael L. Neve** will describe neuropathology observed in transgenic mice expressing fragments of APP. Finally, **Donald L. Price** will present different animal models for the study of the biology of APP and for the analysis of amyloidogenesis.

The presentations will be followed by a session to discuss the advantages and disadvantages of different strategies toward understanding Alzheimer amyloidosis.

311

SYMPOSIUM. CELL BIOLOGY OF THE GROWTH CONE. D.J. Goldberg, Columbia Univ. Coll. of P&S (Chairperson); M.C. Fishman, Harvard Med. Sch. & Mass. Gen. Hosp.; M.P. Sheetz, Duke Univ. Med. Ctr.; D. Bentley, Univ. of California, Berkeley.

Molecular biological and video microscopic techniques have begun to yield insights into the specialized motile ending of a growing neuronal process, the growth cone. The functioning of the cytoskeleton and of signal transduction pathways in the basic behavior of the growth cone and in the modulation of this behavior by environmental cues will be discussed. After a brief introduction, the symposium will progress from the analysis of the role of a single molecule in the growth cone to the eventual consideration of the growth cone in the living animal, all the while focusing on internal events. Fishman will discuss evidence that the protein GAP-43 regulates the sensitivity of growth cone transduction and serves as an intracellular G protein activator. Sheetz will discuss experiments with VEC-DIC microscopy and optical (laser) tweezers that suggest that a membrane myosin controls the rate of elongation of filopodia and that fast axonal transport can control growth cone activity. Goldberg will talk about the potential role of tyrosine phosphorylation in regulating the specialized actin network of the growth cone as well as the subcellular mechanism of action at the growth cone of laminin, a substrate-binding promoter of neurite growth. Bentley will show how video microscopic observation of living growth cones making navigational decisions within the grasshopper embryo reveals how the behavior of microtubules underlies the critical role of filopodia in these decisions.

VISUAL CORTEX: THEORETICAL STUDIES

313.1

THE LONG OPEN TIME OF THE NMDA CHANNEL FACILITATES THE SELF-ORGANIZATION OF INVARIANT OBJECT RESPONSES IN CORTEX

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Each successive area in the "what" pathway between V1 and anterior IT seems to encode visual information about objects with increasing invariance to position and scale (Desimone *et al* 1984; Tanaka *et al* 1991). Foldiak (1991) and Stryker (1991) have proposed that to accomplish such an encoding the nervous system might link successive images of an object as it moves through the field of view. We propose that the long open time of the NMDA channel provides a mechanism to link temporally contiguous firing patterns triggered in a lower visual area as an object moves across the visual field to a more invariant pattern of activity in the subsequent higher area. In order to explore how such a system could produce invariant object coding, a computer simulation of a three-level hierarchy of neocortical areas was developed. A set of simulated 2-dimensional objects was presented to the visual field. The initial coding of form was built into a 16x16 retinotopic array of oriented edge detectors, the simulated primary visual area. Activity in this area was driven by continuous translations of the objects through the visual field, sometimes with rotation and dilation. Excitatory synaptic efficacy was mediated by a mixture of AMPA and NMDA kinetics. Feedforward synapse strengths were initiated with loose divergent retinotopy, and were allowed to self-organize, with induction of plasticity proportional to the open time of the NMDA channels. Local lateral inhibition was present. Preliminary results indicate that the long open time of the NMDA channel, coupled with Hebbian plasticity and some form of firing threshold, provides a physiological mechanism permitting the radically shifting patterns of neuronal activity in lower areas which are triggered by different retinal images of an object to become linked to an increasingly invariant pattern of activity at each successive higher area.

313.2

A SPIKING ATTRACTOR NETWORK MODEL ACCOUNTS FOR BIMODAL STATISTICS OF VISUAL CORTEX NEURONS

G. C. Littlewort, D. Zipser*, P. Perez, J. M. Fuster
Cognitive Science, U.C.S.D., La Jolla, CA 92093

The interspike interval distributions of many cortical neurons, in awake animals observing their environment, is bimodal in the sense that it can be fit by the sum of two exponentials (ignoring refactory times). (Smith et al, Biophys J 5, p47, 1965; Fuster et al, Arch ital Biol 103, p159, 1965). We have extended and confirmed these observations using interspike interval distributions derived from recordings in visual cortex (V1 and IT) in awake behaving monkeys. A spiking neural network was developed to model this bimodal process. It is derived from a logistic neuron with excitatory self-connection and two stable activity levels, or attractors. This one neuron concept is extended to a network of spiking neurons, with many identical units and recurrent connections between all units. The logistic function now represents the probability of producing a spike. The individual units in this network are stochastic and slow firing. The number of units firing per time interval has a bimodal probability distribution. Observation of a single unit in this network provides interspike interval distributions that can be compared with experimental data. The simulated distributions match the real data. This spiking model with feedback can be analysed as a stochastic Markov process, enabling theoretical predictions of higher order correlations in the dynamics of the spike trains, that can be compared with experimental data.

313.3

CORTICAL CELLS DO NOT PERFORM TEMPORAL INTEGRATION OF SMALL EPSP'S W. Softky and C. Koch*, Computation and Neural Systems, California Institute of Technology, Pasadena, CA 91125.

Cortical cells fire almost randomly. For non-bursting cells in visual areas V1 (Knierim & Van Essen 1992) and MT (Newsome *et al.* 1989) of behaving macaque monkey firing at sustained rates up to 300 Hz due to visual stimuli, the rate-normalized Coefficient of Variation (C_V) of the interspike interval distribution is consistent with a completely random process (e.g. $C_V \approx 1$).

This high variability cannot result from small, independent, and random EPSP's converging onto a leaky integrate-and-fire neuron. Both this and related models predict very low firing variability ($C_V \ll 1$) for realistic EPSP depolarizations and membrane time constants. Independent, excitatory synaptic input to a compartmental simulation of an anatomically reconstructed layer V cat visual cortex pyramidal cell also yields $C_V \ll 1$, in agreement with the integrate-and-fire models but in strong disagreement with the majority of our monkey data. The simulated cell only produces highly variable firing when Hodgkin-Huxley-like currents (I_{Na} and very strong I_{DR}) are placed on the distal basal dendrites. Now the simulated neuron acts more as a millisecond-resolution detector of dendritic spike coincidences than as a temporal integrator. Only with such fast and strong dendritic nonlinearities or with strong synchronization among individual synaptic events does our simulated cell exhibit the high variability seen in rapidly firing cortical neurons.

313.4

3-D VISION AND FIGURE-GROUND SEPARATION BY VISUAL CORTEX. Grossberg, S*., Department of Cognitive and Neural Systems, Boston University, Boston, MA 02215.

A neural network model of 3-D visual perception and figure-ground separation by visual cortex is described. A unified explanation is given of how a 2-D image may generate a 3-D percept; how figures pop-out from cluttered backgrounds; how spatially sparse disparity cues can generate continuous surface representations at different perceived depths; how representations of occluded regions can be completed and recognized without usually being seen; how occluded regions can sometimes be seen during percepts of transparency; how high spatial frequency parts of an image may appear closer than low spatial frequency parts; how sharp targets are detected better against a figure and blurred targets are detector better against a background; how low spatial frequency parts of an image may be fused while high spatial frequency parts are rivalrous; how sparse blue cones can generate vivid blue surface percepts; how 3-D neon color spreading, visual phantoms, and tissue contrast percepts are generated; how conjunctions of color-and-depth may rapidly pop-out during visual search. These explanations arise derived from an ecological analysis of how monocularly viewed parts of an image inherit the appropriate depth from contiguous binocularly viewed parts, as during DaVinci stereo. The model predicts the functional role and ordering of multiple interactions within and between the blob and interblob streams of areas V1, V2, and V4. Interactions from cells representing larger scales and disparities to cells representing smaller scales and disparities are of particular importance.

313.5

A CENTER-OF-MASS MODEL PREDICTS JUDGEMENTS OF HUMAN DOT DISPLACEMENT DISCRIMINATION. **Joy Hirsch*. Dept of Ophthalmology and Visual Science, Yale University School of Medicine, New haven, CT 06510.**

One of the unanswered questions in human vision is what regulates the precision of human spatial discriminations. Recently Hirsch and Mjolsness (1992) have demonstrated the success of a center-of-mass, CM, computation in predicting the accuracy of random dot displacement discriminations. Two alternative explanations may account for the success of the model: 1. the CM is the result of a visual search strategy based on uncertainty about the displacement; or 2. the CM is the result of an automatic sequential process that appreciates the dot locations and then identifies the cluster CM. These alternative explanations were tested by psychophysical measurements of dot displacement discrimination using random dot arrays that varied in shape and displacement certainty. Results of the certainty experiments support alternative 2 above. Results of the shape experiments show that location judgements are more precise when the array is round-like rather than elongated. Taken together these data suggest that a very large receptive field may "gather" the individual elements of an array and report the centroid. Further these data lead to the speculation that this "gathering field" may be shape specific.

313.6

DYNAMICAL CONTROL OF VISUAL ATTENTION THROUGH FEEDBACK PATHWAYS: A NETWORK MODEL. K.P. Unnikrishnan* and Janani Janakiraman, GM Research Laboratories, Warren, MI 48090 and The University of Michigan, Ann Arbor, MI 48109.

Attention involves the ability of the visual system to filter out unimportant features, resolve ambiguities and enhance relevant features. Associations learned at higher brain centers play an active role in the dynamics of visual attention, possibly by affecting the processing at lower centers. A model is presented to investigate the role of feedback pathways from association cortical areas to primary visual cortex and lateral geniculate nucleus in attentional mechanisms.

In this model, visual attention is viewed as a unitary process, linking peripheral sensory relays with higher level pattern analyzers through a well established feedback loops (1,2). Previously learned associations between images affect the processing at the lower level neurons through the feedback loop. The Alopex algorithm used in our simulations can easily be carried out by known neuronal circuitry (3).

Computer simulations of the system are presented. These diffuse feedback pathways are shown to be capable of dynamically modifying tuning properties of lower level neurons and hence quicken the convergence properties of these networks. These results show how dynamic changes in the tuning properties of lower level neurons can help the visual system to focus attention rapidly. The change in tuning properties of V4 and IT neurons in the monkey has lead to the hypothesis that these changes play a role in some of the static aspects of attention (4). Our results show that this may instead be a dynamic phenomenon, helping the visual system to focus attention rapidly.

1. Harth, Pandya, & Unnikrishnan, Concepts in Neurosci., 1, 53 (1990). 2. Janakiraman & Unnikrishnan, Proc. IJCNN (1992, in press).

313.7

CONSTRUCTIVE AND CORRELATIVE REENTRY IN THE VISUAL SYSTEM: COMPUTER SIMULATIONS AND PSYCHOPHYSICS. O. Sporns*, G. Tononi, and G.M. Edelman, The Neurosciences Institute, 1230 York Ave., New York, NY 10021.

What neural processes underlie well-known psychophysical phenomena such as the generation of form from motion and motion capture? In order to identify possible neural bases of these phenomena, we constructed a computer model simulating the organization of functionally segregated areas of the visual cortex. These areas are arranged into three parallel streams which deal with different stimulus attributes, such as form, color, and motion, and they are linked by a system of forward, backward, and lateral connections. Areas in the three streams contain simulated neurons with specialized response properties, such as selectivity to component motion, pattern motion, simultaneous color contrast, color constancy, and position-invariant discrimination of different shapes.

In the model, the process of ongoing signaling within and among these areas, called reentry, can give rise to both constructive and correlative properties. These properties are illustrated on the basis of examples taken from psychophysics. We propose that constructive properties, by means of which the activity of "higher" areas is reentered into a "lower" area of a different stream and modifies its responses to an incoming stimulus, underlie the generation of form from motion. Another consequence of such reentry is the emergence of temporal correlations in the activity of neuronal groups within a cortical area as well as between different areas (PNAS 86:7265, 88:129). This is exemplified in the model by the emergence of short-term correlations between different streams that are consistent with psychophysical capture phenomena, such as motion capture.

(Supported by the Neurosciences Research Foundation)

313.8

MODELING INTEGRATION IN THE VISUAL CORTEX G. Tononi, O. Sporns, and G.M. Edelman*, The Neurosciences Institute, 1230 York Ave., New York, NY 10021.

The visual system consists of multiple, anatomically segregated and functionally specialized cortical areas which are linked by a network of reciprocal connections. Although there is no evidence that these areas are coordinated by a higher order center, the visual image that we perceive seems to be unified and coherent. In an extension of our previous work, we address the problem of perceptual integration in the brain based on the notion of reentry, a process of parallel and recursive signaling along ordered anatomical connections within and among maps.

We have developed a computer model simulating nine functionally segregated visual areas organized into three streams for form, color, and motion. The model receives visual input consisting of camera images of objects of different shapes and colors. A computational strategy involving a phase variable is introduced to represent explicitly the dynamics of short-term temporal correlations among thousands of units distributed across different areas. The model solves the so-called "binding problem" through short-term correlations, which serve to link similar object features within a simulated cortical area and to bind multiple attributes of one or more objects across several areas, including a non-topographic one. Integration emerges from cooperative effects within and among the specialized areas, and it leads to a simulated foveation response, that can be used as a basis for conditioning. Reward is simulated through the activation of a saliency system that resembles diffuse projection systems in the brain. Resulting distributed synaptic changes allow the model to successfully discriminate among multiple input stimuli that require the dynamic conjunction of form, color, and location.

(Supported by the Neurosciences Research Foundation)

313.9

OPTIMIZATION ANALYSIS OF THE ORGANIZATION OF CORTICO-CORTICAL CONNECTIONS BETWEEN AREAS OF MONKEY CEREBRAL CORTEX. M.P. Young*, Univ. Lab. of Physiology, Oxford, OX1 3PT, U.K.

The cerebral cortex is composed of many structurally and functionally distinct areas, each of which receives a large number of afferent inputs from other cortical areas and sends a large number of output projections. The cortex is thus served by a very large number of cortico-cortical connections, so that the areas and their inter-connections form a network of remarkable complexity. In fact, at least 834 projections are presently known to interconnect the 70 or so cortical areas of the Macaque, of which at least 305 projections link the 30 or so areas of the monkey visual system. Understanding the gross organization of cortical processing systems hence represents a formidable topological problem.

I have pursued an optimization approach to this topological problem that gives both qualitative and quantitative insight into the connectional topology of the cortical systems. The first step in the analysis is to derive a connection matrix by collating information about connections from the neuroanatomical literature. The second step is to derive by optimization a structure which best explains the variability in the connection matrix by placing areas at optimal points so that the length of connections between them is at a global minimum while the length of non-connections is at a global maximum.

The results show that the visual system is divided into a dorsal 'stream' and a ventral 'stream' with limited cross-talk, that the system is hierarchically organized, that these two streams reconverge in area 46 and in the superior temporal polysensory areas, and that the 'wiring' can be accounted for by a neighbourhood wiring rule together with 3 fascicles. The auditory system is arranged in a single hierarchy with progressively 'higher' stations distributed more rostrally on the superior temporal gyrus. The somatosensory system is another hierarchy, but unlike the other sensory systems, it contains the cortical motor system as a diverticulum about half way up the hierarchy. An analysis of the entire cortico-cortical network showed the limbic structures and most of the prefrontal cortical areas to be topologically closely associated, forming a 'fronto-limbic complex' to which the 'higher' areas of the sensory systems project.

313.10

OPTIMIZATION ANALYSIS OF THE CONNECTIONS BETWEEN AREAS OF CAT CEREBRAL CORTEX. J.W. Scannell, M.P. Young and Colin Blakemore
SPON: Brain Research Association. Univ. Lab. of Physiology, Oxford, OX1 3PT.

The cat cerebral cortex is composed of about 65 areas and is served by at least 1139 cortico-cortical connections. Characterizing a processing system of such complexity represents a formidable topological problem.

We collated cat cortico-cortical connection data and produced a connection matrix which recorded the presence or absence of connections and an estimate of their density. From this we computed a topological map which best explained the variability in the connection matrix (see Young, this volume). To assess the effect of differences in the published descriptions of the density of connections we made a quantitative comparison between the 'best' map (computed from the density-coded matrix) and maps produced from two alternative matrices; in one all connection strengths were made equal, and in the other all the weak connections were discarded. The alternative solutions explained 96% of the variance of the 'best' solution, showing that possible errors in estimating the connection density did not have a major effect on the topological structure.

The results show four topological clusters of areas which correspond to the visual, auditory, somatosensory-motor systems and a 'fronto-limbic' complex. The cat visual system is divided into two streams by virtue of its connectivity with non-visual areas. One stream, containing area 7, the anterior ectosylvian sulcus and the oculomotor areas passes towards the somatosensory-motor system. The other, containing areas PS, 20b and EPp, passes towards the auditory system. Unlike in the monkey, the visual streams do not project densely to the fronto-limbic complex. The auditory system is also a 2-streamed hierarchy. The dorsal auditory areas project more heavily to the dorsal fronto-limbic areas and the ventral auditory areas to the ventral fronto-limbic areas. The somatosensory-motor system is a single hierarchy as in the monkey. The primary motor cortex is closely associated with SI, SII and area 5, and the premotor cortex is closely associated with area 5 and SIV. The higher auditory and somatosensory areas have strong connections with the fronto-limbic complex which is the system most distant from the sensory periphery. Supported by the MRC and the Oxford McDonnell-Pew Centre for Cognitive Neuroscience.

313.11

A COMPUTATIONAL STUDY OF CORTICAL HYPERCOLUMNS AND THE TOPOLOGY OF RANDOM ORIENTATION MAPS Eric L. Schwartz and Alan Rojer*, Computational Neuroscience Lab., NYU Med. Ctr. 550 1 st Ave. NY 10016 and Dept. Computer Science, Courant Institute of Mathematical Sciences, NYU

Smoothing a random pattern of orientations causes the appearance of orientation "vortices." similar to the hypercolumn pattern of monkey visual cortex, and to the "pinwheels" that have been recently observed in cat area 18. The generic presence of such patterns in continuous random orientation maps is a consequence of a basic topological theorem (non-retraction of $R^2 \rightarrow S^1$), as illustrated with computer simulations and animation. Several of the qualitative features of cortical hypercolumn patterns, including the features of the "puff-extra puff" structure of monkey V-1, are evident in our simulations. The principle conclusion of this work is that any measurement procedure, such as optical dye recording, 2-deoxyglucose, or multi-unit recording, which has a measurement resolution in the range of 100μ to 200μ, is insufficient to establish the detailed structure of cortical hypercolumns, and may in fact artifactually produce such hypercolumn structure out of random orientation noise. Consequently, methods such as optical dye recording are not a reliable indicator of these patterns. Since single unit recording has never unambiguously established the structure of the hypercolumn pattern, our current knowledge of this fundamental feature of cortical architecture is called into serious question. And, given a reliable future demonstration of the details of the hypercolumn pattern, it must be remembered that there is no physiological, developmental, or computational mechanism that need be invoked to produce them. They are noise patterns, whose production is a by-product of the definition and the topology of orientation maps.

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313.12

COMPUTER RECONSTRUCTION FROM 2DG SERIAL SECTIONS OF THE TOPOGRAPHIC MAP OF MACAQUE VISUAL CORTEX David Rosenbluth, Amar Munsif, Thomas D. Albright, and Eric L. Schwartz* Comptl. Neuro. Lab., NYU Med. Ctr. 550 1 st Ave. NY 10016, Dept. Comp. Sci., Courant Inst. of Math. Sciences, NYU, and Salk Inst., P.O.Box 85800, San Diego, CA.

We present a quantitative two dimensional estimate of the Macaque V-1 topographic map, the cortical magnification factor, and, for the first time, an estimate of the error in the measured map function. Autoradiograms of coronal sections obtained after 2DG was administered and a stimulus pattern was presented to a paralyzed monkey, were reconstructed into a 3D model of the cortex. This 3D numerical model was then flattened into a 2D model, which was fit with a conformal map using the Nelder simplex algorithm. The fitted map function determined the (small) error in eye position of the paralyzed monkey, and allowed us to compute cortical magnification factor through numerical differentiation. The conformal (locally isotropic) maps generated for two monkeys are in quantitative and qualitative agreement with the experimentally determined V-1 topographic map. The generated maps are in turn similar to the complex log map, which provides a good approximation to the topographic map of Macaque V-1, as suggested in earlier work. Cortical magnification factor derived from these maps is in good agreement with the previous work of the labs of Dow and Van Essen, but is in significant disagreement with the recent measurements of Tootel et al(JNS,6(1989). However, we found that these latter measurements, when corrected for errors introduced by the large step size used in their derivation, are in agreement with our data and other recent estimates. The computer software that was used to perform the 3D reconstructions and 2D flattening has recently been made available to interested workers under support of the System Development Foundation and the NIMH. (Please contact E. Schwartz).

---- Supported by NIMH #45969 and AFOSR Life Sciences #88-0275----

INGESTIVE BEHAVIOR: SALT APPETITE AND FOOD INTAKE

314.1

SODIUM APPETITE IN THE SHAM DRINKING RAT AFTER CHORDA TYMPANI TRANSECTION. S.P. Frankmann[1]*, S.I. Sollars[2] J.D. Dokko[1], D. Veltung[1], and I.L. Bernstein[2]. [1]Bourne Lab, Cornell Medical Center, White Plains, NY 10605 and [2]Dept. of Psychology, University of Washington, Seattle, WA 98195.

The chorda tympani nerve (CT) is a primary gustatory nerve that is maximally responsive to NaCl stimulation. In the rat, CT transection (CTX) delays and blunts the expression of a sodium (Na^+) appetite but does not eliminate it. The role of taste in guiding ingestive behavior can be explored by the "sham drinking" rat, in which stomach contents drain from an implanted gastric cannula. Under these conditions, the Na^+ depleted rat displays remarkably vigorous and prolonged intake of 0.3M NaCl throughout a 2h test. This behavior is viewed as the product of a strong motivational state and potent oropharyngeal stimulation in the absence of postingestive negative feedback.

The present study examined the effect of CTX on "sham drinking" of 0.3M NaCl in Na^+ depleted rats. Wistar rats received either bilateral CTX or sham (intact) surgery and all were implanted with gastric cannulas. They were then Na^+ depleted (10 mg furosemide and overnight Na^+ deficient diet) and their NaCl intakes measured for 2h with the gastric cannula open (sham) or closed (real). As can be seen in the Table below, the sham drinking in the intact group significantly increased the NaCl intake, but failed to do so in the CTX group. In contrast, overnight food deprivation produced similar increases in the sham intake of 0.6M sucrose for both intact and CTX groups.

	0.3M NaCl intake, ml (mean±SEM)			
Time	Intact/Sham	Intact/Real	CTX/Sham	CTX/Real
1 h	35.3±2.8	9.5±2.3	10.7±2.4	6.2±1.1
2 h	46.0±3.4	13.1±2.8	11.9±2.9	9.8±1.6

These results strongly suggest that information provided by the CT is critically important to the strong motivational properties of NaCl after Na^+ depletion. This information is likely to be based on signals in the CT which correspond to activity generated by Na^+ transport through channels in taste buds specifically blocked by the diuretic amiloride.

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314.2

RENIN-ANGIOTENSIN SYSTEM (RAS) BLOCKADE DURING PREWEANLING DEVELOPMENT PRODUCES LONG-TERM INCREASES IN WATER INTAKE IN RATS. **R. Kirby*, M. Henry, A. Alt, C. Novak, A. Revelz and A. Johnson. Dept. of Psychology and the Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.**

The present study examined the influence of RAS blockade during the late preweaning period, when a rat pup initially begins to ingest fluids other than the dam's milk, on the intake of water and hypertonic NaCl solution during the postweaning period. Wistar-Kyoto (WKY) and spontaneously hypertensive rat (SHR) pups were administered losartan potassium (10 mg/kg s.c.), an angiotensin II type I receptor antagonist, daily from postnatal day (PD) 11 through 20. On PD22 animals were weaned to individual cages and intakes of tap water and a 0.3M NaCl solution were monitored daily. Blockade of the RAS produced elevated water intakes through at least 50 days of age, no change in saline intake, and blunted body weight development between PD16 and PD18. To determine if the effects on body weight development were related to the increased water intake, a second study was conducted using the technique of artificial rearing. WKY pups were implanted with gastric catheters on PD11 and infused with a control diet or a diet containing losartan (approximately 10 mg/kg) until PD20. Pups were then weaned to metabolism cages from the artificial rearing procedure and daily measures of water and NaCl intake recorded. Blockade of the RAS increased water intake and urine output. However, there was no effect of losartan treatment on body weight development when intake was fixed using the artificial rearing procedure. These data indicate that RAS activity during the preweaning period has lasting influences on body fluid metabolism.

314.3

EVIDENCE THAT VISCERAL INPUT SENSING REDUCED VASCULAR PRESSURE AND VOLUME FACILITATES ANGIOTENSIN-INDUCED SALT APPETITE. **R.L. Thunhorst* and A.K. Johnson. Dept. of Psychology and Pharmacology and the Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.**

Combined sc administration of the diuretic, furosemide (10 mg/kg) with a low dose of the angiotensin-converting enzyme (ACE) inhibitor, captopril (5 mg/kg), that blocks ACE only in peripheral tissues, rapidly induced ingestion of 0.3 M NaCl solution and water (>4 ml of each in 2 hrs). This treatment increases renin secretion by reducing fluid volume and by interrupting the negative-feedback loop involving angiotensin II (ANG II; Fitts, D.A. & Masson, D.B., Behav. Neurosci., 1989, v.103), and we have found that it reduces mean arterial pressure (MAP) as well, thereby presumably unloading both cardiopulmonary and arterial baroreceptors. Centrally-formed ANG (via unblocked brain ACE) partially mediates ingestion in this protocol because intakes were abolished after blockade of the renin-angiotensin system with a greater dose of captopril (100 mg/kg), that blocks brain ACE as well as peripheral ACE, or with the AT1 receptor blocker, Losartan (DuP 753; 5 or 20 mg/kg, sc). Furthermore, unloading of cardiopulmonary and arterial baroreceptors in this protocol likely facilitates the action of centrally-acting ANG, because central infusions of ANG II (15 or 30 ng/hr) rapidly elicited substantial saline intakes only after fluid volume and MAP were reduced by treatment with furosemide and high dose captopril. Therefore, withdrawal of signals from vascular volume and pressure receptors facilitates salt intake to centrally-acting angiotensin.

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314.4

Angiotensin II Participates In Sodium Intake and C-*Fos* Immunoreactivity In A Rapid Salt Appetite Paradigm. M. Z. Cicha, A. M. Zardetto-Smith*, R. L. Thunhorst, and A. K. Johnson. Depts. of Psychology and the Cardiovascular Center University of Iowa, Iowa City, IA 52242.

Administration of angiotensin elicits salt intake and induces c-*fos* immunoreactivity (*fos*-IR) in forebrain areas associated with body fluid homeostasis. In this study, we employed *fos*-IR as a neuronal marker for areas participating in the behavioral response to sodium depletion, that is, salt appetite. The protocol used in this study produces high levels of circulating angiotensin, and tests were performed to examine the contribution of angiotensin to sodium intake. Rats were given furosemide sc (Furo, 10 mg/kg) followed 5 min later by either a low dose of captopril sc (Lo Cap, 5 mg/kg), which blocks only peripheral converting enzyme, or a high dose of Cap (Hi Cap, 100 mg/kg), which blocks both central and peripheral converting enzyme. Additional animals received DuPont 753, a specific AT1 receptor blocker sc (DuP, 10 mg/kg). Two hr later, animals were euthanized and the brains processed using the avidin-biotin technique for *fos*-IR. Another series of animals received the same treatments, but were allowed access to water and 0.3 M saline to assess intakes. Rats receiving the Furo/Lo Cap combination consumed both saline and water. Rats receiving the Furo/Hi Cap or Furo/Lo Cap/DuP combinations drank neither water nor saline. Rats receiving the Furo/Lo Cap combination demonstrated the heaviest *fos*-IR within the organum vasculosum of the lamina terminalis (OVLT), median preoptic nucleus (MnPO), subfornical organ (SFO), supraoptic (SON) and paraventricular (PVN) nuclei. *Fos*-IR was greatly reduced in the OVLT, MnPO, SFO, PVN and SON in rats receiving Furo/Hi Cap or Furo/Lo Cap/DuP treatments. The results show that *fos*-IR expression is high in areas implicated in the control of body fluid homeostasis following procedures which generate salt appetite, and that the intake and *fos*-IR expression produced by this protocol depend at least in part on AT1 receptors.

Supported by NIH grants HL44546 and HL14388.

314.5

C-FOS EXPRESSION INDUCED IN THE CIRCUMVENTRICULAR ORGANS OF THE LAMINA TERMINALIS BY CIRCULATING ANGIOTENSIN II IN RATS. M.J. McKinley, E. Badoer, R.S. Weisinger, B.J. Oldfield*. Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Vic, Australia 3052.

Thirst and a centrally-mediated pressor response are some of the physiological effects of circulating angiotensin II (ANG II) thought to be mediated by an action of ANG II on cerebral sites, such as the subfornical organ (SFO), organum vasculosum laminae terminalis (OVLT) or area postrema (AP). The aim of the present experiments was to determine whether blood-borne ANG II induced neurons in these regions to increase Fos production. Fos was detected immunohistochemically. Intravenous (IV) ANG II (0.5-5µg/h for 1.5-2h, n=6) in conscious rats induced Fos-immunoreactivity (Fos-IR) in the SFO and OVLT and, to a lesser extent, in the AP, whereas IV isotonic saline (n=4) did not. IV phenylephrine (0.1mg/kg/h for 2h, n=4) which produced a pressor response (~40mmHg) increased Fos-IR in the AP, but not in the SFO or OVLT. Sodium depletion for 2h resulting from furosemide treatment (20mg/kg ip, n=4), which elevates endogenous ANG II levels in blood, increased Fos-IR in the SFO, OVLT and AP. This effect was inhibited in the SFO and OVLT but not in the AP by angiotensin converting enzyme inhibitors. These data support the view that both the SFO and OVLT are sites at which blood-borne ANG II exerts its central actions, whereas Fos-IR induced in the AP in these experiments is not necessarily due to direct actions of circulating ANG II on AP neurons.

314.6

GUSTATORY EXPERIENCE ALTERS TYROSINE PHOSPHORYLATION IN RAT CORTEX. K. Rosenblum, N. Meiri, Y. Hadari, Y. Zick and Y. Dudai*, Weizmann Institute of Science, Rehovot 76100, Israel .

We are investigating short- and long-term experience-dependent modifications in rat cortici that subserve chemical senses. Recently, we have reported that protein synthesis in gustatory cortex is necessary for the formation of long-term taste memory (Rosenblum *et al.* Soc. Neurosci. Abst. 17: 660 and *in press*). In the present study, we have quantified fast alterations in tyrosine phosphorylation of cortical proteins following gustatory experience. Adult male rats were placed on a partial fluid-deprivation schedule (20 ml water allowed only during 10 minutes per day, with food pellets ad libitum). On the day of the experiment, the rats were divided into 4 groups: group 1 was presented with 20 ml water, group 2 with 20 ml 0.1% saccharin solution, group 3 with saccharin followed by an i.p. injection of LiCl (0.15 M, 2% body weight), and group 4 with neither water nor saccharin. The rostral insular cortex (which contains the gustatory cortex) was immediately removed, homogenized, and subjected to SDS-PAGE followed by Western immunoblotting with an anti-phosphotyrosine antibody. A set of phosphoproteins was detected in the MW range of 35-180 kD. Significant differences were found among the experimental groups in the level of tyrosine phosphorylation of several proteins, especially those of MW 110 and 180 kD. The level of tyrosine phosphorylation in rats that drank saccharin was higher than in those that drank water. Phosphorylation was highest in the LiCl treated rats and lowest in those that did not drink at all. Gustatory experience can thus induce fast alterations in tyrosine phosphorylation in brain. (Supported by a grant from the Whitehall Foundation.)

314.7

INTRADUODENAL GLUCOSE ENHANCES INHIBITION OF SHAM FEEDING BY CCK-8. J.E. Cox*, Dept. Psychol., Univ. Alabama-Birmingham, Birmingham, AL 35294.

Impairment of runway performance by cholecystokinin octapeptide (CCK-8) is potentiated by duodenal infusion of 30% sucrose (Behav. Brain Res 38:35-44). The purpose of the research described here was to determine whether this effect generalizes to sham feeding when the infusate is glucose, at concentrations lower than that used previously. Adult, male Sprague-Dawley rats (N=9) sham fed 30% sucrose for 30 min beginning 10 min after intraperitoneal injection of 1 ug/kg CCK-8 or normal saline. Throughout each test, each rat received duodenal infusion of 0.3 M or 0.6 M glucose or saline at 0.388 ml/min or no infusion. A subset (N=5) of the rats underwent additional tests with infusate concentrations of 0.375 M and 0.45 M. Sham feeding was significantly reduced by 1 ug/kg CCK-8 when given in conjunction with glucose, at all concentrations (P's < .05). By contrast, in the absence of duodenal infusions, CCK-8 was without effect. On tests with duodenal saline infusions, CCK-8 was effective only in conjunction with the highest concentration, 0.6 M. Thus, these results demonstrate that (1) a dose of CCK-8 by itself ineffective in reducing sham feeding can be rendered effective by concurrent duodenal infusion of glucose; (2) glucose concentrations at least as low as 0.3 M are sufficient to produce this potentiation; and (3) glucose appears to be more effective in this regard than saline.

314.8

REPEATED MORPHINE IN NUCLEUS ACCUMBENS: CONDITIONING AND SENSITIZATION OF FEEDING. V.P. Bakshi*, A.E. Kelley and S.M. Fleming. Dept. of Psychology, Northeastern University, Boston, MA. 02115.

Acute administration of opioids into the nucleus accumbens (N.Acc.) results in hyperphagia in rats. The present study was conducted to investigate the effect on feeding of multiple opiate injections into this region. On all test days, behavior in sated rats was rated every 10 min for a 2 min period over a total of 4 h immediately following infusion. Food intake and spillage were calculated at the end of each session. Two experiments were completed. In the first, animals were given repeated bilateral infusions of either saline or morphine (10 µg/0.5 µl) into N.Acc. and then challenged with a saline and mock infusion (injector tips were lowered but no solution was delivered). Animals that had received multiple morphine injections exhibited a marked increase in food intake and feeding score in response to both the saline challenge and the mock injection, compared with pre-morphine saline levels. This conditioned feeding effect was present up to 18 d after the final morphine infusion. Repeated morphine administration also resulted in sensitization of feeding; a progressive and robust increase in food intake was noted with successive injections. Multiple saline injections had no behavioral effects. In order to examine the involvement of opiate receptors in these phenomena, a second experiment was conducted in which rats were given repeated injections of either morphine (5 µg/0.5 µl) or a mixture of agonist and antagonist [(5 µg morphine + 5 µg methyl naloxonium)/1.0 µl] followed by a mock infusion. Again, rats that had received multiple morphine injections showed both conditioned and sensitized food intake and feeding. However, co-administration of the opiate antagonist blocked the development of both effects. Interestingly, all conditioned feeding was immediate in onset whereas the acute morphine effect occurred after a delay of approximately 120 min. Results suggest that repeated stimulation of opiate receptors within the N.Acc. leads to a heightened ability of cues (i.e.-the injection procedure) associated with morphine presentation to elicit hyperphagia. This finding may have important implications for understanding disorders of impulse control, and in particular, compulsive eating.

314.9

STEROID RECEPTORS (TYPE I AND II) IN THE PARAVENTRICULAR NUCLEUS (PVN) MODULATE NUTRIENT INTAKE. **D.L. Tempel*, T. Kim, and S.F. Leibowitz, Rockefeller Univ. N.Y. N.Y. 10021.**

Adrenalectomy (ADX) suppresses carbohydrate and fat intake over the 24hr period in the rat. At the start of the dark feeding cycle, the primary effect of ADX is a strong and selective suppression of carbohydrate intake. Subcutaneous (sc) as well as PVN administration of corticosterone (CORT) selectively restores carbohydrate ingestion in ADX rats at this time of the feeding cycle. The present studies, using specific steroid receptor agonists and antagonists, examined the steroid receptor subtype, the type I or type II, which mediates the selective effect of CORT on carbohydrate ingestion at dark onset. Results indicate that in male ADX rats, fed pure macronutrient diets, sc or PVN administration of CORT or the type II agonist RU362 selectively stimulates carbohydrate ingestion at dark onset, (+5.4 and +7.3 Kcals, respectively, $p<0.05$). In contrast, the type I agonist aldosterone (ALDO) predominantly stimulates fat intake in both sham (+4.5 Kcals; $p<0.05$) and ADX (+4.0 Kcals, $p<0.05$) rats. Blockade of PVN type II receptors with the selective antagonist RU486 abolished the carbohydrate-stimulatory effect of both CORT and RU362 but had no effect on the fat-stimulatory effect of ALDO. Conversely, type I steroid receptor blockade with RU318 antagonized ALDO-induced fat intake, while leaving CORT and RU362-induced carbohydrate intake unaffected. In addition, a role for these receptors in mediating natural feeding behavior is suggested by results demonstrating that RU486, in the PVN of adrenal-intact rats, strongly inhibits spontaneous carbohydrate ingestion at dark onset, while RU318 inhibits natural fat intake. These data suggest that PVN type I receptors may be involved in modulating fat ingestion, while PVN type II receptors are important in controlling carbohydrate ingestion specifically at the onset of the dark feeding cycle.

314.10

DOPAMINE IN THE PERIFORNICAL HYPOTHALAMUS ATTENUATES NEUROPEPTIDE Y-INDUCED FEEDING. E.R. Gillard*, D.Q. Dang & B.G. Stanley, Depts. Neuroscience & Psychology, University of California, Riverside, CA 92521.

Mapping studies suggest that the perifornical hypothalamus (PFH) is a primary locus for both the feeding stimulatory effect of neuropeptide Y (NPY) and the anorectic effect of catecholamines (CA), suggesting that NPY and CA may interact antagonistically there. To investigate this, amphetamine (AMPH) was injected through indwelling guide cannulas into the PFH of satiated adult male rats 5 min prior to injection of NPY (78 pmol/0.3 µl) and food intake was measured 1, 2, and 4 hrs later. Amphetamine (50-400 nmol) dose-dependently reduced NPY feeding, usually eliminating it at the higher doses. The receptors mediating this effect were investigated by sequential injection of CA antagonists, AMPH, and NPY into the PFH. Neither the α- nor ß-adrenergic antagonists phentolamine or propranolol (100 nmol) significantly affected AMPH anorexia. In contrast, haloperidol (5 nmol) abolished the AMPH suppression of NPY feeding, suggesting that dopamine (DA) mediates the AMPH effect. To examine this, epinephrine (EPI) and DA (25-200 nmol) were tested for suppression of NPY-induced feeding. While EPI had no significant effect, DA at the maximally effective dose (50 nmol) significantly reduced the feeding response by 33% or more. These findings provide convergent evidence for antagonistic interactions between endogenous DA and NPY in the control of eating behavior.

314.11

ROLE OF AMYGDALOID CATECHOLAMINES IN FEEDING:A MICRO-DIALYSIS STUDY IN FREELY MOVING RATS. L. Lenard, A. Hajnal, Z. Karadi, I. Abraham, I. Vida, A. Czurko & T. R. Scott* (SPON: European Neuroscience Association) Institute of Physiology, Pecs Univ. Med. School, Pecs, H-7643 Hungary

The amygdala (AMY) is innervated by dopaminergic (DA) elements of the mesolimbic system and by noradrenergic (NA) terminals of the coerular-medullary NA cell groups. Our previous data based on different neurotoxic treatments of the AMY showed that a relative DA deficit caused body weight loss and hypophagia, while hyperhagia and weight increase occurred after a relative decrease of NA level. In the present experiments microdialysis probes were implanted into the medial or central part of the AMY and catecholamines (CAs) and their metabolites were determined by HPLC. Measurements were made after food deprivation or satiation and after insulin or glucose challenges. Deprivation was followed by a sharp decline in L-DOPA, NA and DOPAC levels while a significant increase occurred in the extracellular DA content. These changes reversed to initial values after satiation. No significant change of 5-HIAA level was observed after deprivation. Similar changes to those seen after deprivation were observed following insulin challenges, while glucose treatments led to similar response patterns observed after satiation. Our results provide additional support for the role of AMY CA systems in the regulation of feeding and demonstrate that hunger and satiety states are accompanied by opposite changes of AMY CAs and their metabolites.

314.12

LATERAL HYPOTHALAMIC INJECTION OF GLUTAMATE ELICITS EATING IN RATS. Stanley, B.G*., Ha, L.H., Spears, L.C. Depts. Neurosci. & Psych., Univ. California, Riverside, CA 92521.

While lateral hypothalamic (LH) control of eating behavior has been suggested by extensive lesion, electrophysiological, and biochemical data, there is little evidence to specify the neurotransmitters mediating its effects. Glutamate (GLU) is a likely candidate, as it is localized within the LH and has pervasive excitatory effects on neurons there (van den Pol, J. Neurosci., 11, 1991, 2087). To test this, GLU was injected, through indwelling guide cannulas, directly into the LH of satiated adult male rats, and food intake was measured 1, 2, and 4 hrs later. Glutamate (300 to 900 nmol/0.3 μl artificial CSF) produced dose-dependent increases in eating (means of 3.7 to 5.2 gm) only within the first hr of injection. To determine the locus of this effect, GLU was injected into anatomical sites bracketing the LH. In contrast to the eating-stimulatory effect in the LH, injections $\leq$ 1.5 mm anterior, posterior, medial, lateral, or dorsal to this site were ineffective. Finally, as a first step in determining the receptor subtypes mediating the eating response, injections of kainate (KA), NMDA, and AMPA, prototypical agonists of these subtypes, were tested. Although all three agonists produced significant eating, KA was particularly effective. The threshold dose was 0.33 nmol, and at $\geq$ 1.0 nmol KA consistently elicited mean increases of over 9 gm within 1 hr of injection. These findings suggest that glutamate in the LH may be a neurotransmitter in neuronal systems mediating eating behavior.

HUMAN COGNITION: NEUROPSYCHOLOGY

315.1

IMPLICIT MEMORY FOR REPRESENTATIONAL AND NOVEL VISUAL MATERIALS IN PATIENTS WITH ALZHEIMER'S DISEASE. J.D.E. Gabrieli, S. L. Reminger, D.A. Grosse, and R. S. Wilson*. Rush Alzheimer's Disease Center, Chicago, IL 60208.

Patients with Alzheimer's disease (AD) have impaired explicit memory, but intact implicit memory (i.e., repetition priming or the influence of prior processing upon the reprocessing of a stimulus) on some tasks in which they see words or pictures. The pictures in those studies were representational drawings of real objects known premorbidly by the AD patients. The present study examined whether AD patients could show implicit memory for novel visual stimuli. Nine early-stage AD patients and 10 normal control (NC) subjects saw drawings of real objects and novel nonobjects with object-like appearances (from Kroll and Potter, 1984). There were two identical study phases followed by tests of implicit or explicit memory. In both study phases, subjects performed an object decision task in which they saw 20 objects and 20 nonobjects and had to decide whether each drawing was that of an object or a nonobject. In the implicit test, subjects performed the same task with the 40 previously-seen and 40 baseline drawings. In the explicit test, subjects performed a yes/no recognition task with 40 previously seen drawings and 40 foils. All responses were vocal, and response latencies and accuracies were recorded. In the explicit test, subjects recognized objects more accurately than nonobjects, and AD patients were impaired for recognizing both kinds of drawings. In the implicit test, AD patients were slower overall, and both AD and NC subjects categorized objects faster than nonobjects. The AD patients showed repetition priming for objects equivalent to that shown by NC subjects, with both groups faster in categorizing repeated versus baseline objects. The NC subjects also categorized faster repeated versus baseline nonobjects. The AD patients, in contrast, categorized repeated nonobjects slower than baseline nonobjects. Their different response to repeated versus baseline nonobjects indicates that AD patients can show repetition priming (albeit inhibitory in this case) for novel visual stimuli. For the AD patients, it appears that a sense of familiarity generated by implicit memory for repeated nonobjects conflicted with their expected sense of familiarity for only real objects. NC subjects, perhaps due to their explicit memory capacity, could distinguish between familiarity due to study-phase exposure to nonobjects and familiarity due to real-world, pre-experimental experience with the objects shown in representational drawings. Supported by grants from the Illinois Department of Public Health and the NIA P01AGO9466.

315.2

DIMENSIONALITY OF THE SEMANTIC SPACE IN ALZHEIMER'S DISEASE PATIENTS. A. S. Chan, N. Butters *, D. P. Salmon and K. A. McGuire. Depts. of Psychiatry and Neurosciences, Univ. of Cal., San Diego, CA 92093 and VA Medical Center, San Diego, CA 92161.

The organization of semantic memory in Alzheimer's disease (AD) patients was investigated by a triadic comparison task in which subjects chose, among three concepts, the two that are most alike. A multidimensional scaling statistic was used to analyze the proximity data, resulting in a three-dimensional cognitive map for each subject. The cognitive maps, indicating the attributes and the saliency of those features that were used by subjects in categorizing concepts, were compared by discriminant analysis. The results suggest that the structure of semantic memory in AD patients is significantly different than that of NC subjects in two ways. First, AD patients are less consistent in utilizing the attributes of concepts. Second, AD patients focus primarily on concrete conceptual information (size), whereas NC subjects stress abstract conceptual knowledge (domesticity). The performance of AD patients was highly consistent over two sessions within a week. These results not only support the notion that AD patients are characterized by knowledge deterioration, but also suggest that the semantic memory in AD patients may undergo a structural alteration.

315.3

DISTRIBUTED NEURAL NETWORK UNDERLYING MUSICAL SIGHT-READING AND PIANO PERFORMANCE. J. Sergent*, B. MacDonald and S. Terriah. Cognitive Neuroscience Laboratory, Montreal Neurological Institute, McGill University, Montreal, Canada.

Music is a message comprising combinatory acoustical patterns and, like speech, is governed by rules of grammar and syntax, has both an expressive and a receptive side, is made of sounds that can be translated into print, and involves fine motor activity for its production. However, a musical phrase does not convey the same sort of information a verbal sentence does; it evokes feelings and emotions rather than referring to entities in the world; musical notation is grahically, symbolically, and functionally different from the alphabetic writing system. These similarities and differences between the musical and verbal languages may explain why aphasia and amusia are sometimes, but not consistently, associated following brain damage. With the use of MRI and PET measurement of cerebral blood flow, the functional neuroanatomy of musical sight-reading and keyboard performance was studied in ten professional pianists. By comparing through the subtraction method the intensity and spatial pattern of cerebral activation associated with different experimental conditions, the results indicated that reading musical notations and translating these notations into movement patterns on a keyboard produced activation of cerebral areas distinct from, but adjacent to, those underlying corresponding verbal operations.

315.4

CROSS-MODAL ASSOCIATIONS AND THE HUMAN AMYGDALA. F.K.D.Nahm*[1,2], A.R.Damasio[2,3], D.Tranel[3], H.Damasio[3]. Univ. of California, San Diego[1], The Salk Institute[2], and Univ. of Iowa College of Medicine[3].

It has been suggested that damage to the amygdaloid complex of non-human primates impairs tactile-visual cross-modal recognition (CMR). Investigations as to the role of the human amygdala in CMR as well as cross-modal matching (CMM) have been inconclusive. Here we report our attempt to clarify this question by studying two patients: SM-046 who has bilateral damage circumscribed to the amygdala and no damage to the hippocampal formation; and patient Boswell, whose damage in both temporal lobes includes ventral and mesial inferotemporal cortex, amygdala, hippocampus and interconnecting cortices. An Arc-Circle test was used to assess cross-modal matching and cross-modal recognition -- the CMR task included a 60-second delay between sample and match.

Patient SM-046 was not impaired on tactile-visual (T-V) or visual-tactile (V-T) versions of the CMR and CMM tasks as compared to IQ, gender and age-matched controls. Patient Boswell was also intact on the T-V and V-T versions of the CMM task. Taken together, the data indicate that the human amygdala is not involved in either the acquisition or recall of cross-modal associations between perceptually "equivalent" basic stimulus properties. However, since both patients have defects in learning and recalling knowledge which requires integration of higher-order perceptions and internal values (affect and emotion), we suggest that the cross-modal role of the human amygdala may be restricted to associations at this more complex level.

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315.5

DEFECTIVE ESTIMATE OF FUTURE PUNISHMENT IN PATIENTS WITH PREFRONTAL DAMAGE AND IMPAIRED SOCIAL DECISION-MAKING. A. Bechara*, S.W. Anderson, H. Damasio, and A.R. Damasio, Dept. of Neurology, Univ. of Iowa, Iowa City, IA 52242.

Damage to some prefrontal cortices in humans results in impaired decision-making and planning which is most marked in the social realm. It has been proposed that a possible cause of the defect is a failure to re-enact somatic states that would mark (overtly or covertly) negative future consequences of a selected option. Unable to estimate the prospect and magnitude of future punishment, the patients' behavior becomes governed by the presence of immediate reward. As part of a program to test this hypothesis, we developed a card game task, in which the goal was to maximize profit on a loan of play money, and in which response selection was guided by various schedules of immediate reward and future punishment. Subjects were asked to choose one card at a time, from one of four decks; choosing a card was always followed by an immediate reward of play money, but the amount was higher in some decks than in others; at unpredictable points in each deck, choosing a card was followed by money loss, which was greater in the high reward decks than in the low reward ones. The task cannot be solved by mental computation, and relies instead on the ability to develop an estimate of which decks are less risky. Subjects were required to make 100 choices. Control subjects learned to select the most advantageous card decks after about 30 trials. By contrast, EVR (a prototypic patient with "acquired sociopathy" following prefrontal damage and who has superior memory and intellect) persistently selected alternatives which yielded high immediate rewards but brought larger future losses. The results are compatible with the hypothesis that EVR-like patients are defective in their ability to evaluate future risk, independent of their otherwise intact intellectual status.

Supported by NINDS PO1 NS19632.

315.6

PERCEPTUAL FILLING IN OF SCOTOMAS OF CORTICAL AND RETINAL ORIGIN. D. Rogers-Ramachandran*, V. S. Ramachandran, H. Damasio. UCSD and Iowa College of Medicine.

We studied perceptual "filling in" in two patients who had small (6°) parafoveal scotomas caused by right occipital pole damage. We found that: a) A 3° gap in a vertical line was "completed" across the scotoma but the process took 6 seconds. b) when the two halves of the vertical line were misaligned horizontally by 2° they were seen to move vividly towards each other and become collinear before completion occurred. A similar effect occurred with horizontal lines but over much smaller misalignments (<0.5°). c) When viewing dynamic 2-D noise composed of red dots, one of the patients (R.D.) reported that the red color seemed to bleed into the scotoma first so that it was filled in with red for several seconds before being filled in with the noise. d) Coarse checkerboards were completed more readily than fine checkerboards. e) If the corner of a square or arc of a circle fell on the scotoma it was "filled in" (even when physically deleted from the stimulus). This effect cannot be observed in the natural bind spot. f) R.D. also reported that when the perceptually completed vertical line was switched off, he continued to see a persisting "phantom" of the filled-in portion of the line inside his scotoma for about 7 seconds.

None of these effects (except c) were ever observed in two patients with identical scotomas caused by retinal lesions. We suggest that these intriguing filling in effects may be mediated by the kinds of dynamic reorganization of receptive fields that occur when lesions are made in the retina or in area 17.

315.7

PERCEPTUAL "FILLING IN" PHENOMENA IN PHANTOM LIMBS. V. S. Ramachandran*, M. Stewart, D. Rogers-Ramachandran. Brain & Perception Lab, Psychology Department, UCSD, La Jolla, CA 92093.

We studied localization of sensations in a patient whose left arm had been amputated 8 cms above the elbow 4 weeks prior to testing. The patient experienced a "telescoped" phantom hand. We found that: a) A drop of warm water placed on his left cheek or 5 cms above the amputation line caused his phantom hand to feel warm. Water at room temperature was ineffective. Also, a pinprick 5 cm above the amputation line was felt as a distinct pinprick on an individual digit. Thus it would appear that the mislocalization can be *modality specific*. b) We used a Q-tip to study localization of touch. Remarkably, stimuli applied to points remote from the stump were mislocalized to the phantom hand. Furthermore, the distribution of such points was not random -- they appeared to be clustered on the lower left side of the face and there was a surprisingly precise point-to-point "mapping" between points on the face and specific digits (e.g., cheek to thumb; philtrum to index finger; chin to pinkie). It remains to be seen whether this clustering is a general finding or unique to this patient. Sensations from the neck, axilla, trunk, tongue, and contralateral arm were never mislocalized but there was a single point on the contralateral face that elicited referred sensations. c) A second "map" was found on the arm 3 cm above the amputation line with the pinkie represented laterally and thumb medially. The extreme rapidity of functional reorganization (4 weeks) suggests that it is probably subcortical in origin and may be related to the observations of Pons et al (*Science, 1991*). The word "switching" or "disinhibition" might be more appropriate.

315.8

INDEPENDENT TEMPORAL FACTORS UNDERLYING COGNITIVE PROCESSES. N.v.Steinbüchel, E.Pöppel*, B.Hiltbrunner. Institut für Medizinische Psychologie, Universität München, Germany and Ciba-Geigy, Basel, Switzerland.

Previous studies have indicated that cognitive processes are quantized on two different levels, i.e. in the 40 Hz and the 0.3 Hz domain. These quantizations may reflect automatic binding operations and logistical constraints of central information processing. Independent of these automatic timing mechanisms, three different components of processing, reflecting different tasks could now be identified. This conclusion is derived from experiments on single and choice reaction time to auditory and visual stimuli, personal tapping tempo, high speed tapping and sensorimotor synchronization, performed with 36 subjects. With a principle component analysis, it was found that central decision timing, programming of movement times and repetitive timing represent different central timing algorithms. If choice reaction time is decomposed in its central decision component and motor execution, independent factors can be identified. An equivalent observation can be made studying sensorimotor coordination when a sequence of auditory stimuli has to be synchronized with motor responses. Interestingly, fast tapping and a preselected tempo (personal tapping tempo) appear to reflect the same mechanism of dynamic timing.

315.9

CONSEQUENCES OF ORIENTING IN SOMATIC SPACE: A REACTION TIME STUDY. G. Tassinari, D. Campara, G. Berlucchi*. Istituto di Fisiologia Umana, Università di Verona, I-37134 Verona, Italy

It is known that covert orienting to spatial locations can be studied by asking normal subjects to detect targets presented in cued or uncued positions. Cues may either provide valid information about the spatial location of the targets or not. Many previous studies have shown that simple visuomotor reaction time (RT) is lengthened when the target is preceded by a non-informative visual cue on the same side of fixation, as compared to RT to opposite cues and targets. This result has been interpreted as a consequence of inhibition of motor reactions triggered by the cue: subjects are requested to suppress the natural overt orienting towards the cue, so that their general motor set is still affected by this directional vetoing command when the target comes from the same side. In the present experiment, we wanted to learn whether this directional constraint in motor readiness extends to the somatosensory modality by asking subjects to respond by a manual key-pressing to the second of two identical stimuli, delivered to the skin of either the same or opposite shoulders. Non noxious electrical stimulation, 4 msec in duration and about .15mA in intensity, was applied through surface electrodes. Ipsilateral or contralateral stimuli were randomly presented at four different stimulus onset asynchronies (SOA: 335, 850, 1750 and 4250 msec). RTs to ipsilateral combinations were significantly slower than RTs to contralateral combinations at the first three SOAs. A control experiment with the same two electrodes placed on either left or right shoulder at discriminable distances showed that lengthening of ipsilateral responses does not depend on local adaptation but is a true consequence of a lateral unbalance. We conclude that a directional bias of orienting is present also in the somatic space.

315.10

LATERALIZATION OF FRONTAL LOBE FUNCTIONS AND HANDEDNESS. K.Podell and E.Goldberg*, Medical College of PA, Philadelphia, PA 19129

Prior research failed to establish a strong relationship between cognitive measures of hemispheric specialization and handedness. We present the first evidence, to our knowledge, for a robust relationship between handedness and functional lateralization of the frontal lobes in males. We developed a task measuring cognitive bias along the set maintenance (SM)/set shifting (SS) continuum. Low score indicates extreme SM bias, and high score extreme SS bias. Opposite performance patterns emerged when the task was given to right- and nonright-handed males (RHM and NRHM) with left and right frontal lesions (LFL and RFL). RHM with RFL (N=8) and NRHM with LFL (N=3) had low scores (SM bias); and RHM with LFL (N=5) and NRHM with RFL (N=1) had high scores (SS bias). Healthy controls and patients with posterior lesions, both right- and nonright-handed, had intermediate scores.

315.11

MEMORY FOR SPATIAL LOCATIONS AND SENTENCES IN DATA-BASED AND KNOWLEDGE-BASED MEMORY SYSTEMS IN HYPOXIC SUBJECTS. R.O. Hopkins* and R.P. Kesner, Department of Psychology, University of Utah, Salt Lake City, Utah 84112.

Subjects who have experienced a hypoxic episode and college student control subjects were tested for memory of temporal distance information using sentences and spatial locations. Using analogous procedures it was possible to assess memory for temporal distances in both the data-based and knowledge-based memory systems. Memory for knowledge-based information was assessed by the presentation of 8 words that were organized into syntactically and semantically correct sentences and 8 X's that formed a specific spatial pattern. Memory for data-based information was assessed by the presentation of 8 words that were organized into nonsense sentences and 8 X's that formed a random spatial pattern. During the study phase subjects were presented with a list of 8 items which were presented on a computer screen, one at a time, for a duration of 5 seconds. During the test phase subjects were presented with either 2 words or 2 spatial locations (X's) that had occurred in the study phase. Subjects were asked to choose the item that occurred earlier in the sequence. Temporal distance was determined by the number of items that occurred in the study phase between the two test items. Choices occurred across temporal distances of 0, 2, 4 and 6, with 8 observations for each temporal distance. Results indicate that hypoxic subjects were impaired across all distances for new data-based information for both the sentences and spatial locations. However, hypoxic subjects performed similar to control subjects for all distances in the knowledge-based sentences and spatial locations tasks The results suggest that hypoxic subjects can remember knowledge-based information, but have a difficult time remembering new data-based information.

315.12

RIGHT HEMISPHERE COGNITION DOES NOT REQUIRE RIGHT HEMISPHERE LANGUAGE. J.E. Bogen* and D.L. Van Lancker. Depts. of Neurosurgery and Neurology, Univ. of So. Calif., Los Angeles, CA 90033.

It has been asserted (Gazzaniga, 1983 and 1987) that the disconnected right hemisphere without language [in right handers] is cognitively inferior to a chimpanzee or even a monkey. Related is the even stronger claim (Edelman, 1989) that human consciousness requires language. These claims are contradicted by a wealth of evidence. Examples are presented from split-brain subjects, patients rendered aphasic by large left hemisphere lesions, and from two patients hemispherectomized in adulthood after customary development as right-handers. A relevant videotape will be available in the video viewing room.

315.13

THE ABSENCE OF CONTEXTUAL INTERFERENCE IN THE MNEMONIC PERFORMANCE OF AMNESICS. Elizabeth A. Phelps*, William Hirst, Craig Piers, Stephen Hartman, Bradford Richards, and Bruce T. Volpe, Center for Neural Science, New York Univ., N.Y., N.Y. 10003

Studies on experimental animals suggest that the hippocampus may be necessary for forming relations between multiple cues (Eichenbaum et al., 1991, Sutherland & Rudy, 1989), such as contextual cues present during learning. The extension of this hypothesis to human hippocampal function, suggests that changes in context should differentially effect the mnemonic performance of amnesics and normal control subjects. Two studies examined whether this is the case. Amnesics and controls were shown a picture of a family and learned a list of facts about this family. Subjects were then shown two pictures, one of the same family and one of a new family. For each picture the subjects were asked to learn a new set of facts about the family, some of which were semantically related to the previous facts. For the related facts paired with the old family, reinstating the old context improved the mnemonic performance of both amnesics and controls, although amnesics seem to benefit somewhat more than controls. For the related facts paired with the new family, control subjects showed interference in the misleading context of a different family. Amnesic subjects performed similarly on related and unrelated facts paired with the new family. In the second study the control subjects replicated a finding that normal subjects can perform better on tests of cued recall than recognition, when the context of the recognition task is drastically different than the original learning context (Thompson & Tulving, 1972). The amnesic subjects, however, were not effected by the change in context and their recognition performance is superior to their cued recall performance. Both of these studies suggest that while amnesics can use cues present in the testing context to aid mnemonic performance, they have access to these cues only when specifically reinstated and, as a result, do not show the same mnemonic intererence control subjects demonstrate when presented a misleading context during testing.

SOMATOSENSORY SYSTEM

316.1

RESPONSES OF SLOWLY ADAPTING AFFERENT FIBRES IN THE MONKEY TO SPHERICALLY CURVED STIMULI. A.W. Goodwin*, A.S. Browning and H.E. Wheat. Dept. of Anatomy, University of Melbourne, Parkville Vic 3052, Australia.

Neural recordings from monkeys and corresponding psychophysical experiments in humans were used to investigate the role of cutaneous mechanoreceptors in determining the shape of contacted objects. Stimuli consisted of spherically curved segments of different radii presented passively to an immobilized fingerpad. Humans were able to scale for contact force or for curvature, and could discriminate changes in curvature of about 10%. Responses were recorded from single slowly adapting afferent fibers (SAs) in the median nerves of anesthetized monkeys. The stimulus parameters that were controlled were the curvature of the surface, the applied contact force, and the position of the stimulus in the fiber's receptive field.

Responses of SAs increased when the curvature of the surface applied to the center of the receptive field increased, or when the contact force increased. Changing the position of the stimulus in the receptive field also changed the response. The profile of response, as a function of position, reflected the shape of the stimulus, being more peaked and greater in magnitude for more curved surfaces and broader and lower in magnitude for less curved surfaces. Although the stimulus parameters (curvature and contact force) are confounded in individual fibre responses, the profiles of activity across the SA population provide information about each parameter.

316.2

WHISKERS, BARREL FIELDS, AND SENSORY MOTOR PERFORMANCE OF RATS

M. Glickstein*, I. Kralj-Hans, B. Mercier, T. Onyirioha, G. Wynn Dept. of Anatomy & Developmental Biology, University College London, Gower Street, London WC1E 6BT.

Normal rats quickly learned to run rapidly along a one meter straight runway for a food reward. The runway contains a gap along its course whose width can be varied and whose position is not predictable. The animals were reinforced if and only if they ran faster than a pre-set and continously adjusted time. Shaving the large mystacial whiskers abolished the animal's ability to traverse a 14 cm gap successfully when tested in the dark but not in the light. Running was not affected by shorter gaps in either the dark or light. In most animals tested to date bilateral lesions of the barrel fields or unilateral lesions combined with ipsilateral whisker shaving impaired or abolished performance in the dark. Control lesions which spared the most lateral barrels had no effect on performance in the light or dark. Animals with barrel field lesions can sometimes recover successful performance by detecting the leading edge of the gap and jumping maximally. Our anatomical evidence suggests that an efferent pathway from the barrel fields to the cerebellum by way of the pontine nuclei may play a critical role in this simple behaviour. We are currently studying the effects of blocking the efferent pathway to the cerebellum by interrupting corticopontine fibres from the barrel field in the basis pedunculi.

316.3

RECEPTIVE FIELD PROPERTIES OF IPSILATERAL TRIGEMINOTHALAMIC NEURONS IN CATS. Jin Y. Ro, Norman F. Capra, and Revers Donga*. Dept. of Physiology, University of Maryland Dental School, Baltimore MD 21201.

This study describes the distribution, receptive field properties, and antidromic latencies of neurons thought to project to the ipsilateral ventroposteromedial nucleus (VPM) of the thalamus. Previous anatomical studies showed that most of these cells are located in or near the dorsal part of the principal sensory nucleus. In 4 pentobarbital anesthetized cats, each VPM was stimulated in alternate fashion, while recording extracellular responses from the rostral brainstem. Neurons responding with a short-fixed latency to thalamic stimulation were tentatively classified as trigeminothalamic cells. This classification was supported by the ability of these cells to follow high frequencies of thalamic stimulation. The receptive fields of all antidromically activated cells were mapped using natural stimuli such as blowing, brushing, light pressure, pinch, and stretching the jaw. Twenty four trigeminothalamic neurons were identified. Of these, 15 were activated by antidromic stimulation of the ipsilateral thalamus. The mean antidromic latency of these neurons was 0.9 msec. Most neurons were low threshold and had relatively discrete ipsilateral intraoral receptive fields including tongue, palate, and periodontal tissues. However cells with other receptive field characteristics were identified, including one with convergent properties and one that responded to ramp and hold stretches of the jaw. Ipsilateral projection neurons tended to be more medially situated above the trigeminal motor nucleus than contralaterally projecting neurons. These results are consistent with anatomical data reporting the distribution of primary afferent fibers to this region. The more rostral uncrossed projections and the decussating projections from more caudal regions of the trigeminal sensory nuclei provide the oral cavity with significant bilateral representation in more rostral parts of the neuraxis. Supported by DE06027.

316.4

STAINING FOR CALCIUM BINDING PROTEINS REVEALS WHISKER-RELATED PATTERNS, FUNCTIONALLY DISTINCT CELL GROUPS AND PLASTICITY IN TRIGEMINAL (V) NUCLEI. N. Hobart, M.F. Jacquin*, C.A. Bennett-Clarke, R.W. Rhoades & N.L. Chiaia. Dept. Anatomy & Neurobiology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104; Dept. Anatomy, Med. Coll. of Ohio, Toledo, OH 43699.

Calbindin (CA) and parvalbumin (PA) immunostaining and retrograde tracing were used to define the targets of V brainstem cells that stain for these 2 proteins in adult rats. Large #s of PA+ cells occurred in V nucleus principalis (PrV) and they were arranged in a whisker-related pattern. Almost all of these cells projected to thalamus. In interpolaris (SpI), large #s of small-medium cells were PA+ in a whisker-like pattern. None of these cells had long-range targets, but many projected to PrV. SpI also had many large CA+ cells; most projected to thalamus or colliculus; some also projected to PrV. In caudalis (SpC), PA+ cells occurred in inner lamina II, and in layers III+IV where they formed whisker patterns; none had long-range targets. Large #s of CA+ cells occurred throughout lamina II in SpC, with smaller #s in layers I and III-V; a small % projected to thalamus. Thus, PA and CA identify 2 functionally distinct cell groups in V nuclei and the PA cells are somatotopically patterned in PrV, SpI and SpC. PA and CA also distinguish 2 cell groups in superficial dorsal horn. In 7 adults with infraorbital nerve section at birth, PA+ cells were not arranged in a whisker-related pattern and their #s were reduced by the following %s in PrV, SpI, SpC layers III+IV, and I+II, respectively: 20±14, 35±13, 29±21, 0 (mean±SD). CA+ cell #s were reduced in SpI and SpC I+II by 26±21 and 0%. Thus, V inputs are necessary for patterning PA+ cells as well as the survival of some PA+ and CA+ V neurons. DE08971, DE07734, DE07662, NS29885.

316.5

ANALYSIS OF DENDRITES FROM INTRACELLULARLY FILLED THALAMO-CORTICAL PROJECTION (TCP) NEURONS IN THE SOMATOSENSORY THALAMUS OF THE CAT. L.A. Havton and P.T. Ohara* Dept of Anatomy, UCSF, San Francisco, CA 94143. USA.

Standard techniques were used to record receptive fields and label TCP neurons using glass micropipettes containing 4% HRP or 2% Neurobiotin in adult cats anaesthetized with sodium pentobarbitol. The animals were perfused with aldehyde fixatives and 50μm coronal sections were cut through the thalamus. Labeled cells were located in the VPL or VPM and responded to the normal range of non-nociceptive stimuli.

Quantitative analysis of physiologically characterized neurons at the light microscope level suggests that non-nociceptive somatosensory TCP neurons form a homogenous population. 52 dendrites from 5 neurons were fully reconstructed and quantitatively analyzed at the light microscope level. The dendrites ranged in size from 404μm^2 to 14,733μm^2 (mean 4140μm^2, SD 3400) and in number of dendritic end points from 1 to 29 (mean 8.8, SD 6.9). There was also a positive correlation between primary dendritic diameter and total dendrite size.

The pronounced variation in the morphology of individual dendrites suggests that the more stereotyped structure of individual TCP neurons results from a combination of dendrites with different features.

Support: NS23347, NS21445 and NS26488

316.6

A RETICULO-RETICULAR COMMISSURAL PATHWAY IN THE RAT THALAMUS G.Battaglia *, L. Scarpina, C. Colacitti, C. Regondi and R. Spreafico. Dept. of Neurophysiology, Neurological Institute "C. Besta", 20133 Milano, Italy .

The thalamic reticular nucleus (RTN) is known to extensively project to the ipsilateral dorsal thalamic nuclei. A few previous studies reported that RTN as well as other ventral thalamic structures send commissural projections to the contralateral thalamus (Rinvik, 1984; Nakamura and Kawamura, 1988). The present investigation was undertaken to ascertain whether commissural connections exist between the RTN of the two sides of the rat thalamus. Different tracing techniques were employed in deeply anesthetized adult rats: the retrograde transport of WGAapoHRP-gold, combined with GABA immunocytochemistry, and the anterograde transport of biocytin. After WGAapoHRP-gold injections in the rostral pole of RTN, retrogradely labeled neurons were present in the contralateral rostral pole of RTN, concentrated in its medial part. After WGAapoHRP-gold injections in the caudal RTN, retrogradely labeled neuons were present in the contralateral ventromedial part of the caudal RTN, close to the border with the zona incerta. Anterograde tracing findings confirmed the retrograde labeling data: after iontophoretic biocytin injections in the rostral pole of RTN, anterogradely labeled axons and terminals were detected in the medial part of the contralateral rostral pole of RTN, as well as in the contralateral anteromedial and anteroventral thalamic nuclei. The present study suggests that RTN could provide a channel for interthalamic connectivity. Further experiments are in progress to ascertain the quantitative relevance of these thalamic commissural pathways.

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316.7

UNITARY THALAMOCORTICAL SYNAPTIC CURRENTS EVOKED IN LAYER IV CELLS OF NEONATAL MOUSE BARREL CORTEX BY CHEMICAL STIMULATION OF THE THALAMUS IN VITRO. A. Agmon*, R.A. Warren, L. Yang and E.G. Jones. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

We have recently described whole-cell thalamocortical synaptic currents recorded in layer IV neurons of developing mouse barrel cortex, elicited by electrical stimulation of the ventrobasal nucleus of the thalamus (VB) (Agmon and O'Dowd, J. Neurophysiol., in press). However many important issues require data on unitary events, i.e. synaptic responses to activation of individual thalamocortical axons. We have modified our methods in order to evoke and record such events. Tight-seal whole-cell recordings were achieved in layer IV neurons, and the precise region of VB giving rise to monosynaptic excitatory currents (EPSC's) was mapped by electrical stimulation. Small quantities of glutamate (1mM in 0.9% saline) were then pressure-ejected in the mapped region. Responses were dose-dependent and varied from sporadic unitary events to massive excitatory synaptic barrages. Some of the resolved unitary events showed a voltage-dependency typical of NMDA receptor-mediated currents. Ongoing experiments are aimed at providing a detailed characterization of these unitary synaptic inputs. The morphology of the thalamocortical axons involved is being studied by focal injections of Di-I into the mapped locus in VB at the end of the recording session. Supported by BOA-Giannini Foundation and by NS21377.

316.8

CORTICOTHALAMIC SYNAPTIC CURRENTS IN NEURONS OF THE VENTROBASAL NUCLEUS OF THE DEVELOPING MOUSE THALAMUS STUDIED IN VITRO. R.A. Warren*, A. Agmon, M. Spitzer, and E.G. Jones. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA92717.

The corticothalamic projections form one of the major inputs to the dorsal thalamus. They provide both direct excitatory input, and indirect inhibitory input via the reticular thalamic nucleus (RTN). It is thus possible to study both the excitatory and inhibitory influences of the corticothalamic projections on ventrobasal nucleus (VB) relay neurons. We have studied the synaptic inputs evoked in thalamic relay neurons by electrical stimulation of the corticothalamic pathway at different postnatal ages. Whole-cell recordings were performed in a mouse slice preparation with preserved corticothalamic connectivity. Synaptic currents were evoked in VB neurons as early as postnatal day 4 by electrical stimulation in deep layer VI, white matter or internal capsule. Both excitatory and inhibitory synaptic currents were recorded. Inhibitory inputs were often trains of relatively large all-or-none events. These probably originated in RTN neurons since in rodents, VB is virtually devoid of GABAergic interneurons and RTN is the only source of GABAergic inhibition. The electrophysiological and pharmacological characteristics of these responses are currently under investigation. Supported by NS22317 and FRSQ of Canada.

316.9

EPICORTICAL POTENTIALS EVOKED IN GRANULAR AND PERIGRANULAR SOMATOSENSORY CORTEX OF THE RAT BY CONTRALATERAL ELECTRICAL STIMULATION JOSEPH A. KITHAS, SHI DI and DANIEL S. BARTH* *Department of Psychology , University of Colorado, Boulder, CO 80309-0345 (U.S.A)*

In a previous report from our laboratory, stimulation of individual vibrissa was demonstrated to produce epicortical potentials localized to the functionally defined vibrissa/barrel field in the rat. This work utilized electrocorticographic mapping and numerical analysis capable of resolving individual cortical columns.

The present experiment extended these methods to study the influence of transcallosal input on the somatosensory evoked potential complex. While recording from an 8x8 microelectrode array covering a 3.8 x 3.8 mm^2 area of the right vibrissa/barrel field, a similar array was used for electrical stimulation of a homologous region in the left hemisphere. The spatial patterns of potentials evoked by contralateral electrical stimulation and by mechanical stimulation of contralateral vibrissae were compared to cytochrome oxidase stained tangential sections of somatosensory cortex.

Our results indicate that while contralateral whisker stimulation activates populations of cells confined to the anatomically defined barrel field of primary somatosensory cortex, potentials evoked transcallosally by electrical stimulation are more widely distributed and suggest a major input to perigranular cortex posterior and lateral to the barrel field.

316.10

DEVELOPMENTAL REGULATION OF PLASTICITY IN SOMATOSENSORY CORTEX OF KITTENS. S.L. Juliano, D.E. Eslin, R. Code, R. Sonty*. Dept. Anatomy, Cell Biol., & Neurosci., USUHS, Bethesda, MD 20895.

This lab has previously shown that amputation of a single digit (DA) in adult cats causes expansion of stimulus-evoked metabolic activity in the somatosensory cortex (SSC) following stimulation to an adjacent digit. To test the idea that developmental mechanisms may affect the ability of the cortex to reorganize, DAs were performed on kittens 2, 4 or 6 weeks of age. After unilateral amputation of digit 2, animals survived until they were 3-4 months of age. At this time, a 2-deoxyglucose (2DG) experiment was conducted, stimulating digit 3 bilaterally. Digitized maps of metabolic activity reveal that the DA at all ages causes substantial reorganization in the activity pattern. For the animals that received a DA at 6 wks of age, the expanded pattern was like that found in the adult, demonstrating a distribution that was similar to, but larger than, the pattern evoked in the normal hemisphere. When the DAs were conducted at 2 or 4 wks of age, the characteristics of the activity evoked by stimulation to the digit adjacent to the amputation were substantially different from that evoked in the adult or in the 6 wk animal. The metabolic activity pattern contralateral to the DA was severely disorganized compared to that found on the normal side. Rather than occurring as an expanded version of the normal distribution, the evoked activity was located in numerous small foci that covered an expanded cortical territory. Even though these numerous foci were widespread within the SSC, the actual area included in the individual spots was similar to, or only slightly larger than, the cortical area demonstrating increased 2DG uptake on the normal side. Activity patterns evoked in hemispheres contralateral to a DA in adults or 6 wk old treated animals cover about 2 times the cortical area in the normal hemisphere. The disorganized pattern after a DA in young animals may reflect functional and anatomical substrates that are present during development of the cerebral cortex. Supported by NS24014.

316.11

ABNORMAL SOMATOSENSORY CORTICAL ORGANIZATION AND CORTICAL PLASTICITY IN HUMANS REVEALED BY MAGNETOENCEPHALOGRAPHY

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A 14-channel neuromagnetic recording system was used to record and map magnetic evoked responses to vibratory stimulation of the digits in both normal subjects and in a number of patients with injuries and/or congenital malformations of the hand. In 5 normal subjects studied, the fingers of the hand were represented in a somatotopic order superiomedially along the postcentral gyrus (area 3b), with the little finger and the thumb separated by a two-point average distance of 10.6±1.3 mm (mean ± SEM), with the little finger consistently superior to the thumb on the gyrus. In three of the five normal subjects, all five fingers were studied, and the average distance between adjacent fingers was found to be 4.2 ±0.4 mm. We have reported previously (Mogilner et al, Soc. Neurosci. Abstr. 1991) a case of homotransplant of innervated skin from the ring finger to the thumb, without displacement of somatosensory perception. The functional interdigital distance (thumb–ring finger) following surgery, 0.2 mm, was significantly smaller than both the patient's own other interfinger distances (10.6 mm, p=.0016), and the control group's interfinger distances (p=.0326, one-tailed *t*-test), indicating no cortical reorganization in accordance with cognitive findings. Two subjects with syndactyly (digit fusion) were also studied. In one subject with bilateral syndactyly, both hand areas were abnormal – the thumb–little distances, right: 1.6 mm, left: 1.1 mm, were significantly smaller than controls (p=0.026 and 0.023,1-tailed *t*-test). The other patient,with unilateral syndactyly, underwent surgical separation of the digits. The pre-operative organization was grossly abnormal and non-somatotopic (thumb-little distance: 2.4 mm p=.035, little finger located below all fingers except thumb). One month following digit separation, the thumb-little distance had increased to 10.5 mm, and the little finger's location shifted significantly (Θ=3.8°,p=.018). This result indicates the presence of plasticity in the adult somatosensory cortex and its correlation to the new post-surgical functionality.

316.12

ACTIVITY-DEPENDENT CHANGES OF GLUTAMATE-IMMUNOREACTIVITY IN THE SOMATIC SENSORY CORTEX OF MONKEYS FOLLOWING PERIPHERAL AND CENTRAL MANIPULATIONS OF SOMATOSENSORY INPUT. A. Minelli°, L. Danielsson, J. Hahm, T.P. Pons*, and F. Conti°, °Institute of Human Physiology, University of Ancona, Via Ranieri, I-60131 Ancona (Italy), and Laboratory of Neuropsychology, N.I.M.H., Bethesda, MD 20892 (USA)

Previous studies using electrophysiological techniques demonstrated that the adult brain possesses a remarkable capacity for changes in cortical maps in response to experimental perturbations of the sensory input, but little effort has been made to identify the molecular mechanisms underlying this phenomenon. Since the regulation and expression of neurotransmitters and neuromodulators occurs in an activity-dependent manner, such changes could provide a mechanism that might account for alterations in sensory maps following central or peripheral perturbations.

In the present experiments, five monkeys were used to determine whether changes in glutamate (Glu) activity were correlated with presence or lack of cortical reorganization seen in operated animals. Four animals had the three nerves innervating the hand cut and tied, while a fifth animal had the cortical hand representation in SI ablated. Six to eight weeks later, the five animals were perfused transcardially and sections from the spinal cord, dorsal column nuclei, ventroposterior thalamus and the first (SI) and second (SII) somatic sensory cortex were processed for Glu-immunocytochemistry. In the animals with nerve cuts, the pattern of Glu-immunoreactivity in the dorsal horn of the spinal cord, in the dorsal column nuclei, and in the ventrobasal thalamus, and in SI appeared as in unoperated animals. By contrast, the hand representation in SII contained regions with a normal distribution of Glu-positive neurons alternating with regions devoid of Glu-positive neurons. Electrophysiological experiments conducted in the same animals revealed reorganization in SI but not in SII. In the animal with the SI ablation, Glu-immunoreactivity appeared normal throughout SII, and recordings indicate complete reorganization in SII. These results suggest that i) the reduction of Glu levels may contribute to the elimination of SII neural activity after peripheral nerve cuts; and ii) a correlation exists between the presence or absence of map reorganization and Glu levels.

316.13

A PET STUDY IN HUMAN SUBJECTS TO LOCALIZE BRAIN STRUCTURES INVOLVED IN THE SOMATOSENSORY DISCRIMINATION OF TACTILE "ROUGHNESS".

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The regional cerebral blood flow (rCBF) was measured with ^{15}O-butanol and Positron Emission Tomography (PET) in 10 healthy subjects performing a two-alternative forced choice discrimination of roughness using pairs of standardized stimuli (Roland and Mortensen, Brain Res. Rev.12:1-42, 1987). There was a total of 4 activation runs for each subject, consisting of 2 roughness discriminations, a similar task to discriminate length of pairs of similar smooth cylinders, and a control trial which required exploratory finger movements only, without a specific stimulus to feel. Mean subtraction images were computed using the computerized adjustable brain atlas of Bohm and Greitz (1986) and areas of significant blood flow change were identified. Both the roughness and the length discrimination tasks activated cortical fields in the contralateral post-central gyrus and supramarginal gyrus, including the anterior and posterior lip of the post-central gyrus but not extending to the superior or inferior lip of the intraparietal sulcus. These areas were interpreted as being somatosensory association areas engaged in the discrimination and perception of roughness and tactile length. The length discrimination task activated in addition, parts of the angular gyrus bilaterally and several regions in the superior and middle frontal gyri bilaterally.

317.1

EFFECTS OF 5HT MONOAMINE UPTAKE BLOCKERS AND 5HT1A AGONISTS ON SPONTANEOUS FIRING RATE OF PREFRONTAL CORTICAL NEURONS. A. Ceci*, A. Baschirotto and F. Borsini. Boehringer Ingelheim Italia S.p.A. Milan, Italy.

In the present study we have determined the effects of two 5HT monoamine uptake blockers (fluoxetine and imipramine) and two 5HT1a agonists (buspirone and 8OH-DPAT) on spontaneous extracellular activity of 43 neurons within prefrontal cortex (PFC). The rats were anaesthetised with chloral hydrate and only one cell for rat was tested. The drugs were administered i.v. at logarithmic doses. Fluoxetine increased the basal firing rate in dose-dependent manner, dose-range 0.1-1000 μg/kg, with 53% maximum excitatory effect at 1000 μg/kg. Imipramine, at the doses of 1 up to 300 μg/kg, induced an excitation of basal firing rate; the maximum excitatory effect was 30% at 3 and 10 μg/kg; raising the dose up to 1000 μg/kg, an attenuation of the effect was detected. Buspirone produced a dose-related, 0.1-30 μg/kg, excitatory effect on basal firing rate, 54% maximum effect at the dose of 30 μg/kg; increasing the dosage up to 1000 μg/kg a clear attenuation of the excitatory effect was noted. 8OH-DPAT elicited a dose-dependent bimodal effect, which was characterized by an activation of PFC neurons at low doses 0.1-10 μg/kg, followed by an inhibition of basal firing rate with higher doses, 30-300 μg/kg. The basal firing rate between vehicle and drugs was not statistically different. The results are discussed in the terms of preferentially pre-or post-sinaptically activity of the two uptake blockers and the two 5HT1a agonists.

317.2

EFFECTS OF 5-HT_{1A} AND 5 HT_{1B} AGONISTS ON FOREBRAIN 5 HT SYNTHESIS AFTER LOCAL APPLICATION INTO THE MEDIAN RAPHE OR THE NUCLEUS ACCUMBENS IN THE RAT. V. Hillegaart[1]*, A. Wijkström[2] and S. Ahlenius[1]. Department of [1]Behavioural Pharmacology and [2]Bioanalysis, Astra Arcus AB, S-151 85 Södertälje, Sweden.

In a previous study, it was shown that the local application of the 5-HT_{1A} agonist 8-OH-DPAT into the median raphe produced a statistically significant decrease in the nucleus accumbens 5-HT synthesis, as estimated by the 5-HTP accumulation in rats pretreated with the 5-HTP decarboxylase inhibitor NSD-1015 (100 mg/kg IP). In the present series of experiments, the effects of 8-OH-DPAT on 5-HT synthesis in the nucleus accumbens was examined after local application of the compound in the same forebrain area (0.5 and 5.0 ug). There were no effects on forebrain 5-HT synthesis, however, after local application of 8-OH-DPAT in the vicinity of terminal serotonergic autoreceptors, presumed to be of the 5-HT_{1B} subtype. Control 5-HTP accumulation: 1.50±0.27 (mean SD, n=4). The corresponding values after 8-OH-DPAT (0.5 and 5 ug) were 1.47±0.12 and 1.35±0.28, respectively. In order to test the functional coupling of such receptors, nucleus accumbens 5-HTP accumulation was measured after local application of the 5-HT_{1B} receptor agonist CP-93129 into this forebrain area. Preliminary observations, however, suggest that also this compound lacks effects in the nucleus accumbens after local application into this brain area. CP-93129 (1 and 10 ug) 5-HTP accumulation: 1.42±0.14 and 1.57±0.40, respectively. Together, these observations demonstrate that forebrain 5-HT synthesis is regulated by somato-dendritc 5HT_{1A} autoreceptors, whereas the functional coupling of terminal 5-HT_{1B} receptors in the nucleus accumbens could not be demonstrated in the present test system.

317.3

ORIGINS OF SEROTONERGIC AFFERENTS TO THE HYPOGLOSSAL NUCLEUS. **S. Manaker* and L.J. Tischler, Pulmonary and Critical Care Division, Department of Medicine and Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, PA 19104.**

Hypoglossal motoneurons alter their firing rates in response to serotonin (5HT), and the hypoglossal nucleus (Mo12) is densely innervated by 5HT terminals. While several brainstem regions that contain 5HT also project to the Mo12, which of these contributes 5HT afferents to the Mo12 remains to be determined. Fluorogold (FG; 20 nl) was injected into the Mo12 of anesthetized rats, who survived 10 days. After dextran/saline perfusion and paraformaldehyde fixation, the brainstem was removed and frozen. Sections (32 μ) were cut through the entire brainstem, and the FG injection site examined. In cases with the FG injection site restricted to the Mo12, every 6th section was processed for 5HT immunofluorescence (rabbit anti-5HT, 1:2000; Texas Red-conjugated goat anti-rabbit, 1:400). Retrograde labeling by FG was observed in all sources of Mo12 afferents, while 5HT immunoreactivity was present in all major groupings of 5HT somata. Double labeled cells were observed primarily in the nuclei raphe pallidus and obscurus, with fewer double labeled cells in the caudal nucleus raphe magnus and the parapyramidal nucleus. No double labeled neurons were noted in other groupings of 5HT somata, including the rostral nucleus raphe magnus, the dorsal and median raphe nuclei, and the locus coeruleus and laterodorsal tegmental nucleus. These observations suggest that the 5HT innervation of the Mo12 originates exclusively from the caudal raphe and parapyramidal nuclei. (Supported by SCOR HL-42236)

317.4

SEROTONINERGIC AND CHOLINERGIC CONCERT ACTIONS ON ENTORHINAL CORTEX LAYER II NEURONS: AN IN VITRO STUDY. R. Klink* and A. Alonso. Montreal Neurological Institute and McGill University, Montreal, QC, Canada H3A 2B4.

Entorhinal cortex (EC) layer II, a key element in the learning and memory functions of the neocortical-hippocampal circuitry, receives dense acetylcholine (ACh) and serotonin (5-HT) innervation. Both 5-HT and ACh act in concert in cognitive functions. We reported last year on the cholinergic modulation of EC layer II neurons intrinsic excitability (Alonso and Klink, 1991). By means of intracellular recording in rat brain slices we have now extended the analysis to the effects of 5-HT. Bath application (30-60s) of 5-HT (10-30μM) triggered on EC layer II stellate cells a large (50%) increase in membrane conductance frequently associated with a moderate (1-4mV) membrane depolarization which persisted for relatively long periods (>10 min). 5-HT also largely increased (100%) the frequency of both the Na-dependent rhythmic subthreshold oscillations characteristic of these cells (6-12Hz in control) and the repetitive bursting activity (spike "clustering") (13Hz in control) that they develop upon moderate d.c. depolarization. Concomitantly, in the presence of 5HT the intraburst firing frequency increased to about 40 Hz and the bursting activity tended to become more rhythmic. The 5HT-induced depolarizing response is consistent with mediation via 5-HT3 receptors which are present in high concentrations in the EC. As the above-described 5-HT actions largely oppose those of ACh, the interplay between cholinergic and serotoninergic systems in the modulation of EC layer II neurons intrinsic oscillations may be of great significance for understanding the interactions of both systems in learning and other cognitive aspects of hippocampal function.

317.5

DEX-FENFLURAMINE WHEN ADMINISTERED ORALLY IN DOSES IN CONSIDERABLE EXCESS OF THE HUMAN THERAPEUTIC DOSE PRODUCES NO ULTRASTRUCTURAL OR AXONAL TRANSPORT CHANGES IN RAPHE SEROTONERGIC NEURONS OF THE RAT. M. Kalia.* Department of Pharmacology and Neurosurgery, Jefferson Medical College, Philadelphia PA 19107.

We have previously reported that the observed reduction in immunocytochemically labeled nerve fibers in the rat cortex following both short- and long-term oral treatment with dex-fenfluramine (D-FF) is dose dependent and rapidly reversible *(Kalia, M., Brain Res. '91)*, indicating that the loss of immunoreactivity (IR) is merely a function of the loss of serotonin from the nerve terminals as a result of the therapeutic action of the drug (a 5-HT uptake inhibitor and 5-HT releaser). The present study was conducted to confirm this conclusion by using two other indicators of neuronal integrity - electronmicroscopy and the ability of the neuron to transport and accumulate retrogradely transported material. The retrograde transport of cholera-toxin horseradish peroxidase (CTHRP) combined with 5-HT IR was used to examine the functional status of rat cortical serotonergic networks following oral D-FF administration (8 & 16 mg/kg/day) for 4 or 21 days. The animals survived 3 - 5 weeks after cessation of the treatment before the entire cortex was injected with 400 μl of CTHRP under nembutal anesthesia. Following a 3 day survival, the animals were perfused and the brain stem was processed for double-labeling using HRP histochemistry and 5-HT IR *(Rye et al, '85)*. No differences were seen between D-FF treated animals and pair-fed controls. Quantitative analysis was performed with a Bioquant Meg IV image analysis system. Electronmicroscopic examination of 5-HT labeled neurons in D-FF treated animals showed normal morphology. These results indicate that short- and long-term treatment with D-FF does not produce functional or morphological changes in brain serotonergic neurons. (Supported by IRIS).

317.6

EFFECT OF STRESS ON SEROTONIN TRANSMISSION IN THE PREFRONTAL CORTEX AND INTERACTIONS WITH TRYPTOPHAN, DIAZEPAM AND BUSPIRONE; A MICRODIALYSIS STUDY. E. Carboni*, C. Cadoni, G.L. Tanda, and G. Di Chiara. Department of Toxicology, University of Cagliari, Italy

It has been widely reported that serotonin (5-HT) metabolism is increased by stress although generally 5-HT concentrations have not been affected. We use here the transversal dialysis for detecting tryptophan (Try), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) in the prefrontal cortex of freely moving rats. Restraint stress, obtained by taping the rat hind legs for 40 min., increases 5-HT output by 90 %, over basal values, without changing levels of Try and of 5-HIAA. Immobilization for 40 min., causes an increase in 5-HT output by 125 % over basal values affecting also levels of Try and 5-HIAA that were increased respectively by about 250 % and 50 % over basal values. The administration of systemic Try (100 mg/Kg i.p.) just before the application of stress potentiated only serotonin release induced by immobilization. The pretreatment with both diazepam (1 mg/Kg i.p.) and buspirone (1 mg/Kg s.c.) antagonizes the increase in serotonin release induced by both restraint and immobilization.

These results indicate that: a) 5-HT release is affected by plasma and brain Try availability; b) 5-HT in the prefrontal cortex plays an active role in emotional behavior; c) diazepam and buspirone, drugs with anxiolitic properties might interact with stress via 5-HT transmission.

317.7

THE BLOOD-BRAIN BARRIER TRANSPORT OF L-TRP MEASURED AT STEADY-STATE IN RAT. M. Diksic*, A. Takada, and A. Gjedde. Department of Neurology and Neurosurgery, McGill University, Montreal Neurological Institute, Montreal, Canada

The purpose of this study was to establish which fraction of the plasma Trp interchanges with brain Trp under a steady-state. In the past, the blood-brain barrier (BBB) transport of L-Trp was measured under non-steady state conditions. Total plasma Trp was used for data analysis, although a large fraction of Trp is known to be bound to plasma proteins. Six groups of three Wistar rats (190-200 g) were catheterized with femoral vein and artery catheters under anesthesia, allowed to awaken, and injected (i.p.; constant volume) about 2 h later with saline, (control) or 20, 50, 100, 200, 300 and 400 mg/kg of L-Trp methyl ester. One hour after injection of the L-Trp ester, rats were infused with a mixture of L-[^{3}H]Trp (40 μCi) and [^{14}C]butanol (10 μCi) into the femoral vein for 22 sec. Rats were decapitated 20 seconds after the beginning of the tracer infusion, the brain removed and homogenized for radioactivity determination. Arterial blood was collected from the arterial catheter designed to yield about 1 g of blood in 20 sec. The PS product (not corrected for protein binding) was highly correlated with the free plasma fraction of Trp ($p < 0.001$). From the relationship between the PS and the plasma free Trp concentration, an apparent K_m of 27 ± 11 μM, a V_{max} of 6.3 ± 1.6 nmol/g/min, and a diffusion constant of 0.136 ± 0.006 ml/g/min were estimated by non-linear least square. The functional relationship between plasma free and bound Trp yielded a B_{max} of 360 nmol/ml and K_D of 81 ± 10 μM. Under steady-state conditions the plasma free Trp fraction is the one functionally communicating with the brain. The simple diffusion of Trp was higher than previously measured. The V_{max} is far above the requirements of the brain for Trp.

317.8

SEROTONIN SUPPRESSES EPILEPTIFORM BURSTS IN RAT CA1 NEURONS. D. Salgado* and K. A. Alkadhi. Department of Pharmacology, University of Houston, Houston, TX 77204-5515 USA.

Serotonin can evoke multiple responses via activation of the various 5-HT receptor subtypes. In the CA1 region of the hippocampus, where a high density of 5-HT$_{1A}$ receptors are present, serotonin causes an inhibitory response. Previous studies on whole animal preparations done by other investigators suggest that serotonin may be involved in the inhibition of seizure activity. Further, it has been shown that the Genetically Epilepsy Prone Rats are deficient in brain serotonin content. The purpose of this study was to assess the role of 5-HT in bicuculline-induced epileptiform bursts using the rat brain slice preparation. All experiments employed conventional techniques for intracellular recording from CA1 neurons of the hippocampus. Serotonin (20 µM, n=5) suppressed the bicuculline evoked response. Further, serotonin (20 µM) caused a marked hyperpolarization (control: -73.9±1.5 mV; after 5-HT: -82.7±2.3 mV, n=7) accompanied by a decrease in input resistance (control: 52.5±6.1 MΩ; after 5-HT: 31.7±4.9 MΩ, n=4). The selective 5-HT$_{1A}$ agonist 8-OH-DPAT (20 µM) mimicked the effects of serotonin. These preliminary results suggest that serotonin may play a significant role in the inhibition of epileptiform activity and that this should be further investigated focusing specifically on the inhibitory action mediated via the 5-HT$_{1A}$ subtype.

317.9

EFFECTS OF MAO INHIBITORS ON EXTRACELLULAR 5-HT IN THE RAPHE NUCLEI AND FRONTAL CORTEX OF RAT BRAIN. "IN VIVO" MICRODIALYSIS STUDIES IN THE AWAKE RAT. F. Artigas*, P. Celada, N. Bel
Dept. of Neurochemistry, C.S.I.C. Barcelona 08034, Spain

The net effects of MAO inhibitors on aminergic neurotransmission are still poorly known. Electrophysiologcal results suggest a potentiation of 5-HT transmission *via* a desensitization of 5-HT autoreceptors. We have examined the actions of MAO inhibitors on the brain extracellular compartment of 5-HT in frontal cortex and dorsal+median raphe nuclei. Previous studies indicate that the raphe nuclei may be a preferential target for the action of antidepressant drugs. Locally applied, clorgyline (CLG), brofaromine (BRF, a reversible inhibitor of MAO-A) and tranylcypromine (TCP) induce dose-related increases of extracellular 5-HT that were more important in the raphe nuclei. Depreny (DEP) was without effect in both areas at specific doses. Systemically administered, TCP (15 mg/kg) induced huge increases of extracellular 5-HT in the raphe nuclei (60-fold) and frontal cortex (10-fold) while increasing tissue 5-HT by 6-fold. However, CLG and BRF did not increase dialysate 5-HT at doses that inhibited MAO-A by more than 95% and increased tissue 5-HT (CLG) by 4-fold. Extracellular 5-HT increased after a two step inhibition of MAO: in animals pretreated with clorgyline (10 mg/kg) or deprenyl (2.5 mg/kg) the administration of deprenyl or clorgyline (respectively) led to large (ca. 10-fold) and significant increases of extracellular 5-HT after the administration of the second MAO inhibitor. These results show that the complete inhibition of MAO-A and the concurrent increase of intracellular 5-HT do not result in an increase of the 5-HT output, probably because the increase of tissue 5-HT occurs in a non-releasable compartment (e.g. glial cells).

PRESYNAPTIC MECHANISMS IV

318.1

CLONING AND EXPRESSION OF GLYCINE, TAURINE/β-ALANINE, AND TWO GABA TRANSPORTERS. N. Nelson, Q.-R. Liu, B. Lopez-Corcuera, S. Mandiyan, H. Nelson, and J.L. Noebels*. Dept. of Gene Regulation, Roche Institute of Molecular Biology, Nutley, NJ 07110 and Developmental Neurogenetics Laboratory, Baylor College of Medicine, Houston, TX, 77030

Neurotransmission in the CNS is terminated by a rapid reaccumulation of neurotransmitters into presynaptic terminals by transporters. We have isolated cDNA clones from a mouse brain library encoding the sodium dependent, high affinity GABA transporter (GABAT2), a second GABA transporter, a glycine transporter and a taurine/β-alanine transporter. All four transporters share similar amino acid sequences and membrane topography. *Xenopus* oocytes injected with synthetic RNAs from the cDNA clones revealed that the glycine transporter is specific for glycine transport. No other amino acid could be transported by it, nor did they inhibit glycine uptake into oocytes. The taurine transporter takes up β-alanine at about 5-fold lower affinity than taurine. The two GABA transporters differ in their pharmacology as well as their Km for GABA. The tissue distributions of those transporters are under study by Northern blot analysis and at the cellular level. *In situ* hybridization of the ^{35}S-labelled antisense mRNA for the high affinity GABA transporter in mouse brain showed a clear pattern of high expression in neocortex, hippocampal pyramidal cell layer, habenular, periventricular and suprachiasmatic nuclei, zona incerta and cerebellar Purkinje cells. Neither hippocampal nor cerebellar granule cell populations were clearly positive, and there was trace expression over white matter tracts containing oligodendroglia. These tranporters directly regulate inhibitory synaptic transmission, and in the case of glycine, may indirectly regulate NMDA receptor-mediated excitatory amino acid signalling in the CNS.

318.2

LITHIUM POTENTIATES CHOLINERGIC PRESYNAPTIC INHIBITION OF GLUTAMINERGIC, BUT NOT GABAERGIC TRANSMISSION. G.A. Cohen* and D.V. Madison. *Dept of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305-5426.*

We have previously reported that acute bath application of a therapeutically relevant level (2mM) of LiCl strongly potentiates carbachol-induced (1uM) presynaptic inhibition of excitatory transmission in rat hippocampal slices, while LiCl alone only slightly reduces excitatory transmission (Cohen, et al., Neuroscience Abstracts, 1990). Our present experiments center on comparing presynaptic modulatory interactions of these two agents in glutaminergic and GABAergic transmission, and on elucidating the biochemical mechanisms of these effects.

It has been shown that carbachol inhibits both polysynaptic and monosynaptic evoked IPSPs (Cohen, et al., Neuroscience Abstracts, 1991). LiCl (2mM) had a slight inhibitory effect on poly- and mono-synaptic IPSPs. However, LiCl markedly potentiated cholinergic inhibition (carb, 1uM) of polysynaptic IPSPs, but did not potentiate cholinergic inhibition of monosynaptic IPSPs. Since polysynaptic IPSPs differ from monosynaptic IPSPs in that they involve the glutaminergic activation of a GABAergic synapse, our results suggest that lithium interacts with cholinergic inhibition at glutaminergic, but not GABAergic, presynaptic terminals.

Bath application of myo-inositol (5-10mM) did not affect the inhibition of the EPSP induced by carb (1uM), LiCl (2mM), or carb + LiCl. This suggests that the interaction of acutely administrated LiCl with carb is not mediated by a depletion of myo-inositol. We are currently pursuing the signal transduction mechanism involved in presynaptic inhibition induced by carbachol and lithium.

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318.3

PRESYNAPTIC FACILITATION OF GLUTAMATE EXOCYTOSIS MAY BE SUSTAINED BY ACTIVATION OF A PROTEIN KINASE CASCADE. D.M. Terrian[1], D.K. Ways[2], R.V. Dorman*[3] and D.A. Zetts[1]. Depts. [1]Anatomy and [2]Medicine, East Carolina University School of Medicine, Greenville, NC 27858. [3]Dept. Biological Sciences, Kent State Univ., Kent, OH 48504.

Hippocampal mossy fiber (MF) nerve endings contain multiple protein kinase C (PKC) isoforms and the activation of PKC significantly increases the K^+-evoked release of both glutamate and dynorphin B (Terrian et al., Hippocampus 1:303-314, 1991). Here, we report that the persistent activation of PKC leads to a delayed and and sustained enhancement of the K^+-evoked release of endogenous glutamate but not dynorphin B from isolated hippocampal MF synaptosomes. The K^+-evoked release of glutamate was found to be selectively enhanced when MF synaptosomes were preincubated with the PKC activator 4ß-phorbol 12,13-dibutyrate (PDBu) for 15 min, extensively washed with a PDBu-free medium and depolarized. Three lines of evidence indicate that the sustained enhancement of glutamate release observed under this condition may be due to the activation of a phosphorylation cascade that is no longer directly dependent on continued PKC phosphotransferase activity. First, the evoked release of glutamate was desensitized to the potentiating effects of PDBu after a 15 min exposure to the phorbol ester. Second, the potentiation of glutamate release persisted throughout the course of PDBu-induced down-regulation of the α, ß, γ, ε and ζ PKC isoforms. Third, preincubation of MF synaptosomes with PDBu lead to an increase in the basal, PKC-independent, phosphorylation of two endogenous substrates with molecular weights of 45 and 50 kDa. It is concluded that the PKC second messenger system generates a signal, that may be subsequently propagated by the activation of a protein kinase cascade, that is sufficient to enhance K^+-evoked release of glutamate from hippocampal MF synaptosomes. However, it is postulated that the generation of this signal is not sufficient to raise the bulk level of cytosolic free calcium and, therefore, the release of dynorphin B is not affected. AFOSR 89-0531 and NIH R29CA 43823.

318.4

MONOSIALOGANGLIOSIDE (GM1) ENHANCES GLUTAMATERGIC TRANSMISSION IN RAT HIPPOCAMPAL SLICES. P. Miu* and K. Krnjević. Anaesthesia Research Dept. McGill University, 3655 Drummond St., Montréal, Canada, H3G 1Y6.

GM1 can be found abundantly in mammalian nerve terminals, where it may modulate transmitter release. Indeed, GM1 enhances excitatory inputs while suppressing the inhibitory inputs to CA1 cells (Miu and Krnjević, 1991, Can. J. Physiol. Pharmacol., 69:Axxii).

To isolate the excitatory and inhibitory PSPs, we perfused rat hippocampal slices with ACSF containing either bicuculline (10 μM) or kynurenic acid (2 mM) respectively. In addition, spontaneous EPSPs were also measured in the presence of TTX from CA3 cells, where the excitatory mossy synapses are close to the soma.

In CA1 neurons, GM1 enhanced both the frequency and peak amplitude of the spontaneous, TTX- and kynurenate-sensitive EPSPs. In the presence of TTX, GM1 also increased the frequency and peak amplitude of spontaneous EPSPs in CA3 cells, suggesting a direct action on mossy fibre terminals. Monosynaptic IPSPs evoked in the presence of kynurenic acid, however, were not altered by GM1. Hence, these observations suggest that GM1 a) selectively facilitates the excitatory component of the neural circuitry by increasing excitatory transmitter release, b) has little direct effect on inhibitory synapses, c) but depresses the firing of inhibitory interneurons.

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

318.5

REGULATION OF SPONTANEOUS GABA RELEASE IN THE HIPPOCAMPUS. V. A. Doze* and D. V. Madison. *Department of Molecular & Cellular Physiology, Beckman Center, Stanford Univ. School of Medicine, Stanford, CA 94305-5426.*

Synaptic inhibition in the hippocampus arises primarily from GABA-containing interneurons. We have been examing the effects of various neurotransmitters (e.g., epinephrine and baclofen) and changes in extracellular potassium on the spontaneous release of GABA from inhibitory interneurons in the rat hippocampus using whole cell patch-clamp recording techniques.

Epinephrine (1-10 μM) caused a marked increase in both the frequency and amplitude of monosynaptic, action potential-dependent, spontaneous GABAergic inhibitory postsynaptic currents (IPSCs) recorded in CA1 pyramidal neurons in rat hippocampal slices (recorded with KCl and QX-222 in the electrode, and CNQX/APV in the ACSF to monosynaptically isolate action potential-dependent GABA release). In contrast, baclofen (1-10 μM) reduced the frequency and amplitude of these action potential-evoked IPSCs. Interestingly, neither epinephrine or baclofen affected the frequency and amplitude of miniature, action potential-independent, GABA-mediated IPSCs (recorded with KCl and QX-222 in the electrode, and CNQX/APV and TTX in the ACSF to block action potential-dependent release).

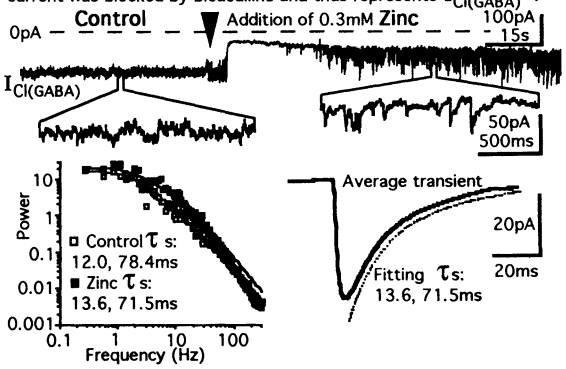
Changes in the extracellular potassium level also caused marked changes in the frequency and amplitude of monosynaptic, action potential-dependent, spontaneous IPSCs. Increasing the extracellular potassium caused a large increase in both the frequency and amplitude of these IPSCs. Decreasing the extracellular potassium resulted in a reduction in the frequency and amplitude of these events. Changes in extracellular potassium also caused changes in the frequency of miniature, action potential-independent, GABA-mediated IPSCs, but did not affect their amplitude.

V. A. D. is a N.I.M.H. Predoctoral Fellow. D. V. M. is a Lucille P. Markey Scholar. This work was supported in part by a grant from the Lucille P. Markey Charitable Trust.

318.6

ZINC TRANSFORMS GABA LEAKAGE INTO QUANTAL RELEASE. J. Vautrin*, A. Schaffner and J.L. Barker.
Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

18 day-old embryonic rat hippocampal neurons cultured for 1-12 days were clamped at -80mV with Cs Cl patch pipettes. The clamp current was blocked by bicuculline and thus represents $I_{Cl(GABA)}$.



Thus, control noise and spontaneous "quanta" exhibit similar kinetics and, Zinc rapidly synchronizes GABA-mediated Cl^- channel openings, suggesting that it transforms continuous leakage into discrete quanta.

318.7

STUDIES ON DYNAMIN FUNCTION IN DROSOPHILA. K.S. Krishnan, S. Rao, A. van der Bliek@,R. B. Kelly#*, and M. Ramaswami # Molecular Biology Unit, Tata Institute of Fundamental Research, Colaba, Bombay 400005, India; @ Division of Biology, Caltech, Pasadena, CA 91125; # U.C S F., Dept of Biochemistry and Biophysics, San Francisco, CA 94143-0534

We have used Drosophila genetics and molecular biology to study the role of dynamin in the nervous system. Multiple subforms of dynamin are encoded by the shibire gene of Drosophila. Several mutant alleles of shibire induce reversible, temperature-dependent paralysis due to a conditional block in synaptic-vesicle recycling. When developing shibire flies are pulsed to high temperatures during varied developmental stages, varying, but remarkably specific defects are observed in diverse tissues. These defects might result from the blockage of endocytosis-dependent, or perhaps microtubule-dependent developmental processes. In an attempt to understand the diverse roles of dynamin in Drosophila, we have isolated a collection of intragenic revertants of the temperature-dependent paralysis induced by the shibire ts2 mutation. All of the revertants appear to restore partial (but not complete) activity to the dynamin forms required for synaptic-vesicle recycling. We are examining these alleles for suppression of other shibire phenotypes. We expect that the sequence of the revertant alleles will also offer some insights into the structure of dynamin; towards this end we will shortly be sequencing the alleles that we have isolated. These and other data will be presented.

318.8

SMALL FUSION PORES PASS LITTLE LIPID. F.W. Tse* & W. Almers. Dept. Physiol. & Biophysics, U. of Washington, Seattle, WA 98195.

We voltage-clamped 3T3 fibroblasts expressing HA (the fusion protein of the infuenza virus) while they fused to single human red blood cells (RBC). The electrical conductance of the cell-cell connection (fusion pore) was calculated from the membrane capacitance (C) measured with a lock-in amplifier (26 - 28 °C). The RBC membrane was loaded with a fluorescent lipid ($DiIC_{18}(5)$) so that movement of lipid into the fibroblast could be detected with an image intensifier and later analyzed digitally. When HA was activated by acidification of the external medium, a small but abrupt increase in C was seen 27 - 159 s later, indicating the opening of the fusion pore. Over the next 2 - 300 s, the pore conductance (g) increased from <0.5 nS to >1 - 2 nS. In 12 cells, we calculated the rate constants of lipid flux from segments of data in which the average value of g could be also calulated as a function of time. Lipid flux was clearly seen when g was >1 - 2 nS, but not when g was <0.5 nS. Flux appears to require that g exceeds a minimum value. This is not expected if the pore is made of mobile lipid, in which case flux should rise as the square root of g. Instead, our data suggest that (1) either the initial fusion pore (<0.5 nS) is proteinaceous, or the lipids in it are essentially immobile (perhaps due to interactions with proteins), and (2) the fusion pore dilates with the addition of lipids.

Supported by GM-39520.

318.9

KINETICS AND SIZE OF CHOLESTEROL LATERAL DOMAINS IN BRAIN SYNAPTOSOMES: MODIFICATION BY SPHINGOMYELINASE AND EFFECTS ON CYTOSOLIC CALCIUM. A. M. Rao, U. Igbavboa, M. Semotuk and W.G. Wood*, VA Medical Center, GRECC, and Dept. of Pharmacology, Univ. of Minnesota, School of Medicine, Minneapolis, MN 55417.

Cholesterol domains have been well-studied in non-neuronal membranes. The purpose of these experiments was to determine if: 1) exchangeable and non-exchangeable cholesterol domains or pools were present in brain synaptosomal membranes; 2) effects of sphingomyelin on cholesterol domains; and 3) cholesterol domains and calcium uptake and cytosolic calcium in synaptosomes. Cholesterol domains were determined using cholesterol exchange between radiolabeled small unilamellar vesicles and mouse synaptosomes. In some experiments, synaptosomes were treated with sphingomyelinase (*Staphylococcus aureus*), that has previously been shown to alter membrane cholesterol in non-neuronal membranes. Calcium uptake and $[Ca^{2+}]_i$ were measured using Fura-2. The size of the exchangeable pool of synaptosomal membrane cholesterol was approximately 50% of total membrane cholesterol when measured at 37°C. The $t_{1/2}$ of cholesterol exchange at 37°C in synaptosomes was approximately 9 hr. Temperature-induced changes in membrane fluidity significantly reduced the size of the exchangeable pool and significantly increased the $t_{1/2}$ of cholesterol exchange. Sphingomyelinase treatment of synaptosomes significantly increased the $t_{1/2}$ of cholesterol exchange but did not modify the exchangeable pool of cholesterol. Calcium uptake into synaptosomes was significantly increased in sphingomyelinase-treated synaptosomes. Cholesterol domains can be described in neuronal tissue and the size and kinetics of those domains were altered by temperature-induced changes in fluidity and hydrolysis of sphingomyelin, that also increased Ca^{2+} uptake. Supported in part by the Dept. of VA and NIAAA 07292 (WGW).

318.10

THE COMBINED EFFECTS OF AGE AND HALOTHANE ON TRANSMITTER RELEASE AT THE NEUROMUSCULAR JUNCTION: M.D. Sokoll*, B. Bhattacharyya, K. Tsen and L.R. Davies, Department of Anesthesiology, University of Iowa College of Medicine, Iowa City, IA 52242

It is now well documented that increasing age into the geriatric range is associated with a decrease in anesthetic requirement. We wished to examine the possible relationships between age and anesthetic at the neuromuscular junction.

METHODS: The phrenic nerve-cut diaphragm muscle preparation of the rat was used. The preparation was placed in a bath (volume 3 ml). Miniature endplate currents MEPC's and endplate currents EPC's were recorded with a two microelectrode voltage clamp. MEPC's and EPC's (0.4 and 40 Hz) were recorded before and during the application of halothane (1 and 2%). MEPC's and EPC's were analyzed for amplitude and time constant of decay (tau). Quantum content of EPC's was calculated by the direct method. Rats aged 6, 18-20 and 30-33 months were studied.

RESULTS: Membrane potential related changes in MEPC amplitude and tau were similar in all groups of rats. Quantum content of the EPC's (0.4Hz) was greater in the young animals (52 vs 44 for 6 and 30 month animals). Halothane decreased the amplitude and tau of both MEPC's and EPC's. Quantum content of EPC's elicited at 0.4Hz was decreased following the application of halothane. The decrease was greater in the older animals. The percentage of rundown of the EPC was similar in all groups and was not altered by the application of anesthetic.

DISCUSSION: Transmitter output at the neuromuscular junction is decreased in older rats. The anesthetic induced decrease is greater in the older animals. The process of mobilization of transmitter in the nerve terminal (relative to EPC#1) appears to be unaltered by either age or the anesthetic halothane. If anesthetics have similar actions in the CNS these may be related to the mechanism of anesthetic action.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION

319.1

NEUROSTEROIDS CAN BE SYNTHESIZED IN SPECIFIC REGIONS OF THE RAT BRAIN BY ADRENAL STEROIDOGENIC ENZYMES. S. H. Mellon and C. F. Deschepper*, Dept. of Ob, Gyn & Repro Sci, Dept. of Physiology, and The Metabolic Research Unit, Univ. of Calif, San Francisco, CA 94143

Neurosteroids are steroids that are made in and act on neural tissues in an auto/paracrine fashion, as opposed to adrenal and gonadal steroids that can act on the CNS in an endocrine fashion. All glandular steroidogenesis is initiated by P450scc which converts cholesterol (Chol) to pregnenolone (Preg). Rat neonatal forebrain glial cells mimic adrenal steroidogenesis by converting ^{3}H mevalonolactone to ^{3}H Chol, ^{3}H Preg, and ^{3}H 20-OH Preg. Although undetectable by RNase protection assays, RNA-based PCR detected mRNAs for P450scc and P450c11β (11β hydroxylase), but not for P450c17 (17α hydroxylase) or P450c11AS (aldosterone synthase) in primary cultures of neonatal forebrain glial cells. Thus the rat brain contains some, but not all, of the steroidogenic enzymes found in glandular endocrine tissues. Both P450scc and P450c11β mRNAs were found in the amygdala, hippocampus and midbrain and were most abundant in the cortex of both male and female rats, but P450c11β mRNA was more abundant in the female than male hippocampus. P450scc mRNA is found in Type-1 astrocytes of primary glial cultures. P450scc protein is almost as abundant in Type-1 astrocytes as in adrenal Y-1 cells, while P450scc mRNA is about 10^3-10^4 less abundant, suggesting that the protein is very stable in the brain. Thus the synthesis of classic (e.g. glucocorticoids and progestins) and nonclassic (e.g. allopregnanolone) neuorsteroids can be regulated *in situ* in the brain, and do not require extra-neural substrates.

319.2

ELEVATED BLOOD CORTICOSTERONE LEVELS IN NEUROLOGIC MUTANT MICE LURCHER AND STAGGERER. F. Frederic, T. Chautard, C. Oliver, E. Wollman, N. Delhaye-Bouchaud and J. Mariani*. Inst.Neurosc. Univ. P. & M. Curie, Paris, INSERM U. 297, Marseille and Inst. G. Roussy, Villejuif, France

We reported recently an hyperproduction of interleukin-1 (IL-1) by the peripheral macrophages of the cerebellar mutant mice *lurcher* and *staggerer*. As IL-1 causes increased ACTH synthesis by the pituitary and consequently increased blood levels of corticosterone (CS) and thymic hypoplasia in mice, we investigated if an IL-1-dependent increased in plasma CS, might be responsible for the thymic hypoplasia also observed in these mutants.

We measured the plasma CS levels of 7±1 week-old mutants and matched controls, at 10 a.m. or 5 p.m., either at rest or after a stress consisting of a change in the housing conditions during increasing periods of time. At 5 p.m, we found a marked increase of nearly 100% of the resting CS levels in the plasma of *staggerer* and *lurcher* as compared with controls. At 10 a.m, the CS levels were 100% higher in *staggerer*, but not in *lurcher*. Both mutants were more reactive to stress than controls, and at any period of the nycthemeral cycle. The over-reaction was also of the 100% range after 15 min of stress. Both sexes were reactive and females had higher levels than males. Plasmatic CS increase after i.p. injections of exogenous IL-1α was similar in mutants and in controls. The hyper-reactivity of the *lurcher* mutant to an adaptative stress was not suppressed by pretreatment by the IL-1 receptor antagonist protein, suggesting that this abnormality is IL-1-independant in *lurcher*. Whatever its origin, currently under investigation, the high plasma CS level of *staggerer* and *lurcher* may play a role in the thymic hypoplasia of these mutants.

319.3

MATERNAL ADRENALECTOMY ELEVATES POMC mRNA, BUT NOT CRF mRNA IN MALE OFFSPRING. E. Redei*, LF. Li, I. Halasz, and F. Aird. Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

The role of maternal glucocorticoids on the integrity of the hypothalamic-pituitary-adrenal function of offspring was investigated. Dams were adrenalectomized or sham-adrenalectomized on day 12 of gestation. Pups were cross-fostered to intact dams. At 20 days of age, male pups were subjected to intermittent footshock stress (7 shocks in 15 sec, 0.2 mA). Pups were decapitated immediately before (t=0), and 5, 20 and 40 min post-stress. Basal ACTH levels were significantly higher in offspring of adrenalectomized dams (ADX) compared to the sham group. The ACTH stress-response and basal corticosterone levels did not differ between the groups, but the immediate corticosterone response to stress (t=5 min) was significantly higher in the ADX group. Hypothalamic CRF mRNA and glucocorticoid receptor (GR) mRNA levels were not affected by maternal adrenalectomy. In the anterior pituitary, however, POMC mRNA was dramatically (p=0.008) increased, and a trend of increased GR mRNA was seen, in offspring of adrenalectomized dams. These results suggest that expression of the POMC gene, but not the CRF gene, is sensitive to glucocorticoid regulation prenatally. Supported in part by MH45862 and AA07389.

319.4

THE EFFECTS OF CHRONIC COLD EXPOSURE ON DIURNAL CORTICOSTERONE AND ALDOSTERONE RHYTHMS IN SPRAGUE-DAWLEY RATS. M.E. Bligh, S.A. Bhagwat, J.R. Thomas, and T.W. Castonguay*, Dept of Human Nutrition and Food Systems, Univ. Maryland, College Park, MD 20742 & Naval Medical Research Inst., Bethesda, MD 20814.

Chronic cold exposure increases plasma corticosterone (CORT) to maximal levels after approximately 7d. CORT levels then approach baseline concentrations observed at ambient (23°C) temperatures. Aldosterone (ALDO) release is also stimulated by cold exposure. The purpose of this study was to investigate the effects of chronic cold exposure on CORT and ALDO diurnal rhythms. Thirteen male Sprague-Dawley rats were individually housed, adapted to a 12/12 light/dark cycle (lights on 0900h) and provided with Purina Chow and water ad lib. Rats were housed at 23°C. On the 7th d tail blood samples were obtained every 4 h for 24 h beginning at 0900h. Blood samples were centrifuged, and plasma CORT and ALDO were measured via RIA. Laboratory temperature was then decreased to 4°C. On the 7th and 14th d of cold exposure, blood was sampled every 4 h as above. Cold exposure resulted in elevated levels of CORT at 0900h after both 7 and 14 d of exposure ($p<.05$). At 1300h and 0500h levels were elevated only after 7 d of cold exposure ($p<.05$). Aldo was significantly elevated at all time periods after 7 d of cold exposure, but after 14 d were only elevated at 0900h, 1300h, and 2100h ($p<.05$). Peak CORT levels occurred at 1700h (180.5±16.8 ng/ml) at 23°C and 2100h at 7 and 14 d at 4°C (202.2±16.4 and 190.7±16.4 ng/ml, respectively). Peak ALDO occurred at 2100h at 23°C (257.9 + 21.3 pg/ml), and 7d (431.9±21.2 pg/ml) and 14d (398.4±21.2 pg/ml)at 4°C. Results suggest that CORT and ALDO are released by different mechanisms throughout the day/night cycle and that cold stimulates ALDO release by a different mechanism than that stimulating CORT release. (Supported in part by NIH grant [illegible] DK42446 and a grant from Maryland Agricultural Experiment Station Competitive Grants Program).

319.5

MR AND GR SYNERGISTICALLY CONTROL ACTH AT THE PEAK OF THE DIURNAL RHYTHM. M. J. Bradbury*, S.F. Akana, §J.P. Herman and M.F.Dallman, U. California-San Francisco, 94143-0444 and §U. Kentucky-Lexington, 40536-0084.

Adrenalectomized (ADX) rats have high plasma ACTH in the absence of corticosterone(B). Sensitivity of inhibition of ACTH by free plasma B is high in the morning (IC_{50} 0.7 nM) and lower in the evening (IC_{50} 3.7 nM). The reported K_Ds for B with type I (MR) and type II (GR) receptors are 0.5 and 2.5 nM, respectively, suggesting that B on MR controls AM ACTH and that B on GR alone, or in conjunction with MR controls PM ACTH. To find out whether, in the evening, the efficacy of GR occupancy on ACTH is affected by MR occupancy, we implanted rats with low doses of B (relative MR agonist) and dexamethasone (relative GR agonist), alone or in combination at the time of ADX. The plasma ACTH and total plasma B 5 days after surgery, 2 hours BEFORE lights out are shown below **in bold** (n=5 or 6). In combination, rats with 15% B and 2.5µg dex/day had lower ACTH than ADX rats (**). However, alone these doses did not lower ACTH. 15% B and 2.5µg dex/day alone did not inhibit expression of AVP mRNA in the parvocellular paraventricular nucleus (PVN); however, CRF mRNA tended to be reduced by both. We conclude that 1) the control of plasma ACTH at the peak of the diurnal rhythm by GR requires the full occupation of MR, an effect initiated outside of the PVN and 2) the level of CRF mRNA in this paradigm does not predict plasma ACTH.

DEX (µg/day)	0%B PELLET	15%B PELLET	25%B PELLET
		PLASMA ACTH (pg/ml)	
0.0	**1099±121**	**1124±230**	**142±21**
0.5	**1197±73**	**867±178**	**94±12**
2.5	**1047±291**	**224±63****	**81±5**
		PLASMA B (µg/dl)	
0	**0.3±0.1**	**1.7±0.6**	**5.3±1.2**

319.6

SYNERGISTIC POSITIVE TRANSCRIPTIONAL REGULATION OF THE HUMAN CORTICOTROPIN-RELEASING HORMONE GENE BY cAMP AND DEPOLARIZATION. H. Guardiola-Diaz* and A.F. Seasholtz, Mental Health Research Institute and Deptartment of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109

Neuronal gene expression can be regulated by synaptic activity and membrane depolarization. The transduction of signals from neurotransmitter receptors and ion channels on the cell membrane to the genome involves activation of several second messenger systems that often interact. In this study we investigate the mechanisms involved in the regulation of the human corticotropin-releasing hormone (hCRH) gene by depolarization. PC-12 cells are transiently transfected with the hCRHCAT1 construct containing 663 bp of 5' flanking DNA and 127 bp of 5' UTR fused to the bacterial CAT gene. This construct is positively regulated by cAMP (10-15 fold) via a cAMP-responsive element (CRE) located 220 to 227 bp 5' to the mRNA cap site. Potassium-mediated depolarization (55mM) results in a synergistic upregulation of hCRHCAT1 activity from forskolin-activated levels in PC-12 cells. The hCRH sequence between -231 and - 179 bp (including the hCRH CRE) confers the positive cAMP regulation and the depolarization-mediated synergistic upregulation of forskolin-activated CAT activity to a heterologous promoter, consistent with previous reports that the CRE mediates regulation by depolarization for the human proenkaphalin and c-fos genes. However, the effects of verapamil and Bay-K 8644 on the synergy between cAMP and depolarization are significantly different between hCRHCAT and enkephalin-CAT reporter plasmids. These findings suggest that an equivalent stimuli can trigger multiple intracellular cascades that may act independently on different genes, having the same ultimate positive transcriptional effect. These independent transduction steps may be targets for regulation and could explain the specificity in induction of neural genes. (Supported by a NARSAD Young Investigator Award and NIH grant DK42730 to A.F.S.)

319.7

HEMORRHAGE-INDUCED *c-fos* PROTEIN EXPRESSION IN RELATION TO HPA AXIS ACTIVITY. K.V. Thrivikraman* and P.M. Plotsky, Clayton Fndn. Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037.

Mapping of *c-fos* protein expression was used to identify neural circuits activated by controlled venous hemorrhage (HEM; 14 ml blood/kgBW over 3 min) in awake male rats. In one group shed blood was retransfused at 10 min (HR), while in the second group blood was not retransfused (HN). A third group of time controls did not sustain blood loss (CON). Plasma ACTH and corticosterone (B) levels in sequential blood samples were determined by RIA. Groups of rats were perfused immediately before or at 30 min intervals after HEM. Coronal 30 µm frozen sections of the brain were cut and immunostained for nuclear *c-fos* protein. Some sections were also stained for cytoplasmic corticotropin releasing factor (CRF). HEM elicited time-dependent increases in plasma ACTH and B. In HR rats, ACTH decreased towards prestimulus values after retransfusion. In contrast, both ACTH and B peaked by 30 min in HN rats, then plasma ACTH decreased whereas B remained elevated. Following HEM, *c-fos* protein was initially visualized in the nucleus of the solitary tract (NTS; A2), a primary hemodynamic sensitive region of the brainstem. At 60 min, *c-fos* immunostaining was observed in cells of the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei. The number of Fos-positive cells in each of these regions was greater in HN rats than in HR rats. Interestingly, at 150 min post-HEM *c-fos* expression was noted in limbic areas (*e.g.* central nucleus of the amygdala, bed nucleus of the stria terminalis, hippocampal CA_3 region) of HN rats but was absent in the HR rats. Nuclear *c-fos* protein staining was co-localized with cytoplasmic CRF immunostaining in the central nucleus of the amygdala as well as in the PVN. These studies complement mapping of hemodynamically sensitive CNS pathways by other techniques and provide new insights into the sequele of their activation. Furthermore, these studies have identified neural circuits potentially involved in stimulus-dependent activation and modulation of hypothalamic-pituitary-adrenal function.

319.8

HIGH FREQUENCY OF HOMOLOGOUS RECOMBINATION AT THE MOUSE POMC GENE LOCUS IN EMBRYONIC STEM CELLS. M.A. Japón, M. Rubinstein and Malcolm J. Low*, Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201.

Proopiomelanocortin (POMC) generates a diverse family of peptides including ACTH, α-MSH and β-endorphin that may function as hormones, mitogens or neurotransmitters. To characterize the physiological role of individual POMC peptides in development and the unique contribution of β-endorphin to the total opioid activity of the brain we disrupted the entire POMC gene or selectively mutated β-endorphin coding sequences by homologous recombination in embryonic stem (ES) cells. The gene replacement vector POMCXX3 contains a 9.8 kb fragment of murine POMC genomic DNA. 0.5 kb of coding sequences were deleted at the 3' end of the third exon abrogating the production of any bioactive POMC peptides and replaced by a neomycin resistance gene (*neo*). To screen for homologous recombination events using the positive and negative selection method, a herpes simplex virus thymidine kinase gene (*tk*) was inserted at the 5' end of the mouse POMC gene fragment. Both *neo* and *tk* cassettes (kindly provided by Dr. P. Soriano) are under the transcriptional control of the phosphoglycerate kinase-1 promoter. The construction POMCX*4 is similar to the one described above but instead of having the 0.5 kb deletion, a single base was inserted in the codon for the N-terminal tyrosine of β-endorphin converting it to a premature termination codon (TAC to TAA). AB1 ES cells, grown on γ-irradiated SNL76/7 mouse fibroblasts (cell lines generously provided by Dr. A. Bradley), were electroporated in the presence of 25 µg of linearized plasmid. Double selection with 300 µg/ml G418 and 2 µM gancyclovir resulted in a two to three fold enrichment over G418 alone. Five positive clones per construction were detected by PCR from 150 double resistant colonies. Southern blot analysis using different probes revealed, in each case, that 4 clones had a single targeted event with the predicted restriction map. Thus, we obtained homologous recombination at the mouse POMC locus with a relatively high frequency of 1/30. Transgenic mice derived from these clones will be used to study the neuroendocrine physiology of the mammalian stress response and the role of β-endorphin in the development of the CNS and the hypothalamic-pituitary axis.

319.9

CHRONIC ANTIDEPRESSANT DRUG (AD) TREATMENT ATTENUATES HYPOTHALAMIC-PITUITARY-ADRENOCORTICAL (HPA) ACTIVITY IN THE RAT. **J.M.H.M. Reul* and F. Holsboer, Max-Planck-Institute of Psychiatry, Clinical Institute, Dept. Neuroendocrinology, 8000 Munich 40, Germany.**

In major depressive illness a defective HPA regulation is often observed, exemplified by increased basal HPA activity and an altered responsiveness in neuroendocrine challenge tests. We have hypothesized that a deterioration of HPA homeostasis may be an etiological factor in this mental disease. Recently, we have embarked on a study to investigate the effects of AD (i.c. amitriptyline) on the HPA system in order to gain insight in the mechanism of action of these drugs.

In rats, 5 weeks of amitriptyline treatment resulted in decreased basal levels of ACTH and corticosterone as well as a decreased adrenal weight. A decline in adrenal weight was not apparent after 2 weeks of AD treatment and no effects were found on body weight. In addition, AD treatment resulted in time-dependent and dose-dependent changes in brain corticosteroid receptor concentrations. As compared to controls, hippocampal mineralocorticoid receptor (MR) levels were elevated by 40-70% after 2 and 5 weeks of treatment. At 7 weeks, the increments in MR were largely diminished. Hypothalamic glucocorticoid receptor (GR) levels were increased by 20-25% at 5 weeks of treatment, but not at 2 or 7 weeks. We interpret these data as providing evidence for the notion that chronic AD treatment results in an attenuation of HPA activity. We postulate, in view of the role of MR and GR in HPA regulation, that AD-induced changes in brain corticosteroid receptor capacity may underlie the attenuation of HPA activity.

319.10

EVIDENCE AGAINST CHANGES IN CORTICOTROPH CRH RECEPTORS IN DEPRESSED PATIENTS. Elizabeth A. Young*, Huda Akil, Roger F. Haskett, and Stanley J. Watson, Department of Psychiatry and Mental Health Research Institute, University of Michigan Medical Center, Ann Arbor, MI 48109, U.S.A.

Previous studies by a number have investigators have documented a decreased ACTH and β-lipotropin/β-endorphin (β-End) response to ovine corticotropin releasing hormone (oCRH) in depressed patients. Since depressed patients demonstrate higher plasma cortisol concentrations at the time of oCRH challenge, it is difficult to determine if the decreased ACTH response is due to enhanced negative feedback of cortisol on ACTH release or an alteration in CRH receptors in depressed patients. To evaluate the response to oCRH in an "open feedback loop" system, we administered metyrapone, 750 mg, at 4 p.m. and 7:30 pm, followed by administration of 0.3µg/kg oCRH at 8 p.m. in 10 normal controls and 10 depressed patients. Administration of metyrapone at this time in the circadian rhythm clamped plasma cortisol concentrations to less than 2 µg/dl but did not result in rebound ACTH or β-End secretion. In control subjects, metyrapone administration produced a 90% blockade of the cortisol response as well as a 2-fold greater β-End response compared to administration of the same dose of oCRH without metyrapone. The 10 depressed patients and their matched controls demonstrated identical β-End response (integrated response for controls=319±63, for patients=325±86) and cortisol response (integrated response for controls=162±38, for patients=215±58) to oCRH following metyrapone pretreatment. These data confirm that corticotroph CRH receptors are normal in depressed patients, and that cortisol feedback plays an essential role in the abnormal ACTH and β-End response to oCRH in depressed patients.

320.1

EFFECTS OF RENAL DENERVATION AND REINNERVATION ON GANGLIONIC GENE EXPRESSION OF NEUROTRANSMITTER PROTEINS AND c-fos. T.L. Krukoff* and Y. Zheng. Dept. of Anatomy & Cell Biol., Fac. of Medicine, Univ. of Alberta, Edmonton, Canada T6G 2H7.

The molecular basis for the reno-renal reflex was studied in paravertebral ganglia (PG, T11 to L2) and the celiac-mesenteric (CM) plexus following kidney denervation to show changes in mRNAs encoding tyrosine hydroxylase (TH), neuropeptide Y (NPY), and c-fos. For TH and NPY, tissues were removed at 4, 14, 21, and 56 days after denervation of the left kidney; for c-fos they were removed at 1 and 4 hours after denervation and Northern blot analyses were carried out. Compared to control animals, levels of TH mRNA in the left PG and the CM plexus were decreased at 4 and 14 days, elevated at 21 days, and similar to controls at 56 days. In the right PG, TH mRNA levels were increased at 4 and 14 days, decreased from this level at 21 days, and similar to controls at 56 days. Changes in c-fos mRNA levels were present only at 1 hour after denervation and paralleled those described for TH. No changes in NPY mRNA were found in any of the ganglia at any stage in the experiment.

We conclude that kidney denervation leads to acute and chronic decreases in ipsilateral ganglionic levels of c-fos and TH mRNAs, respectively, and that levels of TH mRNA return to normal only after reinnervation is known to occur. Furthermore, increases in c-fos and TH mRNA in the contralateral PG, likely induced by the reno-renal reflex, show that the changes have an early onset and that they continue to operate until the compromised kidney is reinnervated.

Supported by the Medical Research Council of Canada.

320.2

ROLE OF SPINAL NMDA RECEPTORS IN THE REGULATION OF SYMPATHETIC NERVE ACTIVITY AND CONTROL OF ARTERIAL PRESSURE. Frank J. Gordon* Dept. of Pharmacology Emory University School of Medicine, Atlanta, GA 30322.

Rats were anesthetized with urethane, paralyzed and artificially ventilated. The NMDA receptor antagonist D-2-amino-7-phosphonoheptanoic acid (D-AP7; 100 nmol/10 ul) was delivered to the spinal cord via an intrathecal (IT) catheter. The rostral ventrolateral medulla (RVLM) was electrically stimulated (0.5 msec pulses, 100 uA) and sympathetic nerve activity (SNA) was recorded from the lumbar chain. Paired-pulse stimulation of the RVLM (150 trials) produced bimodal averaged sympathetic evoked potentials (total duration < 200 msec) that were little affected by IT D-AP7. However, in the same animals, IT D-AP7 virtually eliminated the sustained sympathoexcitation and pressor responses produced by 10 sec trains of RVLM stimulation. In other animals, 1500 msec stimulus trains (50 Hz) were delivered to the RVLM and sympathetic potentials were recorded. IT D-AP7 had little effect on the initial (100-200 msec) increase in SNA, but SNA rapidly diminished and could not be sustained for the remainder of the stimulus train. Additionally, blockade of spinal NMDA receptors had little effect on the baroreflex-mediated periodicity of SNA locked to the cardiac cycle, but abolished reflex-mediated increases in SNA evoked by lowering arterial pressure for 1-2 min with i.v. nitroprusside. These data indicate that synaptic activation of NMDA receptors in the spinal cord is necessary to support sustained increases in SNA that are required to elevate peripheral arterial blood pressure. (Supported by NIH grant HL36907 and American Heart Association)

320.3

SPINAL MECHANISMS UNDERLYING THE SYMPATHOEXCITATORY EFFECTS OF RAPHE PALLIDUS NEURONS. S.F. Morrison* Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Raphe pallidus stimulation elicits a long-latency excitatory potential in rat sympathetic nerves. This excitation appears to be mediated by raphe pallidus neurons which project to the thoracic spinal cord with slowly conducting axons. In the present study, characteristics which distinguish serotonergic neurons in other raphe nuclei were examined for these raphe pallidus neurons. The conduction velocities of the spinal axons of raphe pallidus neurons ranged from 0.4 to 0.8 m/s. The frequency with which such neurons were found to be spontaneously active was increased in unanesthetized, decerebrate rats in comparison to those anesthetized with urethane/chloralose. The rate of discharge of raphe pallidus neurons was slow (< 5Hz), and often characterized by very regular interspike intervals. Their activity was not synchronized to the cardiac cycle and was not altered by stimulation of the baroreceptor reflex during large increases in arterial pressure. Their activity was, however, completely inhibited following intravenous administration (4 g/kg) of 8-OH-DPAT, a 5-HT_{1A} receptor agonist. These results, similar to those in the cat (Brain Res. 477:172-182, 1989), suggest that sympathoexcitatory, raphe pallidus neurons are serotonergic. However, the additional finding that the long-latency excitation of splanchnic sympathetic preganglionic neurons following raphe pallidus stimulation was blocked by iontophoretic application of the glutamate receptor antagonist, kynurenic acid, indicates that the sympathoexcitatory effects mediated by raphe pallidus neurons are dependent on the activation of excitatory amino acid receptors in the intermediolateral nucleus. (Supported by HL 47196)

320.4

ELECTRICAL STIMULATION OF HINDLIMB AFFERENT FIBRES INHIBITS CAROTID SINUS BARORECEPTOR NEURONES IN THE CAT NUCLEUS TRACTUS SOLITARIUS (NTS). S.E. McMahon, P.N. McWilliam*, J. Robertson & J.C. Kaye. Cardiovascular Studies, University of Leeds, Leeds, U.K.

Hindlimb contraction produces an inhibition of the cardiac vagal component of the baroreceptor reflex and a rapid cardiac acceleration mediated by a withdrawal of vagal tone. These effects could be mediated by an inhibition of neurones in the baroreceptor reflex pathway. Experiments were designed to investigate whether baroreceptor neurones in the NTS are inhibited by electrical stimulation of hindlimb afferents in chloralose anaesthetized cats. Extracellular recordings were made from single neurones in the NTS that were activated by stimulation of the carotid sinus nerve. The evoked activity in 22/25 carotid sinus neurones was significantly inhibited by only a single or twin pulse to the ipsilateral peroneal nerve. In the majority of cases the inhibition was mediated by stimulation of group III afferent fibres only, although a small proportion also showed some degree of inhibition by groups I and II fibres. When the ipsilateral isolated carotid sinus was distended these neurones responded with bursts of firing, indicating that they were baroreceptor neurones. In a high proportion of these neurones the inhibition was antagonized by ionophoresis of the GABA antagonist bicuculline. Therefore, baroreceptor neurones in the NTS can be inhibited by peroneal nerve stimulation and this inhibition is mediated at least in part by GABA. This inhibitory mechanism maybe involved in mediating the cardiovascular response to exercise.

320.5

PARTICIPATION OF NMDA AND NON-NMDA RECEPTORS IN BAROREFLEX SIGNAL TRANSDUCTION IN NUCLEUS TRACTUS SOLITARII (NTS) OF RATS. H. Ohta* and W.T. Talman, Department of Neurology, The University of Iowa and VAMC, Iowa City, IA 52242

Excitatory amino acids (EAA) may be important neurotransmitters released from baroreceptor nerve terminals in the NTS. EAA receptors have been categorized by their major agonists as NMDA and non-NMDA receptors. Although, microinjection of EAA agonists into NTS produces depressor and bradycardiac responses similar to those induced by activation of the baroreflex, studies of the effect on the baroreflex of antagonists injected into NTS have been inconsistent. The present study is designed to determine if NMDA or non-NMDA receptors are involved in the baroreflex at the level of NTS. Baroreflex responses elicited by iv administration of phenylephrine and nitroprusside were tested before and after bilateral microinjection into NTS of MK801 (1 nmol) or DNQX (20 pmol) in rats anesthetized with chloralose. Activity of the baroreflex was assessed by sigmoidal curve fitting. Both MK801 and DNQX produced increases in arterial pressure and heart rate. The gain of the baroreflex expressed by the average slope of the reflex curve was similarly reduced by each antagonist:

		Before	After
MK801	(n=7)	-2.32 ± 0.30	-1.38 ± 0.30*
DNQX	(n=6)	-2.57 ± 0.25	-1.22 ± 0.25*

* p<0.05, comparison between before and after.

These results suggest that both NMDA and non-NMDA receptors are involved in baroreflex transmission in NTS. Support: VA Merit Review and Clin. Investigator(WTT), and NIH HL32205 and HL14388.

320.6

MECHANISMS OF PRESSOR AND BRADYCARDIC RESPONSES PRODUCED BY MICROINJECTION OF L-GLUTAMATE (L-GLU) INTO THE NUCLEUS TRACTUS SOLITARII (NTS) OF CONSCIOUS RATS. **B.H.Machado* and E.Colombari. Dept. of Physiology, School of Medicine of Ribeirão Preto, 14.049, Ribeirão Preto, Brazil.**

Microinjection of L-GLU (5nMoles/100nl) into the NTS of conscious rats (n=7) produced pressor (+37±4 mmHg) and bradycardic responses (-114±12 bpm). The pressor and bradycardic responses were sequentially blocked by prazosin (1mg/Kg,i.v.) (+7±3 mmHg and -116±8 bpm) and methyl-atropine (1mg/Kg,i.v.) (+8±4 mmHg and 0±0 bpm), respectively. Microinjection of L-GLU into the NTS after the blockade of glutamatergic receptors with kynurenic acid (10nMoles/100nl) (n=12) produced increase of pressure (+26±3 mmHg) and almost abolished the bradycardic response (-10±3 bpm). The results shown that L-GLU into the NTS produced increase of pressure and bradycardic response, which is not reflex in origin. The data on the pressor response suggest that L-GLU into the NTS is not involved in the reflex inhibition of efferent sympathetic activity, while the bradycardic response to L-GLU seems to be mediated by glutamatergic receptors. (Supported by FAPESP).

320.7

ARE ANGIOTENSIN II AND SUBSTANCE P RECEPTORS ON MEDIAL NTS NEURONS PRE- OR POSTSYNAPTIC? K. L. Barnes,* L. Qu and A. J. McQueeney. Dept. of Brain & Vascular Research, Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195-5286.

We previously discovered a group of medial nucleus tractus solitarii (nTS) neurons that are excited by both angiotensin (Ang) II and substance P (SP), and that Ang II releases SP from medulla slices. Vagal deafferentation suggested that some Ang II receptors in the nTS are presynaptic on vagal afferent fibers, while SP receptors are postsynaptic on intrinsic nTS neurons. Thus the cardiovascular effects of Ang II in the medial nTS might be mediated by Ang II-induced release of SP from vagal afferents. To determine the pre- or postsynaptic location of receptors that mediate the excitation of medial nTS neurons by Ang II or SP, we tested the capacity of each peptide to activate nTS neurons before and after blockade of synaptic transmission in the *in vitro* rat medulla slice. Blockade of synaptic transmission by low Ca^{++} artificial cerebrospinal fluid (aCSF; 0.2 mM Ca^{++}, 5 mM Mg^{++}) was verified by elimination of short latency spikes evoked by solitary tract stimulation, but not postsynaptic activation of the neuron by L-glutamate. Ang II-induced excitation was prevented in 12 of 26 medial nTS neurons when synaptic transmission in the slice was blocked. In contrast, low Ca^{++} aCSF failed to prevent excitation by SP in 25 SP-responsive cells. In 11 of 20 neurons excited by both peptides, low Ca^{++} aCSF blocked excitation by Ang II but not by SP; 9 cells continued to respond to both peptides. These findings suggest that a substantial proportion of nTS neurons excited by both Ang II and SP have presynaptic Ang II receptors and postsynaptic SP receptors, and may be the neuronal substrate for mediation of the cardiovascular effects of Ang II in the medial nTS via release of SP. (Supported by NSF BNS-9109673 and NHLBI HL-6835).

320.8

SOMATIC AFFERENT (SA) INPUTS TO CELLS IN THE NUCLEUS OF THE TRACTUS SOLITARIUS (NTS). S. W. Mifflin,* Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX. 78284-7764

Anatomical studies have shown that NTS neurons receive mono- and poly-synaptic inputs from SA fibers. To examine the integration of SA inputs within the NTS, intracellular recordings were obtained from 14 cells which responded to sciatic nerve (ScN) stimulation in pentobarbital anesthetized, mechanically ventilated and paralyzed cats. Cells responded to single pulse ScN stimulation (500uA, 1.0ms) with an EPSP (n=9; latencies = 7.3-21.8ms) or an EPSP/IPSP (n=5; latencies = 9.1-19.8ms). None of these PSPs exhibited the characteristics of a monosynaptic input. Higher intensity or train ScN stimulation (3-5 pulses, 20ms interval) evoked a longer latency (140-310ms) depolarization in 6 cells. A variety of sensory modalities were represented as cells were activated by manual flexion of the hindlimb (n=4), squeezing of skeletal muscle (n=3), lightly touching the skin (n=2), or pinching the skin (n=4). One cell was activated by both manual flexion of the hindlimb and pinching the skin between the toes. Four cells receiving a ScN input also responded to carotid sinus nerve stimulation with an EPSP (n=2) or an EPSP/IPSP (n=2). SA inputs to NTS neurons might provide a substrate for SA interactions with visceral afferent (e.g. baroreceptor and chemoreceptor) reflexes and might contribute to the reflex responses observed during activation of SA fibers. (Supported by NIH HL-36080)

320.9

NEUROTENSIN PROJECTIONS FROM NUCLEUS OF THE SOLITARY TRACT TO NUCLEUS AMBIGUUS. J. Ciriello* S. Roder and V. Badhwar . Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

We have previously shown that microinjections of neurotensin (NT) into the nucleus ambiguus (AMB) region in spinal animals elicit a vagal bradycardia. However, the sites of origin of the NT pathways to AMB are not known. In this study, experiments were done to investigate whether neurons in the nucleus of the solitary tract (NTS) containing NT innervate AMB in the rat, using the retrograde tracer Fluorogold (FG; 2%) and the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L; 2.5%) in combination with NT immunohistochemistry. FG (20-30nl) injections overlapping the AMB region resulted in retrogradely labelled neurons in NTS. Neurons within the caudal aspects of the dorsolateral (dl) subnucleus of NTS labelled with FG were found to contain NT immunoreactivity. Iontophoretic PHA-L injections within the dlNTS resulted in dense fiber and presumptive terminal labelling in the region of AMB. Using the double labelling immunofluorescence technique for identification of PHA-L and NT, a moderate number of PHA-L/NT fibers were found in and around the AMB. These data suggest that NT pathways from NTS maybe involved in controlling vagal cardiomotor neuronal activity and that these pathways likely function as central components of the baroreceptor reflex arc. (Supported by MRC of Canada and HSFO.)

320.10

EFFECT OF MICROINJECTION OF OPIATE PEPTIDES INTO NUCLEUS AMBIGUUS ON HEART RATE, T. X. Zhang* and J. Ciriello, Department of Physiology University of Western Ontario, London, Canada N6A 5C1

F8F amide (NFF), leu-enkephalin (l-ENK) and met-enkephalin (m-ENK) like immunoreactivity has been demonstrated in the region of nucleus ambiguus (AMB) that is known to contain vagal cardiomotor neurons in the rat. In this study, the effect of microinjection of opiate peptides into AMB on heart rate was investigated. Experiments were done in alpha-chloralose anesthetized and artificially ventilated male wistar rats that had their spinal cord cut at the level of C_2. Microinjections of 20 nl (50 pmol) of NFF into AMB elicited a bradycardia response (-61.4 ± 14.4 bpm) that was not significantly attenuated 5 and 60 min. after the administration of naloxone (10 mg/kg, i.v.) (-41.8 ± 6.3 and -42.7 ± 7.7 bpm, respectively). Microinjections of 20 nl (200 pmol) of l-ENK or m-ENK into AMB also elicited decreases in heart rate of -13.7 ± 1.6 and -23.6 ± 3.7 bpm, respectively. However, these bradycardic responses were blocked by the administration of naloxone. Microinjections of 20 nl (200 pmol) of l-ENK or m-ENK 5 min after the administration of naloxone elicited an increase in heart rate (+13.6 ± 2.3 and +19.8 ± 0.4 bpm, respectively). The responses to microinjection of l-ENK returned to control after 60 min. In addition, it was found that the decrease in heart rate elicited by a 20 nl injection containing 200 pmol of NFF (-76.5 ± 16.3 bpm) was significantly larger than those obtained at the same dosage of l-ENK and m-ENK. Taken together, these data suggest that AMB vagal cardiomotor neurons have receptors that are activated by NFF, l-ENK and m-ENK. These results also suggest that NFF activates receptors different to those of the other opiate peptides. (Supported by the MRC of Canada).

320.11

INNERVATION OF CARDIOMOTOR REGIONS OF THE NUCLEUS AMBIGUUS (AMB) BY DESCENDING PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS (PVH)-OXYTOCIN (OXY) CONTAINING FIBRES. E.T. Kiriakopoulos*, J. Ciriello and M.M. Caverson. Depts. of Anatomy and Physiology, Univ. of Western Ontario, London, Ontario N6A 5C1.

We have previously shown that approximately 5% of PVH neurons projecting to cardiomotor regions of AMB contain OXY. We have also shown that microinjections of OXY into AMB inhibit vagal cardiomotor neurons (VCN). However, it remains equivocal whether PVH-OXY fibres innervate vagal motor neurons (VMN) in AMB. The present study was done to provide a description of the relationship of PVH-OXY fibres to VMN in AMB. Experiments were done in pentobarbital anesthetized male Sprague Dawley rats. Phaseolus vulgaris leucoagglutinin (PHA-L; 2.5%), was iontophoresed into PVH. In the same animals the retrograde tracer Fluorogold (2%) was injected into the cervical vagus nerve to retrogradely label VMN. After 7-14 days the rats were perfused transcardially and forebrain and brainstem sections were processed for the identification of PHA-L and OXY using the double labelling immunofluorescence technique (Vecta). Fibres and presumptive nerve terminals double labelled with PHA-L and OXY were seen throughout the AMB cell column in the compact and external subdivisions. The projections were most dense at intermediate levels of the AMB, from the level of obex to 1.0 mm rostral to obex. PHA-L/OXY immunoreactivity was closely associated with VMN in the external formation of AMB, the principle site of VCN in AMB. These data support the suggestion that PVH-OXY neurons provide an input to cardiomotor regions of AMB and represent the anatomical substrate by which OXY may influence VCN function. (Supported by MRC)

320.12

INCREASED TRH GENE EXPRESSION IN MEDULLARY RAPHE NUCLEI BY HYPOTHYROIDISM: IMPLICATION IN THE REGULATION OF VAGAL OUTFLOW TO THE HEART. H. Yang*, V. Wu, G. Ohning and Y. Taché. CURE/VA Wadsworth Medical Center, Dept. of Medicine and Brain Research Institute, UCLA, Los Angeles, CA 90073.

Hypo- or hyper-thyroidism is accompanied with changes in vagal activity. TRH acts in medullary neurons projecting to the vagus to stimulate vagal activity. Using Northern blot analysis we found that thyroidectomy (Tx) increases medullary TRH gene expression in rats (Gastroenterology, 102:A767,1992). Tx decreased total T_4 serum levels (nmol/l) from 35±4 (control, n=5) to 9±1 (one week post Tx, n=5, $P<0.05$) and throughout the first 5 weeks in rats. TRH mRNA signal in brainstem was significantly increased by 70% (one week) or 2-3 fold (3-5 week) after Tx (n=3,4,4,4) compared with sham operated rats (n=9). This effect was reversed by daily injection of T_4 (4 μg/100 g, ip) in Tx rats. Rats with sham operation or Tx (30-40 days after the surgery) were fasted overnight and anesthetized with urethane. Heart rate (HR, beats/min) was measured by a polygraph through a PE-90 cannula inserted into the cervical artery. Basal HR was 377±25 and 282±7 ($P<0.05$) in sham operated and Tx rats respectively. Gastric distension performed by flushing the stomach with 10 ml saline reduces HR from 261±9 to 171±22 ($P<0.05$) for 4-6 seconds in Tx rats but not in control group. Kainic acid (96 ng/30 nl) injected into the raphe pallidus, where TRH mRNA neurons are located, reduced HR by 34% ($P<0.01$) in Tx rats at 5-15 min post injection period. In control group, HR was not changed. Bilateral microinjection of TRH polyclonal antibody (24 μg/100 nl/side) into the nucleus ambiguus which sends parasympathetic projections to the heart and contains TRH immunoreactive terminals, prevented the decrease in HR in Tx rats. These results suggest that hypothyroid state enhanced medullary TRH gene expression and TRH activity in the nucleus ambiguus which may account for the bradycardia and enhanced vago-vagal reflex.

321.1

NITRIC OXIDE MEDIATES COMPONENTS OF NMDA AND GP120 NEUROTOXICITIES IN PRIMARY STRIATAL CULTURES. V.L. Dawson*, T.M. Dawson, G.R. Uhl, S.H. Snyder. *Lab. Mol. Neurobiol., ARC/NIDA, & Depts. Neuroscience and Neurology, Johns Hopkins School of Medicine, Box 5180, Baltimore, MD. 21224.*

Nitric oxide (NO) mediation of NMDA toxicity in primary neuronal cultures from cerebral cortex and hippocampal slices is supported by studies utilizing NO synthesis inhibitors, NO releasers, and inactivators. We now document roles for NO in neuronal death induced in primary striatal neuronal cultures by NMDA and the HIV coat protein, GP120. In cultures assessed 24 hr following a 5 min exposure to either NMDA (300 μM) or GP120 (100 fM), 60% of neurons fail to exclude trypan blue; fewer than 5% of control cells stain. More than 60% of NMDA toxicity and 50% of toxicity due to GP120 is prevented when NO synthesis inhibitors or inactivators are coapplied to the cells; this blockade is reversible by excess substrate. More modest roles for non-NMDA receptors and voltage-dependent calcium channels in GP120 toxicity were supported by 30-40% reductions in toxicity by NBQX and nifedipine, respectively. These results document important roles for NO, as well as non-NMDA components, in mediating GP120 neurotoxicity. If such mechanisms apply *in vivo*, drugs blocking NO synthesis might slow progression of HIV encephalopathy.

321.2

NITRIC OXIDE SYNTHASE: NMDA AND THE IMMUNOSUPPRESSANT, FK-506, MODULATE PHOSPHORYLATION, FUNCTION AND NEUROTOXICITY T.M. Dawson,*[1] J.P. Steiner,[1] V.L. Dawson,[2] G.R. Uhl[1,2] and S.H. Snyder[1]. [1]Depts. Neurosci. & Neurol., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205 & [2]Lab. Mol. Neurobiol., ARC/NIDA, Baltimore, MD 21224.

Potential modulation by NMDA of the phosphoprotein nitric oxide synthase (NOS) is of interest due to the central role of NO in NMDA-mediated neurotoxicity. Phosphorylation can be modulated by NMDA receptor-mediated calcium fluxes through activation of the calcium-dependent phosphatase, calcineurin. Since phosphorylation can influence the function of many brain proteins, we have examined possible roles for NMDA induced calcium-modulated phosphorylation of NOS. In primary cerebral cortical cultures, NMDA stimulated formation of cGMP via activation of NOS is completely attenuated by 100 nM FK-506, a potent immunosuppressant that interacts with immunophilins to inhibit calcineurin. In contrast, FK-506 fails to inhibit cGMP elevations by NO releasers. Nearly all of the toxicity induced by a 5 min exposure to 500 μM NMDA, assessed 24 hr later by trypan blue exclusion, can be blocked by coapplication 100 nM FK-506. Attenuation of NMDA toxicity by FK-506 occurs in a dose dependent manner with protection occurring with concentrations of FK-506 as low as 100 pM. These results suggest a central role for NOS phosphorylation in mechanisms of NO-mediated NMDA toxicity. If such mechanisms apply *in vivo*, clinically-useful drugs like FK-506 which may inhibit NOS dephosphorylation could alter NO-mediated mechanisms implicated in excitotoxic and neurodegenerative disorders.

321.3

CHRONIC GLUTAMATE TOXICITY IN MOTOR NEURONS FROM ORGANOTYPIC SPINAL CORD CULTURES. RW Kuncl*, Lin Jin, and JD Rothstein. Johns Hopkins University, Dept Neurology, Baltimore, MD 21205.

The pathogenesis of motor neuron death in amyotrophic lateral sclerosis (ALS), is unknown. ALS has been characterized neurochemically by a derangement in the control of neurotransmitter glutamate metabolism: CSF levels of glutamate and aspartate are elevated and their high affinity transporter is defective in brain and spinal cord. Inefficient glutamate transport, and subsequent chronic increase in extracellular glutamate, could be responsible for motor neuron death. To test the hypothesis that chronic defects in glutamate uptake can produce motor neuron toxicity, we developed a tissue culture model employing organotypic rat spinal cord maintained with chronic inhibition of glutamate uptake. Slices (300 μm) of lumbar spinal cord from 8-14 day old rat pups were cultured on Millicell membranes. Using this method, organotypic cultures could be maintained with good morphology for greater than 60 days. Chronic inhibition of glutamate uptake was produced by culturing tissue in the presence of threohydroxyaspartate (THA) or pyrrolidine-dicarboxylic acid (PDC), both specific inhibitors of glutamate transport. THA produced chronic elevation of glutamate in the medium and produced motor neuron toxicity after 25-30 days in culture using 100 μM THA, and after 18 days using 500 μM THA, as determined by assay of tissue choline acetyltransferase (ChAT) activity and by histological analysis of one micron plastic sections. PDC produced similar results. Motor neuron toxicity was completely blocked by the non-NMDA antagonists CNQX and NBQX (10-50 μM), but not by the NMDA antagonists MK-801 and CPP (10-50 μM). Tetrodotoxin blocked THA mediated toxicity, suggesting that presynaptic release was required for cell death. This model demonstrates that the chronic loss of glutamate transport in ALS can produce motor neuron degeneration and that, in organotypic cultures, motor neurons appear to be susceptible to non-NMDA mediated glutamate toxicity.

321.4

IMPAIRED MITOCHONDRIAL METABOLISM REFLECTS THE STRIATAL PATHOLOGY IN HUNTINGTON'S DISEASE. Robert J. Ferrante*, Neil W. Kowall, Emmanuel Brouillet, and M. Flint Beal. Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114

A recent hypothesis in Huntington's disease (HD) suggests that abnormalities in the electron chain may secondarily lead to slow excitotoxic neuronal death by making neurons more vulnerable to endogenous glutamate. The striatal pathology of HD is characterized by relentless neuronal loss and astrogliosis, with selective involvement of spiny projection neurons. Dysmorphic proliferative changes are an early marker of neuronal dysfunction in HD. The systemic administration of the succinate ubiquinol oxidoreductase (Complex II) inhibitor, 3-nitropropionic acid, resulted in specific bilateral striatal lesions in rodents. Combined Nissl/NADPH-diaphorase stains demonstrated differential sparing of aspiny neurons, while GFAP immunochemistry identified marked gliosis. The severity of the pathology was dose dependent. The lesion loci were primarily within the rostral dorso-lateral aspect of the striatum. Golgi staining of lesioned striata identified proliferative dysmorphic alterations in spiny striatal neurons. Antisera against neural cell adhesion molecule and microtubule-associated protein 1B, markers of neurite outgrowth, were increased throughout the lesion site in both the neuropil and surviving neurons. These alterations resemble the pattern of neuronal degeneration observed in HD and strengthen the possibility that there may be a primary defect in mitochondrial energy metabolism in this disorder.

321.5

NMDA RECEPTOR-INDUCED MEMBRANE FLUIDITY CHANGES MAY MODULATE CALCIUM INFLUX IN CORTICAL NEURONS L.L. Dugan*, V.M.G. Bruno, S.M. Amagasu, D.W. Choi, and R.G. Giffard Depts of Anesthesia and Endocrinology, Gerontology, and Metabolism, Stanford University School of Medicine, Stanford, CA 94305; Dept. of Neurology, Washington Univ., St. Louis, MO 63110

Arachidonic acid is released from cortical neurons following N-methyl-D-aspartate (NMDA) receptor stimulation, and has recently been shown to alter NMDA calcium currents (Miller et. al. 1992 Nature **355**:722). Transient alteration of the lipid environment is a possible mechanism for this effect. We have documented significantly increased membrane fluidity following 10 minute exposure of cortical neuronal cultures to 500μM NMDA. Fluorescence anisotropy (r) was decreased by 7% in the NMDA treated samples (n=6, $p<0.05$), indicating a more fluid, less restricted membrane environment for the receptor. In preliminary experiments, ^{45}Ca accumulation was increased in cultures pretreated with arachidonic acid (200μM) or with another polyunsaturated fatty acid, 22:6 (200μM), and this increase was not seen when 50μM diacylglycerol (diolein) was included in the preincubation. The samples treated with arachidonic acid or 22:6 were more fluid than samples exposed to NMDA alone, an effect which was normalized by the co-incubation with diacylglycerol. These results indicate that NMDA receptor stimulation can alter the physical state of the membrane, and that NMDA-induced fluidity changes could be responsible for subsequent augmentation of calcium current after initial stimulation.

321.6

THE PUTATIVE ESSENTIAL NUTRIENT PQQ PROTECTS AGAINST NEURONAL DAMAGE IN AN *IN VIVO* STROKE MODEL. G. Gardner[1]*, P.M. Gallop[1], E. Aizenman[2], P.A. Rosenberg[1] and F.E. Jensen[1]. [1]Children's Hospital and Harvard Medical School, Boston, MA 02115, and [2]Dept. Physiology, University of Pittsburgh Medical School, Pittsburgh, PA 15261.

We have previously shown that the soluble redox cofactor pyrroloquinoline quinone (PQQ) blocks NMDA receptor mediated cell damage in cortical cultures. This protection *in vitro* appears to be caused by direct oxidation of the redox modulatory site on the NMDA receptor. In the present study, we investigated the effects of PQQ pretreatment on neuronal damage in an *in vivo* model of stroke employing a combination of bilateral carotid ligation and hypoxia in immature rats aged postnatal day (P) 7-9. Three to 4 hours after bilateral carotid ligation, rats were treated with either PQQ (10mg/kg i.p.) ($n=19$) or vehicle ($n=14$) 30 min before exposure to hypoxia (10% O_2 for 30 min or 8% O_2 for 25 min). After 48 hours of recovery, the animals were sacrificed and transcardially perfused with 4% paraformaldehyde in PBS. The brains were serially sectioned and stained with cresyl violet. A blinded light microscopic analysis was performed for each of the 2 hypoxic conditions (10%O_2 - moderate, and 8%O_2 - severe) to compare PQQ-treated animals with vehicle-treated littermate controls. In 17/19 matched pairs, the infarct size in the PQQ-treated animal was significantly smaller than the control, and in 2/19 pairs the infarct was judged as the same for the PQQ- and vehicle-treated animals. These data show a significant effect of PQQ treatment in reducing stroke size compared to vehicle-treated controls ($p<0.001$). In addition, the PQQ-treated animals did not show any behavioral side effects at the dose used in this study.

321.7

THIAZIDE DIURETICS REVERSIBLY BLOCK POSTSYNAPTIC GLUTAMATE RECEPTOR DESENSITIZATION IN RAT HIPPOCAMPAL NEURONS. K.A. Yamada*. Deptartments of Pediatrics and Neurology, Washington University School of Medicine, St. Louis, MO 63110

Recently we reported that diazoxide (DZ), a benzthiadiazide drug, reversibly blocked rapid, postsynaptic glutamate (GLU) desensitization in rat hippocampal neurons and thereby enhanced non-NMDA mediated glutamatergic synaptic responses (Yamada and Rothman, *J. Physiol.*, in press). Thiazide diuretics are also benzthiadiazide drugs. These experiments were performed to determine if drugs chemically similar to DZ also block GLU desensitization, using voltage-clamp recording from cultured rat hippocampal neurons and rapid, whole-cell application of quisqualate (QA). QA currents were increased by cyclothiazide (CYZ), hydroflumethazide, trichlormethiazide, hydrochlorothiazide, bendroflumethazide, and DZ. Benzthiazide and chlorothiazide did not increase QA currents, nor did chemically similar non-thiazide drugs quinethazone, chlorthalidone, metolazone, or sulfadiazine. CYZ was the most potent, virtually eliminating desensitization at micromolar concentrations. The steady-state QA current was increased 10-15 fold and the peak QA current 3 fold by 10-100μM CYZ, with EC_{50}s of 0.3μM and 2μM respectively. In addition, 10μM CYZ potentiated spontaneous miniature excitatory postsynaptic currents (mEPSCs). In conclusion, certain benzthiadiazide drugs ("neuroactive thiazides") potently block GLU desensitization. CYZ's effects upon mEPSCs is similar to that observed for DZ on evoked EPSCs, and indicates that these drugs also affect responses produced by synaptically released GLU at synaptic GLU receptors. The activity of several chemically related drugs, and the apparent high affinity, concentration-dependent effect of CYZ suggests that these drugs may act at a novel modulatory site that regulates desensitization of non-NMDA receptors and modulates glutamatergic synapses. (Supported by NIH grant NS01443).

321.8

DESENSITIZATION CHANGES THE PHARMACOLOGY OF GLUTAMATE NEUROTOXICITY. A.M. Moudy, K.A. Yamada and S.M. Rothman*
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In a variety of experimental paradigms, glutamate (Glu) neurotoxicity is largely mediated by activation of NMDA receptors. Even prolonged exposure of cultured hippocampal neurons to Glu is tolerated if NMDA antagonists are present. We have recently shown that some thiazides can dramatically diminish rapid Glu desensitization in voltage-clamp experiments. We now demonstrate that in the presence of these compounds, Glu toxicity is antagonized by an AMPA/kainate antagonist, CNQX.

When cultured hippocampal neurons were exposed to 500 μM Glu for either .5 or 18 hours, over 90% of the cells died. Addition of 10 μM MK-801, an NMDA antagonist increased survival to about 70%. Addition of either 500 μM diazoxide or 10 μM cyclothiazide diminished survival to less than 20%. Addition of 20-50 μM CNQX increased survival back to at least 70%. Neither thiazide was toxic in the absence of Glu. Thiazides which failed to potentiate Glu currents in voltage-clamp experiments had no effect on Glu toxicity. The organic calcium channel antagonists nifedipine and nimodipine did not duplicate the CNQX effect.

In voltage-clamp experiments, CNQX reduced cyclothiazide-enhanced Glu currents, but did not reduce steady-state Glu currents below control values. We also used the calcium-sensitive dye fura-2 to measure the effect of cyclothiazide on intracellular calcium (Ca_i) levels in hippocampal neurons. Cyclothiazide did not elevate Ca_i above the peak level produced by Glu + MK-801 when added during exposure to the latter substances. Neurons exposed to Glu while incubating in cyclothiazide + MK-801 did not have higher Ca_i than cells exposed to Glu in MK-801 alone.

These experiments indicate that desensitization dramatically reduces the toxicity of Glu and that there are circumstances when Glu toxicity can be blocked by AMPA/kainate antagonists. These results offer a potential explanation for recent favorable outcomes in neuroprotection experiments which utilized an AMPA/kainate antagonist.

321.9

NEUROTOXICOLOGICAL EVIDENCE FOR A NOVEL BENZODIAZEPINE SITE WITHIN THE NON-NMDA GLUTAMATE RECEPTOR COMPLEX JW Olney*, MT Price, CF Zorumski, K Yamada . Washington University, St. Louis, MO 63110

Non-NMDA glutamate receptors typically are responsive to either quisqualate (Quis) or kainic acid (KA), but when these agonists are continuously applied, the response to Quis is rapidly desensitizing and to KA is non-desensitizing. Recently a diuretic drug, cyclothiazide, was shown to convert the Quis response to a KA-like non-desensitizing response (Yamada, NS Abst, 1992). Tarnawa et al have described a benzodiazepine, GYKI 52466 (GY), with muscle relaxant properties and apparent antagonist activity at the non-NMDA receptor. In the present experiments, the neurotoxic responses to Quis and KA in the isolated chick embryo retina were blocked by GY at concentrations of 25 μM and 75 μM respectively, whereas the neurotoxic action of NMDA was not blocked by Gy at concentrations up to 250 μM. The neuroprotective action of GY was non-competitive and could not be overcome by 10-fold increases in the concentration of Quis or KA. Against simulated ischemia (oxygen/glucose deprivation), neither the NMDA antagonist MK-801 (1 μM) nor GY (50 - 200 μM) provided more than equivocal protection, but when the two agents were combined (MK-801 at 1 μM + GY at 50-200 μM), they provided complete protection. The antagonist action of GY against Quis neurotoxicity was counteracted by cyclothiazide, and the mechanism of this interaction was competitive in that the action of cyclothiazide was easily overridden by increasing the concentration of GY. These findings, together with those in a companion study (Zorumski et al., NS Abst, 1992), suggest that the non-NMDA receptor ion channel complex may contain a previously unsuspected benzodiazepine recognition site where GY acts to facilitate the desensitizing properties of this receptor and cyclothiazide, a structurally similar molecule, exerts an opposing action. This putative recognition site represents a promising new target for rational development of drugs to treat neurological disorders. (Supported by RSA MH 38894 (JWO).

321.10

ELECTROPHYSIOLOGICAL EVIDENCE FOR A NOVEL BENZODIAZEPINE SITE WITHIN THE NON-NMDA GLUTAMATE RECEPTOR COMPLEX C.F. Zorumski*, K. Yamada, S. Mennerick, & J.W. Olney, Depts. of Psychiatry and Pediatrics, Washington Univ. Medical School, St. Louis MO 63110

The non-NMDA class of glutamate receptors desensitize rapidly when activated by quisqualate (Quis) or AMPA but not when activated by kainate (KA). Certain lectins, aniracetam and thiazide derivatives inhibit this desensitization, but agents augmenting desensitization have not been described. GYKI 52466 (GY) is a novel benzodiazepine (BDZ) which is reported to be an antagonist at non-NMDA receptors. We have investigated the actions of GY on amino acid-gated currents in cultured postnatal rat hippocampal neurons using whole-cell voltage clamp recordings.

At concentrations > 1μM, GY non-competitively inhibits currents gated by KA and Quis with an EC_{50} against KA of 14μM. The EC_{50} for the competitive non-NMDA antagonist, CNQX, is 2μM. When coapplied with KA, GY depresses steady-state currents to a greater extent than peak responses, imparting a desensitizing appearance to the normally non-desensitizing KA current. GY also depresses KA/AMPA responses when applied before the agonist. The effects of GY are weakly voltage-dependent with no effect on the KA equilibrium potential. GY does not alter currents gated by NMDA or GABA. The effects of GY can be overcome by cyclothiazide (CYZ), an agent which inhibits non-NMDA receptor desensitization. GY and CYZ appear to interact competitively suggesting a shared site of action. These observations suggest that desensitization of non-NMDA receptors can be modulated by drugs acting at a novel BDZ site.

321.11

MEMANTINE, A CLINICALLY-TOLERATED NMDA OPEN-CHANNEL BLOCKER, PREVENTS HIV COAT PROTEIN-INDUCED NEURONAL INJURY *IN VITRO* AND *IN VIVO*. Stuart A. Lipton* and Frances E. Jensen. Depts. of Neurology, Children's Hospital, Beth Israel Hospital, Brigham & Women's Hospital, Massachusetts General Hospital, and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Studies on mixed neuronal-glial cultures from rodent brain and retina suggest that at least part of the neurological manifestations of AIDS may stem from neuronal injury mediated indirectly by the HIV-1 coat protein gp120 (reviewed in *Trends in Neurosci.* 1992;15:75-79). Concurrent activation of NMDA receptors is necessary for gp120 to induce neuronal damage, and NMDA antagonists or Ca^{2+} channel antagonists can ameliorate this injury *in vitro*. In the present study, stereotactic injection of gp120 (5-10 pmoles, *n*=5), but not control glycoprotein, into rat pup cortex produced a focal lesion by 2-10 d consisting of foamy macrophages and neuronal damage. Memantine, a drug that blocks NMDA receptor-operated ion channels, was studied for possible protective effects both *in vitro* and *in vivo*. Memantine, a more potent congener of the anti-viral drug amantadine, has been employed clinically as an anti-Parkinsonian agent in Europe for over ten years with therapeutic levels of 2-12 μM. In rat retinal cultures, 2 μM memantine prevented gp120 (20 pM)-induced injury of identified ganglion cells. In the *in vivo* gp120 rat model, either nimodipine (400 μg/kg, ip, *n*=3) or memantine (20 mg/kg, ip, *n*=2) was protective. Taken together, these data suggest that memantine, like nimodipine, has therapeutic potential as a channel antagonist capable of ameliorating neuronal injury associated with exposure to gp120.

321.12

MPP$^+$ INDUCES NEURONAL DEGENERATION IN THE SUBSTANTIA NIGRA PARS COMPACTA BY AN EXCITOTOXIC MECHANISM. Rachana Srivastava, Emmanuel Brouillet, Elsdon Storey*, M. Flint Beal and Bradley T. Hyman. Neurology Service, Mass. General Hospital, Boston, MA 02114.

The neurotoxic effect of MPTP on dopaminergic neurons is mediated by 1-methyl-4-phenylpyridinium (MPP$^+$) which is accumulated by the dopamine uptake system. The mechanism by which MPP$^+$ results in neuronal death may be due to its ability to bind to complex I, which inhibits mitochondrial energy metabolism, and may lead to secondary excitotoxic neuronal death. In the present study we examined neuronal cell death in the substantia nigra (SNc) following intrastriatal injection of 90 nmol of MPP$^+$. Histologic evaluation of the SNc at 1 week showed ipsilateral gliosis. Quantitative cell counts showed a 75% loss of SNc neurons, a 67% decrease in SNc volume, and a 26% decrease in neuronal density. Prior decortication significantly ($p<.01$) protected against these changes, and there was no significant decrease in either neuronal number or density (n=3). Administration of MK-801 for 48 hours by Alzet pump at a dose of 1.2 mg/kg/h also significantly protected against MPP$^+$ induced neuronal loss in the SNc ($p<0.05$) (n=3). These results show that MPP$^+$ induces neuronal degeneration in the SNc by an NMDA receptor mediated excitotoxic process. Supported by the Parkinson's Foundation.

322.1

DIAZEPAM WITHDRAWAL MODULATED BY GLUTAMATE ANTAGONISTS.

K.G. Steppuhn, H.H. Schneider , D.N. Stephens and L. Turski*. Res. Labs of Schering AG, 1000 Berlin 65, Germany.

Long term treatment leads to tolerance to and dependence on benzodiazepines. Adult NMRI mice, 25-30 g in weight, withdrawn from chronic treatment with diazepam (15 mg/kg/day for 12 days) showed a time related evolution of anxiety, muscle rigidity and seizures between days 4 and 21 after discontinuation of diazepam intake. A period between withdrawal days 1 and 3 was symptom-free. During this "silent phase" the susceptibility of mice to quisqualate and kainate seizures, and the magnitude of monosynaptic reflexes mediated by non-N-methyl-D-aspartate (NMDA) mechanisms, were enhanced. In apparent contrast, the "active phase" of withdrawal, between days 4 and 21, was characterized by an increased susceptibility of mice to NMDA seizures and enhanced magnitude of NMDA dependent polysynaptic reflexes. Treatment of mice with the NMDA antagonist CPP (3-((+)-2-carboxypiperazine-4-yl)-propyl-1-phosphonate; 1 mg/kg/day) during chronic administration of diazepam prevented the evolution of tolerance and dependence. The AMPA antagonist NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline; 10 mg/kg/h), but not the NMDA antagonist CPP (10 mg/kg/h) prevented or markedly reduced the evolution of withdrawal symptoms when administered by means of osmotic mini-pumps during the withdrawal days 1 to 3 ("silent phase"). In mice subjected to NBQX treatment during the first three withdrawal days, no muscle rigidity or seizures, and little anxiety were observed up to withdrawal day 21. These observations indicate that the non-NMDA-sensitive phase of withdrawal is short and symptom-free, but essential for triggering clinical symptoms. Interference with non-NMDA mediated mechanisms during the "silent phase" by using AMPA antagonists such as NBQX may offer a therapeutic approach for preventing the evolution of withdrawal symptoms after termination of benzodiazepine treatment. NMDA antagonists may prevent withdrawal when co-administered with the benzodiazepines.

322.2

DEVELOPMENT OF TOLERANCE AND WITHDRAWAL TO THE ACTION OF FULL AND PARTIAL ALLOSTERIC MODULATORS OF $GABA_A$ RECEPTORS.

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Rats receiving protracted daily doses of full (diazepam and alprazolam) and partial (clonazepam and bretazenil) allosteric modulators of $GABA_A$ receptors were tested for tolerance and withdrawal response. The brain content of DBI, the putative ligand of mitochondrial DBI receptors (MDR) was also studied. Benzodiazepines (BZs) were administered by oral gavage three times daily in anticonvulsant doses equipotent to 33 μmol/kg of diazepam. Doses of drugs were progressively increased by 50% every 4 days. After 21 days of treatment, rats allowed an 18 hrs drug free period, were challenged with single, anticonvulsant oral doses of the respective drugs and tolerance tested by seizures elicited with bicuculline infusion. Protracted treatment with diazepam and alprazolam but not clonazepam or bretazenil showed tolerance to the anticonvulsant effect of these drugs. For withdrawal response, rats were left free of drugs for 24 hrs after protracted treatment and then challenged with flumazenil (16.5 μmol/kg, i.v.) before behavioral tests with the Vogel paradigm. Protracted treatment with diazepam but not bretazenil showed a withdrawal effect characterized by an increased sensitivity to the behavioral inhibition of electric shock. Immunohistochemical estimation of DBI-LI content with DBI-antiserum in the cerebellum of rats receiving protracted diazepam and alprazolam, but not bretazenil or clonazepam, revealed a significant (2-3 fold) increase DBI-LI in the Bergmann glial which is not correlated with an increase GFAP-LI.

It can be inferred that tolerance and withdrawal effects that occurs with full but not partial allosteric modulators of $GABA_A$ receptors may be associated with an elevated glial content of DBI. This increase may mediate glial MDRs stimulation and thereby elicit neurosteroid biosynthesis; which may account for the $GABA_A$ receptor desensitization that occurs after protracted treatment with diazepam and alprazolam.

322.3

TRIAZOLAM IS A MAXIMALLY EFFICACIOUS ALLOSTERIC MODULATOR IN A BROAD SPECTRUM OF STRUCTURALLY DIFFERENT RECOMBINANT $GABA_A$ RECEPTORS.

I. Ducic, G. Puia, G. Mereu*, S. Vicini and E. Costa. FGIN, Georgetown University, Washington DC.

Tolerance, sedation, ethanol potentiation and ataxia are unwanted side effects of diazepam and other benzodiazepines (BZD), used in the treatment of anxiety disorders. With triazolam (Halcion) a sleep inducing triazolobenzodiazepine also psychotic symptoms, fast tolerance, amnesia, syncope and paranoid behavior have been reported (Oswald, I., 1991 Lancet, 338:516). In order to elucidate the reason for these severe side effects of triazolam, we compared the facilitatory efficacy of triazolam, diazepam, and other benzodiazepines of GABA induced Cl^- currents on native and recombinant $GABA_A$ receptors. Using the patch clamp technique we employed whole-cell recordings of GABA-activated Cl^- currents in human kidney tumoral cells transiently transfected with cDNAs encoding for various molecular forms of α, β and γ subunits of the $GABA_A$ receptors. Triazolam (10 μM) applied to various recombinant $GABA_A$ receptors showed a greater efficacy (T-test $p<0.05$) on receptors including $\alpha1\beta1\gamma1$, $\alpha1\beta1\gamma2$ and $\alpha1\beta1\gamma3$ subunits, whereas diazepam (10 μM) was more efficacious only on $GABA_A$ receptors assembled with $\alpha3\beta1\gamma2$. We also found that the same concentration of triazolam (10 μM) is the most efficacious (142 ± 8 %, mean ± SD, n=14 cells) in $\alpha1\beta1\gamma1$ subunit containing receptors when compared with similar concentrations of clonazepam (69 ± 10 %, n=6), zolpidem (58 ± 20 %, n=8), alpidem (49 ± 10 %, n=10) and bretazenil (50 ± 12 %, n=10). It seems possible to infer that the triazolam greater efficacy and broad spectrum of action on various molecular forms of $GABA_A$ receptors expressed in vivo (Wisden et al., 1992 J. Neurosci., 12:1043) may account for the peculiarities and severity of its side effects.

322.4

STRUCTURAL CHANGES OF THALAMO-CORTICAL CIRCUITS IN A RAT FOETAL ALCOHOL SYNDROME

D. Minciacchi(*), A. Granato(#), M. Santarelli and A. Sbriccoli Lab of Exp. Neurol., Inst. of Anatomy(#), Catholic University, 00168 Rome, Italy.

Prenatal exposure to ethanol is able to affect the normal development of the cortex in rats. Here we analyze the organization of thalamo-cortical circuits in adult animals exposed to ethanol during prenatal development.

Pregnant rats were given intragastric administrations of ethanol during the third week of gestation. Offsprings underwent either cortical (frontal areas Fr2 and CG1-CG3) or thalamic injections of WGA-HRP.

Results obtained from animals prenatally exposed to ethanol were compared with those from control cases. a) In the frontal cortex of animals prenatally exposed to ethanol a strong reduction of anterograde labeling was present as consequence of contraction of the thalamic-recipient layers. b) Densitometric analysis of the thalamus revealed a considerable reduction of the non-cellular labeling in offsprings of alcohol-dependent rats. c) The overall pattern of retrograde labeling was similar in both offsprings of alcohol-dependent rats and control cases.

We observed major structural changes of the normal organization of thalamo-frontal circuits in animals prenatally exposed to ethanol. The damage seems to involve specifically the terminal arborizations of both thalamo-cortical and cortico-thalamic fibers, sparing apparently their parent cell bodies.

These modifications could represent structural bases for some of the behavioural impairments observed in the human foetal alcohol syndrome.

322.5

ADRENALECTOMY POTENTIATES BEHAVIORAL DEPENDENCE TO ALCOHOL AND INDUCED CORTICAL HYPERVASCULARIZATION IN MALE RATS.

Ph. DE WITTE* and F. LAMBLIN. Lab. Psychobiology Univ. of Louvain, Louvain-la-Neuve, Belgium.

Adrenalectomized and sham-operated rats were exposed to chronic alcoholization using an inhalation procedure. After sejourning 4 weeks into the alcoholization chamber, rats were placed in an apparatus for motility detection and then submitted to a free-choice paradigm (water vs a 10% (v/v) ethanol solution). Another group of experimental rats were perfused with a nuclear emulsion allowing to reveal the cortical vascularization. No differences were observed between adrenalectomized and sham-operated animals in the motility recording for 20 hr after withdrawal of alcohol inhalation. The sham-operated rats presented an alcohol induced behavioral preference towards alcohol lasting 10 days following chronic alcoholization by inhalation while the adrenalectomized rats preferred the alcoholized beverage (mean of 25 ml/day 10% alcohol vs 10 ml/day water) until we sacrificed the rats, i.e. 75 days after withdrawal of inhalated alcohol. As regards the microvascularization of the frontal cortex, alcoholized adrenalectomized rats displayed significant enhanced cortical microvascular network when compared to alcoholized sham-rats. These data show thus that intact adrenals protect animals to behavioral dependence towards alcohol while alcohol impairs the adrenal response.

322.6

GENETIC DIFFERENCES IN THE EFFECTS OF ETHANOL: THEIR RELATION TO INHIBITORY AND EXCITATORY BRAIN NEUROTRANSMITTERS

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Behavioral effects of ethanol were examined in CBA, C57, and BALB mice. In BALB animals, increasing doses of ethanol had a biphasic action on locomotor activity (stimulation followed by inhibition) whereas only inhibition was seen in C57 and CBA mice. Ethanol produced a similar dose-dependent and preferential increase in the DOPAC/DA (dopamine) ratio in the limbic forebrain as compared to striatum in all animals suggesting no direct relationship between ethanol-produced enhancement of DA release and ethanol-induced locomotor stimulation. C57, and especially CBA mice were more sensitive to the sedative actions (sleep time) of ethanol than BALB mice. CBA mice were more sensitive to the convulsant effects of the GABA antagonist picrotoxin whereas no such differences were noted after administration of the specific GABA receptor antagonist bicuculline. In receptor binding studies the modulation of 3H-flunitrazepam binding by GABA and pentobarbital was similar in the forebrain of CBA and C57 mice. Further behavioral studies revealed that CBA mice differed in their response to non-competitive and competitive NMDA receptor antagonists as compared to C57 and BALB mice. In summary, these data suggest that genotypic differences are present in the GABA and glutamate receptor systems and that a complex interplay between various inhibitory and excitatory neurotransmitter systems may determine the pharmacologic profile of ethanol.

322.7

ETHANOL CAUSES INHIBITION OF GRANULE CELLS AND THE DEVELOPMENT OF ACUTE TOLERANCE IN CEREBELLAR SLICES. C.Huang*, C.-F.Hsiao and R.Huang. Univ. Missouri-Kansas City, Kansas City, MO 64110.

Ethanol (0.3-1.2g/kg, IV) caused severe inhibition of cerebellar granule cells in the cat. To determine whether this inhibition was due to the primary effects of ethanol on afferent structures or from direct ethanol actions on the cerebellar cortex, we have investigated the consequences of ethanol on cerebellar granule cells in brain slices. Cerebella from 35-day old rats were cut into sagittal sections. Single granule cells and granule cell clusters were recorded with tungsten or glass microelectrodes. Ethanol concentrations were varied from 5-50mM. Ethanol at 10mM was the lowest concentration which inhibited the spontaneous discharges of granule cells. Ethanol from 20-50mM produced a near-complete inhibition on some granule cells but had little or no effect on other granule cells or Purkinje cells. Inhibition or recovery took place in 2min or as soon as the control solution in the recording chamber was replaced by the ethanol solution or vice versa. In some granule cells, acute tolerance developed within 30-45min so that there was a significant recovery of spontaneous activity even when ethanol was present. We concluded that ethanol had a direct effect on the cerebellar cortex and that rapid development of tolerance occurred. (supported by PHS AA07643)

322.8

THE EFFECTS OF TWO ETHANOL DOSES ON EEG ACTIVITY IN MALES AT RISK FOR ALCOHOLISM. H.L. Cohen*, B.Porjesz and H. Begleiter, Neurodynamics Lab., SUNY Hlth Sci. Ctr., Bklyn, Brooklyn, N.Y. 11203

The present investigation examined the effects of placebo (P), low dose (LD) and high dose (HD) ethanol on EEG activity in adult males at high risk for the development of alcoholism (HR, N = 21, $\bar{X}$ = 22.8 yrs) and in matched, low risk (LR, N = 21, $\bar{X}$ = 22.7 yrs) controls. Only one condition (P, LD, or HD) was presented each day and condition order was randomized. For each subject, both blood alcohol level(s) (BAL) measured via breathalyzer, and EEG activity, using the entire 10/20 International System, were recorded prior to and at intervals of ca. 35, 70, 105 and 140 minutes after P, LD or HD administration. The Fast Fourier Transform (FFT) was used to calculate power spectral densities (PSD). Measures of relative area under the power spectral curve were obtained for each of the following frequency bands: slow alpha (SA, 7.5 - 10 Hz), fast alpha (FA, 10.5 - 13.0 Hz), slow beta (SB, 13.5 - 19.5 Hz) and fast beta (FB, 20 - 26 Hz) at electrodes: F3, F4, C3, C4, P3, P4, O1 and O2. The results of repeated measures MANOVA conducted on the normalized relative area values revealed significant changes in SA activity at each electrode examined. Risk Group differences in SA activity were obtained at F3, F4 and P4, and were due to the differential effects of ethanol rather than to baseline differences in SA activity. Risk Group differences were also observed in the relationship between SA activity and the phases of the blood alcohol curve. HR had significantly greater increases during the ascending phase (acute sensitization) and significantly greater decreases during the descending phase (acute tolerance).

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322.9

OPIATE SUPPRESSION OF THE PROESTRUS LUTEINIZING HORMONE SURGE IN RATS PRENATALLY EXPOSED TO ETHANOL. L.R. Nelson*, R. Poland, S Tritt, A.N. Taylor. Depts. of OB/GYN, Anatomy & Cell Biology, and Psychiatry, UCLA and West LA VAMC, Los Angeles, CA 90024.

Work in our laboratory has shown that adult, female, Sprague-Dawley rats, prenatally exposed to ethanol, have altered endocrinological and behavioral responses to opiate administration and to stress. For example, when these rats are either stressed with intermittent footshock or receive morphine, they have greater elevations in plasma corticosterone and ACTH than do control rats. Since the pituitary release of luteinizing hormone (LH) is known to be depressed by both stress and opiates, we assessed the effect of opiates on the proestrus LH surge in these rats. Timed-pregnant Sprague-Dawley dams were randomly assigned to three groups: Control (N), fetal ethanol-exposed (FAE), or pair-fed (PF). The ethanol dams were fed a liquid diet containing 5% w/v ethanol from gestational day 8 to delivery. With this regimen ethanol consumption averages 11-13g/kg/day and results in maternal blood alcohol levels of between 80 - 120 mg/dl. The pair-fed dams were given an isocaloric liquid diet without ethanol. Female pups were reared in group cages with the following light cycle: on-0500h; off-1900h. Testing was begun at approximately 120 days of age. Vaginal smears were checked in the morning across several cycles to determine the day of proestrus. On the morning of proestrus, rats were smeared and weighed. At 1400h morphine sulfate 10 mg/kg (MS) or saline was administered intraperitoneally (IP). At 1800h, blood was rapidly obtained by cardiac puncture under light halothane anesthesia. Compared to the N and PF groups, FAE rats had significantly lower LH levels following saline administration. All groups had diminished LH levels following MS, and FAE rats had the largest decrement (72.5%) compared to N (40%) and PF (50%). Thus, in this paradigm, FAE rats again demonstrate a potentiated response to MS. In addition, we suggest that the IP saline injection is a significant stressor and as such, depresses the LH levels in FAE rats.

LONG-TERM POTENTIATION III

323.1

CORRELATION BETWEEN IMMEDIATE EARLY GENE INDUCTION AND THE PERSISTENCE OF LTP. W.C. Abraham*[1], J. Demmer[2], S.E. Mason[1], P. Lawlor[3], J. Williams[2], M. Dragunow[3], and W.P. Tate[2]. Depts. of Psychology[1] and Biochemistry[2], Univ. of Otago, Dunedin and Dept. of Pharmacology[3], Univ. Auckland Medical School, Auckland, New Zealand.

Levels of immediate early gene (IEG) induction were correlated with the persistence of LTP across a number of tetanization protocols. Groups of awake rats, which had been chronically implanted with stimulating and recording electrodes in the perforant path and dentate hilus, respectively, were separately tested for LTP persistence (over days or weeks), IEG mRNA levels (20 min post-tetanization) using Northern blots, and IEG protein levels (2 h post-tetanization) using immunohistochemistry. Varying the number of stimulus trains (400 Hz) from 10-50 (on one day) resulted in a gradual increase in the persistence of LTP. The frequency distribution of decay time constants was bimodal, however, with maxima at 5.8 d (LTP2) and 30.7 d (LTP3). LTP was not as long lasting when 10 trains were delivered on consecutive days, compared to the same number of total trains given on one day. The IEGs *zif/268*, c-*jun*, *jun-B*, *jun-D* and Fos-related proteins all showed gradual increases in mRNA and protein levels as the number of trains given on one day was increased. *Zif/268* was the major responding gene after 10 trains, however, and was the only IEG showing repeated responses on consecutive days of stimulation. These results suggest that the pattern and degree of IEG expression influences the stabilization and subsequent maintenance of LTP over days or weeks.

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323.2

EVIDENCE FOR THE TRANSLOCATION OF PKC ISOZYMES BY PHORBOL ESTERS AND DURING THE INDUCTION PHASE OF LTP. Xiaolan Jiang*, Paul Osten and Todd C. Sacktor (SUNY-Brooklyn, NY, 11203)

Protein kinase C has been implicated in both induction and maintenance of LTP because many of the agents that inhibit PKC block LTP and because activators of the enzyme, such as phorbol esters, mimic the potentiation. To investigate the roles of PKC and the individual isozymes of the kinase in synaptic regulation, all known isozymes were assayed simultaneously by Western blot in rat hippocampal slices following conditions that enhance synaptic strength. Phorbol esters, at a dose (5 μM) that causes synaptic enhancement, translocated to membrane all isozymes of PKC in concert, a shift in subcellular distribution associated with the enzyme's activation. A brief high-frequency tetanus to Schaffer collaterals, which in parallel experiments induced LTP in 85% of healthy slices, was also tested. One min after tetanus, a translocation of several isozymes similar to that seen with phorbol esters was observed in CA1. Multiple isozymes of PKC, therefore, may be important in the short-term regulation of synaptic strength induced by synaptic activity. In the accompanying abstract evidence is presented for a role for a single isozyme in LTP maintenance.

323.3

EVIDENCE FOR A CONSTITUTIVELY ACTIVE FORM OF THE ζ ISOZYME OF PKC IN LTP MAINTENANCE. Paul Osten* and Todd C. Sacktor (SUNY-Brooklyn, NY, 11203)

Protein kinases appear to play roles in both induction and maintenance of LTP in the hippocampus, but the relationship and transition between these two phases is not understood. Protein kinase C (PKC) may be activated or modified following an initial translocation to membrane, resulting in a persistent phosphorylation that could contribute to maintenance. These modifications could include permanent insertion into membrane or proteolytic cleavage of the enzyme's regulatory domain, resulting in a constitutively active form of the enzyme. The analysis of PKC, however, is complicated by the heterogeneity of PKC isozymes, each of which may have a distinct role in neuronal function. We used carboxy-terminal antibodies to examine simultaneously by Western blot the known PKC gene family in CA1 of the rat hippocampus following tetanic stimulation of Schaffer collaterals that produced LTP. While most of the isozymes were down-regulated 30 min following tetanization, the ζ isozyme appeared to undergo limited proteolysis to a fragment that by molecular weight would be a constitutively active kinase, not regulated by transitory second messengers. This change may contribute to a mechanism for the maintenance of LTP.

323.4

PERSISTENT PKC ACTIVATION IN THE MAINTENANCE PHASE OF NMDA RECEPTOR-INDEPENDENT LTP (LTP_K). *C. M. Powell*, D. Johnston, & J. D. Sweatt*, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The use of N-methyl-D-aspartate (NMDA) receptor antagonist D,L-2-amino-5-phosphonovaleric acid (APV) has categorized LTP into two types: NMDA receptor-dependent (NMDA-R-*dep*) LTP and NMDA receptor-independent (NMDA-R-*indep*) LTP. We are interested in identifying potential molecular mechanisms for the maintenance of NMDA-R-*indep* LTP. LTP was elicited in area CA1 of intact rat hippocampal slices, in the presence of 50 μM APV, by application of 25 mM tetraethylammonium (TEA) for 10 min plus low frequency stimulation (0.05 Hz) of Schaffer-collaterals (LTP_K; Aniksztejn & Ben-Ari, Nature 349; 67-9). Population synaptic responses (pEPSPs) were potentiated during the 45 min period following TEA washout (pEPSP slope at 45 min 170 ± 12% of baseline, n=14). Slices were frozen and the region between the electrodes was microdissected. Kinase activity was assayed using a peptide substrate selective for protein kinase C (PKC), $neurogranin_{28-43}$. A significant increase in basal (Ca^{2+}-independent) PKC activity was observed with LTP_K (269 ± 74% of control, $p<0.05$). This increase in basal PKC activity was blocked by addition of the selective PKC inhibitor peptide $PKC_{(19-36)}$ (5 μM) to *in vitro* reactions (control 0.39 ± 0.01 pmol/min/μg, LTP_K 0.37 ± 0.08 pmol/min/μg, n=6). No change in Ca^{2+}/PS/DAG-stimulated PKC activity was observed (control 2.77 ± 0.34 pmol/min/μg, LTP_K 3.24 ± 0.34 pmol/min/μg, n=9). These results suggest persistent PKC activation as a mechanism for the maintenance of this form of NMDA-R-*indep* LTP. As persistent PKC activation has previously been described in NMDA-R-*dep* LTP (Klann et al, JBC 266; 24253-6), these two forms of LTP, distinct in their induction mechanisms, may converge on a common biochemical pathway in their maintenance phases.(MH44754)

323.5

LTP-ASSOCIATED PERSISTENT PKC ACTIVATION DETECTED USING A SELECTIVE PEPTIDE SUBSTRATE DERIVED FROM THE SEQUENCE OF NEUROGRANIN AND NEUROMODULIN S.-J.Chen*, E.Klann, and J.D.Sweatt, Division of Neuroscience, Baylor College of Medicine, Houston, Texas 77030.

We have previously observed that the maintenance phase of long-term potentiation (LTP) is associated with an NMDA-receptor dependent persistent kinase activation. Indirect evidence using an inhibitor peptide of PKC suggested the kinase persistently activated in LTP was protein kinase C (PKC). As an additional test for the presence of persistently activated PKC, we developed a selective PKC substrate peptide, $neurogranin_{(28-43)}$ ($NG_{(28-43)}$), and determined the phosphorylation of $NG_{(28-43)}$ in homogenates from control and LTP slices. The synthetic peptide has a sequence (AAKIQASFRGHMARKK) corresponding to the PKC phosphorylation site of native neurogranin (also known as RC3) and has a high degree of sequence homology to neuromodulin (GAP43/F1/B50). $NG_{(28-43)}$ was phosphorylated by purified PKC and PKM with a K_m of about 150 nM and the phosphorylation was sensitive to inhibition by the selective PKC inhibitor peptide $PKC_{(19-36)}$. No significant phosphorylation of $NG_{(28-43)}$ by cAMP-dependent protein kinase or CaM kinase II could be detected. Substituting the Phe^{35} residue with Ala resulted in a dramatic decrease in affinity (K_m 150 μM), indicating a crucial role of the sequence "SFR" in the binding of substrate to PKC. Substituting the Ser^{34} residue with Ala generated a PKC inhibitor peptide with Ki of 25 μM. $NG_{(28-43)}$ was phosphorylated in hippocampal homogenates in the presence of EGTA and addition of calcium and lipid cofactors greatly stimulated the phosphorylation of $NG_{(28-43)}$. We observed an LTP-associated increase (207±51% of control, n=9) in the phosphorylation of $NG_{(28-43)}$ in homogenates of area CA1 of hippocampus. The increased activity was expressed in the presence of EGTA, indicating an increase in calcium-independent PKC activity. The increased $NG_{(28-43)}$ phosphorylation was blocked by the addition of $PKC_{(19-36)}$ to the homogenates. These data strongly support the hypothesis that PKC is persistently activated in the maintenance phase of LTP. (Supported by McKnight and Klingenstein Foundations)

323.6

CHARACTERIZATION OF LTP-ASSOCIATED PERSISTENT PROTEIN KINASE ACTIVATION USING $NEUROGRANIN_{(28-43)}$, A NOVEL SELECTIVE PEPTIDE SUBSTRATE FOR PKC. E. Klann*, S.-J. Chen and J.D. Sweatt, Division of Neuroscience, Baylor College of Medicine, Houston, Texas, 77030.

We have previously described a Ca^{2+}-independent, persistent activation of PKC to be associated with the maintenance phase of LTP. To investigate the mechanism(s) underlying this phenomenon, we used a selective peptide substrate for PKC, $neurogranin_{(28-43)}$, as an exogenous substrate to assay PKC activity. LTP of the Schaffer collateral input into area CA1 of rat hippocampal slices was studied. Slices were frozen, dissected, and homogenized at various time points after the final tetanic stimulation. We detected an increase in Ca^{2+}-independent (basal) PKC activity 45 minutes after tetanus (203±28% of control, n=13). This persistent increase in PKC activity was inhibited by the NMDA receptor antogonist APV (50 μM; 113±12% of control, n=4). Control and LTP homogenates (45 min time point) were centrifuged at 133,000 g for 60 minutes and separated into pellet and soluble fractions. We detected a significant increase in basal PKC activity in the soluble fraction (177±38% of control, n=6). In contrast, no significant increase in the pellet fraction was observed. Control and LTP homogenates were also assayed 45 sec, 2 min, 5 min, 15 min, and 90 min following the final tetanus. We detected significant increases in basal PKC activity at 2 min (161±24% of control, n=10) and 5 min (184±35% of control, n=6). We detected slight, statistically nonsignificant increases at the other time points. 2 minute LTP homogenates also exhibited an increase in total PKC activity (assayed in the presence of Ca^{2+}, phosphatidylserine and oleoylacylglycerol, 174±18% of control, n=10). This finding was not observed at other time points, indicating the possibility of different mechanisms of PKC activation at different time points after tetanus. To begin to address this question, we performed Western blots for PKC using control and LTP homogenates at the 45 minute time point and observed an LTP-associated decrease in PKC immunoreactivity (77±7% of control, n=4). These data are consistent with a post-translational modification of PKC at later time points. Supported by an NIH NRSA (E.K.) and the McKnight and Klingenstein Foundations (J.D.S.)

323.7

PKC(19-13) AND CAMKII(273-302) GIVEN TOGETHER INTRACELLULARLY TO THE POSTSYNAPTIC NEURON SYNERGISTICALLY BLOCK LTP IN HIPPOCAMAPAL CA1 REGION. **T.P.Feng*and J.H.Wang. Shanghai Institute of Physiology, Chinese Academy of Sciences, Shanghai 200031, China**

Inhibition of postsynaptic protein kinase C (PKC) before or after the tetanus has been shown to block the induction or to arrest the maintenance of LTP in rat hippocampal CA1 region respectively (Wang and Feng, PNAS in press). We now report, first, that the specific inhibitor of Ca^{2+}/calmodulin dependent kinase II (CaMKII), CaMKII(273-302), given intracellularly to the postsynaptic neuron before or after the tetanus can likewise block the induction or arrest the maintenance of LTP respectively, and, second, that the specific inhibitor of PKC, PKC(19-31), and CaMKII(273-302) given together strikingly reinforce each other in the production of the inhibitory effects on LTP. This provides direct evidence that both the induction and the maintenance of LTP in the hippocampal CA1 region involve the joint activity of PKC and CaMKII in the postsynaptic neuron. [We thank Drs. Richard Tsien and Howard Schulman for the supply of CaMKII(273-302)]

323.8

MICE MUTANT FOR THE ALPHA CA/CALMODULIN KINASE II HAVE IMPAIRED LTP AND PLACE- LEARNING. Alcino J. Silva , Yanyan Wang@¶ Richard Paylor#, Jeanne Wehner#, Charles Stevens@ and Susumu Tonegawa .
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We have created mice lacking the alpha Ca/calmodulin kinase II (αCAMKII), a major synaptic kinase. Field potential as well as whole cell recordings in the CA1 region suggest that these mutant mice have severely deficient LTP, but normal neurotransmission. Furthermore, the ratio of NMDA to non-NMDA components, as well as the voltage dependence of the NMDA receptor are not affected in the mutant mice. Hence, these mice are an ideal model to study the behavior implications of normal synaptic transmission but deficient LTP. Mice lacking this kinase cannot learn the precise location of a hidden platform within the Morris maze, a sensitive task of hippocampal function. Behavioral analysis shows that the learning deficit is specific, and that it is not due to a motor impairment, a lack of motivation, an inability to see distal cues or recognize that they are relevant to solving the tests. Our results demonstrate that αCAMKII is essential for place-learning, and they strongly support the hypothesis that LTP is an electrophysiological phenomena underlying some forms of learning.

323.9

RECOMBINANT VACCINIA VIRUS INFECTION OF SELECTED TARGETS IN THE HIPPOCAMPAL SLICE AND LTP. D.L. Pettit* & R. Malinow. Neuroscience Program & Physiology and Biophysics Dept., Univ. of Iowa.

LTP is triggered postsynaptically in CA1 hippocampus but the mechanisms underlying its expression remain unclear. Here we describe a new technique that allows expression of recombinant proteins in anatomically selected neuronal populations, while maintaining intact synaptic circuitry and function. A vaccinia virus carrying a gene for ß-galactosidase (ß-gal) was used to infect selected neuronal populations in the rat hippocampal slice. Following extracellular application of virus in the CA3 or CA1 pyramidal cell body layer the slices are incubated overnight to obtain optimal expression of the gene product. ß-gal expression was confirmed by light microscopy after fixing, staining with X-gal, and xylene clearing of the slices. Robust X-gal staining is confined to presynaptic (CA3) or postsynaptic (CA1) injection sites, showing that there is no trans-synaptic infection during the incubation period. Individual cells stain in the apical and basal dendrites as well as a perinuclear ring. Cryostat sections (20μm) made from infected slices show that virtually 100% of the cells in the injected region are infected. Serial sections show uniform infection throughout the thickness of the slice.

We tested the effects of infection and overnight incubation on synaptic transmission and LTP. Whole-cell recordings from an infected region with the in vivo - fluorescent ß-gal marker CMFDG in the pipette confirmed the viability of infected cells. Synaptic transmission and LTP in infected cells were indistinguishable from control recordings. In other experiments, a pathway originating from an infected CA3 region showed LTP of comparable magnitude to that in an uninfected subiculum pathway. Subsequent histological processing revealed strong X-gal staining in the stimulated CA3 region. These control experiments show the feasibility of this technique. We are currently generating recombinant constructs to modify selected protein kinase activities to test their role in the presynaptic terminal.

323.10

A POTENTIAL ROLE FOR AN ADP-RIBOSYLTRANSFERASE (ADPRT) IN HIPPOCAMPAL LONG-TERM POTENTIATION (LTP). E.M. Schuman*, M.K. Meffert#, H. Schulman#, and D.V. Madison. *Dept. of Molecular & Cellular Physiology and Dept. of Pharmacology#, Stanford School of Medicine, Stanford, CA 94305.*

Previous studies have strongly suggested that nitric oxide (NO) production is necessary for the production of LTP in the CA1 region of the hippocampus. However, the molecular target(s) of NO and the mechanisms by which NO brings about synaptic potentiation remain to be elucidated. We have begun to examine these questions by asking if either of two established NO targets, a soluble guanylyl cyclase and a cytosolic mono-ADPRT, plays a role in the production of LTP. In initial studies we have examined whether a membrane permeant analog of cGMP in conjunction with strong presynaptic activity is sufficient to enhance transmission. Tetanic stimulation in the presence of APV (50 μM) and dibutyrl cGMP (100 μM) failed to potentiate synaptic transmission, although LTP could be elicited in the same pathway following wash-out of both agents. In addition, an inhibitor of cGMP-dependent protein kinase, H-8, applied extracellularly before tetanic stimulation failed to reduce LTP. Conversely, extracellular application of three different inhibitors of ADPRT, nicotinamide (NIC; 1 mM), luminol (LUM; 200 μM), and vitamin K (100 μM) were extremely effective blockers of LTP. We attribute the actions of these agents to inhibition of a mono- rather than poly-ADPRT since benzamide (100 μM; IC_{50} poly-ADPRT = 22 μM) did not block LTP. Also, in preliminary experiments postsynaptic injection of NIC did not prevent tetanus-induced LTP of one synaptic pathway, although subsequent bath application of NIC blocked LTP in a second pathway, suggesting that the critical site of ADPRT activity may be in the presynaptic neuron. Finally, in homogenized CA1 tissue incubated with [^{32}P]NAD+, the NO donor sodium nitroprusside (SNP; 300 μM) increased the ADP-ribosylation of ~45 kD and ~116 kD protein(s) and induced the ribosylation of a ~ 40 kD protein(s). The SNP-induced ribosylation of these proteins was blocked by NIC and LUM at the same concentrations that were effective in blocking LTP.

323.11

LTP-INDUCING TETANIC STIMULATION CAUSES A NITRIC OXIDE-MEDIATED INCREASE IN cGMP IN HIPPOCAMPAL AREA CA1. D.M. Chetkovich* and J.D. Sweatt. Div. Neurosci., Baylor Col. Med., Houston, TX 77030

Recent reports have suggested that nitric oxide (NO) may be necessary as a "retrograde messenger" traveling from the postsynaptic to the presynaptic cell in the induction of LTP. Hemoglobin, which absorbs NO in the extracellular space, and NO synthase inhibitors have been shown to block LTP. In light of these findings, we have undertaken a biochemical approach to determine if NO is a transcellular messenger in LTP. One known target of NO action is soluble guanylyl cyclase (GC); NO activates GC, leading to production of cGMP. We measured cGMP levels in area CA1 following LTP-inducing high-frequency stimulation (HFS, 100 Hz, 3x1 sec). We found that HFS in area CA1 leads to an increase in cGMP levels (control: .35±.01; HFS: .44±.03 pmol cGMP/ mg protein, n=8, p<.01). This increase was augmented in the presence of the phosphodiesterase inhibitor IBMX (control: 2.49+.12; HFS: 5.82±.18 pmol cGMP/mg protein, n=7, p<.001), indicating an effect of HFS on cGMP synthesis rather than degradation. Competitive inhibitors of NO synthase, N-methyl-l-arginine and l-nitroarginine, both blocked the increase in cGMP seen with HFS. The effects of both inhibitors were reversed by 1 mM arginine. Hemoglobin markedly attenuated the increase in cGMP seen with HFS, suggesting that NO can cross cell membranes to affect changes on GC. Taken together, these data implicate NO as the mediator of the increases in cGMP caused by HFS. Part of the increase in cGMP was NMDA receptor-dependent, as the NMDA receptor-antagonist APV (100 µM) attenuated the increase in cGMP observed with HFS. NO-mediated increases in cGMP were also dependent upon the presence of extracellular Ca^{++}, as EGTA (1 mM) blocked the increases in cGMP caused by HFS. Interestingly, a significant increase in cGMP was observed with HFS in the presence of 500 µM APV, 100 µM CNQX and 100 µM AP3, indicating that some production of NO may be occuring presynaptically, or postsynaptically via stimulation of non-glutamate receptors. In summary, these data support a role for NO as a transcellular effector in LTP, as well as implicate the cGMP pathway in the induction of LTP.

323.12

ROLE OF NITRIC OXIDE IN NMDA-RECEPTOR MEDIATED NEUROTRANSMITTER RELEASE IN HIPPOCAMPUS

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Nitric oxide (NO) has been implicated as a rapid, diffusible messenger required for the induction of long-term potentiation (LTP). The synthetic enzyme for NO (NOS) is localized in some hippocampal neurons and is activated by increased levels of intracellular calcium subsequent to NMDA receptor activation. We investigated whether NO can link postsynaptic NMDA receptor activation to changes in neurotransmitter release using synaptosomes from hippocampi of anesthetized adult guinea pigs. The ability of the synaptosomes to release preloaded radiolabeled neurotransmitter (^{3}H-D-aspartate or ^{3}H-L-norepinephrine) and endogenous neurotransmitter (L-glutamate) was evaluated by scintillation counting and luminometry, respectively. NMDA (100 uM) caused a large release of neurotransmitter: 60% of available glutamate and aspartate; 20% of available norepinephrine. Release of all three neurotransmitters was blocked in calcium free media and by the NMDA receptor antagonist 5-amino-phosphonovalerate (AP5). Release was also blocked if the synaptosomes were exposed to the competitive blockers of NOS, N^G-monomethyl-L-arginine (100 μM) or N^G-nitro-L-arginine (100 μM); the D-enantiomers did not block release. Moreover, extrasynaptosomal hemoglobin (Hb-20 μM) blocked the NMDA effect. The NOS block was reversible (via 1 mM L-arginine application) and did not effect neurotransmitter release subsequent to K^+ depolarization. Thus, NO may serve to link NMDA receptor activation to changes in neurotransmitter release from both presynaptic glutamatergic and catecholaminergic terminals in a local volume of tissue.

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PEPTIDES: PHYSIOLOGICAL AND BEHAVIORAL EFFECTS

324.1

NEUROTENSIN INDUCES SUSTAINED RHYTHMIC BURSTING ACTIVITY AND IS INTERNALIZED IN BASAL FOREBRAIN CHOLINERGIC NEURONS. A. Alonso*, M.P. Faure and A. Beaudet. Montreal Neurological Institute and McGill University, Montreal, QC, Canada H3A 2B4.

The cholinergic cells of the basal forebrain (BF) play an important role in the control of limbic and neo-cortical activity. Recent evidence indicates that most of these neurons are endowed with neurotensin (NT) receptors. Moreover, ICV application of NT has an awakening effect and enhances EEG theta activity (Latell et al, Peptides, 1989). In the present study we have investigated by intracellular recording in guinea-pig brain slices the effects of NT on the intrinsic excitability of BF (substantia innominata-horizontal limb of the diagonal band) neurons identified as cholinergic by a) their electrophysiological characteristics (Khateb et al, Soc. Neurosci. Abstr., 1992) and b) their ability to internalize NT (Faure et al, Soc. Neurosci. Abstr., 1992). The most salient electrophysiological characteristic of most of these neurons is their ability to generate a Ca-dependent low-threshold burst that can usually repeat 2-4 times when the cells are depolarized from their resting potential (close to -70 mV) to about -60 mV. Bath application of NT or fluorescein-labeled NT (NT-FTC) (1-100nM) caused a depolarization associated with a decrease in membrane conductance and triggered the development of a sustained rhythmic bursting activity (0.5-3Hz) with a robust enhancement of individual burst events. The depolarizing and oscillatory-inducing actions of NT persisted in the presence of TTX. Furthermore, confocal examination of NT-FTC responsive neurons that were intracellularly injected with biocytin revealed that they had internalized the neuropeptide as assessed by double fluorescence labeling. These data demonstrate a potent and direct neuromodulatory action of NT on the intrinsic excitability of BF cholinergic neurons suggesting that the peptide may thereby play a major role in the BF-mediated control of cortical arousal.

324.2

INTERNALIZATION OF NEUROTENSIN IN BASAL FOREBRAIN CHOLINERGIC NEURONS: A CONFOCAL MICROSCOPIC ANALYSIS. M-P. Faure*, A.Alonso, K.Leonard and A.Beaudet. Montreal Neurol Inst., McGill Univ.Montreal, Canada.

We have previously demonstrated an extensive association of specific high affinity neutotensin (NT) binding sites with acetylcholinesterase positive neurons in both rat and human basal forebrain. Furthermore, hybrid cells derived from medial septal cholinergic neurons were found to bind and internalize exogenous NT. To test the hypothesis that NT is similarly internalized in basal forebrain cholinergic neurons themselves, we have resorted to a fluorescein thiocarbamyl conjugate of NT (FTC-NT) shown to exert the same electrophysiological effects as unlabeled NT (Alonso et al, Soc. Neurosc. Abst.1992) and to retain high binding affinity for brain NT receptors. Slices (350µm thick) of guinea pig basal forebrain, maintained at 35°C in oxygenated Ringer, were preloaded with 100nM FTC-NT for 3 min, superfused with Ringer for 15,30,60 and 120 additionnal min, fixed with 4% paraformaldehyde and sectionned at 50µm for choline acetyltranferase (ChAT) immunostaining. Confocal laser microscopic (CLSM, Leica) examination of 25 optical sections taken at 0.25µm intervals showed intense specific intracellular FTC-NT labeling at all wash-out times. The labeling pattern was initialy diffuse and predominantly dendritic, becoming more granular and perikarial with time. At 120 min the label was mainly confined to the perinuclear area within endosome-like particules. Treatment with 0.2% triton for 15 min did not affect this pattern, confirming the intracellular localization of the label. Merged confocal images of perikarial FTC-NT and ChAT-Texas red immunofluorescence indicated that the label had been selectively internalized within ACh neurons. These results confirm the selective association of NT receptors with basal forebrain cholinergic neurons and demonstrate the existence of a somatodendritic internalization of the peptide within these cells.

324.3

PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) FACILITATES THE INDUCTION OF TYROSINE HYDROXYLASE (TH) BY NICOTINE IN BOVINE ADRENAL CHROMAFFIN CELLS (BAC). R.A. Rius, A.M. Szekely*, A. Guidotti, E. Costa. Fidia-Georgetown Institute for the Neurosciences, Georgetown University, School of Medicine, Washington, DC 20007.

PACAP is a novel peptide present with VIP in cholinergic neurons (Reg. Pept. 37: 287, 1992). Thus, PACAP, similar to VIP, might be a good candidate for acting as a cotransmitter of acetylcholine in the cascade of events operative in the transsynaptic induction of TH gene expression. The present study was designed to investigate whether nicotine and PACAP are cotransmitters in TH induction. In BAC, PACAP (10^{-10} to 10^{-7}M) produced a dose-dependent increase of TH *Vmax* when measured after 48 hrs of treatment. This effect was preceded by an increase in TH mRNA content that starts after 2 hrs and peaks at 12 hrs. A significant induction (35%) in TH was already observed with $5x10^{-10}$M PACAP and maximal increase (3 fold) was observed with 10^{-7}M PACAP. VIP and forskolin also induced TH in BAC, however, they were 3-4 orders of magnitude less potent and VIP also less efficacious. In BAC, TH can be induced after a short (20 minutes) pulse of exposure to PACAP. The potency of PACAP was increased in presence of the phosphodiesterase inhibitor HL 725 (trequinsin 10^{-5}M) indicating a role for cAMP in the TH induction by PACAP. In addition to increasing cAMP, PACAP increased the expression of several immediate early genes including c-*fos* and *jun-B*. Nicotine (10^{-6}M) applied for 48 hrs produced a modest increase (20-30%) in TH. Moreover, in the presence of nicotine, the action of PACAP or VIP in TH induction was facilitated. It remains to be studied whether PACAP and VIP facilitates TH induction triggered by splanchnic nerve stimulation.

324.4

COMPARISON OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) AND ACETYLCHOLINE (ACh) IN STIMULATING CATECHOLAMINE (CA) SECRETION FROM RAT ADRENAL GLAND. Xie Guo, John Haycock† and Arun R. Wakade*. Dept. Pharmacol. Wayne State Univ., Detroit, MI & †Dept. Biochem. Molec. Biol., Louisiana State Univ. Med. Ctr., New Orleans, LA

The role of vasoactive intestinal polypeptide (VIP) as a non-cholinergic neurotransmitter is well-established in rat adrenal medulla. PACAP is a new member of the VIP/secretin/glucagon family having 68% homology with VIP in the N-terminal 28 amino acid residues. High-affinity receptors for PACAP are present in the rat adrenal medulla, and we have previously demonstrated that stimulation of splanchnic nerves releases PACAP from perfused rat adrenal gland (basal, 47 $\pm$ 15 pg; stimulated, 130 $\pm$ 40 pg). Here we compared the stimulatory actions of PACAP and ACh on secretion of epinephrine (EPI) and norepinephrine (NE). CA secretion was increased by as little as 1-3 nM PACAP as opposed to 2-3 μM ACh. Secretions of CA upto 250 ng/4 min were produced by 1 μM PACAP whereas 30 μM ACh was required to produce similar increases. Different proportions of the CAs were released in response to PACAP vs. ACh. The ratio of EPI to NE was 4:1 for ACh but increased to 7:1 for PACAP. CA secretion returned to basal levels within 5 min after treatment with 30 μM ACh whereas the secretory response to 10 nM PACAP continued for over 30 min after removal of PACAP. Omission of Ca^{2+} (plus 1 mM EGTA) prevented the increases in CA secretion produced by either ACh or PACAP, and removal of Ca^{2+} from the perfusion medium reversed the post-treatment increase in CA secretion produced by PACAP. These date indicate that PACAP and VIP could both function as non-cholinergic transmitters in splanchnic nerve/chromaffin cell synapses.

324.5

IN SITU HYBRIDIZATION ANALYSIS OF PREPROTACHYKININ mRNAs IN DISCRETE AREAS OF THE RAT BRAIN : AN INVOLVEMENT OF TACHYKININS IN DIFFERENT NATRIOREXIGENIC TREATMENTS. P. Pompei, A.M. Magarinos*, J. Angulo and B. McEwen. Lab. of Neuroendocrinology, the Rockefeller University, New York, NY 10021.

Previous studies have shown that the bed nucleus of the stria terminalis (BNST) plays a major role as a site of action for the inhibitory effect of intracerebroventricularly (icv) injected tachykinins (Tks) on salt appetite. This investigation examined the presence and abundance of preprotachykinin (PPT) A-gene mRNA transcripts in BNST and in the caudate-putamen (CP) of sodium depleted (DEP) (furosemide-induced natriuresis) and adrenalectomized (ADX) rats. In situ hydridization with synthetic oligodeoxyribonucleotide probes specific for PPT A-gene was used. Quantitative analysis revealed dispersed PPT A labelled cells in sections from rat CP and BNST. Furthermore, the concentration of PPT A mRNAs in the ventral CP was denser than in the dorsal CP. The intensity of silver grains in both regions of the brain was greater in DEP than in control animals. On the other hand, both CP and BNST showed a markedly lower level of PPT A mRNAs in ADX rather than sham operated rats.

These findings, in relation to the enhanced PPT gene expression in CP and BNST of DEP rats, are provocative expecially in light of the antinatriorexigenic effect of these peptides and they also extend previous functional considerations on the primary role that Tks exert on sodium homeostasis. (Supported by MH 43787).

324.6

NEUROPEPTIDE K (NPK) STIMULATES GH RELEASE IN THE RAT. V. Rettori, L. Debeljuk, M.C. Aguila, A. Bartke and S.M. McCann*. Dept. of Physiology, UT Southwestern Medical Center, Dallas, Texas 75235.

NPK is a 36-amino acid peptide synthesized within the precursor polypeptide ß-preprotachykinin A, which contains neurokinin A (NKA) as a C-terminal decapeptide. To evaluate the possible role of NPK on GH secretion, it was microinjected into the third cerebral ventricle (3V) of conscious, freely moving, ovariectomized rats. Blood samples were taken from previously implanted external jugular catheters. NPK (0.5 μg/3 μl saline (0.9% NaCl)) significantly increased plasma levels of GH beginning at 70 min, reaching a peak at 90 min and declining until 120 min as compared to 0 time and saline-injected controls. To elucidate the site of action of NPK, anterior pituitaries from male rats were incubated for 2 hrs in the presence of NPK (10^{-9} to 10^{-7}M). None of the doses used modified the release of GH. Therefore, we studied the effect of NPK (10^{-8}-10^{-7}M) on somatostatin release from mediobasal hypothalami (MBH) in vitro. There was no significant effect of NPK on somatostatin release. We conclude that the stimulatory action of NPK on GH release is at the hypothalamic level probably mediated by GRH release. The delay in the effect suggests that NPK may be converted to an active fragment; however, this fragment is not NKA since it was inactive at equivalent in vivo doses (supported by DK40994).

324.7

INFUSION OF OXYTOCIN ANTAGONISTS INTO THE VTA OR MPOA BLOCKS THE POSTPARTUM ONSET OF MATERNAL BEHAVIOR. Cort A. Pedersen*, Jack D. Caldwell and Gail Ayers. Dept. of Psychiatry, BDRC, Neurobiology Curriculum, The University of North Carolina School of Medicine, Chapel Hill, NC 27599-7160.

We have recently reported a significant increase in oxytocin (OT) binding at parturition in the ventral tegmental area (VTA) and the medial preoptic area (MPOA). We have, therefore, tested whether antagonism of OT in these sites blocks the onset of maternal behavior (MB) occurring at parturition. Primiparous rats received either PPTPO (a specific OT antagonist), a specific V_1 antagonist (1ug/0.25ul/side) or NS bilaterally into the VTA region, OTA (another OT antagonist) or NS bilaterally into the MPOA (0.25ug/0.25ul/side) or PPTPO (2ug/2ul), OTA (0.5ug/2ul) or NS into the lateral cerebral ventricles after delivery of the first pup, 1 hr later, 15 and 40 min after delivery of the last pup. Each pup was removed from its mother's cage after it was delivered and licked clean of blood and birth fluids. 40 min after delivery of the final pup and immediately following the last central infusion, 8 of their offspring were returned to each rat and behavioral observations were conducted over a 30 min period. Among animals receiving PPTPO into the VTA only 1/10 retrieved pups or crouched in a nursing posture >1100 sec ($p<.05$ compared to other treatments). Among animals receiving OTA into the MPOA 0/7 retrieved pups or crouched >1100 sec ($p<.05$ compared to other treatments). ICV administration of PPTPO or OTA had no significant effect on MB. The VTA and MPOA appear to be sites of endogenous OT activation of MB. Supported by HD25255 and MH33127.

324.8

EFFECTS OF AN OXYTOCIN-ANTAGONIST (OT-A) ON SUCKLING-INDUCED RELEASE OF OXYTOCIN (OT) INTO THE SUPRAOPTIC NUCLEUS (SON) AND BLOOD. I. Neumann[1], R. Landgraf[1], E. Koehler[2], J.A. Russell[3] and J. Summy-Long[2]*. Univ. of Leipzig, Germany[1], M.S. Hershey Med. Ctr., Hershey, PA[2], Univ. of Edinburgh, U.K.[3].

OT released within the SON during suckling may stimulate release of more OT within the nucleus as well as into blood. To examine this, the SON area of conscious lactating rats was microdialyzed (MD) with artificial CSF (aCSF, 3.9 ul/min) on 2 days, collecting 30-min samples before, during and after suckling. On day 1 vehicle (1.5 ul) was infused adjacent to the MD probe 5 min before returning the pups. This was repeated on day 2 except half the rats received OT-A (50 ng/1.5 ul). On day 1, OT (pg/30 min;$\bar{X}\pm$sem;n9,10) in dialysates increased ($P<0.05$) during suckling in vehicle (0.5+0.1 vs 2.0+0.4) and OT-A (0.6+0.1 vs 2.8+1.0) groups. On day 2, however, suckling elevated ($P<0.05$) OT in dialysates only from rats given vehicle (0.6+0.1 vs 2.2+0.4) but not OT-A (0.9+0.2 vs 1.1+0.2). On day 3, OT-A did not alter intranuclear release of OT by MD of 1M NaCl in aCSF. In other studies, OT-A (50 ng/1 ul) infused into both SONs before suckling lowered ($P<0.02$; vs vehicle) plasma OT (15.3+1.5 vs 29.6+5.2 pg/ml) and weight gain of the litters (1.6+0.9 vs 5.8+1.2 g). These data indicate a receptor-mediated, positive feedback of OT on it's own release in the SON to facilitate OT secretion into blood during suckling. Funded by VW-Stiftung(I/67301) and RO1HD 25498.

324.9

SENSITIVITY OF THE NEUROTENSIN RESPONSE OF RAT SUBSTANTIA NIGRA NEURONES *IN VITRO* TO TOXINS AND ION CHANNEL BLOCKERS

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Parke-Davis Neuroscience Research Centre, Addenbrookes Hospital Site, Hills Road, Cambridge CB2 2QB

Two populations of neurones were observed in the substantia nigra, pars compacta. Of 1 group of neurones, 38 responded to neurotensin (0.1-1.OμM) with a depolarization and increase in firing rate. The response to NT was dose dependent and lasted for between 3 and 15 mins. The depolarizing effects of NT persisted in the presence of TTX. Current voltage relationships determined under current and voltage clamp conditions showed an increase in membrane resistance occurred during the NT response. The effects of NT persisted in the presence of high magnesium, cobalt and manganese although in the presence of manganese the duration of the response was reduced. Addition of apamin (1μM) and charybdotoxin (300nM) had no blocking action on the NT response although the input resistance of the neurone increased slightly after five minutes exposure to these agents. Addition of 2mM caesium had no effect on the NT response (n=3) whilst addition of 30Oμm barium (n=3) or replacement of potassium with caesium (n=3) blocked the NT response. The effect of all these agents was fully reversible on washout. 100-3OOnM α-dendrotoxin caused a partial block of the NT response which washed out. The results of the present study suggest that the depolarizing response to NT is mediated via a postsynaptic receptor on a subpopulation of dopamine sensitive neurones in the substantia nigra. The ability of either barium, replacement of potassium with caesium or a-dendrotoxin to reduce or abolish the NT response suggests a potassium conductance is involved in the depolarising response.

324.10

NEUROPEPTIDE AND NE EFFECTS IN PGE2-SENSITIVE mPOA HEAT GAIN SITES OF THE RAT. C.W. Simpson* and G.E. Resch. Schl. Biol. Sci., Univ. Missouri at Kansas City, Kansas City MO 64108.

In previous reports we presented data supporting neurochemical differentiation of adjacent PGE_2-sensitive mPOA sites in which a variety of neurotransmitters and ligands elicited colonic temperature (Tc) increases in ketamine anesthetized rats and hamsters. In this report these investigations have been extended to evaluate effects of four peptides (α-MSH, AVP, ß-End, and NPY) and NE in unanesthetized rats. Each was injected in 1ul volumes into previously identified PGE_2-sites as previously described. Each peptide elicted a change in Tc: α-MSH, ß-End, and NPY increased Tc 0.49° to 0.80°C; and AVP decreased Tc -0.8°C. Each value represents the mean of values measured in 7 to 9 rats. To the best of our knowledge, this is the first demonstration of AVP induced hypothermia in contrast to other reported attenuations from a febrile state. There may be an expanded role for AVP beyond antipyresis. Threshold doses determined for each peptide: α-MSH and NPY, 10^{-12}gm; AVP and ß-End, 10^{-6}gm. The threshold dose for NE was 10^{-8}gm. Data from NE injections were consistent with the reports of others, showing primarily decreases and some increases in Tc. Similarly for the peptides instances of opposite responses occurred. Such response patterns are consistent with site differences reported by Boulant in vitro, and Beckman in vivo and consistent with different routes of administration (icv vs tissue injections) reported by Lipton. These data indicate these peptides modify Tc at these PGE_2-sensitive mPOA heat gain sites. In addition the data imply that several parameters may modify the expression of responses to any given ligand; eg. fever, internal/external heat load, and anesthesia. AVP attenuates fever but did not induce hypothermia in the ventral septal area. Internal heat load blocked heat gain pathways but this did not occur in normo- and hypothermic animals. Finally, NE did not elicit a fall in Tc in ketamine anesthatized rats but did in the unanesthetized rat. Supported by AFOSR 870295.

324.11

IMMUNONEUTRALISATION OF NEUROPEPTIDE Y REDUCES FAST-INDUCED FEEDING IN THE RAT - EVIDENCE OF A PHYSIOLOGICAL ROLE IN FEEDING BEHAVIOUR. P.D.Lambert, J.P.H.Wilding, S.G.Gilbey, E.Comoy, C.Bohuon and S.R.Bloom. (SPON: Brain Research Association) Endocrine Dept., Hammersmith Hosp., London. U.K. *Institute Gustave-Roussy, Paris. France.

Central administration of NPY produces a robust feeding response in the rat, and raised levels of hypothalamic NPY and NPY mRNA have been shown following food deprivation and in animal models of obesity and diabetes. These observations suggest that NPY may have a physiological role in feeding. However, the important question is whether or not changes in the level of endogenous, central NPY mediate changes in feeding. We therefore investigated the effects of intracerebroventricular injection of a monoclonal antibody to NPY (NPYAb), prepared from mouse ascites, on NPY-induced and fast-induced feeding. We also studied the feeding response to central injections of NPY and NPYAb in streptozotocin-induced diabetic rats. All studies were of crossover design comparing the effects of NPYAb with an antibody to chloraquine which had been prepared in the same way and had no affinity for NPY. Intracerebroventricular pretreatment with NPYAb (5ul) reduced NPY-induced feeding by 74±6% ($p<0.001$, n=6). Pretreatment with NPYAb 10 minutes before 24 hour fasted animals were presented with food reduced their food intake by 29±6% ($p<0.02$, n=9). NPY-induced (2.4nmols) food intake in diabetic animals was 52±10% lower than seen in controls ($p<0.04$, n=6). The food intake of diabetic animals following a 24 hour fast could not be reduced by central injection of NPYAb. These data suggest a significant, physiological role for NPY in the control of feeding after food deprivation in the rat. There was reduced sensitivity to the central effect of NPY on feeding in animals with insulin-deficient diabetes, suggesting that the NPY system is excessively stimulated in this model.

324.12

L-365,260, A CCK_B ANTAGONIST, BLOCKS CCK-4-PANIC J. Bradwejn*, D. Koszycki, A. Couetoux duTertre, H. van Megen, J. den Boer, H. Westenberg, C. Karkanias, J. Haigh. McGill Univ., Montreal, Canada; Univ. of Utrecht, The Netherlands; Merck Sharp & Dohme, USA and UK.

CCK-4, a CCK_B receptor agonist, induces panic attacks in panic disorder (PD) and normals. This study investigated the effects of pre-treatment with L-365,260, a CCK_B antagonist, on CCK-4-panic in PD. Patients were treated with L-365,260 (10 or 50 mg PO) or placebo 90 mins before 20 ug of CCK-4 I.V.. 24 patients were entered; 3 were challenged once and 21 were challenged twice in a double blind, incomplete block design. The panic rate after CCK-4 was: 13/15 (placebo), 5/15 (10 mg), and 0/15 (50 mg); ($P<.01$ for 10 mg and 50 mg against placebo). The adjusted mean (±SD) sum intensity scores on the Panic Symptom Scale were 37.2±15.6 (placebo), 25.7±16.3 (10 mg) and 12.4±15.9 (50 mg). Differences between 50 mg and placebo were statistically significant ($P<.01$) and differences between 10 mg and placebo approached significance ($P=.07$). These data demonstrate that L-365,260 can block CCK-4-panic. The clinical efficacy of this CCK_B antagonist in PD warrants investigation.

324.13

REGULATION OF PEPTIDES AND PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE (PAM) ACTIVITY IN THE PITUITARY AND BRAIN OF HIBERNATING GROUND SQUIRRELS. G.P. Mueller, T.A. Ford, T.L. Stanton and A.L. Beckman*. Uniformed Services University of the Health Sciences, Bethesda, MD 20814 and California State University, Long Beach, CA 90840.

Morphine physical dependence is significantly reduced in the hibernating state of the ground squirrel, Citellus lateralis. This effect may be mediated by changes in endogenous beta-endorphin (β-END) and cholecystokinin octapeptide (CCK-8) systems, which have reciprocal influences on opiate analgesia in awake rodents. To investigate this, we examined the effects of hibernation on: (1) levels of pituitary β-END and brain CCK-8 and (2) the activity of PAM, the enzyme that catalyzes formation of bioactive α-amidated CCK-8. We observed that the concentration of β-END in the pituitary anterior (AL) and neurointermediate (NIL) lobes, and CCK-8 in cerebral cortex, was decreased by 50% ($p<0.05$) compared to non-hibernating animals. Conversely, hibernation was associated with a nearly 2-fold increase in the activity of PAM (membrane bound and soluble) in the AL, due to an increase in the V_{max} (no apparent change in K_m). PAM activity in the NIL and cerebral cortex, however, was not altered by hibernation, indicating that different mechanisms control PAM activity in these tissues. Thus, hibernation differentially alters neuropeptides and processing enzymes involved in analgesia. Changes in the balance of these systems may contribute to the hibernation-related reduction in morphine dependence.

324.14

BOMBESIN-LIKE PEPTIDES (BLP) ARE ENDOGENOUS REGULATORS OF FERTILIZATION OF OVUM BY SPERM: EXPRESSION OF LIGANDS AND RECEPTORS IN TESTES AND THEIR ROLE IN FERTILIZATION. S.R. Nagalla, S.Vijayaraghavan, K.Li, A. Archibong, D.P.Wolf, E.R.Spindel*. Div of Neurosci, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Bombesin-like peptides are important neurotransmitters, paracrine hormones and autocrine growth factors. The best characterized mammalian BLP is gastrin-releasing peptide (GRP) which is widely distributed in brain, GI tract and lung. Here we report evidence for a potentially important role of a BLP to effect fertilization through regulation of the acrosome reaction in primate sperm. RNA blot analysis showed the presence of GRP and GRP receptor mRNA in rhesus testes at levels higher than we have previously observed in any normal adult tissues. *In situ* hybridization localized the GRP mRNA to the spermatogenic cells in the seminiferous tubules. Sequence analysis of partial cDNAs isolated from a rhesus cDNA testes library showed high homology with the 3'-end of the human GRP mRNA though PCR suggests that the 5'-end of the mRNA may be different. As a necessary part of successful fertilization, sperm undergo an exocytic process called the acrosome reaction. Concentrations of GRP as low as 1 nM significantly stimulated the acrosome reaction (AR) of both human and monkey sperm. The stimulation of the AR by GRP could be blocked by the bombesin antagonist [D-Phe^6] bombesin (6-13) propylamide (D-Phe^6Bn) which is selective for the GRP-preferring bombesin receptor subtype. D-Phe^6Bn also blocked the AR induced by progesterone another known mediator of the AR. Finally, D-Phe^6Bn blocked the *in vitro* fertilization of monkey ovum by monkey sperm. Thus a BLP, most likely GRP itself, plays an essential role in fertilization. This suggests that appropriate bombesin agonists may have utility for *in vitro* fertilization procedures and that appropriate bombesin antagonists may have potential as contraceptive agents.

325.1

TRANSLATION AND PROCESSING OF AMYLOID PRECURSOR PROTEIN ARE DIFFERENT IN RAT CORTICAL NEURONS, ASTROCYTES AND MICROGLIAL CELLS. LeBlanc AC*, Xue R, Gambetti, P. Inst. Pathol. Case Western Reserve U. Cleveland, OH 44106

Translational and post-translational regulation of amyloid precursor protein (APP) gene expression was studied in primary cultures of rat cerebral cortex neurons, astrocytes and microglia as well as in cultures of whole cerebral cortex by pulse-chase experiments. Detergent soluble (cytosolic and membrane-associated proteins) and detergent insoluble cellular proteins (cytoskeleton and nuclei fraction) as well as secreted proteins were immunoprecipitated with antibodies to various regions of the APP. The rate of synthesis of detergent soluble cellular APP was different in the various cell types. The most abundant synthesis was found in astrocytes despite their relatively low levels of APP mRNA. Chase experiments after 60 min. pulses, revealed that the half-life of detergent soluble APP also differed in each cell type. In addition, longer pulse times of 6 hours followed by chase, revealed a pool of detergent soluble APP which appear to be very stable and were detectable even after a 24 hour chase. Secretion was more rapid in mixed cerebral cortex cultures compared with isolated cells but slowed down considerably with time in vitro. The detergent insoluble cellular proteins are also being investigated. The results of these experiments emphasize differences in the rate of translation, distribution and post-translational processing of the APP among the main cell types of the CNS. Supported by NIH NIA RO1 AGNS 08155, Britton fund and NIH NIA AGO8992-01.

325.2

THE ~11.4 KD COOH-TERMINAL APP DERIVATIVE IN TRANSFECTED 293 CELLS HAS THE AMYLOID ß-PROTEIN AT ITS AMINO TERMINUS. T. Cheung*, M. Shoji, J. Ghizo**, S. Estus, T. Golde, S. Gandy***, P. Greengard***, B. Frangione**, and S. G. Younkin. Case Western Reserve University, Cleveland, OH 44106 ; **NY Univ. NY, NY 10012; ***The Rockefeller University, NY, NY

The 39-43 residue amyloid ß protein (ßAP) deposited in Alzheimer's disease is derived from membrane-associated glycoproteins referred to as the amyloid ß protein precursor (APP). Using antisera to the COOH-terminus of the APP, we previously isolated a set of 8-12 kD proteins from human brain and embryonic kidney 293 cells, employed augmentation after transfection and pulse chase studies to show that all of the proteins in this set are authentic APP derivatives, and performed epitope mapping to obtain strong evidence that the largest (~11.4 and ~11.8 kD) proteins in this set are potentially amyloidogenic COOH-terminal derivatives that have the entire ßAP at or near their NH_2-terminus (Estus et al., Science, 255,726,1992). To better identify the cleavage that produces the ~11.4 kD ßAP-bearing derivative, we radiolabelled 293 cells transfected with a $ßAPP_{695}$ expression construct with ^{35}S-Met and ^{3}H-Phe, immunoprecipitated with 369 (anti-$ßAPP_{645-694}$), separated the radiolabeled proteins by 10/16% tris/tricine SDS/PAGE, transferred to immobilon, and sequenced. To validate this method, the major ~8.7 kD COOH-terminal derivative isolated with the ~11.4 kD fragment was sequenced, shown to begin at $ßAP_{17}$ as previously reported (Esch et al., Science 248,1122,1990), and ^{3}H Phe and ^{35}S-Met were recovered only at the appropriate cycles. Although only a small fraction of the cpm in the isolated 11.4 kD protein were recovered in the sequinator, ^{3}H-Phe was identified only at cycles 4, 19, and 20 as expected for a COOH-terminal derivative beginning at $ßAP_1$. Thus our results indicate that the 11.4 kD COOH-terminal APP derivative has the amyloid ß-protein at its amino terminus.

325.3

SEARCH FOR MUTATIONS IN THE β-AMYLOID PRECURSOR PROTEIN (βAPP) BY DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE). M.B. Podlisny*, B.L. Ostaszewski, E. Abrams[1], D.R. Tolan[2], D.J. Selkoe. Harvard Medical School, MIT[1] and Boston Univ.[2], Boston, MA.

Missense mutations in βAPP discovered in a few kindreds with familial Alzheimer's disease (FAD) have provided the first specific molecular cause of AD. Because FAD is a common, multi-ethnic autosomal dominant disorder, it is likely that additional point mutations (or other defects) in the βAPP gene will be found in some other FAD families. We have established a sensitive and efficient procedure for screening large numbers of putative FAD subjects for βAPP mutations that utilizes DGGE. Exonic segments of genomic DNA are amplified by PCR using primers designed by computer simulation to encompass a single DNA melting domain; one primer is joined to a GC-clamp. Any mutations in the amplified exonic fragments should be detected with >98% efficiency (Sheffield et al., PNAS 86:232, 1989). Fragments are electrophoresed on polyacrylamide gels containing a gradient of urea/formamide denaturant dictated by the individual melting characteristics of each exonic segment. Gels of positive control DNA from an HCHWA-Dutch patient with the $βAPP_{693}$ mutation and an FAD patient with the $βAPP_{717}$ mutation always showed the expected 2 heterodimers and 1 or 2 homodimers of double-stranded DNA. As positive controls for those exons in which no DNA mutation has yet been identified, we have utilized mixed samples of genomic DNA from human and monkey. These species differ by 1-4 bases in most βAPP exons, and these substitutions are readily detectable in our DGGE system. Analysis to date on βAPP exons 16 and 17 (comprising β/A4) and exon 18 (carboxyl terminus) in 51 AD subjects having 1 or more first degree relatives with a history of AD have not revealed any base substitutions. The age of onset in these putative familial cases range from 44 - 83 years, but most are >65 yr old. We are currently extending our DGGE analysis to other βAPP exons and additional patients. Mutations in exons 16, 17 and 18 of βAPP appear to be an infrequent cause of AD.

325.4

ß/A4 FROM DIFFUSE PLAQUE AMYLOID IS CLOSELY SIMILAR TO COMPACTED PLAQUE AMYLOID. S.J. Frucht, D.A. Teplow, D.J. Selkoe, E.H. Koo*. Harvard Medical School and Brigham and Women's Hospital, Boston, MA

Diffuse ß-amyloid plaques are believed to be an early stage in senile plaque evolution. We have biochemically characterized diffuse plaques from cerebellum in AD cases and from cortices of AD and aged individuals with abundant diffuse plaques but lacking senile plaques or significant vascular amyloid. On centrifugation, diffuse plaque material requires much higher speeds to pellet than compacted cores and fractionates differently on sucrose density gradients. In a slot blot immunoassay, diffuse plaque amyloid and isolated compacted amyloid cores are virtually identical as regards to their sensitivities to a variety of proteases and chaotropic agents. Following SDS extraction and formic acid solubilization, cortical and cerebellar diffuse plaque amyloid migrates as a ~4 kDa doublet. Moreover, partially purified diffuse plaque amyloid reacts specifically with a variety of ß/A4 antibodies. Homogenates from AD cases with both diffuse and compacted plaques show the same ~4 kDa doublet and a prominent ~16 kDa tetrameric form. Whereas a tetrameric form of ß/A4 is occasionally detected in the diffuse plaque material, we have been unable to demonstrate longer APP-derived fragments using antibodies directed to regions N- or C-terminal to the ß/A4 domain. Our results to date suggest that although diffuse plaques are morphologically different from senile plaques, their biochemical properties and major amyloid protein species are closely similar.

325.5

ALZHEIMER'S β/A_4 PROTEIN FORMS A FORMATE ESTER WITH SERINE-26 IN CONCENTRATED FORMIC ACID.

W.E. Klunk, MD, PhD*, C. Xu, PhD, and J.W. Pettegrew, MD. *Lab. of Neurophysics, Univ. of Pittsburgh, WPIC, Pittsburgh, PA 15261*

Deposition of β/A_4 may play an role in the pathogenesis of AD and has been the subject of intense investigation. The insoluble nature of this peptide has hampered work in this area and has necessitated the use of harsh chemical methods of solubilization. A common method was the use of concentrated formic acid. We have previously reported that this technique covalently modifies the peptide (Klunk et al. *J. Neurochem. 54:* 2050-2056; 1990). In this study, we use a combination of one- and two-dimensional ^{1}H NMR techniques to show that the modification occurs via formation of a formic acid ester of a serine residue in the peptide. One dimensional NMR comparison of the formylated and native peptide shows the appearance of a singlet at 8.3 ppm, consistent with a formate proton. After sequential assignment of all peaks by two-dimensional NMR, it was clearly seen that the only major changes in chemical shift occurred at the lone serine in the peptide fragment studied. Relayed COSY experiments showed a cross peak between the formate proton and the serine beta protons, further demonstrating the nature of this covalent attachment. This data, combined with our previous study, conclusively demonstrates that dissolution of β/A_4 in concentrated formic acid results in covalent modification of this peptide through formic acid esterification of a serine residue and makes this procedure unsuitable for many studies.

325.6

SITE PROTECTED, CATIONIZED MONOCLONAL ANTIBODY AGAINST BETA AMYLOID AS A POTENTIAL DIAGNOSTIC IMAGING TECHNIQUE FOR ALZHEIMER'S DISEASE. **U. Bickel, Y.M.-Y. Lee, J.Q. Trojanowski, and W.M. Pardridge*. Departments of Medicine and Neurology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA, 90024, and The University of Pennsylvania School of Medicine, Philadelphia, PA, 19104.**

While cerebrovascular amyloid and senile plaques containing beta amyloid (βA4) are widely accepted as the most consistent morphological markers of senile dementia of Alzheimer's type (SDAT), the only positive ante mortem diagnosis available to date is by brain biopsy. One approach to a diagnostic test for cerebral amyloid would be the application of radiolabeled antibodies and an imaging technique such as single photon emission tomography. However, it is first necessary to enable the antibody to pass through the blood-brain barrier, which generally is intact in SDAT. Cationization has recently been shown to profoundly enhance brain uptake of immunoglobulin G (Triguero et al, P.N.A.S. 86, 4761, 1989).

In the present study, a well-defined monoclonal antibody, AMY33 (Stern et al, Am. J. Pathol. 134:973, 1989), raised against a synthetic βA4 peptide, was used. AMY33 IgG was affinity purified from mouse ascites on protein G-Sepharose. Site protected cationization in the presence of a molar excess of synthetic βA4 was performed using hexamethylenediamine and carbodi-imide, followed by gel chromatographic purification at acid pH to remove βA4 peptide. Isoelectric focusing proved that the pI of cationized AMY33 was shifted from 7 to 9. Retained affinity of the cationized antibody to βA4 peptide was demonstrated with ELISA. In immunocytochemistry on SDAT brain sections the modified antibody still recognized cerebrovascular and plaque amyloid. ^{125}I-labeled cationized AMY33 is taken up by isolated bovine brain capillaries in vitro and can now be evaluated in vivo for its ability to detect amyloid deposits.

325.7

LAMINAR AND SIZE DISTRIBUTION OF SENILE PLAQUES IN AD, CRITICAL CORONARY ARTERY DISEASE (cCAD) AND NORMAL CONTROLS.
D. Larry Sparks* and Huaichen Liu. UKMC Lexington KY 40536-0230

We have previously reported the enhanced prevalence of senile plaques (SP) in non-demented individuals with cCAD compared to age-matched non-demented non-heart disease (non-HD) controls. Furthermore, we have found an age-related increase in the occurrence of SP in both cCAD and non-HD individuals, although it is shifted to younger ages in cCAD by approximately 40 years. The youngest cCAD patient exhibiting cortical SP was 26 years of age.

Previous studies in AD patients have shown increased numbers of SP in superficial layers of cortex (I-III) compared to deep layers (IV-VI) with larger SP being found in layers II,III and V. We have investigated the density, and size and laminar distribution of SP in young and old AD, cCAD and non-HD subjects. Utilizing automated image analysis (Kontron) and manual counting methods we have accessed SP in frontal pole stained by the Bielschowsky method from 12 AD, 12 cCAD and 11 non-HD individuals. All of the individuals investigated exhibited SP in frontal pole, which predicated their selection. In each case, three columns of non-overlaping images perpendicular to both the pia and the white matter were analyzed for the full thickness of the cortical mantle. Each individual Kontron image measured 537.9 μm in width by 616.4 μm in depth.

An age-related decrease in the depth of the cortex was found in the cCAD and non-HD subjects, and SP tended to be larger in the young cCAD and non-HD subjects. Except for the increased number of SP in AD ($p < 0.001$), there were no significant differences among the elderly groups. In the younger groups, SP density in cCAD was between AD and non-HD. In all three groups, SP density was greatest in the superficial cortical layers, predominently layers II and III. SP size was determined between 200 (the lower limit cut off) and 10200 μm^2 in 500 μm^2 increments, where >90% of the SP were between 200 and 2700 μm^2 in all groups and in all layers of cortex. Increased numbers of large SP in cCAD and non-HD may reflect the prevalence of the diffuse form.

325.8

TRANSMITTER- AND PHF-IMMUNOLABELED DYSTROPHIC NEURITES ARE NOT SEEN IN OTHER NEURODEGENERATIVE DISEASES EXCEPT IN THE PRESENCE OF β-AMYLOID DEPOSITION.
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We sought to determine whether the dystrophic neurites which we observed related to senile plaques in patients with Alzheimer's disease (AD) were in fact unique to AD or whether they were characteristic of a more generalized process of neurodegeneration. To accomplish this, we examined the brains of cases with Parkinson's disease (PD), PD with concurrent AD (PD-AD), PD that were demented but not with concurrent AD (PD-DEM), Huntington's disease (HD), and Pick's disease and compared these various cases to normal controls and AD cases using antibodies directed against tyrosine hydroxylase, substance P, neurotensin, somatostatin, amyloid-ß protein (AβP), and paired helical filaments.

Additionally, all tissue sections were counterstained with thioflavine-S. Inclusive within our tissue sections was the basal ganglia, basal forebrain, amygdala, entorhinal, temporal, and insular cortices. Within these various brain regions we found that immunolabeled dystrophic neurites, characteristic of AD, were seen only in regions where AβP deposition was present. These observations held true even in areas known to display severe degenerative changes such as the caudate in the HD cases and the temporal cortex in the Pick's, as well as severe afferent degeneration such as the caudate and putamen in all three PD groups. While these findings did not rule out that other forms of neuritic degeneration were accompanying the degeneration seen in these latter diseases, it did suggest that the enlarged immunolabeled neurites so frequently observed in AD brains are unique to regions of senile plaque formation and probably are forming in response to ß-amyloid deposition.

325.9

ALZHEIMER AMYLOID PRECURSOR PROTEIN CAN BE RELEASED FROM HIPPOCAMPAL SLICES *IN VITRO*.
Steven A. Farber*, Roger M. Nitsch and Richard J. Wurtman. Dept. of Brain and Cognitive Sciences, M.I.T. Cambridge, MA 02139, and Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Abnormal processing of the amyloid precursor protein (APP) is a central event in the formation of the amyloid deposits in Alzheimer's disease brain. APP is a membrane glycoprotein which can either be secreted or internalized and degraded by lysosomal proteolysis. We have shown that APP secretion in transfected cell lines is stimulated by activation of cell-surface neurotransmitter receptors (Nitsch, R.M. *et al.*, this meeting). To examine the physiological relevance of this finding, we tested the hypothesis that APP processing can be affected by neuronal activity. Hippocampal slices from adult rats (6-8 months) were prepared at 4°C, and equilibrated in specially designed superfusion chambers (50min, 37°C) with oxygenated artificial CSF (0.8ml/min). The slices were then stimulated electrically (30Hz, 0.5A/cm^2, 1ms pulse duration) for 50min; APP in the superfusate was purified using ultrafiltration and dialysis, and measured by densitometric Western blot analysis (mAb 22C11). Electrical stimulation caused a significant 2-fold increase in APP release ($p<0.01$, n=10 rats per group) as compared to unstimulated controls in parallel chambers. This increase in APP release was proportionate to stimulation frequency in the range of 0, 10, 20, and 30 Hz. These data show for the first time that APP is released from hippocampus *in vitro*, and that release can be increased by neuronal depolarization. They thus suggest that APP processing in the brain is regulated by neuronal activity. Supported by NIMH, NIA and the Center of Brain Sciences and Metabolism Charitable Trust.

325.10

HIGH CONCENTRATIONS OF ALUMINUM OR IRON PROMOTE AGGREGATION OF HUMAN ß-AMYLOID PEPTIDE
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The salient pathological feature of Alzheimer disease (AD) is the presence of a high density of amyloid plaques in the brain tissue of victims. The plaques are predominantly composed of human ß-amyloid peptide (ßA4), a 40-mer whose neurotoxicity may be related to its aggregation. Aluminum is a proposed risk factor in the etiology of AD, but the mechanism by which aluminum or other metals exert their effects remain unclear. Radioiodinated human ßA4 was used to assess the effects metals had in inhibiting or promoting ßA4 aggregation. Whereas 0.001M calcium, cobalt, copper, manganese or EDTA had no effect on ßA4 aggregation, at the same concentration, aluminum and iron strongly promote ßA4 aggregation. These results demonstrate that high concentrations of aluminum and iron promote ßA4 aggregation, and suggests a mechanism by which aluminum and iron may play a role in the pathogenesis of AD. Supported by the NIH and the VA.

325.11

ULTRASTRUCTURAL LOCALIZATION OF COMPLEMENT PROTEINS TO NEURONAL MEMBRANES AND β-AMYLOID PEPTIDE CONTAINING ALZHEIMER'S DISEASE PATHOLOGY.
S.D. Webster*, L.-F. Lue, M. McKinley, and J. Rogers. Sun Health Research Institute, Sun City, Arizona 85351.

At the light microscopic level, proteins of the classical complement pathway have been demonstrated to co-localize with neuritic plaques, neurofibrillary tangles, and neuropil threads in Alzheimer's disease (AD) brain tissue. However, ultrastructural studies are important in demonstrating a functional role of complement cytolytic processes in AD pathogenesis. AD and control brain tissue was processed according to standard immunocytochemical techniques for electron microscopic examination, and antibodies recognizing C1q, C3d, C4d, and C5b-9 (the membrane attack complex) were visualized by colloidal gold labelling. Complement immunoreactivity was observed at sites of AD pathology on elements with the ultrastructural characteristics of myelinated and nonmyelinated neuronal processes, many of which had degenerative morphology. These findings may be relevant to the generalized neuronal and neuritic loss characteristic of AD.

325.12

COMPLEMENT CASCADE INHIBITORS IN ALZHEIMER'S DISEASE.
J. Schultz*, L.-F. Lue, L. Brachova, J. Rogers, and W.H. Civin. Sun Health Research Institute, Sun City, AZ 85372.

We and others have reported profuse expression of classical pathway complement proteins in Alzheimer's disease (AD) but not nondemented elderly (ND) brain. These proteins are highly co-localized with β-amyloid peptide (β-AP) containing AD pathology. We have also demonstrated that β-AP may be capable of activating the classical pathway directly, without the usual mediation of Ig. In the periphery, non-Ig mediated complement activation by isolated peptides is handled by C1 inhibitor (C1 INH). Utilizing Western blot analysis, ELISA, and immunohistochemical techniques we have determined that C1 INH levels do not differ in AD and ND patient sera, and that C1 INH is not present in brain. The absence of C1 INH in AD brain would strongly favor non-Ig mediated complement activation by β-AP, as well as activation by serum amyloid P, another protein known to be co-localized with AD pathology and known to activate complement without Ig mediation. Once complement activation has occurred, cells under attack may increase expression of complement inhibitory mechanisms. Our studies show that C4 binding protein, which mediates one such mechanism, is immunohistochemically co-localized with neuritic plaques, neurofibrillary tangles, and neuropil threads in the AD brain. These studies therefore may help explain how β-AP is able to activate complement in the AD brain without Ig mediation, as well as provide evidence that such activation is of sufficient pathologic significance as to warrant the mounting of complement cascade inhibitory mechanisms.

325.13

Reduced Levels of Secreted Amyloid β-Protein Precursor in CSF of Pathologically Confirmed Alzheimer's Disease Patients.

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Pathologically, Alzheimer's disease (AD) is characterized by deposition of amyloidotic material in both the cerebral vasculature and the parenchyma of the brain. Using a highly specific monoclonal antibody (mAbp2-1) raised against native human PN-2, which cross-reacts with all secreted AβPP isoforms, we measured cerebrospinal fluid (CSF) levels of secreted AβPP in seven patients diagnosed as probable AD who have since expired and were pathologically confirmed as having AD. In addition, we analyzed CSF for secreted AβPP levels in four live probable AD patients who were neuropathologically confirmed as having AD via biopsy. Finally, we analyzed CSF for secreted AβPP levels in three individuals with a single amino acid substitution (Phe for Val) in the transmembrane domain of the AβPP. All patients who were clinically symptomatic for AD and histopathologically shown to contain senile plaques in conjunction with moderate to severe cerebral amyloid angiopathy (CAA) had at least two to three fold lower secreted AβPP levels in the CSF ($\bar{x}=0.7 \pm 0.4$ μg/ml) than did the non-AD control patients ($\bar{x}=3.5 \pm 0.4$ μg/ml). AD patients either without or with only sparse CAA had slightly higher secreted AβPP CSF levels ($\bar{x}=2.0 \pm 0.8$ μg/ml) than did those AD patients with moderate to severe CAA. Our data suggests that low levels of secreted AβPP in the CSF may be an indication of an abundance of certain amyloidotic lesions in the CNS, especially CAA.

325.14

ALZHEIMER AMYLOID PRECURSOR FORMS AND A4/ß PROTEIN IN MICROVESSELS ISOLATED FROM HUMAN BRAIN. R.N. Kalaria*, S. N. Kroon and I. Lieberburg. Dept. of Neurology, CWRU Sch Med, Cleveland, OH 44106 & Athena Neurosciences, S. San Francisco, CA 94080.

The amyloid A4/ß protein involved in Alzheimer's disease and Down syndrome is derived from a membrane associated precursor protein (APP). It is not known how amyloid deposits in the vasculature which is commonly involved in the pathology of these diseases. It is also not clear whether vascular APP processing is independent from that in senile plaques. To address these issues, we have used relatively simple *in vitro* studies employing immunochemical methods on isolated cerebral microvessel (CMV) fractions containing mainly capillaries obtained postmortem from cortex of AD subjects and aged controls. Using antibodies to various domains of APP and to ß protein we found that the full length APP and its smaller fragments and ß protein are readily detectable in solubilized fractions of CMV from AD subjects. Immunocytochemical studies on isolated CMV preparations showed anti-ß protein reactive deposits along the vessel length. However, larger vessels with smooth muscle showed typical concentric deposits described previously. To demonstrate the localization of ß protein deposits and whether they could be removed by digesting the basement membrane (BM), we pretreated CMV with collagenase (0.1-1%; 1 hr at 37°C). These treatments showed that ß protein immunoreactivity was largely removed, however, there was distinct appearance of a 5-6 kD fragment evident with antibodies to the carboxyl terminal of APP containing the ß protein domain. These experiments suggest that the vascular BM is inherently involved in amyloid deposition and that in addition to larger arterioles with smooth muscle cerebral capillaries readily metabolize APP and also contain amyloid deposits.

NEURAL-IMMUNE INTERACTIONS

326.1

EVIDENCE FOR IN VITRO AND IN VIVO REGULATION OF CYTOKINES BY THYROTROPIN RELEASING HORMONE (TRH). D.V. Harbour*, M.Jyoti, W.L.Kang. Dept HBC&G, UTMB, Galveston, TX 77550.

We studied effects of TRH on the release of Interferon (IFN)-gamma and Interleukin (IL)-2. IL-2 and IFN- were significantly increased by human PBLs and Molt 4 T-cells after treatment with TRH but not mock alone. Both effects could be abbrogated by pretreatment with antisera to IFN- . The mRNA levels for IL-2 rose 2-4 fold after 4-6 hours TRH treatment then declined. The TRH induced IFN had antiviral activity of up to 100 U/μl. To test the in vivo relevance of this data, we performed in vivo TRH induction tests on Balb/c mice. The mice were normal (N), hypothyroid (HO), hyperthyroid (HY) or thymectomized [TMX (lowered T cell number)]. The TSH RIA levels verified that the TRH tests were working. Spleen mRNA levels for IL-2 from the HO mice was stable and 2-3 fold higher than N; but, HY mRNA was less than half of the N mRNA level. Immunofluorescent (IF) studies correlated with in vivo serum levels. Monoclonal abs to IL-2 and IFN- , showed a significant reduction of IL-2, and IFN- from N spleen cells from time 0 to 60 minutes. The HO mice showed very high basal levels of IL-2 and IFN- and declined after treatment with TRH. The HY mice showed no IF.

326.2

MORPHINE ATTENUATES INTERLEUKIN-1 ACTIVATION OF C-FOS EXPRESSION IN THE RAT HYPOTHALAMUS. S.L. Chang[1]*, T.T. Tao[1], J. Zadina[3] and J. Thompson[2]. [1]Dept. of Physiology, [2]Dept. of MIP, Louisiana State Univ. Med. Ctr., [3]VA Med. Ctr. and Tulane Med. Center, New Orleans, LA. 70119.

Interleukin-1 (IL-1) is now known to be synthesized by neuronal cells. IL-1 receptors have been shown to occur throughout the rat brain. Several studies have suggested overlapping effects of opioid and IL-1 in CNS. In the present studies, we examined IL-1 regulation of c-FOS expression; morphine attenuates this regulation. The c-fos mRNA concentration of rat hypothalamus was elevated ~ 100% ($p < 0.05$) 30 min after direct intracerebroventricular (ICV) injection of IL-1α (200 ng) and returned to near the control level 60 min after the injection. In a parallel study, the cannulated rats were grouped randomly to receive either IL-1α (200 ng), IL-1ß (200 ng), control vehicle (100 mM Tris pH 7.6, 1% BSA) or nothing through cannulation for 3 hrs. At the end of treatment, the rats were perfused for immunocytochemistry of FOS protein. The FOS immunoreactivity was markedly increased by both IL-1α and IL-1ß in the paraventricular nucleus (PVN) and arcuate nucleus (ARC) of the hypothalamus. In a second parallel study, chronic morphine pelleting appeared to prevent IL-1α activation of c-fos mRNA in the hypothalamus. These data suggest that CNS action of IL-1 on the hypothalamus-pituitary adrenal axis may work through the regulation of FOS protein and morphine exposure attenuates this regulation.

326.3

INTERLEUKIN-1ß INDUCES THE RELEASE OF 5-HYDROXYINDOLEACETIC ACID (5-HIAA) FROM THE HYPOTHALAMUS *IN VIVO* P.S. Mohankumar, S. Thyagarajan and S.K. Quadri*. Neuroendocrine Research Laboratory, Kansas State University, Manhattan, KS 66506.

Interleukin-1ß (IL-1ß), a lymphokine, stimulates the release of hypothalamic and pituitary hormones. The mechanism of this action is not clear. There is evidence that the effects of IL-1ß on the neuroendocrine system are mediated through neurotransmitters. We have shown before that IL-1ß stimulates the release of norepinephrine and dopamine from the hypothalamus (HT) both *in vitro* and *in vivo*. Recently, it has been reported that interleukin increases the extracellular levels of 5-HIAA in the HT. Except for this study, there are no reports on the effects of IL-1ß on the indoleamine metabolism. In the present study, we investigated the effects of IL-1ß on the release of 5-HIAA from the arcuate-ventromedial area (ARC-VMN) of the HT using push-pull perfusion technique. Push-pull cannulae were implanted stereotaxically in the ARC-VMN of adult male rats. The hypothalamic nuclei were infused through the cannulae with 100 ng, 50 ng, 25 ng of IL-1ß or the vehicle (PBS-BSA). The perfusate samples were analyzed for 5-HIAA using HPLC. There were no differences in the pre-treatment release of 5-HIAA among the various groups. In the groups treated with 100 ng and 50 ng of IL-1ß, the average release of 5-HIAA during the post-treatment period increased significantly (220% and 47% respectively) compared to the pre-treatment release ($p<0.001$). There was no significant change in the release of 5-HIAA in the control and 25 ng IL-1ß treated groups in the post-treatment period. In the group treated with 100 ng of IL-1ß, 5-HIAA release increased significantly within 25 minutes and continued to increase even after 325 minutes ($p<0.05$). In the 50 ng IL-1ß treated group 5-HIAA release increased within 25 minutes (14%) but decreased to the pre-treatment levels within 50 minutes ($p<0.05$). This data suggest that IL-1ß has significant effects on the indoleamine metabolism of the hypothalamus.

326.4

EXCITATORY AMINO ACID INVOLVEMENT IN THE INTERLEUKIN 1β AND TACHYKININ-INDUCED EFFECTS ON LH RELEASE IN CASTRATED MALE RATS. J.J. Bonavera, S.P. Kalra and P.S. Kalra*, Department of Obstetrics and Gynecology, University of Florida College of Medicine, Gainesville, FL 32610

Evidence suggests that cytokines and tachykinins may interact to modulate neuroendocrine responses. Interleukin-1β (IL-1β) and the tachykinin, neuropeptide K (NPK), have been shown to markedly suppress LH release in gonadectomized rats. Since N-methyl-D-aspartic acid (NMDA) is excitatory to LH secretion, we tested the hypothesis that IL-1β and NPK-induced LH suppression may be due to diminution in the action of this excitatory signal. Adult male rats were castrated and implanted with permanent indwelling cannulae in the third cerebral ventricle. Two weeks later rats were injected with either saline (control, 3 μl), IL-1β (100 ng/3μl saline) or NPK (2.5 nmol/3 μl saline) intracerebroventricularly at time '0' min. Each of these treatments was followed by four iv injections of NMDA (5 mg/kg body wt.) or saline at 0, 30, 60 and 90 mins. Blood samples were withdrawn for LH analysis at different intervals from an indwelling intra-atrial cannula for 120 mins. NMDA treatment alone did not alter LH levels in control castrated rats, whereas both IL-1β and NPK markedly suppressed LH release starting at 70 and 30 mins post-injection, respectively. Treatment of IL-1β- and NPK-injected rats with NMDA completely prevented this LH suppression. These results are suggestive of a commonality of neural pathways in the inhibitory effects of hypothalamic cytokines and tachykinins involving an excitatory NMDA receptor. (Supported by NIH HD 11362).

326.5

TWENTY-FOUR-HOUR CIRCADIAN RHYTHMS OF CIRCULATING INTERLEUKIN-1α IN HEALTHY FEMALE VOLUNTEERS. J. Licinio*, M. Wong, M. Altemus, L. Tamarkin, P.W. Gold. Clinical Neuroendocrinology Branch, NIMH Intramural Research Program, Bldg. 10/3S231, Bethesda, MD 20892.

Interleukin-1α (IL-1α) is pleiotropic cytokine released by various cells in response to inflammation and stress. Even though it is widely known that IL-1α acts locally at multiple sites, including the brain, it is still uncertain whether IL-1α is a classical hormone, circulating systemically. We have tried to elucidate this point by measuring circadian rhythms of IL-1α plasma levels. **Methods:** 6 female healthy volunteers (ages 18-35) were admitted to the NIH Clinical Center for 48 hours. After one night acclimatization, we conducted a 24-hour blood collection at 15-min intervals. Plasma was assayed by enzyme-linked immunoassay (Assay Research, Inc., College Park, MD), with a limit of detection of 25 pg/ml (intra-assay C.V.=7%, inter-assay C.V.=17%). **Results:** All subjects had detectable levels of IL-1α through the 24-hour period. A pulse analysis reveals that IL-1α levels seem to have discrete peaks and circadian variation. The integrated 24-hour IL-1α levels were 3.11.7±0.65 (mean±SEM) $\mu g.ml^{-1}.24\text{-}hours^{-1}$, with a range of 4.40 (1.55 min., 5.95, max.). **Implications:** Our data show that IL-1α is present in the systemic circulation throughout the 24-hour period in healthy females. IL-1α levels exhibit circadian variation, which suggests that this cytokine may be a classical hormone, being secreted in the blood stream, and acting at distant sites, possibly as a key element in the modulation of the response to stress and to inflammation. The source of systemic IL-1α remains to be determined.

326.6

TRANSGENIC EXPRESSION OF IFN-γ IN MURINE CNS.
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IFN-γ has been implicated in the induction of diabetes and EAE in mice. To determine the effect of IFN-γ expression in the CNS, we have produced (C3H X C57BL/6) transgenic mice using a DNA construct that contained mouse IFN-γ cDNA, with the MBP promoter. This promoter had been shown to direct expression to oligodendrocytes *in vivo*. Transfection of our construct into an oligodendrocyte cell line induced secretion of biologically active IFN-γ. Of eight transgenic founders, only one showed IFN-γ RNA expression in the CNS. IFN-γ message was expressed in the CNS at levels comparable to those found in mice with severe EAE, whereas CNS from non-transgenic littermates showed no IFN-γ message. Expression in the spinal cord was significantly higher than in the brain. Expression of IFN-γ in non-CNS tissue was comparable to that in normal mice. Mice from this transgenic line have been observed for several months and have not revealed neurological symptoms even at peak ages for EAE susceptibility or for development of IFN-γ-induced diabetes, neither has histological evaluation of CNS tissue showed significant abnormalities. Therefore, unlike studies in which IFN-γ was transgenically expressed in the pancreas, transgenic expression of IFN-γ in the CNS was not sufficient to produce autoimmune disease. We are currently backcrossing our mice to the EAE-susceptible strain, SJL/J, and also generating homozygous transgenics, to further define the role of IFN-γ in neurological autoimmune disease.

326.7

KINETIC ANALYSIS OF ADHESION-MOLECULE EXPRESSION IN THE CNS OF EAE MICE. J.M. Dopp[1]* and J.A. Olschowka[2]. Depts. of Microbiology and Immunology[1], and Neurobiology and Anatomy[2], University of Rochester School of Medicine and Dentistry, Rochester, NY, 14642.

Leukocyte migration into the murine CNS increases dramatically during the course of experimental allergic encephalomyelitis (EAE). Leukocytes extravasate out of the bloodstream and into the CNS parenchyma, initiating an inflammatory cascade which leads to paralysis. Extravasation is facilitated by leukocyte expression of adhesion molecules which bind to counterpart ligands on CNS vascular endothelial cells (CVECs). We used immunocytochemistry to analyze leukocyte and CVEC adhesion-molecule expression during EAE.

SWXJ mice (n = 3) were injected twice s.c. with an emulsion containing 5 mg CNS homogenate in adjuvant, and thrice i.v. with 3.3×10^8 heat-killed Pertussis bacteria. Control mice received identical injections lacking CNS homogenate. 8, 11, and 14 days later, mice were perfused with 4% PF, and sections from brain, spleen, and spinal cord were stained for intercellular adhesion molecule-1 (ICAM-1), leukocyte-endothelial cell adhesion molecule-1 (LECAM-1), and leukocyte markers.

A low constitutive level of CVEC ICAM-1 expression in major CNS vessels was upregulated on days 11 and 14 in EAE mice. ICAM-1 was also expressed by CVECs of smaller capillaries and Ia+/GFAP(-) glial cells in EAE mice. LECAM-1 expression by neutrophils in the choroid plexus of control mice was faint and scattered at all timepoints, while EAE mice showed foci of intensely-staining neutrophils in the choroid plexus, as well as perivascular staining around major vessels on all days. ICAM-1 and LECAM-1 expression preceded clinical symptoms and was likely integral in initiating CNS inflammation. Ongoing experiments involve the analysis of additional adhesion molecules, cell markers, and cytokines using this model.

Supported by PHS grant NS29400.

326.8

CD4 EXPRESSION IN MOUSE AND HUMAN BRAIN.
B. Pessac*, B. Omri, F. Alliot, P. Crisanti, M.C. Marty, J. Rutin, and A. Privat. Centre de Biologie Cellulaire C.N.R.S. , 67 rue Maurice Günsbourg, 94205 IVRY cedex, FRANCE.

CD4 and its mouse equivalent L3T4 are members of the immunoglobulin gene superfamily expressed on the surface of most thymocytes and of a subset of T lymphocytes. We have observed, in the developing and adult mouse brain, a 55 kD molecule indistinguishable in Western blots and in 2D immunoelectrofocusing from the thymus L3T4 molecule. In young SCID mice, the L3T4 protein is not detected in thymus, but is clearly present in brain. *In situ* hybridization and immunocytochemical experiments show that the full length L3T4 transcript and the L3T4 protein are expressed coordinately in neurons throughout the brain and in distinct neural structures including hippocampus, cerebellum and olfactory bulb. In addition, L3T4 immunoreactivity is present in brain small vessel walls, ependymal cells and choroid plexus. We are currently investigating the presence of the CD4 protein in various regions of *post-mortem* human brain samples. Western blot show a 55 kD protein immunoreactive to a novel CD4 monoclonal antibody (a gift from A.Bernard, Nice, France) in extracts of distinct human brains. Current immunocytochemical experiments suggest CD4 expression in astrocytes as well as in neurons.

(This work was supported by grants from CNRS and ARNS).

326.9

THE ESTABLISHMENT OF A LATENT HSV-1 INFECTION IN THE CENTRAL NERVOUS SYSTEM REQUIRES A FUNCTIONAL IMMUNE RESPONSE.
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Following intra-ocular injection, HSV-1 (strains F and KOS) enters the CNS through classical visual synaptic connections to the lateral geniculate and superior colliculus. After infecting neurons in these primary ocular targets, strain F established a latent infection by 6-7 days post-infection, while the KOS strain invariably produced lethal infections. Through immunohistochemical analysis of the CNS using antibodies to antigens including HSV-1, CD4, CD8, Mac-1, F480 and I-E (MHC class II), we have found that the response of the CNS to the presence of HSV-1 includes and goes beyond a general inflammatory response. The inflammatory response is characterized by the infiltration of macrophages and activation of resident microglia in the regions of virus infection. In addition, following either KOS or F infection of the CNS, CD4+ and CD8+ lymphocytes were specifically recruited to the sites of viral infection. While MHC class II protein synthesis was induced in anitgen presenting cells during both the productive (KOS) and latent (F) infections, a striking difference in the I-E antigen expression was observed. In the KOS viral infection centers the MHC class II antigen was primarily expressed in the cytoplasm/nucleus of the microglia and macrophages. By constrast, during the establishment of a latent infection the MHC class II antigen was expressed at the cell surface of the antigen presenting cells. These data suggest that the antigen presentation step in the immune response is interrupted during a KOS infection, but not during the F infection. Thus, it is quite likely that the full cascade of the T-cell mediated immune response to virus is require to establish a latent viral infection in neuronal cells.

326.10

PERIPHERAL METABOLIC AND ENDOCRINE RESPONSES INDUCED BY CENTRALLY ADMINISTERED INTERLEUKIN-1β IN THE RAT.
R.D. Stith, L. Templer and R.W. Blair*. Dept. Physiology and Biophysics, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

This study was based on the hypothesis that centrally-acting interleukin-1β (IL1) affects peripheral carbohydrate metabolism. Fasted, male Sprague-Dawley rats (275-400 g) were administered 1 uL of IL1 or sterile PBS via a 3rd ventricle cannula. Core body temperature (Tc) was constantly monitored and blood samples were obtained over 360 min from awake, freely moving rats via a jugular cannula implanted two days prior; liver tissue was obtained at the time of death. Peak Tc (39.6^{o}, 39.3^{o}, and 38.2^{o}; control=37.6^{o}) and plasma corticosterone (cort) levels (685, 666, 682 ng/ml; >300% of control) were measured after 15, 25, and 50 ng IL1, respectively. Plasma glucose was elevated in a dose-related manner: the peak of the response (100-105 mg/dL; 125% of control) was earlier (300 min @ 15 ng and 120 min @ 50 ng) and its duration was longer with increasing doses. Hepatic glycogen content was diminished to 50% of control by 15 ng, 40% of control by 25 ng IL1, and to 70% of control by 50 ng. The plasma insulin response to 15 ng IL1 (from 25 uU/ml to 80 uU/ml) paralleled the increase in plasma glucose. After 25 and 50 ng, however, plasma insulin levels remained at control basal levels in the face of elevated plasma glucose. No significant changes in plasma glucagon were observed. When the same doses of IL1 were injected iv, no changes in Tc or plasma cort were found. These data reveal that in addition to fever, anorexia, somnolence, etc., central IL1 induces changes in peripheral metabolism.

326.11

INHIBITION OF INTERLEUKIN-1-INDUCED CORTICOTROPIN-RELEASING FACTOR SECRETION BY ALPHA-MELANOCYTE-STIMULATING HORMONE IN VITRO. K. Lyson* and S.M. McCann. Department of Physiology, UT Southwestern Medical Center, Dallas, TX 75235-9040.

Interleukin-1 (IL-1) and interleukin-6 (IL-6) are potent stimulators of corticotropin-releasing factor (CRF) release from the hypothalamus, which can be responsible for the immunosuppressive effects of centrally administered cytokines. Since we demonstrated that alpha-melanocyte-stimulating hormone (α-MSH), an antipyretic compound in cytokine-mediated fever, could inhibit IL-6-induced CRF release from medial basal hypothalamus (MBH), we examined if such α-MSH action applies also to IL-1-induced CRF release. After 60 min preincubation in Krebs-Ringer bicarbonate buffer (KRB), MBHs were incubated for 30 min with KRB or IL-1 and/or α-MSH (10^{-15} - 10^{-11}M). CRF release into the incubation medium was measured by RIA. As reported previously, none of the α-MSH concentrations used changed basal CRF release significantly. IL-1 evoked a bell-shaped, dose-dependent increased CRF secretion with a maximal effective dose of 10^{-15}M ($p<0.025$), 100 times lower than that of IL-6, which was significantly suppressed, in a dose-dependent fashion, by α-MSH at concentrations of 10^{-14} and 10^{-13}M, with the maximal inhibitory effect at 10^{-13}M α-MSH ($p<0.001$). The results show that α-MSH is a potent inhibitor of IL-1-induced CRF release from the hypothalamus in vitro, which provides another evidence for the central antagonistic action of α-MSH against cytokines.

GENESIS OF NEURONS AND GLIA II

327.1

IDENTIFICATION OF A CHICKEN ACHAETE-SCUTE HOMOLOG EXPRESSED IN BRAIN AND RETINA. C.L.Jasoni, M.B.Walker, and T.A.Reh*. Department of Biological Structure, University of Washington, Seattle, WA 98195.

The *achaete-scute* genes of *Drosophila melanogaster* are members of the basic helix-loop-helix (bHLH) family of transcription factors, mammalian members of which include the c-myc proto-oncogene, several muscle determination genes, and mammalian *achaete-scute* homologs (MASH)-1 and -2. The *achaete-scute* complex genes, and presumably their vertebrate counterparts, are believed to play a role in the specification of neuronal identity. We employed a degenerate polymerase chain reaction (PCR) strategy to identify a chicken *achaete-scute* homolog (CASH-1). Fully degenerate PCR primers were designed based on conserved amino acid residues in the basic region and second helix of the bHLH motif. PCR, of random hexamer reverse transcribed total RNA extracted from stage 24 retina, yielded a single band of ≈140bp. Sequence analysis revealed the PCR fragment to be 88% identical to MASH-1 at the nucleotide level and 100% identical at the deduced amino acid level. Northern blot analysis, using cloned CASH-1 PCR fragment as a probe, revealed the presence of an mRNA of 1.4kb. CASH-1 mRNA is expressed in all CNS regions examined -- forebrain, midbrain, hindbrain, and retina -- but not in liver and heart. CASH-1 mRNA is expressed from embryonic day 3.5 through E8.5. The pattern and timing of expression of CASH-1 is similar to that found for MASH-1, and the high degree of sequence conservation between MASH -1 and CASH-1, suggests that these molecules have important functions in early neural development. Supported by NSF BNS-9110676.

327.2

ANALYSIS OF A DROSOPHILA GENE CLUSTER EXPRESSED DURING CNS DEVELOPMENT S.D.Zhang, D.Mellerick-Dressler, H.Gainer * W.Odenwald and J.Kassis # Lab. of Neurochemistry,NINDS,NIH and # Ctr. for Biologics Evaluation and Research, FDA, Bethesda, MD 20892

Enhancer-detection screening has led to the discovery of two closely spaced Drosophila genes, *castor* and *pollux*, that are expressed in the developing CNS. The two genes, separated by 99 bp, are located at the chromosomal subdivision 83c and are transcribed in converging directions.

castor encodes a putative transcription factor containing an acidic domain, multiple homopolymeric amino acid stretches and four linked TFIIIA like zinc fingers. Its expression is restricted to a subset of CNS neuroblasts and to ventral midline glial precursor cells during the intermediate stages of neurogenesis. The expression pattern of the segmentation gene *engrailed* is altered in the CNS of *castor* null mutant embryos, suggesting that *castor* is required for the proper differentiation of a subset of neuronal precursor cells.

DNA sequence analysis of *pollux* indicates that it encodes a leucine-rich protein, containing a putative leucine zipper motif. *pollux* transcripts are detected in oocyte nurse cells and are ubiquitous in early embryos. Late in embryonic development, *pollux* is expressed in CNS neurons. The analysis of *pollux* mutants will be reported.

327.3

Cell cycle-specific expression of the neuronal phenotype in an immortal hypothalamic cell line. **V. Quiñones-Jenab*, S. Meiners, M. Marone, and H. M. Geller. UMDNJ-Robert Wood Johnson Medical School and The Graduate School, Rutgers University, Piscataway, NJ 08854**

The *ts*V1 cells were derived by infecting E14 mouse hypothalamic cells with a replication defective retroviral construct containing the SV40 *ts*A58 large T antigen (T-ag). This cell line contains cells with two different morphologies: the vast majority of cells are flat and stellate; and a smaller number are phase-bright, process-bearing-round cells. The *ts*V1 cells express astrocyte-specific markers including: GFAP, S100, and tenascin. When the *ts*V1 cells enter the mitotic phase of their cell cycle, the flat cells become phase-bright and round and in some cases extend process. These cells have condensed chromosomes and also express many typical neuronal proteins, such as: neurofilament (NF) 200, NF-160, NF-68, tau, microtubule associated protein 2 (MAP2), GAP-43, and neuron-specific enolase. Other cells with similar morphology during mitosis, such as HeLa and C6 cell lines, did not express any of these neuronal markers. This would suggest that the *ts*V1 cell line may be a neuro-glial precursor expressing different phenotypes during the cell cycle. Alternately, these proteins may have a functional role in mitosis. We believe that this system is a good model to understand the control of cell fate, mitosis and maturation of hypothalamic glial and neuronal cells. Supported in part by NIH P01 NS 21469.

327.4

IMMORTALIZED STRIATAL AND MESENCEPHALIC CELLS EXPRESS D2-DOPAMINE RECEPTORS AND GAD_{67}. D. C. Chugani*, J. Segovia, A. J. Tobin, M. Mahmoudi, H. Roseboro. UCLA Departments of Radiological Sciences and Biology and The Brain Research Institute, Los Angeles, CA 90024

We have produced reversibly immortalized cells from embryonic day 10 rat mesencephalon and striatum using a retroviral vector containing the genes for temperature sensitive large T antigen of SV40 and neomycin resistance (G418) (obtained from R. McKay, MIT). Following selection in G418, surviving cells were cloned by dilution and grown at 33°C. At 39°C the cells stopped dividing, but displayed fibroblast-like morphology. Addition of bFGF, BDNF or NT3 together with IBMX and db-cAMP resulted in differentiation of cells into neuron-like morphology. Cell lines expressing neuronal morphology were screened under different growth conditions by both immunocytochemistry and PCR for brain cell markers and for neurotransmitter receptors and synthetic enzymes. We have identified six cell lines that express the neuron specific immunochemical markers, neuron specific enolase and neurofilament 68. These cells were screened by PCR for the D2 dopamine receptor and the GABA synthetic enzyme GAD_{67}.Three cell lines expressing neuron specific markers were found to contain mRNA for the D2 dopamine receptor and 2 of the cell lines, in addition, contained mRNA for GAD_{67}. These cell lines now provide us with a valuable tool for the study of regulation of the D2 receptor and GAD in response to pharmacological treatments.(Supported by NS 15654, DOE DE-FC03-87ER60615 and NS 22256)

327.5

HUMAN GAP43: MULTIPLE 5'UNTRANSLATED REGIONS AND DISTINCT REGULATION IN NEUROBLASTOMA CELL LINES: E Örtoft*, C Betsholtz, S Påhlman and U Hammerling, Dept of Pathology, University Hospital, S-751 85 Uppsala, Sweden.

The neuronal protein GAP43 is preferentially located in growth cone membranes of the elongating exon. Northern analysis revealed two GAP43 hybridizing bands of 1.4 kb and 1.6 kb in human neuroblastoma cell lines. The temporal expression of the two clusters of GAP43 mRNA differed during phorbolester-induced neuronal maturation of SH-SY5Y and LA-N-5 neuroblastoma cells. cDNA clones, encompassing overlapping stretches of the GAP43 transcribed regions, were isolated from SH-SY5Y cells. No heterogeneity was found in the open reading frame. A genomic clone of circa 1050 bp, overlapping the major transcription initiation sites, was also isolated. By RNase protection several GAP43 transcript species were identified, and two clusters of cap sites located circa 200 bp apart were found. The region 5'of the coding sequence was devoid of appropriately positioned consensus TATA- or CAAT-motifs. It is concluded that the synthesis of the larger GAP43 species is explained by alternative initiation of transcription.

327.6

A NEW METHOD FOR CULTURING ORGANOTYPIC CEREBELLAR SLICES: MATURATION, GROWTH AND NEURONAL MAPPING USING IMMUNOPEROXIDASE METHOD.

D.K. Racker*, D.J. LaPorte, P.E. Hockberger and J.C. Houk, *Dept. of Physiology, Northwestern Univ. Medical School, Chicago, IL 60611.*

A simplified method for culturing organotypic cerebellar slices (OTCS) is described utilizing glass coverslips and 12 well culture plates, as opposed to collagen gels or plasma clots and Maximow chambers (Seils, Rev. Neurosci., 4:329-3423, 1979) or roller tubes (Gahwiler, Neurosci. Methods, 4:329-342,1981).

Cerebelli of postnatal day 1 Sprague-Dawley rats were parasagitally sectioned into 1 mm segments (Microblades, Fine Science Tools) with the choroid plexus attached. Excised cerebelli and slices were kept cold throughout the procedure (Cryoplate, Streck Industries). Slices were allowed to recover in cold medium [40% fetal calf serum, 30% Hanks solution, 30% Dulbecco's modified Eagles medium, 1 mM L-glutamine, 6.6 g/L glucose, 26 mM $NaHCO_3$, 10 mM Hepes, 0.2 units/ml insulin and 1% antibiotic antimycotic (Sigma)] for 30 min, then washed in fresh medium, plated into separate wells containing 1 ml medium and pretreated coverslips, incubated at 37 °C with 95% humidity and 7% CO_2 and fed once weekly in medium without antibiotics. Coverslips (Gold seal, VWR) were scrubbed (Microclean, Cole Palmer), acid etched, and coated with 0.5 mg/ml polylysine (PL) or with PL and 10 µg/ml laminin or 50 µg/ml fibronectin. Organogenesis was evaluated in live and 4% paraformaldyhyde fixed OTCS at 1.5, 2, 3, 4 and 5 weeks in vitro and compared to perfused-fixed frozen sections of age-related littermates. Immunoreactivity specific for: Purkinje cells (PC, mono- and polyclonal calbindin, Sigma and S. Christakos); PCs and Deep cerebellar nuclei (DCN) neurons (302, S. Hockfield); Golgi cells and DCN (303, S. Hockfield); neuron specific enolase and astrocytes (NSE and GFAP, Dako) was visualized using the LSAB kit (Dako) along with Thionin for Nissl and Holmes stain for myelin.

Temporal development of cell types and myelination followed closely that in vivo. OTCSs thinned so that individual neurons were visible by direct observation and could be photograhed throughout the culture period. The choroid plexus was a topographical marker for the caudal cerebellar and DCN regions. Supported by NIH NS17489

327.7

HIGH CONCENTRATIONS OF MORPHINE INHIBIT PURKINJE CELL MORPHOGENESIS AND SURVIVAL IN ORGANOTYPIC CULTURES OF THE MOUSE CEREBELLUM.

K.F. Hauser*. Dept. of Anat. and Neurobiol., Univ. Kentucky Sch. of Med., Lexington, KY 40536-0084.

To determine whether opiates can intrinsically affect neuronal maturation in the cerebellum, the effects of morphine on Purkinje cell numbers and dendritic differentiation were examined in developing cerebellar explant cultures. Monoclonal antibodies against calbindin-D_{28k}, a cell-type-specific marker that labels the axon, soma, and dendrites of Purkinje cells, were used to immunolocalize Purkinje cells in permeabilized, whole-mount explants. Opiate-dependent changes in the maturation of calbindin-D_{28k}-immunoreactive Purkinje cells were examined in paired, symmetrical (right versus left) organotypic cultures isolated from 1- and 7-day-old cerebella from thirty-six male ICR mice. One explant from each pair was continuously exposed to 10 µM morphine, 10 µM morphine plus 30 µM (–)-naloxone, or 30 µM (–)-naloxone alone for 7-10 days in vitro, while the other explant served as a control. Purkinje cells were categorized as immature or mature depending on whether they had primary dendrites. In explants derived from 1-day-old mice, continuous morphine treatment significantly reduced the number of immature Purkinje cells and decreased the total dendritic length of mature Purkinje cells. Both effects were completely blocked by concomitant treatment with naloxone. This suggests that the cellular response to morphine was not a result of generalized toxicity but mediated through specific opioid receptors. Morphine treatment did not affect the number or dendritic length of Purkinje cells in explants derived from 7-day-old mice. Collectively, the results suggest that at high concentrations opiates can inhibit Purkinje cell morphogenesis and survival in a selective, age-dependent manner. These effects are likely to be mediated through a direct action on endogenous opioid systems within developing cerebellar cultures. Supported by NIDA grant DA 06204.

327.8

OPIATES DO NOT AFFECT ASTROCYTE DEATH IN DEVELOPING MIXED-GLIAL CULTURES.

J.A. Gurwell* and K.F. Hauser. Dept. Anat. and Neurobiol., Univ. Kentucky Sch. of Med., Lexington, KY 40536-0084.

Opiates reduce the number of cells in mixed-glial cultures in part by inhibiting the rate of proliferation of type 1 astrocytes. High concentrations (1 µM) of morphine, however, cause a complete cessation in the net production of cells, despite continued DNA synthesis, suggesting that morphine is killing cells. To determine whether morphine affects glial survival, dissociated mixed-glial cultures of ICR mouse cerebra were maintained in DMEM with 10% fetal calf serum and continuously exposed to 1 µM morphine, 1 µM morphine plus 3 µM naloxone, or 3 µM naloxone alone. Cell death was assessed using fluorescent markers for living (calcein AM) and dead (ethidium homodimer) cells (Live/Dead Viability/Cytotoxicity Assay; Molecular Probes Inc., Eugene, OR) at 3, 5, and 7 days *in vitro*. After a 40 min. incubation, live cells fluoresced green (an indication of esterase activity), whereas the nuclei of dead cells fluoresced red. Cell counts indicated that there was a significant loss of cells in all treatment groups. However, opiate treatment did not affect the number of dying cells compared to control values at 3, 5, or 7 days *in vitro*. Thus, the rate of cell death was always similar in control and treated cultures. When the identity of the dying cells was investigated by combining the Live/Dead Assay with immunocytochemical markers for astrocytes (GFAP) and bipotential O-2A glial progenitors (A2B5), GFAP+ type 1 astrocytes were not found to be dying, whereas 1-4% of the A2B5+ cells were dying (per 40 min. labeling period) at varying times *in vitro*. These and previous findings suggest that opiates decrease the number of type 1 astrocytes solely by inhibiting proliferation and not by altering their survival. The loss of O-2A progenitors occurs irrespective of opiate treatment and reflects the developmental dynamics occurring in these cultures. Supported by NIDA grant DA 06204.

327.9

DEVELOPMENTAL PROFILE OF GFAP IN THE RAT BRAIN: INCREASED REGIONAL GFAP FOLLOWING PRENATAL EXPOSURE TO METHYLAZOXYMETHANOL.

E. S. Goldey[1], S. Barone, Jr.[2], K. M. Crofton[1] and J. P. O'Callaghan*[1]. [1] Neurotoxicology Division, U.S. Environmental Protection Agency and [2] ManTech Technologies, RTP, NC 27711.

Reactive gliosis, as evidenced by qualitative and quantitative increases in glial fibrillary acidic protein (GFAP), is a universal response to neural damage of the adult CNS. This same response only rarely has been demonstrated following damage to the fetal or neonatal CNS. Here we examined: 1) the ontogenetic profile of GFAP immunoreactivity and content in specific regions of rat brain from birth to adulthood and 2) the temporal and regional profile of GFAP immunoreactivity and content following prenatal exposure to the developmental neurotoxicant methylazoxymethanol (MAM). Rats were given a single i.p. injection of saline or MAM (30 mg/kg) on day 15 of gestation. GFAP content was determined in nine brain regions collected from pups on each of postnatal days (PND) 1, 4, 10, 21, and 84. GFAP concentrations increased throughout brain development and reached near adult levels by PND 21. A consistent regional GFAP concentration gradient was revealed (high to low concentration): brain stem > hippocampus > cerebellum = olfactory bulbs = diencephalon = mesencephalon > cerebral cortex > striatum. MAM exposure caused significantly elevated GFAP concentrations compared to controls only in the cortex and hippocampus, regions previously demonstrated to be extensively damaged by MAM. GFAP was significantly elevated in the rostral half of the cortex on PND 1, and by PND 4 rostral cortex, caudal cortex and hippocampus all showed significantly elevated GFAP concentrations relative to controls. In addition, GFAP concentrations were significantly elevated in the cortex samples collected from mature (PND 84) MAM-treated animals. These results provide a profile of the developmental increase in GFAP in specific brain regions and indicate that neurotoxicant exposure of the fetal CNS induces a rapid and long-lasting elevation of GFAP in the damaged brain regions.

327.10

ASTROCYTES EXPRESS GABA, NSE AND EARLY GLIAL IMMUNOREACTIVITIES AFTER ISCHEMIC- OR LESION-INDUCED INJURY IN THE ADULT FOREBRAIN.

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Transformation of normal astrocytes to reactive astrocytes after injury in the adult brain is a well- known phenomenum. Recently, we have shown that reactive astrocytes express GABA but not its synthesizing enzyme-glutamic acid decarboxylase (GAD) immunoreactivity in the gerbil forebrain after transient ischemia. The expression of GABA immunoreactivity in reactive astrocytes begins around 4-7 days and lasts for 3 months after ischemia. Using a double immunofluorescent method, we have found that these cells are not only immunoreactive to GABA but also to GFAP or vimentin, confirming their glial identity.

Previous in vitro tissue culture studies have shown that 02A progenitor cells are immunoreactive to GABA but not GAD. This raises the question of whether reactive astrocytes in the adult brain may re- express fetal trait(s) of astrocytes after injury. To further elucidate the phenotype of astrocytes after injury in the adult brain, we made lesions in the forebrain using either transient ischemia or thermal lesions as our experimental models. We then used a panel of antibodies including A2B5 (a marker for 02A progenitors and type 2 astrocytes), galactocerebroside (GalC; a marker for 02A progenitors and oligodendrocytes) and neuron specific enolase (NSE; a marker for neurons) to study the identity of reactive astrocytes. Using immunofluorescent techniques, we found that reactive astrocytes immunostained with GABA are also immunohistochemically labeled with NSE, A2B5 or GalC. These preliminary results further support the notion that reactive astrocytes in the adult brain can re-express fetal trait(s) of undifferentiated glial cells after injury. The functional significance of the aberrant expression of GABA and NSE immunoreactivity by astrocytes will be further investigated with physiological and pharmocological approaches.

327.11

MET-ENKEPHALIN IMMUNOREACTIVITY IN HIPPOCAMPAL ASTROCYTES FOLLOWING BILATERAL DENTATE GRANULE CELL LESIONS. L. Thai*, C. Mitchell, M. Barns, and J.S. Hong. Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599; LMIN, NIEHS/NIH, Research Triangle Park, NC 27709.

In the primary hippocampal culture system, proenkephalin and its bioactive peptides are present in the type I astrocytes in great abundance. Hauser et al., (1987) suggested that enkephalin secreted from glial cells may suppress cell growth. Up to this time, however, enkephalin has not been localized within glial cells in the hippocampus. The purpose of this study was to examine whether enkephalin peptides are present in glial cells within injured or regenerative areas. Colchicine was injected bilaterally into the hippocampus to selectively degenerate dentate granule cells. Met-enkephalin immunoreactivity was determined by using antibodies against Met-enkephalin in two-week and two-month post-lesion animals. As revealed by glial filament acetic protein (GFAP) antibody staining, glial cells were found in higher number in the lesioned hippocampi at the two-week compared to the two-month time point. Met-enkephalin immuno- reactivity correlated positively with the GFAP labeling. A shrinkage of the hippocampi was also observed in the two-month post-lesion animals. The emergence of Met-enkephalin in glial cells following colchicine lesion suggests a possible role of Met-enkephalin for regulating glial cell growth in injured brain regions.

327.12

LESION-ENHANCED NEURONAL INCORPORATION OF REPLICATION MARKERS INTO THE ADULT RAT BRAIN. D.G. Herrera*, M. Skup, A. Ribeiro-da-Silva and A.C. Cuello. McGill University, Department of Pharmacology, 3655 Drummond Street, Montréal, Québec, Canada H3G 1Y6.

We investigated whether spontaneous or lesion-induced neuronal reproduction may occur in the adult rats by using two markers of cell division: tritiated thymidine (^{3}H-Thy) and bromodeoxyuridine (BrdUrd). Experiments were performed on both control (i.e. unoperated), and fimbria-fornix (FF) transected animals. One hundread twenty nanograms (100 μCi) of ^{3}H-Thy or one hundread micrograms of BrdUrd were administered intracerebroventricularly immediately after FF transection. Ten days or 1 month after onset of administration brains were fixed and processed for autoradiography (^{3}H-Thy) or immunocytochemistry (BrdUrd). Light and electron microscopic studies revealed that ^{3}H-Thy and BrdUrd were incorporated into the nuclei of neuron-like cells localised predominantly in the granular cell layer of the dentate gyrus (DG) of the hippocampal formation. Most of the neuron-like cells analyzed by E.M. proved to be neurons. Unilateral FF lesion enhanced the incorporation of both markers into neuron-like cells in DG. This was more evident one month after the lesion. These results suggest that under certain conditions DNA synthesis in the adult mammalian brain may be stimulated. (Funded by the Medical Research Council of Canada and the Centres of Excellence Network for Neural Regeneration and Functional Recovery).

327.13

DISCRETE BRAIN LESIONS PROMOTE NEUROGENESIS IN ADULT RING DOVES--A POSSIBLE MECHANISM UNDERLYING BEHAVIOR RECOVERY? C. Ling*, M. Chaiken and M.-F. Cheng. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102

Brain lesions are known to induce reactive gliogenesis throughout life. However, there have been no reports about the relationship between brain lesions and neurogenesis in higher vertebrates. It was thought that neurogenesis stops after birth in birds and mammals. Studies of songbirds have revealed that neurogenesis occurs in adult birds, and we have recently demonstrated that neurogenesis persists throughout life in ring doves. In this study we explore the effects of brain lesions on neurogenesis and the relationship between lesion-induced neurogenesis and behavioral recovery in adult doves. Twenty doves received electrolytic lesions in the Nucleus Taeniae (avian Amygdala) of the telencephalon, and then received daily [3H]thymidine injections for seven days, starting 2 days after lesioning. The birds were sacrificed 2 months after the last injection. The courting and mating behavior of these birds was observed before and after electrolytic lesions or sham lesions. We found that discrete brain lesions promote neurogenesis--the number of new neurons per brain section was doubled, and new neurons concentrated around the lesion site. Changes in courtship behaviors after lesioning were correlated with the time course of neurogenesis.

327.14

IN VITRO NEUROGENESIS BY MULTIPOTENTIAL PRECURSOR CELLS OF THE ADULT AVIAN BRAIN S. A. Goldman*[1], A. Zukhar[1], T. Mikawa[2] Depts. of [1]Neurology and [2]Cell Biology, Cornell Univ. Medical College, NYC, NY 10021.

The adult songbird brain generates neurons throughout life, from stem cells located in the forebrain ventricular zone. We previously found that explants derived from one such region, the vocal control nucleus HVC, continue to generate neurons *in vitro* when cultured under relatively low serum conditions. This paradigm has allowed us to study the ontogeny of the new adult neurons, by using retroviral-mediated insertion of *lacZ* into cultured HVC ventricular zone cells, followed by an analysis of the progeny generated *in vitro*. Presumptive HVc ventricular zone from 3-7 day old canaries was dissociated into low-serum media, plated upon laminin, and then exposed to the *lacZ*-bearing retrovirus CXL. After fixation 6 days later, clonally-derived cells were identified by β–galactosidase expression. Single ventricular zone cells yielded clusters of co-derived neurons and radial cells, whose identities were confirmed by immunolocalization of MAP-2 and vimentin, respectively. Among 301 β-galactosidase$^+$ clones examined, 19 included neurons; of these, none contained more than 1 neuron (median 4 cells/cluster, range 1-6 cells). Several clones included both MAP-2$^+$ neurons and vimentin/mAb 3A7$^+$ ependymoglia and radial cells (mAb 3A7 courtesy of V. Lemmon). These results suggest the *in vitro* division of single *lacZ*-infected precursors, and the asymmetric differentiation of their progeny into both neurons and non-neuronal siblings. The newly produced neurons and radial guide cells of the postnatal avian brain may thus arise from a common, pluripotential stem cell, capable of generating both neurons and their substrate cells. The multipotentiality of these stem cells suggests that adult neurogenesis may be restricted not only by a humoral inhibition of precursor cell mitosis, but also by a diversion of daughter cell phenotype into non-neuronal lineages.

327.15

DEVELOPMENT OF PERIPHERAL SPINAL PROJECTIONS: EPIGENETIC INFLUENCES ON THE EXPRESSION OF SPINAL NEUROTRANSMITTERS. N. Leblanc, G. Audet, R. Marchand and E. Philippe*. Centre de recherche en Neurobiologie, Univ. Laval, Québec, Canada, G1J 1Z4.

During the development of a neuronal population, the microenvironment may play a major role in initiating and maintaining the expression of phenotypes such as γ-aminobutyric acid (GABA) and dopamine. In order to determine what factors could induce expression of these transmitters in spinal motoneurons, we will verify at what embryonic stage the efferent contacts are established as well as the stage at which these motoneurons are contacted by the peripheral, propriospinal and supraspinal afferences.

DiI crystals (a fluorescent tracer) deposited in the leg of embryonic chicken fixed at different stages, allows us to study the development of the peripheral sensitive and motor projections.

When DiI crystals are implanted at E4.5, fluorescence is visible at the lombo-sacral level in the dorsal and ventral roots, the motoneurons and the spinal ganglions. An oval longitudinal bundle is observed, on transverse sections, in the white substance of the dorsal horn. This bundle extends to adjacent segments. At later stages (E5, E6), the bundle is broader and subdivided, suggesting an increase in the number of axons. At theses stages we also notice that DiI is progressively seen in small nervous fibres of the intermedio-lateral region of the grey substance.

Knowing that the expression of various phenotypes is initiated at these embryonic stages, it would be interesting to determine if the initiation of the contacts described above have an influence on the appearance of GABA and dopamine in the spinal neurons. (Supported by l'Association de l'ataxie de Friedrich and the F.R.S.Q.)

327.16

A TEMPORAL RELATIONSHIP BETWEEN NEUROGENESIS AND NEURONAL DEATH IN THE HIGH VOCAL CENTER OF THE ADULT CANARY BRAIN. J.R. Kirn*, S. Kasparian & F. Nottebohm. The Rockefeller University Field Research Center, Millbrook, NY 12545.

The high vocal center (HVC) in songbirds continues to receive new neurons in adulthood (Goldman &Nottebohm, 1983). In canaries, neurogenesis is coupled to neuron loss (Alvarez-Buylla et al., 1990; Kirn & Nottebohm, 1991). However, little is known about the extent to which neuron production and death vary throughout the year --- information necessary to understand how adult neuronal replacement is regulated. We used ^{3}H-thymidine to quantify neurogenesis and pyknotic cell counts to measure cell death at monthly intervals for a period of 1 year. Adult male canaries (15-28 months old) received 4 days of ^{3}H-thymidine injections as described elsewhere (Kirn & Nottebohm, 1991). Birds (n=2-5 / month) were sacrificed 27 days later. Brains were processed for autoradiography and counterstained with cresyl violet. ^{3}H-labelled neurons and pyknotic cells were found in HVC at all times. Sept. and Feb. showed the highest incorporation rates of new neurons. Pyknotic cell densities were also highest in Sept. and in Jan.-Feb. In additional birds injected in RA (robustus archistriatalis) with fluorogold (FL) in Oct.-Feb. or in May (3-5 birds / group), 54% of pyknotic cells in HVC were FL-labelled and therefore were degenerating RA-projecting HVC neurons. Collectively, these results show that adult neurogenesis and neuronal death are temporally related. Testosterone levels drop sharply prior to the observed peaks in HVC neuronal turnover (Nottebohm et al., 1987). There may be a relationship between neurogenesis, neuron death and endocrine state. (Supported by NIH #MH18343 & NS29843)

327.17

GENERATION AND MOVEMENT OF CELLS IN THE DEVELOPING FROG CEREBELLUM. N.J. Uray*. Dept. of Anatomy, Kirksville College of Osteo. Med., Kirksville, MO 63501.

Normally developing and thyroxine-induced (100 ppm) metamorphosing bullfrog tadpoles were used in this study. Routine histological sections and ^{3}H-thymidine (10μCi/g) autoradiograms of brains were examined to analyze cerebellar cell generation and movement using the method devised by Rüdeberg.

The most useful data came from the thyroxine-induced metamorphosing animals. In these specimens, Rüdeberg's migrations A, A_1 and B could be clearly detected and demarcated. Analysis of the composition and cell movement of migration B, however, is difficult and complicated. In the frog, cells which are homologous to the deep cerebellar nuclei of mammals are mostly ventral to the cerebellar plate, and a distinct migration layer (nuclear transitory zone of Altman and Bayer) is not present in the cerebellar plate, nor does a transposition of these cells and Purkinje cells (cortical transitory zone) occur as described in the rat. Migration B, however, can be subdivided into two separate zones, the superficial one being composed of Purkinje cells and a deep one, formed by small cells whose identity is not clearly understood. Overall, cerebellar formation in the frog has many similarities to the process observed in mammals, but appears to be more simple and less organized. Supported by NIAAA Grant AA97537.

327.18

BIRTH AND FATE OF B & C NEURONS AND GLIA IN BULLFROG SYMPATHETIC GANGLIA. WD Stofer* and JP Horn Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Times of origin of sympathetic neurons and glia in paravertebral ganglia 9 & 10 of the bullfrog were determined by injecting tadpoles repeatedly with 5'-bromodeoxyuridine (BRDU) for 1 - 6 developmental stages between III and XXI. Our goal was to determine whether the birthdates of sympathetic neurons were related to their subsequent fates as cutaneous B cells or vasomotor C cells. After allowing BRDU-injected tadpoles to enter late metamorphic stages (XX-XXV), the ganglia were double-immunostained for BRDU and neuropeptide Y (NPY) using distinct black and brown HRP reaction products. BRDU was localized in nuclei of labeled neurons and glia. NPY was localized in perinuclear cytoplasm of C neurons. Cell counts showed that at least 21% of ganglionic neurons are born during tadpole stages and that neurogenesis ceases during early foot stages (XII-XIV), a time coinciding with the onset of NPY expression. By contrast, the labeling of non-neuronal satellite cells with BRDU was most common during later tadpole stages. Irrespective of their birthdates, about half of the BRDU-labeled neurons were also positive for NPY. This proportion of NPY-positive neurons is indistinguishable from that in adult ganglia.

The results show that: i) early tadpole ganglia contain neuronal and glial precursors, ii) birthdates are different for neurons and glia in this system, and iii) birthdates are not determinants for phenotypic subclasses of sympathetic neurons.

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327.19

SEGMENT SPECIFIC CELL DEATH IN THE PROGENY OF HOMOLOGOUS NEUROBLASTS IN THE GRASSHOPPER. Karen J. Thompson and Melody V.S. Siegler* Dept. of Biology, Emory Univ., Atlanta GA 30322

The median neuroblasts (MNBs) in grasshoppers are identified neuronal stem cells. Each segment of the embryonic ventral nerve cord has a posterior MNB which gives rise to a midline group of neurons. The number of MNB progeny differs between segments. In the metathoracic segment (T3), about 95 neural progeny from the MNB survive embryonic development, whereas in the next posterior segment, the first abdominal (A1), only about 60 neural progeny survive. Segment specific patterns of development account for these differences in neuronal number. In T3, the MNB arises at 29% of embryogenesis and dies at 78%, whereas in A1, the MNB arises at 30% and dies at 73%. In T3, the number of MNB progeny increases at a steady rate, with about 10 cells being added to the midline group per 5% of embryogenesis until 70%. Between 70% and 80%, growth tapers off: although the T3 MNB continues to divide, cell death occurs at the same time, specifically removing the last born progeny. By contrast, in A1, the number of MNB progeny increases in two distinct phases, one from 30% to 45% and the other from 60% to 75%, again at the rate of 10 cells per 5%. Between the two phases, the population is stable: the A1 MNB continues to divide, but cell death occurs coincidentally with the effect of removing cells from the population at the same rate they are being added. Cell death specifically removes earlier born progeny in A1. Thus, the lifespan of the MNB, and the timing and magnitude of cell death among its progeny, differ between the T3 and A1 segments, and result in differences in the number and composition by cell type of the midline groups of T3 and A1.

327.20

RETINAL AFFERENTS AFFECT MITOTIC ACTIVITY AND CELL DEATH IN THE DEVELOPING OPTIC LOBES OF THE MOTH, MANDUCA SEXTA. S.A. Monsma* and R. Booker. Section of Neurobiology and Behavior, Cornell University, Ithaca NY 14853.

The two outer optic ganglia of holometabolous insects, the lamina and the medulla, are produced during metamorphosis from a single structure, the outer optic anlage (OOA). Neuroblasts (Nbs) in the OOA undergo asymmetric divisions to yield an Nb and a ganglion mother cell (GMC); the GMCs divide symmetrically to yield two ganglion cells. Medulla ganglion cells are produced by Nbs and GMCs in the medial half of the OOA, while lamina ganglion cells are produced by Nbs and GMCs in the distal half. Cell death is apparent in the developing medulla cortex as well as in the lamina cortex, usually in a zone adjacent to the OAA.

To examine the influence of retinal afferents on mitotic activity and cell death, we sectioned the optic nerve on one side at pupation, leaving the contralateral side as the unoperated control. Denervation did not affect the mitotic activity of the medulla Nbs, GMCs, or the lamina Nbs. However, the mitotic activity of lamina GMCs was completely abolished within 24 hr of denervation. Further growth of the lamina was completely blocked, and an ectopic population of lamina GMCs built up. These ectopic GMCs degenerated 1-2 d before the OAA degenerated.

Cell death in the already-present lamina continued for up to 3 d after denervation. Cell death in the medulla continued at a normal rate until 6 d after denervation. Thereafter, cell death in the medulla cortex climbed to approximately twice the level of the control side, and remained elevated until ~2 d after the OAA degenerated. These results suggest that retinal afferents can influence both mitotic activity of precursors and cell death.

DEVELOPMENT OF NEUROTRANSMITTER SYSTEMS: CATECHOLAMINES AND NEUROPEPTIDES

328.1

PRENATAL EXPOSURE TO DESIPRAMINE CHANGES THE DEVELOPMENT OF BETA RECEPTORS IN RAT CORTEX X.-K.Gao, A. J. Friedhoff and K. A. Bonnet* Millhauser Lab., N.Y.U.Sch. Med., New York, N.Y. 10016

Prenatal exposure to desipramine (DMI) at 10 or 25 mg/kg was effected via pregnant rat dams, in drinking water, at gestation days (GD) 7-13. DMI at 10 mg/kg caused a significant decrease in 3H-dihyrdroalprenolol (3H-DHA) binding sites in brain cortex of the 14 day old offspring, and later postnatal increase in 3H-DHA binding sites in 28 and 56 day old offspring. DMI at 25 mg/kg caused a significant increase in 3H-DHA binding sites in 7 day old offspring. Since fetal brain begins to show uptake of NE at presynaptic sites at GD18, the effect of DMI may not involve direct presynaptic effects of fetal brain. We observed a dose-dependent increase in NE in blood serum of pregnant mothers during and after treatment with DMI. Since different doses of DMI have differential effects on the development of brain b-receptors, mothers' indirect effects on fetus does not explain the differential effects of DMI. This suggests that prenatal exposure to DMI may have a direct effect on postsynaptic programmatic development in fetal brain.

Supported by Grants MH-08618 and the Courtney Block Fund

328.2

EFFECTS OF PERINATAL EXPOSURE TO COCAINE ON [^{3}H]YM-09151-2 BINDING IN ADULT RAT BRAIN D.L. Dow-Edwards*, B. Landwehrmeyer, and J.M. Palacios. Lab. of Cerebral Metabolism, Dept. of Pharmacology, SUNY-Brooklyn, NY and Sandoz Pharma Ltd., Basel, Switzerland.

Previous studies from this lab have shown that perinatal exposure to cocaine results in alterations in brain glucose metabolism which can be identified in adult male and female rats (e.g. Dev. Brain Research 42:137, 1988). Since many of the regions affected are rich in dopaminergic projections, we examined the brains of similarly treated rats for the concentrations of dopamine receptors. For the present study, rat pups were injected with either cocaine HCl at 50mg/kg/day or vehicle during postnatal days 1-10 or 11-20. At 60 days of age, rats were sacrificed and the brains processed for autoradiography. Sections were incubated for 30 min at RT with 1nM [^{3}H] YM-09151-2 (New England Nuclear), a ligand with high specificity for the D2 receptor. Sections were apposed to [^{3}H] hyperfilm for 2 weeks along with [^{3}H] standards from Amersham. Computerized image analysis using the Loats Imaging System shows that the alterations in YM-09151-2 binding produced by cocaine depend upon the period of drug exposure and the gender of the animal. From these data as well as the data of others, one can conclude that developmental exposure to cocaine has long term effects on the function of central dopamine systems.

Supported by NIDA grant DA04118.

328.3

DEVELOPMENT OF HUMAN BRAIN DOPAMINE D_2 RECEPTORS. J.R. Atashi[1*], C.P. Lawler[1,2,4], C.A. Mathis[5], R.B. Mailman[1,2,3,4] and J.H. Gilmore[1,2,4]. Curriculum in Neurobiology[1], Departments of Psychiatry[2] and Pharmacology[3], and the Brain and Development Research Center[4], University of North Carolina, Chapel Hill, NC, 27514-7250 and Department of Radiology, University of Pittsburgh[5], Pittsburgh PA, 15213.

Alterations in the development of dopamine neurotransmission have been hypothesized to play a role in the pathogenesis of several neuropsychiatric disorders. The present studies examined several aspects of development and localization of dopamine D_2 receptors in post-mortem human brain tissue, using quantitative receptor autoradiography with the selective D_2 benzamide ligand [^{125}I]-epidepride. Time course studies were conducted at ambient temperature. In tissue from both adult and infant brain, maximal binding was reached by ca. 2.5 hr, and remained relatively stable for at least another 10 hr. Preliminary saturation analyses using non-linear regression have been completed in post-mortem brain from one infant (3 mo old, 24 hr post-mortem interval) and one adult brain (53 yr, 16 hr post-mortem interval). The densities of [^{125}I]-epidepride receptors were not significantly different either between the caudate and putamen, or between the adult and infant. On the other hand, in this experiment the K_D was significantly higher in both adult regions compared to the same regions in the infant. In the caudate, D_2 receptors were found to be present in a heterogeneous pattern of density, with areas of low D_2 binding corresponding to acetylcholinesterase-poor zones as defined by histochemistry in adjacent sections. This pattern was present in adult caudate, as well as at six weeks, three months, four months, and four years of age. This pattern appeared in a distinct medial-lateral gradient in adult, but was less defined in the younger subjects relative to the adult. These studies provide further evidence that [^{125}I]-epidepride appears to be a useful ligand to study the localization of D_2 receptor development in human brain. These data also suggest that D_2 receptors undergo significant and specific changes in localization during development.

(Supported, in part, by PHS Grants MH40537 and the Foundation of Hope).

328.4

EFFECTS OF ADULT AND NEONATAL HEMIDECORTICATION ON MUSCARINIC AND D2 RECEPTOR BINDING IN THE CAT NEOSTRIATUM A. Ebrahim [§], K.J. Tatsukawa[Δ§], L.D. Loopuijt[Δ], S Chen [§], D.A. Hovda[†], J.R. Villablanca[Δ], H.T. Chugani*[§] UCLA School of Medicine, [Δ]Ment. Retard. Res. Ctr., [†]Div. Neurosurg., [§]Nuc.Medicine.

This study examined the influence of hemidecortication performed at two different developmental stages on D2 receptors and muscarinic binding in the cat neostriatum. Receptors were measured in the lateral, intermediate and medial caudate, putamen and accumbens nuclei (at rostral and caudal levels) by means of quantitative autoradiography. Tissue was incubated using 1nM [3H]spiperone in the presence of 10μm ketanserin for D2 receptor binding, and in 2nM [3H]quinuclidinyl benzilate for muscarinic receptor binding. Three groups of animals were used: cats hemidecorticated in adulthood (AH, N=3) or at 8-13 day old (NH, N=4) and intact controls (C, N=4).

The muscarinic receptor binding showed no differences between the 3 groups and no right vs. left asymmetries within each group [ipsilateral/left: anterior lateral caudate: NH 622.8 (range 620.5-625.2), AH 650.8 (range 442.1-908.1), C 527.2 (range 493.0-563.1) fmol/mg tissue; contralateral/right: NH 632.9 (range 597.9-667.8), AH 628.5 (range 412.0-754.4), C 585.8 (range 548.4-654.5) fmol/mg tissue]. Dopamine D2 receptor binding also showed no differences between the three groups with one exception; in NH the density in the anterior (lateral) caudate and putamen was higher ipsilaterally compared to C (ipsilateral/left NH 86.4 (range 32.3-75.8), C 38.2 (range 20.0-60.2) fmol/mg tissue. These findings indicate that the deafferentation of the caudate-putamen has no influence on the density of the muscarinic receptors, but results in an increase of D2 receptor binding in parts of caudate and putamen in the NH. Since the increase does not occur in AH, this reflects a permanent change due to plasticity in early development and may represent part of the D2 binding that is not functionally coupled to inhibition of acetylcholine release. Grants USPHS HD-05958,HD-04612, 2P01-NS15654-13.

328.5

TRANSITORY DETECTION OF DOPAMINE IMMUNOREACTIVITY IN DIFFERENCIATING NEURONS OF EMBRYONIC AND LARVAL LOBSTERS. I. Cournil* and M. Moulins. Lab. Physiol. Neurobiol. Comp., Université de Bordeaux I et CNRS, Arcachon, 33120, France.

We were aiming to understand the timing and mechanisms involved in the selection of neurotransmitter phenotypes in the developing nervous system of *Homarus gammarus*. Dopamine and its rate limiting enzyme, tyrosine hydroxylase, are widely distributed in the adult lobster nervous system and can be readily vizualized immunocytochemically.

To trace the development of dopamine containing neurons in embryos and larvae, whole animals at various developmental stages were fixed, embedded in paraffin, and sectioned prior to staining. The adult pattern of dopamine staining appears progressively from 50% development, and once differentiated, most dopamine- containing neurons are maintained throughout subsequent ontogeny of the embryonic lobster. However an intense immunoreactivity which can be detected early in the embryonic neurons of the suboesophageal ganglion and stomatogastric nervous system (innervating pericardial muscles and foregut muscles respectively), has disappeared by the adult stage. We are currently investigating this phenomenon to determine (1) whether it is due to cell death in the adult or to the transitory expression of dopamine by still surviving neurons, and (2) its possible significance for the development of target muscles.

328.6

LONGTERM ALTERATIONS IN NEUROTRANSMITTER BIOSYNTHETIC ENZYMES IN RESPONSE TO NEURONAL INJURY. M. Weiser*, H. Baker, T. Wessel and T.H. Joh Lab. Molec. Neurobiol. Cornell Univ. Med. Coll., Burke Med. Res. Inst., White Plains, NY 10605

Alterations in the levels of neurotransmitter biosynthetic enzymes are a concomitant of many neurodegenerative disorders. To determine the longterm alterations in the levels of neurotransmitter biosynthetic enzymes in response to axotomy, a microknife lesion was placed in either: (1) the posterolateral hypothalamus transecting the axons of both the dopaminergic neurons in the substantia nigra pars compacta (SNc) and the noradrenergic neurons in the locus ceruleus (LC); or (2) more rostrally in the anterior forebrain severing the axons of cholinergic neurons in the horizontal limb of the diagonal band (HLDB). At timepoints from 7 days to 6 weeks, the changes in the mRNA and protein levels for tyrosine hydroxylase (TH) and choline acetyltransferase (ChAT) were examined in Sprague-Dawley rats by *in situ* hybridization and immunohistochemistry. Following unilateral axotomy, TH mRNA and protein levels in the LC were reduced at 14 days and returned to controls levels by 28 days. In contrast, TH mRNA and protein levels in the SNc were irreversibly decreased in a majority of the remaining neurons at both 14 days and 6 weeks. However, a few neurons in the SNc had normal or high levels of TH mRNA. In comparison, in the HLDB, while ChAT mRNA levels increased at 7 days following axonal transection, ChAT protein levels were reduced below control values. Both ChAT protein and mRNA levels were reduced to at least 40% of control values at 6 weeks. These data suggest that the neuronal response to axotomy is phenotype specific and may depend on the axonal arborization pattern of a neuron. Supported by MH44043 and AG08702.

328.7

INDUCTION OF NADPH DIAPHORASE ACTIVITY IN CULTURED PRINCIPAL NEURONS OF THE SUPERIOR CERVICAL GANGLION (SCG) OF THE POSTNATAL RAT.

P.B. Senatus, M.D. Johnson and D.D. Potter*. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

In adult rat neurons, reduced nicotinamide adenine dinucleotide phosphate diaphorase activity (NADPH-DA) has been correlated with the presence of neuronal nitric oxide (NO) synthase (e.g. Hope et al., 1991). Principal neurons of the SCG have been reported to be unreactive (e.g. Grozdanovich et al, 1992), and we have confirmed this finding using NADPH diaphorase histochemistry in 40 um thick frozen sections of SCG's from adult and postnatal Long Evans rats of both sexes. However, sympathetic neurons dissociated from postnatal SCG's became visibly reactive for NADPH-DA after a few days in culture (less than 1% at the time of plating; about 80% after 1 week), and the reactivity was strongly enhanced if 5% adult rat serum was present in the culture medium. Neurons containing NADPH-DA retained immunoreactivity for tyrosine hydroxylase.

About 80% of postnatal SCG principal neurons co-microcultured with dissociated hippocampal cells develop cholinergic function in the first three weeks in culture (Johnson and Potter, 1989). Although most co-microcultured SCG neurons became visibly reactive for NADPH-DA, no correlation was found between NADPH-DA and cholinergic function when synaptic function was assayed using intracellular electrophysiological methods. These data indicate that NADPH-DA can be induced in sympathetic neurons, and raise the possibility that expression of NO synthase can be regulated in the nervous system. Supported by the Freudenberger Fund and NS 02253-32. P.S. is a recipient of a MARC Fellowship. M.J. is a recipient of an American Psychological Assoc. Minority Fellowship and a Harvard Ryan Fellowship.

328.8

NEUROPEPTIDE EXPRESSION BY SYMPATHETIC NEUROBLASTS. S. Tyrrell* and S. C. Landis. Dept. of Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

During development, neurotransmitters and their synthetic enzymes are not normally expressed until after neuroblasts have undergone their final division. One exception is the expression of adrenergic properties, tyrosine hydroxylase (TH) and catecholamine histofluorescence, by sympathoadrenal (SA) precursors and the neural crest cells that colonize the gut. Since many sympathetic neurons contain neuropeptides as well as catecholamines, we examined embryonic sympathetic ganglia containing SA precursor cells for the presence of neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP). At embryonic day (E)11.5, TH immunoreactivity (-IR) was present in the developing superior cervical (SCG) and stellate ganglia but neuropeptide-IR was undetectable. One day later, at E12.5 when only a small number of sympathetic neurons have undergone their final division, virtually all TH-IR cells contained low levels of NPY-IR. The intensity of immunoreactivity increased uniformly over the next two days of gestation. VIP-IR first appeared in approximately one-third of the TH-IR cells in the stellate ganglion at E 14.5 but was not evident in the SCG at any age. Localization of peptide mRNAs by *in situ* hybridization at E14.5, during the peak of neurogenesis, revealed uniform binding of the NPY mRNA probe throughout the SCG and stellate and of the VIP mRNA probe throughout the stellate ganglion, suggesting that many cells in the embryonic ganglia synthesize NPY and VIP. To confirm that neuroblasts contain neuropeptide-IR, we injected pregnant rats with the thymidine analogue BrdU on gestational day 14 and removed sympathetic ganglia from the embryos four hours after the first injection. Sections of these ganglia were triple labeled using TH, BrdU, and NPY or VIP antisera. At E14, many cells exhibited -IR for NPY as well as TH and BrdU. In addition, some cells at E14 contained VIP, TH and BrdU. Sympathetic neuroblasts contain neuropeptides as well as catecholamines, thus, these precursors express multiple aspects of the mature sympathetic phenotype.

328.9

NEUROPEPTIDE Y IMMUNOREACTIVITY IN CAT CORPUS CALLOSUM AXONS DURING DEVELOPMENT. S.-L. Ding* and A.J. Elberger. Dept. of Anatomy and Neurobiology, The University of Tennessee Memphis, College of Medicine, Memphis TN 38163.

It has been demonstrated that many transitory corpus callosum (CC) connections exist throughout visual cortex during normal development in the cat (Elberger, Soc. Neurosci. Abstr. 16:495), but little is known about the chemical nature of CC connections in the developing cat brain. In 100 μm thick coronal sections from normal cats postnatal day (PND) 2 to adult, CC axons were identified immunocytochemically with an antiserum (Incstar) against Neuropeptide Y (NPY) using a biotin-avidin substrate. The density of NPY^+ CC axons increased slightly in postnatal weeks (PNW) 1-2, remained stable in PNW 3-4, and decreased gradually during PNW 5-7. From PNW 8 to adult the density of NPY^+ CC axons was stable below what existed at PND 2. The highest and lowest densities of NPY^+ CC axons occurred at PNW 3-4 and PNW 8 to adult, respectively. Therefore, at least 2 types of NPY^+ CC axons (i.e., transitory and permanent) exist in the developing cat brain. The majority of transitory NPY^+ CC axons, existing until PNW 7, are present while cortical connectivity is being established; therefore, transitory NPY^+ CC axons may play a role in cortical development in the cat.

Many previous studies of CC development show a gradual postnatal decline in the extent of connectivity. However, in PNW 1-2 several NPY^+ CC axons had growth cones located around the CC midline, suggesting that subsets of CC connections may have different developmental timetables. The simultaneous trends of growth and elimination among different subsets of CC axons show the CC to be a complex, dynamic structure. Support by State of Tenn. Neuroscience Center of Excellence (SLD) and NIH grant EY08466 (AJE).

328.10

TISSUE DISTRIBUTION OF DIFFERENT VIP TRANSCRIPTS AS DETECTED BY RT-PCR. A. Orosz[1], J.A. Waschek[2] and D.V. Agoston[1]* [1]Laboratory of Developmental Neurobiology, NICHD, National Institutes of Health, Bethesda, MD 20892 and [2]N.P.I, UCLA, Los Angeles, CA 90024

Vasoactive Intestinal Peptide (VIP) is a 28 amino acid long peptide neurotransmitter with broad physiological effects (reviewed by Waschek, 1992). Among the several functions of VIP, the neurotransmitter, the neuromodulator but also the autocrine trophic function have been demonstrated. The expression of the VIP gene is regulated by neuronal activity during development in the CNS and by soluble factors released from target tissues in the PNS. The expression of some neuropeptide genes, including preproenkephalin, is not restricted to the nervous system; enkephalin transcripts are temporarily present in various tissues during organogenesis. Here we report, that using reverse transcription followed by PCR amplification (RT-PCR) with primers specific to different parts of the VIP-gene, we were able to detect distinct sizes of VIP transcripts in various tissues. Total RNA, derived from tissues dissected from mice of various age were reverse transcribed and PCR amplified. dsDNA fragments were separated on agarose gel, stained with ethidium bromide and transferred to Nytrane membranes for Southern-hydridization. The expected size of message was found in the cerebral cortex and various parts of the CNS, but not in the cerebellum. Various parts of the gastrointestinal tract also contained the correct size of message, but the adrenals, kidney, liver, skeletal muscle, heart, lung, thymus, eyes, lymph nodes were negative. The uterus and the spleen expressed a much larger size of VIP message, whereas in mesenterium both, the normal and extended forms, were detected. The extended form of VIP-message in the spleen is seemed to be more abundant than that of the neuronal form present cerebral cortex. Using the sequence information available on the mouse VIP cDNA, we hypothesize, that the extended form of VIP-message in certain tissues can be generated either by alternative splicing or different transcriptional initiation. This extended form of transcript may encode a distinct molecular form of VIP with a yet unknown function.

328.11

SOMATOSTATIN EXPRESSION AND PLASTICITY IN THE PIGEON VISUAL SYSTEM. [1]G.Fontanesi, [3]G.Traina, [2]M.Molnar and [3]P.Bagnoli*. [1]Dept.of Physiology and Biochemistry, Pisa Univ., [2]VAMC, West LA, Los Angeles, CA and [3]Dept.of Environmental Sci., Univ.of Tuscia, Viterbo, Italy.

The pigeon visual system serves as useful model for studying neurotransmitter expression and maturation since its delayed maturation offers the possibility of a persisting plasticity into postnatal life. We have used the mouse monoclonal antibody S8 (provided by the Canada Regulatory Peptide Group) to map the distribution of somatostatin-immunoreactivity (SS-ir) during development and in adults with unilateral and bilateral retina ablation. Protocols for animal use are in compliance with NIH guidelines. The adult retina contains significant amount of SS-ir. A prominent population of amacrine cells are present in the INL and diffusely arborize in the IPL. Developmental changes include modifications in the density of cell bodies and processes. The adult pattern coincides with the onset of light-driven activity. SS-ir is present in the optic tectum, the n.of the basal optic root, the visual Wulst and the ectostriatum. The chemical differentiation of SS-positive neurons is characterized by drastic variations in their numerical density and their total number as determined by quantitative analysis. Incoming retinal fibers play a role in regulating SS expression since the final distribution of SS-ir is altered by unilateral and bilateral retina removal. Our results demonstrate that developing visual neurons transiently express SS in association with their retention of plasticity into postnatal life.

328.12

DEVELOPMENTAL DISSOCIATION OF R15 NEUROPEPTIDES IN *APLYSIA*. M.W. Cooper, A. Ljungqren, L.H.Dickert & T.J.Carew*. Dept. Psychol. and Biol., Yale Univ. New Haven, Ct. 06520.

The R15 gene in *Aplysia* encodes two alternatively spliced mRNAs, R15-1 and R15-2 (Buck et al., 1987). The peptides encoded by these mRNAs are expressed differently in individual neurons: The R15α1 peptide (encoded by the R15-2 mRNA) is expressed in cell R15 in the abdominal ganglion; this cell is thought to be involved in homeostasis (Kupfermann & Weiss, 1976). Another peptide, R15α2 (encoded by the R15-1 mRNA) is expressed in a number of neurons in the abdominal, pedal and cerebral ganglia (Alevizos et al., 1991). These neurons are thought to comprise part of a neural circuit also involved in homeostatic regulation (Koester, 1991).

To gain insights into the functional significance of the alternative splicing of the R15 mRNAs, we examined the expression of the R15 peptides during development using antibodies specific for the R15α1 and R15α2 neuropeptides (Ab I and Ab I/II respectively). Ab I labels only R15α1, while AbI/II labels both R15α1 and R15α2 (Alevizos et al., 1991). We found that AbI labeled R15 as early as juvenile stage 10; labeling continued to adulthood. R15 was the only cell labeled with Ab I at any stage. AbI/II also labeled R15 from stage 10 until the adult. However, cells other than R15 were not labeled with Ab I/II until 3-4 weeks later (in stage E12). At that time labeling appeared (1) in clusters of pedal cells, (2) in a pair of cells in the cerebral ganglion, and (3) in an oval shaped cell (L40) in the abdominal ganglion. Several weeks later (stage L12), Ab I/II labeling appeared in identified cell RBHE, as well as other neurons, in the abdominal ganglion.

Our data show that the R15α1 peptide is synthesized exclusively by R15 very early in juvenile development. Other cells synthesize only R15α2, and begin expression of this peptide at a later time, suggesting the hypothesis that two peptides resulting from the alternative splicing of the R15 mRNAs are expressed according to different developmental timetables.

328.13

A Cell Type-Specific Enhancer within the *FMRFamide* Neuropeptide Gene Promoter of *Drosophila*. Paul H. Taghert*, Dusan Zitnan and Marie Roberts., Dept. of Anat. and Neurobiol., Washington Univ. Sch. Med., 660 S. Euclid Ave., St. Louis, MO, 63110.

We have used *lacZ* reporter gene constructs to study the promoter and enhancer regions of the *Drosophila FMRFamide* neuropeptide gene in germ line transformants. *FMRFamide* is normally expressed in ~60 diverse neurons of the larval central nervous system that represent ~15 distinct cell types. We reported last year that an 8 kilobase *FMRFamide* DNA fragment (including 5 kilobases of 5' upstream sequence) was sufficient to produce *lacZ* expression in a pattern that mimicked nearly all spatial aspects of the normal pattern. This result indicates that the cell-specific regulation of *FMRFamide* expression is largely generated by transcriptional mechanisms. We have extended this analysis by testing smaller DNA fragments in a variety of vector constructs. Reporter gene expression was lost from selected cell types when smaller fragments were tested: sequences between -5000 bp and -4500 bp were necessary for expression by the interneuronal SE2 cells; sequences between -922 and -162 were necessary for expression by the SP4, Tv and OL2 neurons. Expression in the other ~11 cell types that normally express *FMRFamide* was correlated with inclusion of sequences near the transcription start site and/or within the single intron. A 300 bp fragment (-490 to -162) displayed cell type-specific enhancer activity: when placed adjacent to a heterologous promoter, it directed *lacZ* expression in the OL2 visual system neurons exclusively. This fragment contains 4 regions that display sequence conservation between different species of *Drosophila*; one such fragment contains both an E box and an octamer motif. These results suggest that, in the mature nervous system, the complex pattern of *FMRFamide* neuropeptide gene expression derives from the activity of cell type-specific enhancers that are broadly distributed within the vicinity of the gene and that are independently regulated.

329.1

LOSS OF CHAT- AND NGF RECEPTOR-IMMUNOREACTIVE BASAL FOREBRAIN NEURONS FOLLOWING EXCITOXIC HIPPOCAMPAL LESIONS IN THE DEVELOPING RAT. M.A. Burke-Watson*, E.J. Mufson [1], B.H. Wainer [2], and J.H. Kordower [1], Dept. of Anatomy and Cell Biology; Univ. Illinois Sch. Med., 1 Dept. of Neurological Sciences, Rush Presbyterian Med Ctr., 2 Dept. of Pharmacology, Univ. Chicago Sch. Med., Chicago, IL. 60612 USA.

Adult cholinergic septal/diagonal band neurons persist following elimination of growth factor-producing target neurons within the hippocampus (Sofroniew et al., *Science*, 247: 338-342, 1990; Kordower et al., *Exp. Neurol.*, in press). To determine whether the sustained viability of these neurons is the result of target contact, postnatal day 7 (PN7) rat pups received injections of ibotenic acid into the hippocampus (10 µg/ul; 1 µl injection) and were sacrificed 4 weeks later. As seen previously in adult rats, no reductions in ChAT-immunoreactive neurons were observed within the septum ipsilateral to the lesion ($T(7)=1.97$; $p>.05$). In contrast, PN7-lesioned rats displayed a 28% decrease in ChAT-immunoreactive neurons within the ipsilateral vertical limb of the diagonal band (VLDB) relative to the contralateral side ($T(7)=8.41$; $p<.0001$). Similar results were obtained on adjacent sections immunoreacted for the p75 NGF receptor. To assess the extent of septal/diagonal band connectivity within the hippocampus at this time point in development, intact rat pups were injected at PN7 with fluorogold (2%; 0.2 µl) and sacrificed 48h later. Numerous neurons were retrogradely labeled within the septum. In contrast, only a few magnocellular neurons were retrogradely labeled within the VLDB. This suggests that trophic factor-producing target neurons may be required for basal forebrain viability during the development of afferent fiber innervation. (AG09466, NS25985).

329.2

NGF RESTORES THE AREA OF CHAT-POSITIVE CORTICAL FIBERS IN NBM-LESIONED RATS WITHOUT AFFECTING CHOLINERGIC INTERNEURONS. Ad J. Dekker* and Leon J. Thal, Dept. Neurosciences, UCSD and Dept. Neurology, VAMC, San Diego, CA92161.

Rats received bilateral lesions of the nucleus basalis magnocellularis (NBM) by infusion of ibotenic acid (28 nmol in 2 injections per side). Two weeks after the lesion, osmotic minipumps were implanted that released 10 µg NGF or 0.3 µg Cytochrome-C per day through intraventricular cannulas. Treatment lasted for 6 weeks. Coronal sections of the frontal neocortex were stained for choline acetyltransferase (ChAT). The number of cortical neurons showing ChAT immunoreactivity was enhanced in lesioned animals (+42%), but was not further increased by NGF (+35%). The cross-area of the neurons was not affected by either the lesion or treatment with NGF. However, lesions of the NBM reduced the area of ChAT-positive fibers by 55%, while treatment with NGF restored the area to 95% of control. These results suggest that NGF restores cholinergic innervation to the cortex from remaining NBM neurons, rather than stimulating compensation by local interneurons.

329.3

ULTRASTRUCTURAL THREE DIMENSIONAL RECONSTRUCTION OF CORTICAL ChAT IMMUNOREACTIVE VARICOSITIES: EFFECTS OF LESION, GM1 AND/OR NGF TREATMENT. L. Garofalo,* A. Ribeiro-da-Silva and A.C. Cuello, Dept. Pharmacology, McGill University, Montréal, Québec, Canada.

NGF treatment preserves cholinergic fiber length and produces a significant hypertrophy of cortical presynaptic terminals in layer V of the remaining rat cortex adjacent to a lesion site (PNAS 89, 2639-2643, 1992). We further studied whether such effects could be potentiated by the monosialoganglioside GM1. Adult male Wistar rats were cortically lesioned and treated as previously described (PNAS 86,2056-2060,1989). Thirty days post-lesion, the rats were processed for ChAT immunocytochemical electron microscopic quantitative analysis. A total of 96 varicosities per group were automatically detected and measured by an image analysis system (Quantimet 920). In control unoperated rats the mean cross sectional area of layer V cortical ChAT immunoreactive (ChAT-IR) varicosities was 0.223 ± 0.013 μm^2. Confirming previous results, a remarkable shrinkage (40%) of these boutons was noted in lesion vehicle treated rats while, NGF treatment significantly augmented bouton area 20% above control values. This cholinergic terminal hypertrophy was even more dramatic (52% above control values) in rats treated with both GM1 and NGF. By contrast, GM1 treatment alone failed to prevent bouton shrinkage in lesioned rats. Furthermore, a higher percentage of boutons with synaptic contacts were noted in NGF and NGF/GM1 treated lesioned rats. Three to four representative ChAT-IR cortical varicosities from each group were then examined serially and reconstructed by image analysis in order to quantify bouton volume and synaptic area. The effect of NGF and its potentiation by GM1 was more apparent here, where an increase in bouton volume of 110% and 154% respectively, above control values was observed. A trend towards an increase in synaptic area per varicosity in NGF and NGF/GM1 treated lesioned animals was also noted. The results suggest that NGF can significantly alter presynaptic terminal fields and synaptic connections in the fully differentiated CNS; effects which can be potentiated by the monosialoganglioside GM1. Supported by MRC (Canada) and the Canadian Network of Centres of Excellence for Neural Repair and Functional Recovery.

329.4

CORTICAL DEVASCULARIZING LESION MODEL IN PRIMATES: LONG TERM EFFECT OF HUMAN RECOMBINANT NERVE GROWTH FACTOR AND MONOSIALOGANGLIOSIDE TREATMENT. P. Liberini*[1], E.P. Pioro[1], D. Maysinger[1], F.D. Ervin[2] and A.C. Cuello[1]. Dept. of Pharmacology & Therapeutics[1], and Dept. of Psychiatry[2], McGill University, Montreal, P.Q., Canada, H3G 1Y6.

In the present study, the cortical devascularizing lesion model well established in rat has been reproduced in nonhuman primate (Coercopithecus aethiops). Thirty adult male monkeys randomly divided in four groups underwent surgeries and treatment with recombinant human nerve growth factor (rhNGF) alone or in combination with the monosialoganglioside GM1 (sham operated [7], lesioned/vehicle-treated [7], lesioned/rhNGF-treated [8], lesioned/rhNGF+GM1-treated [8]). After a 6-months survival the animals were processed either for biochemistry (Choline acetyltransferase - ChAT - assay) or for immunocytochemistry (ChAT and NGF-receptor immunostainig). In lesioned/vehicle-treated monkeys the nucleus basalis of Meynert (nbM) underwent retrograde degeneration. ChAT activity significantly ($p<0.05$) decreased to 69 ±5% of sham operated value. The morphometrical analysis revealed a significant shrinkage of ChAT immunoreactive (IR) neurons in the intermediate region of the nbM (61 ±1.4% of sham operated cell size, $p<0.01$). The reported decrease of ChAT activity was fully prevented with the administration of rhNGF alone or in combination with GM1. However, the shrinkage of nbM ChAT IR neurons was fully prevented only in animals receiving both rhNGF and GM1 (89 ± 3.6% of sham operated cell size, $p>0.05$). (Supported by the Medical Research Council of Canada, the Centres of Excellence Network for Neural Regeneration and Functional Recovery and Fidia Research Labs).

329.5

CHRONIC NGF TREATMENT ENHANCES MUSCARINIC M1 RECEPTOR FUNCTION IN ADULT RATS FOLLOWING PARTIAL FIMBRIAL TRANSECTIONS. F. Hefti*, D.M. Araujo, and P.A. Lapchak. USC, Andrus Ger. Ctr., Los Angeles, CA, 90089-0191.

Effects of chronic recombinant human (rh) NGF treatment (icv, 1.0 µg qod for 21 days) on hippocampal muscarinic receptor densities and muscarinic receptor-linked second messenger systems were determined in adult rats 21 days following fimbrial transections. First, quantitative autoradiographic analysis of muscarinic receptors was carried out using [^{3}H]pirenzepine for M1 receptors and [^{3}H]AF-DX 384 for M2 receptors. Partial fimbrial transections did not alter the density of the M1 or M2 receptor population in the dorsal or ventral hippocampus and there was no effect of chronic rhNGF treatment. In contrast, in animals receiving full fimbrial transections which by themselves did not alter muscarinic receptor density, rhNGF treatment increased the density of M1 and M2 receptors in the CA1 region by 35%. Secondly, we determined the effect of chronic rhNGF on muscarinic receptor-mediated second messenger production in rats receiving partial fimbrial transections. The muscarinic M1-receptor mediated response, oxotremorine-induced IP_3 production by hippocampal slices was increased by 61% on the lesioned side of animals treated with a control protein. Chronic NGF treatment prevented the lesion-induced supersensitivity of M1 muscarinic receptors. In rhNGF-treated animals IP_3 production was similar to values on unlesioned control sides. Neither fimbrial transections nor NGF treatment altered oxotremorine-induced changes in hippo-campal cGMP levels. The findings of the present study suggest that rhNGF-induced enhancement of presynaptic cholinergic function(s) translate into functional changes at the level of postsynaptic hippocampal muscarinic receptors.

329.6

DEATH OF SEPTAL CHOLINERGIC NEURONS FOLLOWING ABLATION OF TARGET NEURONS IN NEONATAL RATS. J.D. Cooper* and M.V. Sofroniew. Department of Anatomy, University of Cambridge, Cambridge, U.K.

Following ablation of their target hippocampal neurons in adult or aged animals, the cholinergic neurons of the septal region do not die, but persist in an atrophied state. These observations suggest that target-derived neurotrophins do not precisely regulate neuronal survival in the adult CNS, but influence neuronal structural and chemical phenotype. To investigate the effect of target ablation in the *developing* CNS, the hippocampus was excitotoxically ablated in anaesthetised 9 day old Wistar rat pups. After aspiration of the overlying cortex the exposed hippocampus was injected into 4 sites with NMDA or vehicle. After 28 days animals were perfused and the extent of the lesion and its effect upon afferent septal neurons assessed histologically. Injections of NMDA resulted in the ablation of over 90% of hippocampal tissue. Comparable lesions in adults abolish NGF and BDNF mRNA production and lower septal NGF levels. In lesioned neonates, the number of septal neurons stained for ChAT or the low affinity neurotrophin receptor ($p75^{NGFR}$) declined significantly by over 65%, as compared with a 30% decline in vehicle injected animals (which showed some hippocampal damage). These findings indicate that developing cholinergic neurons are lost from the septum in the absence of their target neurons. This observation suggests that target-derived neurotrophins regulate septal cholinergic neuronal survival during early post-natal development.

329.7

AMELIORATION OF SEPTAL CHOLINERGIC NEURONAL DEGENERATION AFTER NERVE GROWTH FACTOR DEPRIVATION BY CYTOKINE-ACTIVATED ASTROCYTES. K. YOSHIDA*, M. SAGOH, H. WAKAMOTO,T. YAZAKI, M. OTANI, S. TOYA and F. H. GAGE†: Dept. of Neurosurg., Keio Univ., Shinjuku-ku, Tokyo 160, Japan; †Dept. of Neurosci., UCSD, La Jolla, CA 92193.

Nerve growth factor (NGF) synthesis and secretion from astrocytes is cooperatively regulated by various cytokines including fibroblast growth factors, interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and transforming growth factor-β1 (TGF-β1) [Yoshida and Gage: 1991, 1992]. The present study was performed to determine the effect of NGF produced by cytokine-activated astrocytes on NGF-deprived septal neurons *in vitro*. Enzymatically dissociated septal neurons from E-16 rat fetuses were grown on monolayered astrocytes obtained from neonatal rat hippocampi in a serum-free defined medium. Effects of the various combinations of IL-1β, TNF-α and TGF-β1 on septal cholinergic neurons cocultured with astrocytes were examined. A combination of IL-1β and TNF-α increased choline-acetyltransferase (CAT) activity via NGF produced by astrocytes. NGF-treated septal cholinergic neurons were found to degenerate following NGF deprivation. However, a combination of IL-1β and TNF-α significantly reduced the degeneration of NGF-deprived septal cholinergic neurons by activating astrocytes. The cytokines, which regulate NGF synthesis and secretion in astrocytes, are considered to play an important role in neuronal regeneration following brain injury.

329.8

Differential Actions of Neurotrophins in the Locus Coeruleus and Basal Forebrain. W.J. Friedman*, C.F. Ibáñez[1], F. Hallböök[1], H. Persson[1], L.D. Cain, C.F. Dreyfus, and I.B. Black. Department of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854, USA, and [1]Department of Medical Chemistry, Laboratory of Molecular Neurobiology, Karolinska Institute, Box 60400, S10401 Stockholm, Sweden

We have compared different neurotrophins in the regulation of neuronal survival and function using dissociated embryonic cell cultures from two brain regions, the basal forebrain (BF) and locus coeruleus (LC). In the BF, nerve growth factor (NGF) increased choline acetyl transferase (ChAT) activity, but did not influence cholinergic cell survival. In contrast to NGF, BDNF, NT-3, and the novel neurotrophin, NT-4, all increased ChAT activity and cholinergic cell survival.

We also examined embryonic locus coeruleus (LC) neurons in culture. LC neurons are unresponsive to NGF. In contrast, NT-3 and NT-4 elicited significant increases in survival of noradrenergic LC neurons, the first demonstration of trophic effects in this critical brain region. Identification of factors supporting coeruleal and basal forebrain neuronal survival may provide insight into degeneration of these disparate structures in disorders such as Alzheimer's disease. Supported by NIH grants NS 10259 and HD 23315-05, and DA 05132.

329.9

EXPRESSION OF NEUROTROPHIN AND TRK mRNA IN ALS AND AXOTOMIZED RAT SPINAL CORD AND PERIPHERAL TISSUE. J. L. Seeburger*, T.C. Cope, and J.E. Springer, Departments of Neurology (JLS and JES) and Physiology and Biophysics (TCC and JES), Hahnemann University School of Medicine, Philadelphia, PA 19102.

Peripheral nerve crush or transection in adult experimental animals results in the increased expression of nerve growth factor receptor (p75NGFR) mRNA in spinal cord alpha motoneurons and NGF mRNA in peripheral nerve and muscle. We recently found increased expression of p75NGFR and NGF mRNA in postmortem ALS spinal cord motoneurons and muscle, respectively. We propose that the increase in p75NGFR expression in ALS spinal cord may be associated with compensatory growth responses in minimally or unaffected alpha motoneurons.

Recent evidence has supported the involvement of membrane receptor tyrosine kinase (trk) molecules in high affinity neurotrophin binding associated with biological activity. To determine if alpha motoneurons are potentially responsive to target-derived neurotrophins, we have begun to examine the expression of trkA and trkB mRNA in ALS spinal cord and spinal cord from rats receiving sciatic nerve damage. We hypothesize that if neurotrophins are involved in compensatory growth responses in ALS and/or experimental animals, alterations in trkA and/or trkB should be observed. Peripheral nerve axotomy is a well studied procedure for analyzing events associated with muscle denervation and reinnervation. Also, there is substantial evidence demonstrating compensatory growth responses of motoneurons in patients with ALS. We have used cRNA probes in conjunction with in situ hybridization to localize cells in ALS and rat spinal cord sections that may express trkA or trkB mRNA. In addition, a nuclease protection assay was used to analyze the levels of NGF and BDNF mRNA in skeletal muscle and peripheral nerve samples from ALS patients and nerve-transected animals. Although no neurotrophic effects of NGF on alpha motoneurons have been documented, it is possible that NGF and/or other neurotrophins could play a role in compensatory growth events associated with motorneuron sprouting and reinnervation. Supported by PHS grants AG-08969, NS-30248, and the Philadelphia ALS Association (JES).

329.10

DE NOVO EXPRESSION OF THE p75 NGF RECEPTOR FOLLOWING HYPOGLOSSAL NERVE TRANSECTION IN NONHUMAN PRIMATES. **C. Del Rosario, K. Bankiewicz*1, E.J. Mufson, and J.H. Kordower, Dept. of Neurological Sciences, Rush Presbyterian Med. Ctr., Chicago Ill. and 1Surgical Neurology Branch, NIH.**

Few examples of trophic factor-mediated neuronal plasticity exist in the primate brain. In nonprimates, transection of peripheral nerve segments have been demonstrated to induce a *de novo* synthesis of the p75 receptor for nerve growth factor (NGFr). As part of a larger study in our laboratory, Cebus and Rhesus monkeys received complete unilateral transections of the hypoglossal nerve as it courses across the carotid bifurcation. We examined the immunohistochemical expression of NGFr within the hypoglossal nucleus in these monkeys which were sacrificed 1 (n=1), 4 (n=2), 10 (n=2), and 14 (n=1) weeks after surgery. They were compared to monkeys treated identically except for sparing the hypoglossal nerve (operated controls; n=4) and unoperated controls (n=2). The hypoglossal nucleus was devoid of NGFr-immunoreactivity (NGFr-ir) in both control groups. In contrast, all monkeys undergoing hypoglossal nerve transection displayed robust NGFr-ir within neurons of the hypoglossal nucleus ipsilateral to the lesion. Interestingly, the contralateral hypoglossal nucleus also display a few NGFr-ir neurons. These effects were consistent at each time point examined except for a decrease in the number of NGFr-ir neurons in the monkey sacrificed 14 weeks post lesion. The *de novo* expression of other trophic factors are currently being investigated in this nonhuman primate model system. (NS25985).

329.11

NEUROTROPHIN AND *trk* TYROSINE KINASE RECEPTOR mRNA EXPRESSION IN KINDLING IS DEPENDENT ON SEIZURE DURATION. Z. Kokaia[1], J. Bengzon [1], P. Ernfors[2], M. Kokaia[1], G. Leanza*[3], O. Nilsson[3], H. Persson[2] and O. Lindvall[1].

[1]Restorative Neurology Unit, Department of Neurology, University Hospital, S-221 85 Lund, Sweden; [2]Department of Medical Chemistry, Laboratory of Molecular Neurobiology, Karolinska Institute, S-104 01 Stockholm, Sweden; [3]Department of Medical Cell Research, University of Lund, S-223 62 Lund, Sweden.

Expression of mRNAs for nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and the tyrosine kinase receptors *trkA*, *trkB* and *trkC* has been studied using *in situ* hybridization in the rat brain 2 h after kindling-induced seizures. Epileptiform activity produced by hippocampal stimulation (once daily) and exceeding 70 s increased BDNF, NGF, *trkB* and *trkC* mRNA expression in the dentate granule cells irrespective of seizure grade. BDNF mRNA levels were also elevated bilaterally in the CA1-CA3 regions, amygdala and the piriform, entorhinal, perirhinal, retrosplenial and temporal cortices after generalized seizures. The magnitude of the increases was similar throughout kindling development and in the fully kindled brain. *TrkA* mRNA levels were unchanged after seizures. Intraventricular injection of 6-hydroxydopamine or a bilateral fimbria-fornix lesion did not alter seizure-evoked mRNA levels for the neurotrophins or the *trk* receptors, but increased the number of animals with elevated levels in dentate granule cells after the first stimulation, probably due to a prolongation of seizure activity. Both in sham-operated and fimbria-fornix lesioned rats seizure activity caused a significant reduction of NT-3 mRNA levels in dentate granule cells. The results indicate that the induction of BDNF mRNA is an "all-or-none" type of response and dependent on seizure duration but not the grade of kindling development. Changes of BDNF, NGF, NT-3 and *trkB* and *trkC* were observed concomitantly in the dentate gyrus, which suggests, that seizure activity triggers a cascade of genomic events in dentate granule cells, probably regulated via the same mechanism.

329.12

ADRENALECTOMY DISRUPTS ASPECTS OF NEUROTROPHIN EXPRESSION IN HIPPOCAMPUS. J.C. Lauterborn* and C.M. Gall, Dept. of Anatomy & Neurobiology, Univ. of California, Irvine CA. 92717.

Glucocorticoids have been shown to regulate the expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in hippocampus but cellular localization of these changes have not been investigated. Further, it has not been determined if glucocorticoids affect neurotrophin-3 (NT-3) expression. Using *in situ* hybridization and ^{35}-labeled cRNA probes, the effect of adrenalectomy (ADX) on levels of mRNA coding for NT-3, BDNF, and NGF in rat hippocampus was evaluated by film densitometry. Rats were adrenalectomized and killed 10-14 days later with paired controls. NT-3 cRNA hybridization in control rats was dense in CA2 stratum pyramidale and in the dentate gyrus stratum granulosum. Following ADX, NT-3 cRNA hybridization was decreased in CA2 stratum pyramidale to 10-20% of control levels. In stratum granulosum, NT-3 cRNA hybridization was only slightly decreased to ≈80% of control levels. In contrast, in stratum granulosum hybridization to NGF mRNA was markedly decreased to ≈30% of control levels. Hybridization to BDNF mRNA in CA3 stratum pyramidale and in stratum granulosum was unaffected by ADX; in both control and ADX rats these lamina were heavily labeled. These results indicate that within hippocampus basal expression of some neurotrophins by particular cell types (e.g., NT-3 in CA2 pyramidale cells) is largely dependent upon corticosteroids, whereas other aspects of neurotrophin expression are not glucocorticoid dependent. Supported by NS26748 to C.M.G.

329.13

SEIZURE-INDUCED CHANGES IN NEUROTROPHIN mRNA EXPRESSION ARE NOT DISRUPTED BY ADRENALECTOMY. C.M. Gall* and J.C. Lauterborn. Dept of Anatomy and Neurobiology, Univ. of California, Irvine, CA 92717.

Recurrent limbic seizures cause different changes in the expression of mRNAs for nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) in hippocampus and cortex, including increases in NGF mRNA and decreases in NT-3 mRNA occurring after the period of seizure activity. In the present study, the involvement of glucocorticoids in these effects was assessed by quantitative in situ hybridization. Seizure-producing hilus lesions were placed in untreated rats and rats adrenalectomized (ADX) 10 days earlier. In both groups, seizures caused a marked increase in BDNF mRNA throughout the granule and pyramidal cell layers of hippocampus and in both superficial and deep layers of cortex that peaked by 12 hrs and had largely declined by 24 hrs postlesion. In control and ADX rats, NGF mRNA in stratum granulosum rapidly increased over 8-fold by 6 hrs postlesion, declined to near control levels by 12 hrs, and then increased to a second peak at 24 hrs. In both groups, neocortical NGF mRNA was markedly increased 12-24 hrs postlesion. Seizures decreased NT-3 mRNA in hippocampal stratum granulosum to virtually undetectable levels by 12 and 24 hrs postlesion in both control and ADX rats. Thus, although adrenalectomy alone reduces basal levels of NGF mRNA and virtually eliminates NT-3 mRNA expression in some forebrain neurons, this manipulation does not significantly disrupt the pattern of changes in neurotrophin expression induced by limbic seizures in the hilus lesion paradigm. Supported by NS26748 to C.M.G.

329.14

MODULATION OF LOW-AFFINITY NGF RECEPTOR IN ADULT SPINAL MOTONEURONS AFTER TRAUMATIC AND NON TRAUMATIC LESIONS OF THE PERIPHERAL NERVE M.Rende,C.Provenzano and G.De Renzis* Inst. of Human Anatomy, UCSC, Rome, Italy

Adult rat spinal motoneurons (SMN) express low-affinity NGF receptor (LNGFR) after their axotomy (cut or crush).We have analyzed the modulation and the time-course of LNGFR expression in lumbar SMN following (a)traumatic injures of the sciatic nerve that allow a regeneration delayed relative to crush (implantation of a silicone chamber, repeated crushes and delayed repair of a ligated nerve);(b)toxic or metabolic injures without any direct trauma of the nerve (experimental diabetogenesis,Botulinum and Bungarotoxin intoxication, 2,5-Hexanedione intoxication) and (c) block of the axoplasmatic transport by topical applications of Vincristine combined with a nerve crush.The results we present are consistent with the idea that (1)LNGFR immunoreactivity in adult SMN is a specific marker for SMN that are attending an axonal outgrowth and not a generic signal of cellular damage; (2) LNGFR expression in these SMN is related and strict parallel the outgrowth process time-frame and (3) the signal/s that trigger and sustain this expression are retrogradely transported from the periphery. Supported by a Telethon-Italy grant.

329.15

TROPHIC FACTOR PREVENTION OF DIABETIC NEUROPATHY IN A STREPTOZOCIN RAT MODEL. S. C. Apfel*, J. C. Arezzo, M. Brownlee, M. Moran, P. K. Barnecott, and J. A. Kessler. Albert Einstein College of Medicine, Bronx, New York 10461

Peripheral neuropathy is a common debilitating complication of diabetic mellitus. Typically a combination of sensory, motor, and autonomic nerves may be affected. This broad spectrum of neuronal involvement suggests that administration of any one neurotrophic factor may provide inadequate protection. We have established a model of diabetic neuropathy in streptozocin treated rats using a combination of behavioral, biochemical and electrophysiological techniques to document abnormal nerve function. In a series of trials we have tested the ability of several neurotrophic factors including nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), and Org 2766 to prevent the manifestations of neuropathy seen in our model. NGF prevented behavioral and biochemical manifestations of a small fiber neuropathy, but failed to fully prevent slowing of nerve conduction velocity. CNTF had striking effects on sensory neuropeptide and electrophysiology, but failed to prevent the diabetes-induced changes. No one agent prevented all the diabetic changes. Ultimately the ideal therapy may involve using combinations of trophic factors.

329.16

SYSTEMIC NGF AFFECTS NGF RECEPTOR EXPRESSION IN DORSAL ROOT GANGLIA (DRG) OF DIABETIC RAT. B. Figliomeni, C. Panozzo, G. Vantini*, F. Biasiolo, R. Rubini C. Triban and F. Di Gregorio. Fidia Research Labs, Abano Terme, Italy.

Sensory and sympathetic neurons, known to be continually dependent on NGF and/or NGF-like molecules, are adversely affected at an early stage in diabetes mellitus. A diabetes-induced altered synthesis and/or an impaired signal transduction mechanism of these trophic factors may well underlie the clinical symptoms of diabetic neuropathy. To gain further insights into this problem, Northern blot analysis was used to evaluate the levels of mRNA encoding the low-affinity NGF receptor ($p75^{NGFR}$) in L_4 and L_5 DRG of streptozotocin-induced diabetic rat, receiving a continuous subcutaneous infusion of NGF or the corresponding vehicle via osmotic minipumps (Alzet, model 2002). Diabetic rats showed a progressive decrease of mRNA $p75^{NGFR}$ levels in DRG which was counteracted by NGF treatment. These data support the notion that diabetic sensory neurons remain responsive to NGF, suggesting that NGF itself may rapresent a therapeutic agent in diabetic neuropathy.

329.17

SERUM LEVELS OF NGF IN ADULT RATS FOLLOWING SUBCUTANEOUS INFUSION OF NGF. M.A. Tria, B. Figliomeni, M. Fusco*, N. Schiavo, S. Cereser, P. Marini, F. Fogarolo and G. Vantini. Fidia Research Labs, Abano Terme, Italy.

Sympathetic and sensory neurons retain responsiveness to NGF and/or NGF-like molecules throughout their lifetimes. Dysfunctions in trophic factor availability may thus have a pathophysiological role in neuropathies involving these neurons. In this regard, studies concerning NGF and related molecules may open new concepts for treatment of peripheral neuropathies including the possible use of NGF itself as a therapeutic agent. Here we have evaluated NGF levels in serum of rats receiving a continuous subcutaneous infusion of NGF (3-30μg/day, for 2-4 weeks) via osmotic minipumps (Alzet, model 2002). Fairly stable levels of NGF in serum were attained within a few days following pump implantation. The apparent steady-state serum NGF levels showed progressive increments as a function of NGF infusion rate. The serum concentrations of NGF obtained were in the picomolar range, and are therefore compatible with the administered trophic factor having a physiological/pharmacological role exerted via interaction with high-affinity receptors (K_D about 10pM) (see Figliomeni et al., this meeting).

329.18

TREATMENT WITH ACETYL-L-CARNITINE (ALCAR) ELEVATES NGF LEVELS IN THE CNS OF AGED RATS. G. Taglialatela*, D. Navarra, R. Cruciani, M.T. Ramacci, L. Angelucci[1]. Institute for Research on Senescence, Sigma Tau, Pomezia, Rome; [1]Institute of Pharmacology II, La Sapienza Univ. of Rome, Italy.

Some age-related impairments of the CNS in senescent rats, which may be due to reduction of neurotrophic support to neurons, are prevented by chronic treatment with ALCAR. Also, ALCAR potentiates NGF action on PC12 cells and prevents the reduction of NGF receptors in the CNS of aged rats. On the basis of these findings, a study was carried out on the effects of an 8-day treatment with ALCAR (200 mg/kg i.p.) on the NGF levels in the various brain structures of adult and aged rats. Although no differences were found between adult and aged control rats, a significant increase of NGF levels in hippocampus and basal forebrain of the aged rats only was observed following treatment with ALCAR. ALCAR did not affect the NGF levels in frontal cortex and cerebellum. These data are suggestive of a strategy of possible therapeutical relevance to increase NGF content in those CNS areas that are impaired in aged subjects, especially in the light of previous findings of an i.c.v. treatment with NGF rescuing atrophic cholinergic neurons of the basal forebrain and septum in aged rats.

329.19

CORRELATION OF PHARMACOLOGICAL INDUCTION OF NGF *IN VIVO* AND *IN VITRO*. J. L. Vaught*, M.E. Lewis, M.S. Saporito, H. Wilcox, K. Clopton-Hartpence,and S. Carswell. Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380.

Work from several laboratories has shown that NGF can be induced by structurally diverse compounds in various types of primary and established cell lines, as well as in the CNS. To examine whether induction of NGF in cell culture has predictive value for *in vivo* activity, we compared the effects of 4-methylcatechol and 1,25 dihydroxy-vitamin D3 on NGF mRNA expression in mouse fibroblast L929 cells and in rat hippocampus after ICV injection. NGF mRNA was analyzed by Northern blot analysis in L929 cells and RNase protection in rat brain (see accompanying abstracts by Carswell et al. and Saporito et al.). Dose-response studies indicated a similar relative potency in both systems. Similar efficacies were also observed, with each compound stimulating NGF RNA levels between 2- and 4-fold both *in vivo* and *in vitro*. Time course studies of induction using maximally-inducing doses revealed comparable kinetics in cell culture and *in vivo*. In both models, peak levels of induction were observed between 4 and 8 hours after treatment with either compound, but by 24 hours these levels had returned to baseline with 4-methylcatechol and were sustained with 1,25 dihydroxy-vitamin D3. Taken together, the results indicate that NGF induction in L929 cells can be used with at least some classes of compounds to predict both qualitiative and quantitative parameters of activity in the brain.

329.20

MOLECULAR MECHANISMS OF NERVE GROWTH FACTOR INDUCTION BY 4-METHYLCATECHOL. E. K. Hoffman, M. S. Saporito, H. M. Wilcox, K. C. Hartpence, M. E. Lewis* and S. Carswell. Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380.

Nerve growth factor (NGF) has been proposed as a candidate for use as a therapeutic in the treatment of Alzheimer's disease (AD) due to its actions as a neurotrophic agent for basal forebrain cholinergic neurons. Augmentation of endogenous levels of NGF by compounds capable of crossing the blood-brain barrier may provide a novel alternative therapeutic strategy. To test the feasibility of this approach, we have been studying the induction of NGF expression by the catecholamine derivative 4-methylcatechol (4-MC). Treatment of mouse L929 fibroblasts with 75 uM 4-MC results in a 2- to 3-fold induction of NGF protein and RNA levels. Similiarly, ICV injection of 4-MC into adult rat brain leads to an elevation of hippocampal NGF RNA levels. These findings suggest that NGF induction by 4-MC may be mediated at the level of transcription. To address this possibility, we have analyzed the effect of 4-MC on the NGF promoter region by using the bacterial chloramphenicol acetyltransferase (CAT) gene fused to NGF promoter deletion fragments as a reporter system in transient expression assays. Further, we have studied the effect of 4-MC on the initiation and stability of the endogenous NGF mRNA transcripts. The results of these studies indicate that the induction of NGF by 4-MC is complex, and likely involves the participation of multiple mechanisms which together lead to an overall enhancement of NGF levels.

329.21

MULTIPLE CELLULAR PATHWAYS OF INDUCTION OF NGF BY 1,25 DIHYDROXYVITAMIN D3, TPA, SERUM, AND 4-METHYLCATECHOL. S. Carswell*, H. Wilcox, K. Clopton-Hartpence, M.S. Saporito, M.E. Lewis, and J.L. Vaught. Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380.

In an effort to characterize the mechanisms underlying the regulation of NGF expression, we recently showed that isoproterenol, 4-methylcatechol and serum induce NGF by distinct pathways in mouse L929 cells (Carswell et al., *Mol. Brain Res.*, in press). These investigations have now been extended to compare the mechanisms by which the phorbol ester TPA, 4-methylcatechol, serum, and 1,25-dihydroxyvitamin D3 stimulate NGF synthesis. Various combinations of these compounds at maximally-inducing concentrations (50 ng/ml TPA, 50 µM 4-methylcatechol, 10% serum, and 10 nM 1,25 dihydroxyvitamin D3) revealed additive effects on secreted NGF levels from L929 cells. Each of these compounds has been shown previously to induce NGF mRNA, and preliminary data suggest that the additivity observed in NGF protein can be accounted for by commensurate changes in NGF mRNA levels. The goal of this work is now to identify molecular and cellular parameters which discriminate these differing mechanisms. Studies measuring the effects of these compounds on NGF mRNA stability and rate of transcription initiation are in progress, as well as experiments using various metabolic inhibitors. In parallel work, we are testing whether these results can be replicated in the CNS (see abstracts by Saporito et al. and Vaught et al.).

329.22

PHARMACOLOGICAL INDUCTION OF NERVE GROWTH FACTOR mRNA IN ADULT RAT BRAIN. M.S. Saporito*, K.C. Hartpence, E.M Reilly, E. Robbins, C. Steffler, H. M. Wilcox, J.L. Vaught, M.E. Lewis and S. Carswell. Cephalon, Inc. 145 Brandywine Parkway, West Chester, PA 19380.

Nerve growth factor (NGF) may be an effective treatment for Alzheimer's disease based on its ability to maintain cholinergic neurons in animal models of neurodegeneration. However, NGF does not cross the blood-brain barrier making direct administration into the brain the only viable means of delivery. An alternative approach is to upregulate NGF gene expression in the CNS. In the present study, compounds shown to induce NGF in other systems were assessed for their ability to induce NGF in rat CNS. Rats were injected ICV with test compounds and sacrificed between 4 and 24 hrs after injection. NGF mRNA levels were quantified by an RNAse protection assay. Interleukin-1ß, 4-methyl catechol and 1,25 dihydroxyvitamin D3 all induced NGF mRNA in the hippocampus and cortex by 2 to 4 fold after administration to anesthetized rats. Dexamethasone, which reduces NGF levels in L929 cells, increased NGF mRNA in these two brain regions. Induction of NGF mRNA was dose-dependent, with interleukin-1ß and 1,25 dihydroxyvitamin D3 being the most potent. Peak levels of NGF mRNA occurred between 4 and 8 hrs after injection, and the duration of NGF induction was dependent on the dose and the compound administered. These studies demonstrate induction of NGF mRNA in adult rat brain following administration of structurally diverse compounds. Additional studies are focused on determining if these compounds induce NGF to a sufficient level to increase cholinergic function and other NGF-mediated responses .

DEVELOPMENT OF CEREBRAL CORTEX AND LIMBIC SYSTEM I

330.1

EFFECT OF DEPLETION OF LAYER IV ON DORSAL LATERAL GENICULATE CELL NUMBER AND INGROWTH OF GENICULO-CORTICAL AXONS. T.-U. Woo*, J. K. Niederer, and B. L. Finlay. Biopsychology Laboratory, Cornell University, Ithaca, New York 14853.

Previous studies have shown that ablation of the dorsal lateral geniculate nucleus (dLGN) leads to the disappearance of layer IV in visual cortex in the hamster, suggesting a crucial role of the thalamus in specifying or maintaining neurons with stellate morphology. To further explore the trophic relationship of the two structures, we depleted layer IV using the mitotic inhibitor methylazoxymethanol (MAM) and evaluated the effect of this depletion on the dLGN and the pattern of innervation of geniculocortical afferents.

MAM was injected into pregnant hamsters (20 mg/Kg) on embryonic day 14 when the major population of layer IV neurons are generated but after the generation of dLGN. The absolute number of cells in layer IV was reduced by approximately 60%. Examination of brains of animals treated with MAM and labeled with bromodeoxyuridine (BrdU) showed depression of mitosis lasting at most 33 hours after MAM injections. The volume (normal:0.163±0.006 mm^3, n=2; MAM:0.160±0.014mm^3, n=3), neuronal density (normal:129,700±290 /mm^3; MAM: 142,000±7,700/mm^3) and number (normal:20,800±600; MAM:22,700±1,600) of the dLGN of postnatal day 20 animals were not changed significantly by this manipulation. Thalamocortical projections, assessed with HRP, terminated in the dorsal aspect of the remaining cortex with less dense arborizations. There was no compensatory increase in density of terminals in the subplate and layer VI.

Our results have demonstrated that thalamic neurons show surprising independence of layer IV neurons for their survival; this could be due to the fact that stellate neurons are not the sole targets for thalamocortical afferents. Studies in progress are examining thalamocortical axonal development after MAM treatment with the fluorescent axon tracer, DiI.

Supported by NIH Grant R01 NS19245.

330.2

STAGE-SPECIFIC PRODUCTION OF FIBRONECTIN IN NEOCORTICAL DEVELOPMENT: EVIDENCE FOR SYNTHESIS BY BOTH NEURONS AND GLIA. A.L. Pearlman*, T.J. Broekelmann, J.A. McDonald[1], and A.M. Sheppard. Depts. of Cell Biol., Neurol. and Med., Washington Univ. Sch. of Med., St. Louis, MO 63110, and Mayo Clinic[1], Scottsdale, AZ 85259

The distribution of immunolabeling for fibronectin (FN) changes rapidly during early development of the cerebral cortex, but FN remains closely associated with both radial glia and preplate/subplate neurons throughout its transient period of expression (Stewart and Pearlman, 1987; Chun and Shatz, 1988; Sheppard et al., 1991). In the present study, we used *in situ* hybridization with a riboprobe generated from a partial cDNA for human FN (Kornblihtt et al., 1985) to analyze FN production in the neocortex of the embryonic mouse (E11-E16). FN mRNA is evident in two different locations at temporally distinct stages. Before the first postmitotic neurons form the preplate, FN mRNA levels are highest deep in the proliferative epithelium of the ventricular zone, then decrease substanially as the preplate forms. A second stage of FN production begins with the emergence of the cortical plate, when FN mRNA is prominent in the upper intermediate zone and subplate. To identify the cellular sources of FN, we used brefeldin A to block protein secretion by cortical cells in culture, then double-immunolabeled the cells with antibodies to FN and to cell-type specific intermediate filaments. Both neurons, identified with antibodies to neurofilaments, and glia, identified with MAb RC1, produce FN in culture. Taken together, our findings suggest that FN is produced first by cells of the neuroepithelium, the most mature of which are radial glia. The later production appears to be by migrating neurons in the upper intermediate zone, and perhaps by the more mature neurons of the subplate as well.

330.3

EXPRESSION OF TWO CHONDROITIN SULFATE PROTEOGLYCAN CORE PROTEINS IN THE SUBPLATE PATHWAY OF EARLY CORTICAL AFFERENTS. B. Miller*, A.M. Sheppard and A. L. Pearlman, Depts. of Cell Biology and Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

In prior studies we demonstrated that chondroitin sulfate proteoglycans (CSPGs), immunolabeled with antibodies to their glycosaminoglycan side chains, are prominent in the extracellular matrix (ECM) of the marginal zone and subplate in the developing neocortex of the mouse (Sheppard et al., J. Neurosci.11:3928, 1991), and that thalamic afferents travel directly through the CSPG-rich subplate, while cortical efferents cross it (Bicknese et al., Neurosci. Abst., 1991). To identify the CSPGs present in these locations, we used monoclonal antibodies to the core protein of selected CSPGs to immunolabel cortex in the developing rat and mouse. Immunolabeling for two brain-derived core proteins, 1D1 and 3F8 (245kD and 400kD; Rauch et al., J. Biol. Chem., 266:14785, 1991; provided by R. Margolis) appears in the subplate as it forms and before thalamic afferents arrive. Labeling for 1D1 is both more prominent and more restricted spatially than 3F8, which is also faintly evident in the ventricular zone. Immunolabeling for both 1D1 and 3F8 subsequently appears in the layers of the cortex as they mature. Immunolabeling for NG2 (300kD, provided by W.B. Stallcup) is restricted to blood vessels and presumptive 02A precursor cells, and 2D6, an antibody to a basement membrane CSPG (150kD; provided by J.R. Couchman) labels only blood vessels. Thus, two CSPG core proteins identifiable in developing cortex (1D1 and 3F8) are components of the subplate-associated extracellular matrix that may form a pathway for thalamocortical axons. In contrast, the distribution of the other core proteins we have examined does not suggest a role in the formation of axonal pathways or layers in early cortical development.

330.4

GROWING CORTICOTHALAMIC AND THALAMOCORTICAL AXONS INTERDIGITATE IN A RESTRICTED PORTION OF THE FORMING INTERNAL CAPSULE. A.R. Bicknese* and A.L. Pearlman, Depts. of Neurol., Peds., and Cell Biol., Washington Univ. Sch. of Med., St. Louis, MO 63110

Axons from preplate/subplate cells, some destined for the thalamus, are the first to leave the developing neocortex, while axons from the thalamus are the first to arrive. In prior studies from this laboratory, we established that afferents travel within the chondroitin sulfate proteoglycan (CSPG)-rich subplate once they reach the neocortex, while efferents cross the subplate and leave in the intermediate zone which contains much less CSPG (Bicknese et al., Soc. Neurosci. Abst., 1991). Here we examine the relationship of these two sets of fibers to each other and to CSPG as they form the internal capsule. We used single and double labeling with DiI and DiA to trace axons in fixed embryonic mice, and an antibody to the glycosaminoglycan side chains to label CSPG. Cortical efferents leave the cerebral wall in a broad front, traveling through a zone of moderate CSPG immunolabeling until they reach the striatum, which contains little CSPG. Axons leaving the thalamus also travel through a zone containing moderate CSPG, then cross through the striatum in very close proximity to striatal neurons to meet the efferents on the other side. Cortical axons interdigitate with thalamic axons that have crossed the striatum, but the two pathways separate on the cortical side of the interdigitation. Both afferents and efferents subsequently form bundles, suggesting that later arriving axons fasciculate selectively with earlier axons of the same type. Thus axons have several potential guidance cues available to bridge the gap between thalamus and cortex, including selective responsiveness to extracellular matrix molecules, interactions with striatal neurons, and fasciculation with other axons of the same type. If interdigitation of dissimilar axons (e.g. efferents on afferents) is a guidance mechanism, it is only operative over a restricted segment of the axonal trajectory.

330.5

HOW ARE THALAMOCORTICAL AXONS GUIDED IN THE REELER MOUSE? Zoltán Molnár* and Colin Blakemore. Univ. Lab. of Physiology, Parks Road, Oxford, OX1 3PT, UK.

In Reeler mouse, where the layers of the cortex are inverted, thalamic axons nevertheless innervate the correct cortical regions (see Caviness *et al., Cerebral Cort*, 1988, 7, 59). We examined early projections in Reeler, using carbocyanine dyes to trace axons in fixed tissue, viewed by conventional and confocal microscopy. The formation of the preplate, outgrowth of an array of topographically ordered, descending, corticofugal pioneer axons, the synchronous growth of pioneer thalamic axons through the primitive internal capsule and their tangential distribution over the scaffold of corticofugal fibres, all occur indistinguishably in Reeler and normal animals. By the time thalamic axons arrive under their target areas (E15 for occipital cortex), the cortical plate itself has started to form. In Reeler, the plate forms *under* the 'superplate' cells, whose pioneer axons come to lie in oblique fascicles, running through the thickening plate. In normal mice, most thalamic axons accumulate in the subplate below. By contrast, in Reeler, thalamic axons run up diagonally in fascicles *through* the plate, to gather and wait over the 'superplate' cells above.

The puzzling appearance of thalamic fibres in adult Reeler, looping up to layer 1 before ending lower in the cortex, is explained by those axons following precisely the same algorithm of development as in the normal animal, but in relation to the displaced cells of the 'superplate'. Our findings support the hypothesis that early postmitotic cells play a crucial role in guiding and holding thalamic axons (Shatz *et al.* and Blakemore & Molnar, *Cold Spring Harb Symp Quant Biol*, 1990, 55: 469 and 491). We thank André Goffinet for providing Reeler specimens and the Wellcome Trust, the MRC and the Human Frontier Science Program for support.

330.6

GENICULOCORTICAL AXON INGROWTH & MORPHOGENESIS OF CORTICAL NEURONS IN RAT AUDITORY AND VISUAL CORTICES. G.H. Kageyama*, M.Beilstein, L. Hoang, S. Catalano, J. Yu & R.T. Robertson. Dept. Anat. & Neurobiol., Univ. of Calif., Irvine, CA 92717.

The temporal relation between the development of the basilar dendrites of cortical pyramidal cells (PC) and ingrowth geniculocortical (GC) axons was examined in the auditory and visual cortices of prefixed neonatal (E17-P7) rat brains. Laminar identity of labeled PCs was revealed with bisbenzimide. DiI placed in the MGN or LGN for 2+ weeks at 37°C resulted in labeling of developing medial and lateral GC axons, respectively. Although ingrowing medial GC axons reach the intermediate zone and subplate (SP) in auditory cortex one day earlier than lateral GC axons to visual cortex, the timing of their ingrowth into the developing cortical layers was more closely synchonized, so that both medial and lateral GC axons grow into SP on E17-E18, deep VI on E19, middle VI on E20, superficial VI on E21, layer V on E22/P0, the deep half of the cell dense cortical plate (CP, future layer IV) on P1. Layer IV formed on P2. The GC axons grow continuously into the developing deep cortical layers without "waiting" as was previously described for thalamocortical axons growing into S1 (Catalano et al,'91). Golgi-like staining of cortical neurons with DiI revealed that apical dendrites developed well in advance of basar dendrites. Dendrites of SP neurons were already present on E17. The basal dendrites of layer VI, V, IV, and III PCs developed in the same inside-out order of cortical neurogenesis, migration, and settling, closely coinciding with GC ingrowth. Basal dendrites of VI PCs began forming on E18-20, those of V PCs on E20-21, those of IV PCs on P1, and those of III PCs on P2-3. The results indicate that developing GC axons may influence the elaboration of basal dendrites as each laminar cohort of PCs begins to differentiate subjacent to the cell dense CP. It is possible that GC axons may also play an important role in cortical histogenesis and/or topographic specification. Supported by NSF 87-08515 and NIH NS 25674.

330.7

AFFERENTS TO PREPLATE NEURONS IN EMBRYONIC MOUSE CORTEX. J.E. Crandall*, L.C. Hassinger and J. Bonaccorso. E.K. Shriver Center, Waltham, MA 02254 and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

We have been studying the differentiation of early generated neurons that form the preplate and the development of their afferents in normal and reeler mutant mouse cortex (Crandall et al., *Dev. Brain Res.*, 1986, 28:127). We are determining when and where synaptic contacts reach different morphologic types of preplate neurons by photoconverting the fluorescent lipophilic dye, DiI, into reaction product appropriate for electron microscopy. Embryos from timed-pregnant B6C3 mice were perfused intracardially with mixed aldehydes. Crystals of DiI were placed into one of three potential sources of cortical afferents: brainstem, thalamus and cortex. After brains were stored at 37°C for 1-3 weeks, 50-100 μm thick sections were cut with a Vibratome and fluorescently labeled cells and fibers were photoconverted to an electron dense DAB reaction product according to Sandell &Masland (1988). At E13 we have not as yet detected thalamic or brainstem fibers that contact preplate cells. However, both medial and lateral cortical areas send relatively long fibers within the preplate and outer regions of the intermediate zone to adjacent cortical areas. Labeled preplate neurons and their processes present good membrane preservation. To date, labeled cortical fibers occasionally tipped with growth cones contact preplate cells but do not yet show synaptic specializations. By extending the findings of others on embryonic rat cortex to embryonic mice, we can take advantage of the genetic mutant reeler to determine the effect of cell position on afferent ingrowth. Supported by MR Core Grant HD-04147 and the Dept. of Mental Retardation of the Commonwealth of Massachusetts (Contract:1002-20023-SC).

330.8

SYNAPTOPHYSIN IMMUNOHISTOCHEMISTRY REVEALS INSIDE-OUT PATTERN OF EARLY SYNAPTOGENESIS IN FERRET CEREBRAL CORTEX. T. Voigt*, A. D. de Lima, M. Beckmann. Max-Planck-Institut für Entwicklungsbiologie 7400 Tübingen, Germany

Synaptogenesis in the ferret cerebral cortex was examined from the day of birth to adulthood with an antibody against synaptophysin at the light and electron microscopic level. Due to the premature birth of ferrets, the generation of cells destined to the upper cortical layers and their subsequent migration to their final positions in the cortical plate are largely postnatal events. Throughout the newborn ferret cerebral cortex, a high amount of synaptophysin immunoreactivity was present within the marginal zone and subplate region. Staining was also conspicuous within the forming cortical plate. The typical layering pattern of synaptophysin immunoreactivity in the developing cortical plate correlated with the migration pattern of cortical neurons. The synaptic density was lowest directly below the marginal zone where the youngest neurons just stopped their migration. Below this zone the density of the synaptic staining increased gradually towards lower (and older) cortical plate layers. As the cortex expanded, the synaptophysin immunoreactivity pattern closely followed the expansion, suggesting that synapses were formed in a given layer shortly after the cells migrating to this layer reached their final position. As soon as cell migration had finished, the entire cortical plate contained dense synaptophysin immunoreactivity, in a pattern similar to that observed in the adult animal. At any given time, rostral and lateral regions of the cerebral cortex were more advanced in their development than caudal and dorsal regions. Electron microscopic examination of synaptophysin immunoreactivity confirmed that labeling was solely associated with synaptic vesicles. A systematic analysis of P0 and P7 samples showed that synaptic profiles distribution completely matched the distribution of synaptophysin immunoreactivity seen in the light microscope. In conclusion, early synaptogenesis in the developing ferret cerebral cortex is predetermined by neurogenesis and closely follows its inside-out pattern as well as the rostrocaudal and laterodorsal gradients.

330.9

POSTNATAL DEVELOPMENT OF THALAMOCORTICAL AFFERENT "PATCHES" IN AUDITORY NEOCORTEX. R.K. de Venecia* and N.T McMullen. Department of Anatomy, University of Arizona College of Medicine, Tucson, AZ 85724.

Thalamic afferents to rabbit auditory neocortex were labeled with biocytin iontophoretically injected into the medial geniculate body (MGB) at successive postnatal ages. Immunohistochemical methods were used to visualize biocytin-labeled axons in 100 um thick serial coronal sections. Selected axons were serially reconstructed using a computer microscope. In young adults, focal biocytin injections within the MGB labeled two to five "patches" of thalamocortical axons along the dorsal-ventral tonotopic axis within the auditory cortex. Thalamocortical patches approximately 500 um in width arose from the convergence of numerous fine axons (< 1 um diameter) studded with varicosities. The patches were elongated (up to 2 mm) in the anterior-posterior axis, parallel to isofrequency contours. We report that patches are evident as early as postnatal day (PD) 3, almost four days prior to the onset of hearing. At PD-3, axons entered the cortical plate tangentially and gave rise to branches which radiated to the full width of the cortical plate and extended into lamina I. Growth cone-like structures were usually observed at the termination of axons at this age, but appeared less frequently at older ages. By the second postnatal week, axonal arbors had shifted to a predominantly vertical orientation and were characterized by repeated dichotomous branching in laminae III and IV. During the third postnatal week, there was a progressive redistribution of axons from upper cortical layers to lamina III/IV. By PD-21, thalamic axons terminated in 3 zones: lamina VI, lamina III-IV, and lamina I, with terminal branches most densely distributed in III/IV. The major changes in axonal orientation and redistribution of terminal branches at later ages suggests significant afferent remodelling during postnatal development. (Supported by NIH and ADCRC).

330.10

SIMILARITY IN THE PROPORTION OF GABA(+) NEURONS IN 3 AREAS OF THE RAT CEREBRAL CORTEX. C. Beaulieu* and C. Crevier. Département de pathologie, Université de Montréal, Montréal, (Qué) CANADA, H3C 3J7

The numerical density (N_V) and the number of neurons beneath 1 mm^2 of cortical surface (N_C) were estimated in individual laminae of the monocular segment of visual area (OC1M), the somatosensory barrelfield, and a frontal area (FR1) of the rat cerebral cortex by using the disector method. GABA(+) neurons were identified with a postembedding method on semithin sections.

For the total cortical depth, the N_V and the N_C for both GABA(+) and the overall population of neurons were significantly different in the 3 areas. Since the variation in the N_V and in the N_C of GABA(+) neurons among the cortical areas followed a pattern similar to that of the overall population, the proportion of GABA(+) neurons remained similar (15%). It thus appears that when more neurons are added to a cortical area, the basic cortical circuit retains the same proportion of inhibitory neurons. Furthermore, differences found in the N_C for the 2 neuronal populations were due to significant changes of their numbers in layer IV, the main recipient lamina of thalamic axons.

Even though the proportion of GABA(+) neurons was similar in the 3 examined areas of the rat cerebral cortex, it remained lower than that previously found in areas of the cat or monkey cortex in which GABA(+) neurons represented 20-25% of all cortical neurons.

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330.11

DEVELOPMENT OF BASAL FOREBRAIN PROJECTIONS TO CEREBRAL CORTEX: EFFECTS OF IMMUNOTOXIN INDUCED CHOLINERGIC LESIONS AS VISUALIZED BY AChE AND DiI STUDIES IN RATS. K.A. Gallardo[1], R.T. Robertson[1], R.G. Wiley[3], and J.Yu*[2]. Depts. of Anatomy and Neurobiology[1] & Physical Medicine and Rehabilitation[2], University of California, Irvine, CA 92717; Lab. of Experimental Neurology[3], DVAMC, Nashville, TN 37212.

We are studying the development of basal forebrain (BFB) projections to cerebral cortex to determine the timecourse of the ingrowth of these projections, and to determine the possible role of cholinergic afferents in cortical development.

In the present studies we attempted to selectively lesion the NGF-sensitive cholinergic neurons of the basal forebrain in infant Sprague-Dawley rats. Intraventricular injections of 130 to 400 ng of the immunotoxin 192-IgG saporin were made in animals at postnatal day 0 (PND-0) through PND-4. The 192-IgG saporin is an antibody against the low affinity NGF receptor, as described (Wiley et al. *Brain Research* **562**: 149-153, 1991). Following survival periods that varied from 2-10 days, the brains of aldehyde perfused animals were processed for AChE histochemistry. Other brains received placements of DiI into cerebral cortex or the diagonal band of Broca.

Preliminary results show a loss of AChE positive axons in all layers of cerebral cortex and of hippocampus. Decrease in AChE positive axons was evident as early as 2 days after injection and appeared to become more severe with longer survival periods. AChE positive neurons in thalamus appeared not to be affected. Retrograde labeling of BFB neurons following DiI placements in cortex was reduced in treated animals. Fewer neurons were labeled, relative to controls, and some labeled neurons appeared atrophic. These results indicate that basal forebrain cholinergic neurons may be selectively eliminated during development by the use of the 192-IgG saporin antibody.

Supported by NIH grant NS25674 and Alzheimer Foundation grant 90-082.

330.12

DISTRIBUTION OF ACETYLCHOLINESTERASE IN THE DEVELOPING VISUAL CORTEX IN NEONATALLY HEMIDECORTICATE RATS. M. Vinette, D. Boire ,M. Ptito*, F. Leporé, J.-P. Guillemot. Dépt. de Psychologie, Université de Montréal et Dépt de Kinanthropologie, Univ. du Québec à Montréal, Canada.

The primary visual cortex (17) in neonatally hemidecorticate rats was examined in order to investigate the morphological and neurochemical development of the remaining cortex. In normal development, primary sensory cortices display a transient pattern of acetylcholinesterase (AChE) during the second and third postnatal weeks. Areal and laminar distributions of transient AChE correspond to the distribution of geniculo-cortical axons (Robertson, 1988). It has been proposed that transient AChE may play a morphogenic role in the development of these projections (Kristt, 1979, Robertson, 1987).

Wistar rats received a right hemidecortication at postnatal day (PND) 4 and were sacrificed at 3 days interval until PND 27 and in adulthood. Brains were processed for AChE histochemistry and compared to age-matched controls. Results show no apparent modification in the areal and laminar distribution of AChE during the development of visual cortex following hemidecortication. The transient expression of AChE remains confined to layer IV and deep layer III, layer I and layer VI, and its time-course comparable to controls. This suggests that hemidecortication does not affect the time-course nor the distribution of geniculo-cortical projections.

(Supported by FCAR and NSERC)

330.13

POSTNATAL EXPRESSION OF α7 NEURONAL NICOTINIC RECEPTOR mRNA IN RAT SENSORY CORTEX AND THALAMUS. R.S. Broide* and F.M. Leslie. Dept. of Pharmacology, Univ. of California, Irvine, CA 92717.

Sensory areas of the rat neocortex are differentially innervated by thalamic afferents that appear to play a critical role in the determination of cell fate within these regions. α-Bungarotoxin, which is used extensively to study nicotinic receptors at the neuromuscular junction, exhibits a unique, transient developmental binding pattern within areas of sensory cortex, which peaks at postnatal day ten. Recent studies indicate that, in the CNS, the toxin binds to the α7 subunit of the neuronal nicotinic receptor and have suggested that this binding site may be involved in the formation of neural connections. It is thus important to determine whether this site is found on thalamic terminals or on their cortical targets during the early stages of synaptogenesis. The distribution of α7 mRNA has been shown to strongly correlate with that of α-bungarotoxin binding sites in adult rat brain. However, the developmental appearance of the message has yet to be characterized. We have used *in situ* hybridization and receptor binding methodolgy to compare the distribution of α7 message to that of α-bungarotoxin binding sites in postnatal rat brain. In somatosensory cortex, at postnatal day ten, mRNA distribution delineated barrel columns, similar to the binding site distribution. mRNA was most apparent in layers II-III, IV, deep V, and superficial VI, but was present at lower levels in layers of superficial V, deep VI and the subplate. Visual and auditory cortex also showed a high correlation between mRNA and binding site distribution. Within the thalamic nuclei innervating these regions, the ventroposterior medial and lateral geniculate nuclei, but not the medial geniculate, were densely labeled with both markers. Therefore, the α7 subunit may be localized both pre and post-synaptically in somatosensory and visual cortex, but only present post-synaptically in auditory cortex. This suggests a putative role for this site in the formation of thalamocortical synapses. *Supported by PHS grant #DC00450*

330.14

UNIQUE DEPLOYMENT OF α2 and β ADRENERGIC RECEPTORS IN THE SUBPLATE AND INTERMEDIATE ZONE OF DEVELOPING PRIMATE CEREBRUM M.S. Lidow* and P. Rakic *Section of Neurobiology Yale University, School of Medicine, New Haven, CT 06510*

Quantitative receptor autoradiography was used to study the emergence and distribution of adrenergic, dopaminergic, serotonergic, cholinergic, GABAergic and excitatory amino acid receptors in the cerebral wall of developing rhesus monkeys ranging in age from 65th embryonic day (E65) to two postnatal months. During the entire period examined, the majority of neurotransmitter receptors were localized in the cortical plate with little or no presence in the subjacent strata. The notable exception were α2 and β adrenergic sites which were transiently abundant in the embryonic subplate and intermediate zone. The α2 receptors were labeled with [^{3}H]- and [^{125}I]clonidine, and the specificity of binding was assured by its complete displacement with 100μM noradrenaline. The β receptors were labeled with [^{125}I]pindolol in the presence of phentolamine. This binding was also displaceable with 100μM noradrenaline. From the earliest age studied, α2 sites were present in the cortical layer I and throughout the subplate and intermediate zone with the highest density (ranging from 43 to 17 fmol/mm^3 depending on embryonic age) found in three narrow, clearly delineated bands situated close to the cerebral ventricle. On the other hand, β receptors were seen in the entire cortical plate, the subplate and in a separate band situated near the cerebral ventricle (where their density reached 24 fmol/mm^3 at E115). After birth, the density of α2 and β receptors below the cortex declined rapidly and by the 2nd month, the densities of these sites in the white matter were below detection by the methodology employed. In contrast to α2 and β receptors, α1 sites, labeled with [^{3}H]prazosin, were present exclusively in the cortical plate during the entire developmental period studied. The high density of α2 and β adrenergic receptors in subcortical embryonic zones suggest their unique role in modulation, maintenance, transfer and/or elimination of transient neurons and afferents in the embryonic subplate zone. Supported by NS22807 and EY02593.

330.15

AMINO ACID NEUROTRANSMITTERS REGULATE THE PROLIFERATION OF NEOCORTICAL PROGENITORS.

Joseph J. Lo Turco* & Arnold R. Kriegstein. Dept. of Genetics, Harvard Medical School, Boston, MA 02115, & Dept. of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Cell to cell interactions are thought to direct the proliferation and differentiation of neocortical cells. It is critical, therefore, to specifically identify how immature cortical cells interact with each other and with their environments. Using patch-clamp techniques we found that dividing cells in the ventricular zone of developing rat neocortex express functional GABA receptors and kainate receptors. To test the hypothesis that activation of these receptors influences the proliferation of cortical progenitors, we determined the effects of GABA and glutamate agonists and antagonists on the incorporation of [H3]thymidine into embryonic cortex. Both kainate and GABA reversibly decreased [H3]thymidine incorporation in E16 and E19 explants, but failed to affect younger (E14) explants. The decreased incorporation could be blocked by the antagonists BMI and CNQX. In fact, antagonists to GABA or kainate receptors increased [H3]thymidine incorporation in E19 explants but not in E16 explants. The effects of the antagonists indicate that endogenous amino acid receptor agonists act to decrease the proliferation of cortical progenitors. Such regulation may operate as a feedback control mechanism between differentiating cells and dividing cells in developing neocortex.

330.16

GLUTAMATE REGULATION OF GENE EXPRESSION IN DEVELOPING CORTICAL NEURONS. A. Ghosh and M. E. Greenberg, Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115

The molecular pathways by which glutamate receptor activation affects the formation of appropriate synaptic connections in the developing cortex are not understood. To begin to understand how stimulation of glutamate receptors can lead to long term adaptive changes in cortical neurons, we have initiated experiments to examine whether glutamate stimulation can affect the expression of (i) transcription factors (such as c-fos) that can potentially regulate downstream gene expression, and (ii) neurotrophic factors and their receptors since it has been proposed that the formation of appropriate synaptic connections may involve an activity-dependent competition for trophic factors.

Experiments were performed on E18 rat cortex cultures (5 to 15 days in-vitro). Cells were stimulated for 10 minutes with 10 μM l-glutamate in the presence of 1 μM TTX (to prevent trans-synaptic stimulation), following which kynurenic acid (1mM) was added to the medium to prevent further receptor activation. To examine the pharmocology of receptor activated gene expression, various receptor blockers were added to the medium 5 minutes prior to stimulation. 1 hour after stimulation RNA was extracted for Northern analysis. Our initial experiments indicate that glutamate stimulation leads to a robust increase in c-fos expression. This increase is almost completely blocked by the NMDA receptor antagonist APV (100 μM), but is not significantly affected by the non-NMDA receptor blocker CNQX (40 μM). Furthermore, the glutamate induced expression of c-fos is not significantly attenuated by the L-type calcium channel blocker Nifedipine (5μM). Of the molecules that may be involved in neurotrophic interactions, BDNF and its putative receptor, trk-B, are expressed in our cultures. We have found that BDNF mRNA levels are also enhanced by glutamate stimulation. This increase, as in the case of c-fos, is specifically APV sensitive. Our observations suggest that NMDA receptor activation is likely to be required for the expression of rapidly induced transcription factors and also for the regulation of neurotrophic factors such as BDNF in developing cortical neurons.

(Damon Runyon-Walter Winchell Cancer Research Fund Fellowship, DRG-1147)

330.17

EFFECTS OF LOW-DOSES OF IONIZING RADIATION ON DEVELOPING RAT CEREBRAL CORTEX, WITH SPECIAL REFERENCE TO NEURONAL MIGRATION AND EXPRESSION OF N-CAM. S.Fushiki*[1], K.Matsushita[1], A.Yoshiki[1] and W.J.Schull[2]. [1]Dept.of Dynamic Pathol.,Res.Inst.for Neurol.Diseases & Geriatrics,Kyoto Pref.Univ.Med.,Kyoto 602, Japan, [2]Univ. Texas Graduate School of Biomed.Sci., Houston, Texas 77225, U.S.A.

To elucidate the effects of low-doses of ionizing radiation we first investigated the pattern of migration of cortical neurons in the fetal rat brain that was irradiated in utero. The cells were labeled with ^{3}H-thymidine at E16. The doses of cobalt gamma rays were 5, 10,15 and 20cGy. The number of heavily labeled cells within each zone of the cortex was counted in autoradiographic sections and analyzed in relation to dose. Secondly, molecular changes appearing in the fetal cortex after irradiation were immunohistochemically examined with antibodies for N-CAM, L1, MAPs and neurofilaments. Our studies indicate that 1) exposure to doses of 15cGy significantly slows neuronal migration without increasing cell death, and 2) exposure to doses of 15-20cGy results in decreased immunoreactivities of N-CAM in the matrix cell zone. These observations may suggest a presumptive role of N-CAM in cortical histogenesis. (supported in part by a grant from the Atomic Energy Control Board of Canada.)

330.18

A METHOD FOR ABLATING DISCRETE CELL TYPES *IN VIVO*; A MEANS FOR BLOCKING CELL-CELL INTERACTIONS IN DEVELOPING CIRCUITS. S.A. Nirenberg, D.M. Fekete* and C.L. Cepko. Dept. of Genetics, Harvard Med. Sch., Boston, MA 02115.

The development of a neural circuit involves a complex series of cell-cell interactions. One approach to unravelling these interactions is to systematically ablate discrete classes of cells from the circuit as it is being formed. By removing a class of cells, one can determine its role(s) in development. In addition, by ablating an individual cell class at different times during development, one can examine separately the different roles it may play at sequential stages (e.g., regulating proliferation, cell fate, neurite guidance). We describe a technique for ablating discrete classes of cells *in vivo* or in tissue slices. The targeted class of cells is first engineered to express the gene for the enzyme ß-galactosidase. This is achieved by generating transgenic animals in which the ß-gal gene is regulated by a cell type-specific promoter. The cells are then selectively labelled with a photoactivatable dye by applying a ß-galactosidase substrate that releases the dye when enzymatically cleaved. The dye-labelled cells are then photoablated. This approach differs from other ablation methods that have been applied to vertebrates in that it can be used on a broad range of cell types, and a given cell type can be ablated at different developmental times, or in the adult. We demonstrate the use of this technique *in vivo* on several cell types in the mouse retina and cerebral cortex, as well as in the zebrafish embryo.

330.19

NMDA AND NON-NMDA RECEPTOR-MEDIATED SYNAPTIC POTENTIALS IN IMMATURE (P0-8) RAT NEOCORTICAL NEURONS. B.W. Connors*, H.G. Kim, and K. Fox, Dept. of Neuroscience, Brown University, RI 02912.

The most dynamic phase of synaptic development in rat neocortex occurs during the early postnatal period. However, the physiology of the earliest synapses has not been well characterized. Whole-cell current- and voltage-clamp recordings were obtained from developing neurons (P0 to P8) in slices of rat parietal neocortex. Input resistances were 500 MΩ to 2 GΩ, and resting membrane potentials were -40 to -60 mV. Glutamate antagonists hyperpolarized the cells and increased input resistance, suggesting tonic receptor activation at rest. Stimulating the subplate region with single shocks at 1 min^{-1} evoked excitatory postsynaptic events from animals as young as P0. EPSPs were quite variable, often lasted over hundreds of msec, and sometimes occurred repetitively following a single stimulus. They were blocked by TTX and reversed polarity around 0 mV. Synaptic currents were carried by both NMDA- and nonNMDA-specific channels, as judged by relative sensitivities to APV, CNQX and glycine, and the nonlinearity of I-V curves. Unlike mature neurons, NMDA currents predominated in most young neurons. We did not observe any inhibitory inputs before P6. Biocytin-staining showed that EPSPs were obtained from small pyramidal neurons and other immature cell types in the cortical plate and Cajal-Retzius cells in layer I. The presence of large, prolonged NMDA receptor-mediated currents could have significant developmental consequences.

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330.20

BICOMMISSURAL NEURONS IN THE CORTEX OF DEVELOPING HAMSTERS. C. Hedin-Pereira, D. Uziel and R. Lent*. Instituto de Biofísica Carlos Chagas Filho, U.F.R.J., 21941 Rio de Janeiro, Brazil.

In the course of a study on the development of callosal axons originating in dorsal sectors of neocortex, we noticed the existence of some callosal fibers extending long within the white matter into the anterior commissure (AC). To clarify the origin of this bicommissural projection, two series of experiments were done in hamster pups aged P2 through P9 (P1 = day of birth). In the first series, fixed brains were bisected along the midplane, and crystals of DiI and DiA were placed one into each transected commissure, to test for the presence of doubly-labeled bicommissural cells. In the second series a piece of ventromedial forebrain was disected out of one hemisphere exposing the AC for DiI placement, to label AC fibers extending into the callosum (CC), and thus test for the presence of bicommissural neurons in the opposite dorsal cortex. In both cases the brains were cut coronally several months after tracer placement, and all sections were examined, charted, and photographed under a fluorescence microscope coupled to a microcomputer. A few doubly-labeled neurons were found in the lateral transition cortex in most P5 and P7 brains, after tracer placement in both commissures. On P9, however, only a single doubly-labeled cell was found in one of the brains. No labeled neurons were found in the opposite dorsal cortex after tracer placement in the AC. We concluded that a group of cells in the lateral cortex extend a branched axon that grows transiently through both the CC and the AC.

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331.1

PARTICLE-MEDIATED GENE TRANSFER AND EXPRESSION IN RAT BRAIN TISSUES. N.-S. Yang, S.S. Jiao, L. Cheng, F. Siegel* and J. Wolff. Agracetus, Inc., Middleton, WI 53562 & Depts. of Pediatrics & Medical Genetics, Waisman Center, University of Wisconsin, Madison, WI 53705.

We have previously demonstrated *Accell*TM particle-mediated gene delivery process provides a useful means for transduction of various cell types in culture. Here we evaluated application to both fetal and adult rat brain cells. Using a CMVL gene as a reporter, we obtained high levels of transient gene expression in primary culture of fetal brain cells. Reduced but significant levels were also detected in adult brain cell cultures. Both neurons and glia cells were transduced using this technique. The transient gene expression level obtained with the *Accell* technology was found to be at least 100-fold higher than those obtained with three other tested gene transfer methods. The relative strengths of four cellular and seven viral promoters were evaluated in these cultures. *Ex-vivo* transduced fetal brain cells were transplanted into adult host rat brains. *In vivo* expression of transgenes was observed for up to two months, including detection of CMVL activity in the grafts and of human growth hormone (hGH) released into the cerebrospinal fluid (CSF) of host brains. We thus suggest that the *Accell* technology of particle bombardment can be employed as an effective method for *ex vivo* gene transfer into brain tissue. Implications and the current limitations of this technology for gene therapy of certain neurological diseases will be discussed.

331.2

USE OF RECONSTITUTED SENDAI VIRAL ENVELOPES TO DELIVER A NPY-CONTAINING ADENO-ASSOCIATED VIRUS VECTOR TO NEUROBLASTOMA AND GLIAL CELLS IN CULTURE. C.M. de Fiebre*, D. Notabartolo, P. Wu and E.M. Meyer. Dept. of Pharmacology and Therapeutics, Univ. of Florida College of Medicine, Gainesville, FL 32610.

A reconstituted viral envelope system has been used as a vehicle to deliver foreign DNA to cells. These so-called virosomes are reconstituted proteoliposomes derived from the Sendai virus without intrinsic genetic material. Intact viruses are solubilized in Triton X-100. Foreign DNA is added and encapsulated by removing the detergent with SM-2 Biobeads in the presence of exogenous phosphatidylcholine. Previous studies in our laboratory have displayed that virosomes are capable of infusing exogenous substances into neocortical-derived synaptosomes as well as primary neuronal and glial cultures. In the present study, we used this system to encapsulate an adenoassociated virus vector, pJDT95, into which we subcloned the gene for NPY (cDNA). These virosomes were then used to deliver the vector to a neuroblastoma cell line, SH-SY5Y, and to glial cells in culture. Preliminary results suggest that the vector increased message for NPY; however, NPY levels were not increased. SH-SY5Y cells were killed by the vector, but not by the virosomes, potentially because of deleterious effects of transiently increased levels of NPY or because of anti-oncogenic effects of the pJDT95. Glial cells may not have displayed increased levels of NPY due to lack of post-translational processing capabilities.

Supported in part by AG-06226.

331.3

SPINAL IMPLANTS OF CELLS GENETICALLY MODIFIED TO PRODUCE ENKEPHALIN REDUCE NOCICEPTIVE SENSITIVITY IN HOST MICE. H. Wu*, S. C. McLoon and G. L. Wilcox. Department of Pharmacology and Department of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

The aim of the current study was to determine whether AtT-20/hENK cells, an AtT-20 cell line transfected with a proenkephalin gene, implanted around mouse spinal cord would produce an antinociceptive effect. 100,000 cells in 5 µl of medium were injected into mouse lumbar subarachnoid space. Control animals were injected with medium alone. The animals' response to thermal nociceptive stimuli was tested by tail flick and hot plate assays. The mouse spinal cords were also examined histologically for the presence of implanted cells. Three days after implantation, mice were treated with an intrathecal injection of isoproterenol to stimulate opioid release from the cell implants. Isoproterenol produced dose-related antinociception only in the mice with cell implants. Sections of spinal cords from these animals processed for enkephalin immunohistochemistry revealed the presence of enkephalin positive cells surrounding the cord. The broad spectrum opioid antagonist naloxone, but not the µ opioid antagonist β-funaltrexamine, blocked the antinociceptive effect of the implanted cells. This would suggest that the antinociceptive effect of these genetically modified cells was mediated through δ opioid receptors. Enkephalin is generally believed to act via δ opioid receptors, further implying that enkephalins released by the implanted cells were responsible for the observed effect. By 7 days post-implantation, enkephalin positive cells were still present around the spinal cord in the cell-implanted animals. Intrathecal injection of the δ opioid agonist, DPDPE, resulted in a reduced antinociceptive effect in mice with cell implants as compared with the control group. This suggests that the chronic release of enkephalins from the implanted cells produced δ opioid receptor tolerance. These results show that cells genetically modified to secrete enkephalin, when implanted adjacent to the spinal cord, can release enkephalins both tonically and acutely under adrenergic stimulation and can reduce pain sensitivity. (DA-01933 to GLW and EY-05371, EY-09537 and EY-07133 to SCM).

331.4

IMMORTALIZED PANCREATIC ß-CELL LINES AS GABA-RELEASING NEURAL IMPLANTS T. K. Swoboda*, A. P. Signore, B. Zielinski, J. Tseng, T. Kiezeloff, P. A. Tresco, P. Aebischer, Section of Artificial Organs, Biomaterials, and Cellular Technology, Brown University, Providence, R.I. 02912

A neural graft releasing γ-aminobutyric acid (GABA) may be useful in the treatment of a variety of CNS disorders including Huntington's disease, Parkinson's disease, and epilepsy. Pancreatic ß-cells have been shown to express glutamic acid decarboxylase (GAD). This suggested that immortalized ß-cells may release GABA, and therefore avoid the problem of loss of gene expression encountered with the use of genetically engineered cell lines. HPLC analysis of two such cell lines, the NIT-1 (mouse derived) and the RIN-1046.38 (rat derived) revealed that both release GABA. Under static incubation conditions, NIT-1 cells released 11.7±0.26, and RIN cells 21.3±0.80 fg GABA/cell/min. The potential of these cell lines as neural implants is being assessed with encapsulation technology, which circumvents the possibility of tumor formation and host graft rejection. NIT-1 and RIN cells suspended in a hydrogel were loaded into acrylic copolymer capsules. Capsules maintained in vitro for up to 4 weeks showed GABA release in the range of 60 - 160 pg/capsule/minute for both types of cell lines. Histologic analysis revealed viable NIT-1 and RIN cells. Three male Sprague-Dawley rats received intracerebral NIT-1 containing capsules to assess short term viability of the encapsulated cell line in vivo. Viable cells were present upon histological examination 4 weeks post implantation. The data indicates that encapsulated cell lines derived from the pancreatic ß-cells may be useful as a GABA-delivery system within the CNS.

331.5

A POSSIBLE THERAPEUTIC APPLICATION OF CELL LINES ENGINEERED TO PRODUCE GABA. J. Segovia*[1], L.J. Fisher[5], A. Sandrasagra[1], C.H.J. Ruppert[1], B. Anton[2], C.J. Evans[2], F.H. Gage[3] and A.J. Tobin[1,3,4]. Departments of [1]Biology and [2]Psychiatry and Biobehavioral Sciences, [3]Molecular Biology Institute and [4]Brain Research Institute, University of California, Los Angeles, CA, 90024 and [5] Department of Neuroscience, University of California, San Diego, La Jolla, CA, 92093.

Huntington's disease (HD) causes a severe neuronal degeneration in striatum, which is accompanied by decreases in the striatal content of several neurotransmitters, including GABA. Several animal models mimic the pathophysiology and some of the biochemical alterations of HD, as well as inducing motor and learning deficits in the lesioned animals. Transplantation of fetal tissue in rodent models of HD has shown that the tissue survives, integrates into the host brain and induces recovery of some of the motor and learning deficits produced by the lesion. With a view to developing new therapeutic approaches to HD and other neurological disorders with deficits in GABA production, we have established cell lines that synthesize and secrete GABA. To obtain such cell lines we have infected rat primary fibroblasts with GAD_{65} cDNA in a retroviral vector. The engineered fibroblasts, but not the uninfected fibroblasts, express GAD_{65} mRNA, the GAD polypeptide and GAD enzymatic activity. Furthermore, we have shown, in vitro, that the infected fibroblasts secrete GABA into the surrounding medium (and that GABA overflow increases 6 fold in response to K^+-induced depolarization). We are currently employing kainic acid lesioned rats to assess the effect of grafting GABA-producing fibroblasts into the lesioned striatum. (Supported by NS 22256 and The Scottish Rite Schizophrenia Research Program)

331.6

Improved killing of glioma cells in culture and *in vivo* using retrovirus vector with herpes simplex virus thymidine kinase gene and wild type retrovirus. Y Takamiya, MP Short*, F Moolten, C Fleet, XO Breakefield, RL Martuza. Neuroscience Ctr, MGH, Charlestown, MA 02129; Neurosci Prog, Harvard Med Sch, Boston, MA 02115; and Dept of Neurosurg, Georgetown U, Washing, DC 20008.

Previously we demonstrated that the efficiency of retrovirus-mediated delivery of genes sensitizing tumor cells to nucleoside analogues could be increased by adding helper wild type virus (MoMLV). In the present study, packaging cells (psi 2-STK) releasing a replicative-defective retroviral vector encoding the herpes simplex thymidine kinase (HSV-TK) gene under the transcriptional control of the SV40 promoter-enhancer element were infected with MoMLV. These infected packaging cells (psi2-STKWT) release both wild type retrovirus and the retrovirus vector containing the HSV-TK gene. In culture, psi2-STKWT cells were several thousandfold more sensitive to the toxic effects of the nucleoside analogue, ganciclovir (GCV), than were control cells of the psi2 packaging line. This sensitivity surpassed previous results obtained with a retroviral rather than SV40 promoter. Psi2-STKWT and psi2-STK cells did not differ significantly in GCV sensitivity, suggesting that in this system wild type retrovirus exerted no independent debilitating effect on cell growth. When psi2-STKWT cells were co-cultured with glioma cells (C6BU1pBAG cells), the glioma cells (although not carrying the HSV-TK gene themselves) were rendered 1000-fold more sensitive to GCV treatment than glioma cells co-cultured with control psi2 cells in similar ratio. Regression of tumors in response to GCV therapy was also noted in nude mice inoculated subcutaneously with a combination of psi2-STKWT cells and C6BU1pBAG cells. These findings suggest that the efficiency of retrovirus-mediated transfer of GCV sensitivity is increased by placing the HSV-TK gene under the transcriptional control of the SV40 promoter-enhancer, and that cells releasing potent vectors can exert lethal effects *in vitro* and *in vivo* on tumor cells in their vicinity in conjunction with a drug that is otherwise innocuous.

331.7

LONG-TERM SURVIVAL AND MATURATION OF NEURONS DERIVED FROM THE HUMAN CELL LINE N-TERA 2 AFTER TRANSPLANTATION INTO NUDE MOUSE BRAIN S.R.Kleppner*, J.Q.Trojanowski, V.M.-Y.Lee Inst. of Neurological Sciences and Dept. of Path. and Lab. Med., Univ. of PA, Philadelphia, PA 19104

We have previously shown that the N-Tera 2 (NT2) cell line, derived from a human teratocarcinoma, can give rise to pure cultures of fetal cholinergic neurons after treatment with retinoic acid. Having described the *in vitro* phenotype of these neurons, we were interested in phenotypic modifications of these neurons in a CNS environment. When implanted into adult athymic mice these neurons are viable for at least 6 months and show no signs of tumorogenesis. Using a panel of human specific antibodies, we have characterized the phenotype of these cells over the course of 6 months. Before implantation, cells were immunopositive for N-CAM, MAP2, Tau, vimentin, NF-M, NF-L and exhibited low levels of NF-H. Within three weeks after implantation, the grafts exhibited process outgrowth, the extent of which may depend on the exact location of the graft. After 6 months *in vivo*, all markers remained the same except for NF-H which increased expression dramatically by 6 months post-implant. While the continued presence of vimentin indicates that these cells partially maintain a fetal phenotype *in vivo*, the appearance of NF-H may be a sign that they are beginning to mature. Additionally, preliminary results show that NT2-neurons transfected with a β-gal expression plasmid continue to express β-gal at low levels 6 months after transplantation suggesting that the NT2 neurons retained the expression of foreign genes after stable transfection into the undifferentiated NT2 cells. Thus, NT2 neurons exhibit long-term survival in a CNS environment and stable expression of a transfected gene, making them an ideal system for further studies utilizing gene therapy.

331.8

RECOVERY IN THE RAT 6-HYDROXYDOPAMINE MODEL OF PARKINSON'S DISEASE BY DIRECT INTRASTRIATAL INJECTION OF HSV-1 VECTORS WHICH EXPRESS THE HUMAN TYROSINE HYDROXYLASE GENE. M.J. During*, A.I. Geller, A.Y. Deutch, K.L. O'Malley. Sect. Neurosurg. & Neuroend., Yale Univ. Sch. Med., New Haven, CT; Div. End., Children's Hospital, Boston, MA; Dept. Psych., Yale Univ. Sch. Med.; Dept. Neurobiol. & Anatomy, Washington Univ. Sch. Med., St. Louis, MO.

We have developed Herpes Simplex Virus (HSV-1) vectors that stably express a gene in cultured neurons, and *in vivo*, following stereotactic injection into the adult rat brain. A HSV-1 vector that stably expresses human tyrosine hydroxylase (pHSVth) in cultured striatal cells directs regulated release of L-dopa and dopamine from these cells.

The efficacy of intrastriatal injection of pHSVth on recovery in the 6-hydroxydopamine rodent model of PD was assessed using apomorphine-induced asymmetrical rotation. Groups of lesioned rats received intrastriatal stereotactic injection with pHSVth or pHSVlac (expresses *E. Coli* β-galactosidase), or saline. Rats which received pHSVth had an 80±10% reduction in apomorphine-induced contralateral rotational behavior, whereas both control groups maintained baseline rates of rotation. Furthermore, the reduction in rotational behavior persisted for the entire 6 month duration of followup. *In vivo* expression of TH in striatal neurons and glia surrounding the injection site and biochemical recovery of striatal dopamine neurotransmission are currently being investigated using immunohistochemistry and microdialysis.

331.9

ADRENAL CHROMAFFIN CELLS AND TRANSGENIC NGF-PRODUCING ASTROCYTE CO-GRAFTS PARTIALLY RESTORE FUNCTION IN THE UNILATERAL 6-OHDA RAT MODEL OF PARKINSON'S DISEASE. L.A. Cunningham*, M.P. Short[1], X.O. Breakefield[1], M.C. Bohn. Dept. of Neurobiology and Anatomy, University of Rochester Medical School, Rochester, NY 14642 and [1]Molecular Neurogenetics Unit, MGH, Harvard Medical School, Boston, MA 02114.

Our previous studies demonstrated that astrocytes genetically modified to constitutively express a mouse ß-NGF transgene support the survival and neuronal transdifferentiation of intrastriatal adrenal chromaffin cell grafts at two weeks (*Brain Res.* 561:192-202). The aims of the present study were to determine whether these effects would be maintained for longer post-grafting periods and whether the grafts would reduce apomorphine-induced turning behavior in the unilaterally 6-OHDA-lesioned rat. Suspensions of postnatal day 12 adrenal chromaffin cells and transgenic astrocytes (AC + AsN.8) were mixed 1:1 (160,000 total cells) and grafted into the dopamine-denervated striatum of the adult Fischer 344 rat (n=7). Chromaffin cells grafted alone (AC, n=4) or with normal astrocytes (AC + As, n=4) served as controls. When co-grafted with the transgenic astrocytes, the survival of the adrenal chromaffin cells was enhanced 5-12 fold over controls as assessed by tyrosine hydroxylase immunoreactivity 10 weeks post-grafting (1168 ± 143 vs. 221 ± 128 and 99 ± 5 TH-IR cells; AC + AsN.8 vs. AC, and AC + As grafts, respectively, $p<0.01$). Furthermore, 36% of the TH-IR cells in the transgenic co-grafts displayed a phenotype characteristic of differentiated sympathetic neurons, i.e., large soma (30-40 µm in diameter), TH-IR neuronal processes and extensive process elongation. The effects of the transgenic astrocytes on chromaffin cell survival and morphology were paralleled by a 40% reduction in apomorphine-induced rotation, suggesting a partial restoration of striatal function. The results of this study demonstrate that genetically engineered astrocytes are an effective vehicle for delivering ß-NGF trophic support. Supported by NS08906 and the PEW Charitable Trust

331.10

CHROMAFFIN CELLS COGRAFTED WITH NGF-PRODUCING FIBROBLASTS EXHIBIT NEURONAL FEATURES

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Adrenal chromaffin cells (ACC) can differentiate *in vitro* into sympathetic neurons under the influence of nerve growth factor (NGF). Such a phenotypic change, and whether transformed neonatal ACC are functional in a rat model of catecholamine function, was examined. Adult rats were unilaterally lesioned with 6-hydroxydopamine injections into the substantia nigra and assessed with apomorphine (APO) and amphetamine (AMP). Rats then received an injection of either: 1) ACC+NGF-producing fibroblasts, 2) ACC+control fibroblasts, 3) NGF-producing fibroblasts, or 4) control fibroblasts into the denervated striatum, and were behaviorally tested for 8 wks. At 2 months grafts were processed histochemically for neurofilament, MAP2, NGF receptor, SCG10 (indicators of a neuronal phenotype), chromogranin A (CGA, indicator of ACC phenotype) and tyrosine hydroxylase (TH) immunoreactivity (IR). ACC+NGF grafts showed extensive TH, NGF receptor and neurofilament IR throughout the graft, and MAP2 and SCG10 expression in ACC bodies. CGA expression was negative. ACC+control fibroblast grafts showed TH-IR limited to ACC bodies only, and very limited IR to other neuronal markers. The NGF-producing fibroblast grafts showed positive IR for some neuronal markers, reflecting a slight ingrowth of host fibers. No clear differences between the groups were observed in APO or AMP induced rotational behavior. These behavioral observations are being evaluated further.

331.11

INTRACEREBRAL TRANSPLANTATION OF GENETICALLY ENGINEERED MUSCLE CELLS REDUCES EXPERIMENTAL PARKINSONISM IN RATS. S.S. Jiao*, P. Williams and J.A. Wolff. Department of Pediatrics & Medical Genetics, Waisman Center, University of Wisconsin, Madison, WI 53706.

The intracerebral transplantation of genetically modified cells has been proposed for the treatment of several genetic and acquired neurologic disorders. Our previous work has shown that primary cultured muscle cell allografts survive in the rat brain longterm in a healthy and differentiated state and might be a useful "platform" for transgene expression in the brain. The present work indicates that primary cultured muscle cells lipofected with pCMVTH gene expressed tyrosine hydroxylase (TH). In rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal dopamine pathway, grafts of pCMVTH-transfected muscle cells transplanted into the caudate nucleus or in the lateral ventricle reduced apomorphine-induced rotational behavior for at least two months. TH gene expression was localized to the muscle-brain grafts. These results explore the ability of genetically engineered primary muscle cells to serve as a feasible approach to gene therapy for the rat model of Parkinson's disease. The use of plasmids avoids the potential deleterious effects of retroviral vectors and the unstable expression of retrovirally transduced genes.

331.12

TEMPERATURE-SENSITIVE SUBSTANTIA NIGRA NEURAL CELLS FOR CNS TRANSPLANATION: *IN VITRO* ANALYSIS AND GRAFTING INTO PARKINSONIAN RATS AND MONKEYS. J.H. Kordower*, S.B. Schueler, C.H. Markan[1], W. Melega[1], R. Anton[1], and D. Bredesen[1]. Dept. Neurological Sci. Rush Presbyterian Med. Ctr., Chicago Illinois 60612 and [1] U.C.L.A., Los Angeles CA, 90024.

Temperature-sensitive immortalized tyrosine hydroxylase (TH) positive rat nigral neural cells (Durand et. al., Soc. Neurosci. Abstr., 16: 40, 1990) were infected with recombinant retroviruses effecting the expression of increased levels of TH. These cells proliferate at the permissive temperature of 33°C but are terminally differentiated at the restrictive temperature of 38°C. The parental line produced low levels of TH mRNA, TH protein, and <5 ng dopamine/mg protein as assessed by northern blot analysis, immunohistochemistry, and HPLC respectively. In contrast, a retrovirus-infected line produced higher levels of TH mRNA and protein, as well as 2200 ng dopamine/mg protein.

Instrastriatal transplants of the parental cell line (at 38° C) only reduced apomorphine-induced rotation by 30% in unilateral nigrostriatal lesioned rats. In contrast, the retrovirus-infected cell line reduced apomorphine-induced rotations by 65%. Cells survived implantation as determined by prelabeling grafted neurons with Hoechst dye. No tumor formation occurred. However *in vivo*, these cells appeared to have down regulated TH production. Numerous temperature sensitive cell survived grafting for 1 month in immunosuppressed MPTP treated monkeys as identified by both Hoechst labeling and TH-immunohistochemistry. Glial fibrillary acidic protein-immunohistochemistry revealed only a limited host astrocytic response to the implant. These data provide further evidence that conditionally immortalized cells of neural CNS origin are potentially valuable as genetically engineered neural transplants.

331.13

TRANSPLANTS OF THE SYMPATHOADRENAL (MAH-B2) CELL LINE SURVIVE AND DIFFERENTIATE WITHIN THE ADULT PERIPHERAL NERVOUS SYSTEM.

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A current focus of transplantation research centers on the delivery of specific products by transplants of genetically modified cells. The sympathoadrenal cell line (MAH-B2) has been established by the immortalization of embryonic rat adrenal cells with a recombinant murine retrovirus containing the v-*myc* oncogene.

We examined the survival of MAH-B2 cells after transplantation into the brain and the peripheral nervous system (PNS). Cells were grown *in vitro* at high density for 3-5 days, collected and then injected as suspensions into the striatum or the sciatic nerves of adult Wistar rats. Quantitative and qualitative aspects of the transplants were assessed by immunocytochemistry.

No significant survival of the cells was seen in the striatum. In contrast, the peripheral nerves contained 3.0-5.0 x 10^3 MAH-B2 cells/graft. After 2-3 weeks post-grafting, the small, rounded cells differentiated into large sympathetic-like neurons aligned parallel to the Schwann cell columns. Grafted MAH-B2 cells expressed MAP2, neurofilament subtypes and catecholamine enzymes (DBH, TH). There was no evidence of uncontrolled growth of the cells within the nerves (5 months longest time point).

These experiments indicate that the MAH-B2 cells survive well and differentiate when grafted into the PNS. Combined peripheral nerve/MAH-B2 cell transplants will be studied in animals with striatal dopamine depletion. **(Supported by The Parkinson Foundation of Canada)**

331.14

DIFFERENTIATION OF AN IMMORTALIZED NEURONAL CELL LINE RENDERS THEM LESS SUSCEPTIBLE TO CYTOTOXIC T CELL LYSIS *IN VITRO*.

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The expression of class I antigens of the major histocompatibility complex (MHC) allows cells to be recognized and killed by cytotoxic T lymphocytes (CTL) in cases of allogeneic graft rejection. Recent evidence has suggested that CNS neurons are resistant to CTL-mediated lysis (Keane et al., Transplantation, in press), and we tested whether a CNS-derived neuronal cell line showed similar properties. The temperature-sensitive, neuronal cell line RN33B, derived from E12 Sprague Dawley medullary raphe, expresses the RT1.$A^{c/u}$ class I, but not class II, MHC antigens in the undifferentiated state. Neuronally differentiated RN33B cells do not express class I MHC antigens, although treatment with interferon-γ (IFN-γ) can induce class I, but not class II, MHC expression. To determine whether class I MHC expression is a requirement for RN33B cell killing, we performed standard in vitro cytotoxicity assays using ^{51}Cr release as an index of target cell lysis. Our results showed that undifferentiated RN33B cells were killed by alloantigen-specific CTLs, whereas differentiated RN33B cells were markedly less susceptible to CTL-mediated lysis, even after induction of class I MHC expression. These studies suggest that resistance of CNS neurons to CTL-mediated cell killing depends on the degree of neuronal differentiation rather than the level of class I MHC expression. Whether RN33B cells are refractory to lysis after transplantation into allogeneic CNS is examined in the adjacent poster (Shihabuddin et al.). Supported by The Miami Project and NS26887.

331.15

SURVIVAL OF NEURONAL CELL LINE IMPLANTS IN THE CNS OF HISTOINCOMPATIBLE RAT HOSTS.

L.S. Shihabuddin*, L.A. White, S.M. Onifer, S.R. Whittemore and V.R. Holets. The Miami Project, Univ. of Miami School of Medicine, Miami, FL 33136.

Our previous studies demonstrated that the temperature-sensitive neuronal cell line RN33B was able to survive and differentiate with varied morphologies depending on the site of integration. In the present study, we addressed the issue of immunological rejection of RN33B transplants into the CNS, as in vitro studies had suggested that neuronal differentiation of RN33B cells made them less susceptible to cytotoxic T cell lysis (White et al., adjacent poster). RN33B cells were derived from Sprague-Dawley rats and expressed the RT1.$A^{c/u}$ class I MHC epitopes. Allogeneic transplants of ß-galactosidase labeled RN33B cells were unilaterally made into the hippocampus and cerebral cortex of histoincompatible Lewis rats (RT1.A^{l}). Two weeks later, 4 transplanted Lewis rats were challanged intraperitoneally with RN33B cells pretreated with γIFN, which upregulated class I MHC expression. The animals were allowed to survive 3 weeks. In parallel, 4 control Lewis rats also received identical transplants and were sacrificed after 5 weeks. The appearance of the transplanted RN33B cells in the two groups was compared by immunocytochemical and morphological criteria. RN33B cells were not detected in the challanged hosts. In contrast, cells in the unchallanged hosts survived in both sites and differentiated with multiple neuritic processes. Our results suggest that while allogeneic RN33B cell transplants can be induced to be immunologically rejected, neuronal cell line transplants may prove to be useful in replacing damaged endogenous CNS neurons. The survival of chronic RN33B cell transplants is presently under investigation.

Supported by The Miami Project to Cure Paralysis and NS26887.

331.16

ION CHANNEL CURRENTS IN CULTURED HCN-1A NEURONS FROM HUMAN CORTEX.

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A neuronal cell line (HCN-1A) was recently established from human cortical tissue obtained from a megalencephalic child (Ronnett et al, Science 248:603-605, 1990). Immunocytochemical evidence indicating a neuronal origin was presented, but expression of ion channels was not demonstrated. For the present study, HCN-1A cells were cultured in DMEM with 15% fetal calf serum and induced to differentiate with nerve growth factor 10 ng/ml, forskolin 1 mM, and dibutyryl-cAMP 1 mM. Cells or membrane patches were voltage clamped using conventional patch clamp methods. Using appropriate recording solutions, we identified and characterized TTX-sensitive Na^+ currents, two types of Ca^{2+} channel currents, including L-type and possibly N-type currents, and three types of K^+ currents, including a voltage dependent outward rectifier, a TEA-sensitive 146 pS Ca^{2+}-activated K^+ channel, and a 23 pS outward current channel. These data provide additional evidence for a neuronal phenotype, and demonstrate functional potential that will be important in transplant experiments.

331.17

INTERMITTENT IMMUNOSUPPRESSION ENHANCES SURVIVAL OF INTRACEREBRAL TRANSPLANTS OF THE A7 IMMORTALIZED CELLS.

G. S. Okoye*, W. J. Freed[†] and H. M. Geller. Neurosurgical Research Laboratory, UMDNJ-Robert Wood Johnson Medical School, and The Graduate School, Rutgers University, Piscataway, NJ 08854 and [†]NIMH Neurosciences Center at St. Elizabeth, Washington, DC 20032.

We have previously shown that A7 immortalized glial cells survive as an intracerebral implant and promote neurite outgrowth, at least for a short period of time. In this study, we evaluated 1) the ability of intermittent short-term immunosuppression to prolong the survival of A7 cells and 2) the host brain reaction to the implant. We implanted 10^5 A7 cells as previously described in male rats randomized to either control conditions or immunosuppressed using cyclosporine (12.5 mg/kg daily starting 1 day before transplantation for 7 days, followed by intermittent daily dose of 12.5 mg/kg). After a period of from 1 week to 12 weeks, animals were sacrificed and the number and location of surviving A7 cells were evaluated using antibodies to SV40 large T antigen. The dosing of the 12 weeks rats stopped 7 weeks post-transplantation. The reaction of the host brain was evaluated using antibodies recognizing neuronal and glial-specific proteins, as well as tenascin and lymphocyte markers. Preliminiary results indicated significant increase of the survival of A7 cells in the immunosuppressed rat brain as compared to the control. Some immunoreactivity to tenascin, CD-4 and CD-8 lymphocyte markers were evident in the host brain. These preliminary results suggest that short-term intermittent immunosuppression may prolong survival of A7 cells in the rats brain. In addition, there is some host brain reaction in both control and immunosuppressed rats. Supported by NIH P01 NS 21469.

332.1

THE EFFECT OF GLUTAMATE RECEPTOR ANTAGONISTS AND AN UPTAKE BLOCKER ON GLYCOGEN CONTENT AND CELL VOLUME IN CULTURED ASTROCYTES. R.S. Dombro*, A.S. Bender, A. Padron, D.G. Hutson and M.D. Norenberg. VAMC & Depts. of Surg. & Pathol. Univ. Miami School of Medicine, Miami, FL 33101.

Changes in astrocyte glycogen levels often accompany alterations in cell volume. Glutamate (GLU) causes both an increase in astrocyte glycogen content and cell volume. To investigate the mechanism of the GLU-stimulated glycogen increase and its relation to changes in cell volume, the effect of various GLU receptor blockers and the GLU uptake inhibitor, trans-4-carboxy-L-proline (t-PDC) were tested. GLU (1 mM) increased glycogen content by 54% and cell volume by 27% after 90 min of treatment. The following GLU receptor blockers were tested: 50 μM DNQX, 50 μM NBQX, 0.5 mM APH and 0.5 mM MK-801. None of these blockers had any effect on glycogen content or volume except for MK-801 which reduced glycogen by 34% and volume by 27%. Since NMDA receptors are not present on astrocytes, the effect of MK-801 must not be that of a GLU receptor antagonist. The uptake inhibitor, t-PDC, increased glycogen content by 70% and cell volume by 47%. This "paradoxical" action may have been due to its acting as a GLU analog rather than an uptake inhibitor. Thus, GLU-induced changes in astrocyte glycogen content are associated with parallel changes in cell volume. However, these effects do not appear to be receptor-mediated. (Supported by VA Medical Research Service and NIH grant AM 38153).

332.2

ASTROCYTE MODULATION OF CEREBRAL VESSEL FUNCTION VIA EICOSANOIDS AND NITROSYL FACTORS. S. Murphy*, S. Kardos, S.A. Moore, K.I. Orgren and F.M. Faraci. Depts. Pharmacology, Pathology and Internal Medicine, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

Astrocytes release eicosanoids and also nitrosyl factors. To reveal whether these factors influence vascular function, astrocyte-conditioned medium was superfused onto cerebral artery segments and microvessel-derived endothelial and smooth muscle cells in culture. Astrocytes induced relaxation in preconstricted basilar arteries, dependent upon the number of cells added but not on prostanoid synthesis. Relaxation was inhibited by arginine analogs and depended upon intact endothelium, suggesting the latter released a nitrosyl factor. Addition of agents (quisqualate, bradykinin) that evoke release of an astrocyte nitrosyl product to denuded vessels in the presence of astrocytes caused marked relaxation (20%), which was reversed by arginine analogs.

In studies with cerebral microvessel cells, addition of the thromboxane analog U46619 caused dose-dependent release of the vasodilator prostacyclin. In addition, rapid transfer of conditioned medium from ATP-stimulated astrocytes caused similar increases in prostacyclin release from ATP-insensitive endothelial/smooth muscle cells. These results suggest that evoked release of astrocyte eicosanoid and nitrosyl factors influence adjacent vascular cells, indicating the potential role of astrocytes in modulating cerebral vessel function. Supported by NS 24621.

332.3

ETHANOL-INDUCED RELEASE OF ASPARTATE AND TAURINE IN PRIMARY ASTROCYTE CULTURES
H.K. Kimelberg*, M. Cheema, H. Tong[1], E. O'Connor and S.K. Goderie, Division of Neurosurgery, Albany Medical College, Albany, NY 12208, and [1]Rensselaer Polytechnic Institute, Troy NY 12180

Exposure of primary astrocyte cultures to isoosmotic ethanol from 10 to 100 mM leads to release of the preloaded amino-acids [^{3}H] taurine and [^{3}H] D-aspartate, whereas hyperosmotic ethanol causes no release. Volume studies on astrocyte monolayers show that isomotic ethanol results in cell swelling (i.e. it is hypotonic) while hyperosmotic ethanol causes no change in volume (i.e. it is isoosmotic). The release is inhibited by an anion channel blocker, L-644,711, shown to inhibit release of amino-acids due to hypoosmotic media- or potassium-induced swelling. The release is not dependent upon extracellular calcium and shows a decline in response to successive exposures to ethanol, i.e. apparent "desensitization". Thus, the swelling-induced release of amino-acids by ethanol corresponds in its properties to swelling-induced release in astrocytes due to hypotonic or high K^+ media. Interestingly, if one combines an exposure to hyperosmotic ethanol, which normally has no effect, with high K^+ medium, the high K^+ medium-induced release of [^{3}H] D-aspartate is potentiated. Release due to isoosmotic ethanol or the potentiation of the high K^+ response by isoosmotic or hyperosmotic ethanol may contribute to the evolving pathology in the CNS during acute or chronic exposure to ethanol or in conjunction with traumatic and/or ischemic brain injury. Supported by NS 23750 and 30303.

332.4

EFFECTS OF EXTRACELLULAR ATP AND GROWTH FACTORS ON DNA SYNTHESIS IN CULTURED ASTROCYTES. JT Neary*, Q Zhu, SR Whittemore[1] and MD Norenberg. Lab Neuropathol, VA Med Ctr, Dept Pathol & The Miami Project[1], Univ Miami Sch Med, Miami, FL 33101.

Astrocyte proliferation is one of the characteristics of reactive gliosis which occurs following CNS injury. Growth factors such as bFGF and EGF stimulate astrocyte proliferation, and bFGF levels increase following CNS injury. Recently it has been reported that ATP, which is released extracellularly following tissue injury, also increases DNA synthesis in astrocytes. We have now studied the effects of extracellular ATP in combination with growth factors on astrocytic DNA synthesis. Agents tested included bFGF (50 ng/ml), EGF (10 ng/ml), PDGF (2.5 ng/ml), and NGF (100 ng/ml). Cultures were treated with media containing 0.5% horse serum for 2 days, ATP (50-1000 μM) and/or growth factors were then added for 18 hr followed by an additional incubation in ^{3}H-thymidine for 4 hr. Under these conditions, ^{3}H-thymidine incorporation was increased 30-60% by ATP and 4-8 fold by bFGF, EGF, and PDGF; NGF was slightly inhibitory (75% of control). When ATP was added in the presence of bFGF, ^{3}H-thymidine incorporation was markedly increased in a synergistic manner (20-fold stimulation) in mature astrocytes (28 div). Smaller effects were observed in immature cells (15 div). Synergism was not observed with the other growth factors. These findings indicate that ATP markedly enhances bFGF-stimulated DNA synthesis in astrocytes and suggest that ATP may be an important determinant of the astrocytic response following CNS injury. (Supported by Dept. of Veterans Affairs.)

332.5

CHARACTERISTICS OF TAURINE UPTAKE IN HYPEROSMOTICALLY TREATED CEREBRAL ASTROCYTE CULTURES. **J.W. Beetsch* and J.E. Olson. Department of Emergency Medicine, Wright State University School of Medicine, Dayton, OH 45401**

^{3}H-Taurine uptake was characterized in astrocytes from rat cerebral cortex grown in normal and hyperosmotic culture conditions to investigate mechanisms of cell volume regulation and adaptation to states of altered osmolality. With high concentrations of taurine (1mM), uptake is linear for both conditions for at least 30 min. This uptake rate was not affected by exposure to hyperosmotic conditions. The mean ± SEM apparent binding constant for carrier-mediated transport, K_m, was not altered by hyperosmotic conditions (14.1 ± 3.2 μM in isoosmotic media, 13.9 ± 5.6 μM in hyperosmotic media). The maximal velocity of uptake, V_{max}, (mean ± SEM) of taurine was significantly ($p<0.05$) larger in isoosmotically treated astrocytes (1.13 ± 0.10 mg·min/nmol) compared with that of hyperosmotically treated astrocytes (0.62 ± 0.10 mg·min/nmol). The diffusional transport rate was significantly larger in cells grown in hyperosmotic conditions (0.71 ± 0.12 μl/mg·min) compared with cells grown in isoosmotic conditions (0.20 ± 0.12 μl/mg·min). Thus, accumulation of taurine by cultured cerebral astrocytes exposed to hyperosmotic conditions is not due to an increased uptake rate. Other mechanisms, such as decreased taurine release or increased synthetic enzyme activity, may lead to the accumulation of taurine in cerebral astrocytes *in vitro* and, perhaps *in situ*. Supported by NS 23218.

332.6

GLIAL RESPONSE TO GLUTAMATE AND GABA IN THE ISOLATED INTACT MOUSE OPTIC NERVE. Arthur M. Butt* and M.G.Tutton. Dept. Physiology, UMDS, St. Thomas's Hospital, London SE1 7EH, U.K.

Experiments were carried out on isolated intact optic nerves from 23-59 day old mice. Glial cells were impaled with microelectrodes back-filled with 2% horseradish peroxidase in 0.5M KCl. Membrane potentials (E_m) were measured and the response to high [K^+], 1mM glutamate, or 1mM GABA was recorded, and cells were then dye-injected for subsequent identification as astrocytes or oligodendrocytes. Astrocytes have a significantly ($p<0.001$) higher E_m (-69 ± 1 mV, n=193) than oligodendrocytes (-64 ± 1 mV, n=34). Both cell types depolarize in high [K^+] in a Nernst-like manner. Both astrocytes and oligodendrocytes respond to glutamate and GABA in one of four ways: 1) respond to both glutamate and GABA; 2) respond to glutamate but not GABA; 3) respond to GABA but not glutamate; and 4) respond to neither glutamate or GABA. The response, where it occurs, is a 2-5 mV depolarization which is reversible, and ongoing experiments indicate both the glutamate and GABA responses are Na^+-dependent. We are currently investigating the basis of the variation between responses in astrocytes and oligodendrocytes.

This work is supported by the Wellcome Trust (AMB) and the Smith and Nephew Foundation (MGT).

332.7

INVOLVEMENT OF ARACHIDONIC ACID IN THE STIMULATION OF 2-DEOXY-D-[1-^{3}H] GLUCOSE UPTAKE EVOKED BY NORADRENALINE IN ASTROCYTE CULTURES N. Yu[1], J.-L. Martin[1], N. Stella[1,2], L. Pellerin*[1] and P.J. Magistretti[1] Institut de Physiologie, Université de Lausanne, Lausanne, Switzerland[1] and INSERM U114, Collège de France, Paris, France[2].

Noradrenaline (NA) and VIP have been shown to promote the hydrolysis of glycogen in primary cultures of mouse cerebral cortical astrocytes (Sorg and Magistretti, 1991). In view of this action on energy metabolism we have examined the effect of NA and VIP on 2-deoxy-D-[1-^{3}H] glucose (2-DG) uptake. Basal 2-DG uptake by cultured astrocytes corresponds to 3.5 ± 0.3 nmol/mg prot/min of glucose. NA stimulates in a time- and concentration-dependent manner 2-DG uptake. This effect is inhibited by 90 % in the presence of 10 µM cytochalasin B, a specific glucose transporter inhibitor. The EC_{50} of NA is 0.5 µM with a maximal effect resulting in a 2-fold increase over basal level at 10 µM. A time lag of ≈ 5 min occurs between NA application and stimulation of 2-DG uptake. This contrasts with the rapid glycogenolytic action of NA which is half-maximal within 5 min and with the NA-evoked increase in glucose-6-phosphate intracellular level which raises from 2.2 ± 0.3 to 3.5 ± 0.3 nmol/mg prot within 2 min. In contrast, despite its glycogenolytic action, VIP does not influence 2-DG uptake. Application of arachidonic acid (AA) also stimulates 2-DG uptake in a cytochalasin B-sensitive manner, with an EC_{50} of ≈ 40 µM. These observations correlate well with the fact that NA but not VIP stimulates ^{3}H-AA release in the same preparation. These observations suggest that the release of AA may mediate the stimulation of 2-DG uptake evoked by NA in cultured astrocytes.

Sorg and Magistretti, Br. Res. 563 227-233, 1991.

332.8

VIP AND NORADRENALINE EXERT A LONG-TERM CONTROL ON GLYCOGEN LEVELS IN ASTROCYTES : BLOCKADE BY PROTEIN SYNTHESIS INHIBITION. O. Sorg, M. Stolz, S. Beggah and P.J. Magistretti*. Institut de Physiologie, Université de Lausanne, Switzerland.

VIP and noradrenaline (NA) have been previously shown to promote glycogenolysis in primary cultures of mouse cerebral cortical astrocytes[1]. We report now, in the same cultures, a second, temporally-delayed, action of VIP or NA : thus following glycogenolysis, an induction of glycogen resynthesis is observed, resulting, within 9 hours, in glycogen levels that are 6 to 10 times higher than those measured before the application of either neurotransmitter. VIP pulses as short as one minute are sufficient to double, 9 hours, later glycogen levels. The induction of glycogen resynthesis triggered by VIP or NA is dependent on protein synthesis, since both cycloheximide and actinomycin D abolish it entirely. The ability to elicit glycogenolysis is not sufficient *per se* to trigger the induction of glycogen resynthesis : thus two glycogenolytic agents such as methoxamine and phorbol 12,13-dibutyrate, both acting via protein kinase C activation, are unable to induce glycogen resynthesis. This observation, taken together with the fact that dibutyryl-cAMP application also results in enhanced glycogen resynthesis, strongly suggests that the long-term effect of VIP or NA is mediated by the cAMP second-messenger pathway.

Adenosine is also glycogenolytic[1]; however it only marginally induces glycogen resynthesis. We therefore further characterized the glycogenolytic action of adenosine : 2-chloroadenosine promotes glycogen breakdown with an EC_{50} of 3 µM. In addition, ATP and its non-hydrolysable analog ATP-γ-S also promote glycogenolysis, both with EC_{50}s in the low µM range, thus indicating a new functional role of ATP receptors in astrocytes.

[1] Sorg and Magistretti, Br. Res. 563 227-233, 1991.

332.9

PHARMACOLOGICAL PROPERTIES OF THE $GABA_A$ RECEPTOR OF ACUTELY ISOLATED HIPPOCAMPAL ASTROCYTES S. Duffy*[1], D.D. Fraser[1], D. Hoppe[2], H. Kettenmann[2], and B.A. MacVicar[1]. [1]Neuroscience Research Group, U. of Calgary, AB, Canada and [2]Dept. Neurobiol., U. Heidelberg, Heidelberg, Germany.

GABA-evoked responses were studied in astrocytes acutely isolated from rat hippocampus using either patch clamping or fluorometric measurements of $[Ca^{2+}]_i$. GABA (100 µM) evoked inward currents in virtually all cells held at -80 mV to -100 mV (n=50, range of 50-300 pA). The mean E_{rev} was -38 mV, corresponding to the E_{Cl}, indicating that GABA opened Cl^--selective channels. The threshold [GABA] was 1µM. Currents were maximal between 100 µM - 1 mM and desensitized rapidly at these concentrations. The Cl^- current was also activated by the $GABA_A$ agonist muscimol but not by the $GABA_B$ agonist baclofen (both 100 µM, n=5). The GABA-evoked currents were blocked by the $GABA_A$ antagonist bicuculline, and by the Cl channel blocker picrotoxin, but not by the $GABA_B$ antagonist 2-OH saclofen (all 100 µM, n=5). GABA-evoked current was potentiated by the barbiturate pentobarbital (100 µM, n=4), by diazepam (20 µM, n=3) and by the inverse agonist DMCM (20 µM, n=6/8). GABA would be expected to depolarize astrocytes because E_{Cl} is positive to E_m. To determine whether these depolarizations were sufficient to open voltage-dependent Ca channels, astrocytes were loaded with the Ca^{2+}-sensitive dye indo-1. In 30 % of the cells, GABA (1 mM) evoked a transient $[Ca^{2+}]_i$ increase which was blocked by picrotoxin (100 µM, n=2) and by the Ca channel blocker verapamil (100 µM, n=4). Verapamil had no effect on the GABA-evoked current (n=3), indicating that abolition of the $[Ca^{2+}]_i$ signal was due to block of voltage-gated Ca channels. These data indicate that astrocytes express $GABA_A$ receptors *in situ* that may play a significant role in interstitial Cl^- homeostasis and $[Ca^{2+}]_i$-dependent processes.

332.10

STEROIDOGENIC ACTIVITY OF RAT CEREBELLAR ASTROCYTES. R.J. Gasperini, C.R. Anderson*, T.G. Watson.
Dept. of Biological Sciences, Deakin University, Geelong, Vic. 3217, Australia

Primary cultures of astrocytes from neonatal (0-3 day old) rat cerebella have been used to investigate the presence and cellular localization of various steroidogenic enzymes. Monolayer cultures have been established with a simple technique utilizing a rapid trypsinization and trituration of excised rat cerebella. The cells obtained were cultured in Minimal Essential Medium (MEM) supplemented with 2 mM L-glutamine, penicillin/streptomycin and 5-10% fetal calf serum (FCS). After 2-3 weeks growth in 5%CO_2/95%air (or when confluency was reached) the cultures were shown to be significantly free (>95% astrocytes) of contaminating cell types (such as neurons and oligodendrocytes) as determined by glial fibrillary acidic protein (GFAP) and galactocerebroside-c (GAL-c) immunoreactivity and general microhistological criteria for astrocytes.

Incubation of these cultures in serum-free medium for 24 - 48hr. with the radiolabelled steroids [^{14}C]-Progesterone, [^{14}C]-Androstendione, [^{14}C]-Testosterone and [^{3}H]-Pregnenolone have yielded significant amounts of products which, upon purification and identification by TLC and recrystalization to specific activity, suggest an astrocytic localization of the enzymes 17ß-hydroxysteroid dehydrogenase isomerase (17ß-HSD), 3ß-hydroxysteroid dehydrogenase isomerase (3ß-HSD), 20ß-hydroxysteroid oxidoreductase (20ß-HOR), with specific activities significantly higher than those found in whole brain homogenates. The results indicate that primary monolayer cultures of astrocytes provide a well-defined tissue source for the demonstration of a possible *de novo* biosynthesis of steroid entities in the neonatal rat brain.

332.11

HIPPOCAMPAL DEAFFERENTATION INCREASES ^{3}H-THYMIDINE INCORPORATION BY ASTROCYTES *IN VITRO*. J.R. Day*, I. Rozovsky and C.E. Finch. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

Unilateral lesioning of the rat entorhinal cortex (ECL) induces astrocytic changes and reactive synaptogenesis in the adult hippocampus. Astrocyte reactivity, assayed by increased glial fibrillary acidic protein ensues rapidly after ECL. Reports indicate that astrocyte reactivity induced *in vivo* may persist *in vitro* (Lindsay et al., 1982). This study determined if astrocytes cultured at various times after ECL reflected changes paralleling to those *in vivo*. Astrocytes from cortex and hippocampus were cultured separately for 4 days, transfered to serum free medium (24 h), and labelled with 10 µCi ^{3}H-T/ml (24 h). The same number of viable cells was initially plated in all groups, yet at 4 and 14 days after lesion, the ipsilateral hippocampus and cortex contained more adherent cells than the non-lesioned side. The proportion of ^{3}H-T labelled cells from ipsilateral hippocampus was the same at both of these times (80%). The proportion of labelled astrocytes from the unlesioned hippocampus and both cortical hemispheres decreased from 80% (4d) to 65% (14d) *in vitro*. This equivalent incorporation by astrocytes cultured 4 and 14 days after lesion from the deafferented hippocampus may reflect the extended reactivity displayed by these cells *in vivo* in response to the ongoing synaptic reorganization induced by the lesion. Thus, hippocampal deafferentation induces an astrocytic response which persists up to 14d *in vitro*. These results suggest that gene expression is a target of this signal as shown by the enhanced mitogenic response *in vitro*. These data support the hypothesis that ongoing neurodegenerative processes can significantly alter astrocyte gene expression for extended periods of time. (Supported by: J.D. & C.T. MacArthur Program in Successful Aging; NIA 07909)

332.12

NEUROGLIAL RESPONSES TO THE DOPAMINERGIC NEUROTOXIN MPTP IN THE MOUSE STRIATUM. J.W. Francis*, J. Von Visger, G.J. Markelonis, and T.H. Oh. Dept. Anatomy, Univ. Maryland Sch. Med., Baltimore, MD 21201.

Treatment of C57/BL6 mice with MPTP results in the degeneration of nigrostriatal dopaminergic neurons followed by a reactive gliosis in the striatum. We examined both astroglial and microglial/macrophage responses to dopaminergic denervation of the striatum by MPTP. Following treatment, GFAP immunoreactivity increased, reached a peak at two days and persisted for at least six weeks in striatal astrocytes. Vimentin was also markedly increased in astrocytes 48 hr after treatment. There was no appreciable difference in immunoreactivity to laminin between normal and MPTP-treated striata. GFAP protein levels increased only 100% and 190% above normal levels 24 and 48 hr after MPTP treatment, respectively. By contrast, GFAP mRNA levels were increased 4-fold and 15-fold above the control 24 and 48 hr after treatment, respectively. Immunocytochemical studies failed to detect either Mac-1-positive or IL-1ß-positive microglia or macrophages in normal or MPTP-treated striatum. Both Western and Northern blot analyses failed to show measurable IL-1ß protein or IL-1ß mRNA in normal or MPTP-treated striata. Our results suggest that a factor(s) other than IL-1ß may initiate reactive astrogliosis in this neurotoxic model of glial scarring (Supported by NIH grant NS 15013).

332.13

INVOLVEMENT OF PROTEIN PHOSPHATASE IN ASTROCYTE VOLUME REGULATION. A.S. Bender* and M.D. Norenberg. Lab Neuropath Univ Miami Sch Med & Vet Admin Med Ctr Miami, FL 33101.

During exposure to hypoosmotic stress, astrocytes regulate their volume by activating the KCl cotransporter, inducing the efflux of K^+ and Cl^- with obliged water resulting in regulatory volume decrease (RVD). The activation of the KCl cotransporter depends on a phosphorylation-dephosphorylation cycle, mediated by kinases and phosphatases. During hypoosmotic stress there is a reduction in ^{32}P incorporation in 24 and 21 kDa proteins, and during RVD ^{32}P incorporation in these two proteins returns to normal levels (Neary et al., 1990; Soc. Neurosci. Abstr. 17, 1147). Okadaic acid (OA), a highly specific inhibitor of protein phosphatase 1 (PP1) and 2A (PP2A), was used to examine the role of phosphatases in activating the KCl cotransporter and in volume regulation. Following hypoosmotic stress, OA (100 nM) inhibited K^+ release, increased the amount of K^+ accumulation and inhibited RVD. Since these events were not inhibited at 1 nM (when PP2A is completely inhibited) and only partialy at 100 nM, it suggests that PP1 (which is completely inhibited at 1 μM) is inhibited by OA and may be involved in the activation of KCl cotransporter. Our results indicate that activation of the KCl cotransporter during hypoosmotic stress is associated with a net dephosphorylation which may be responsible for RVD. (Supported by USPHS grants NS-30291 and AM-38153 and the Department of Veterans Affairs).

332.14

myo-INOSITOL LEVELS DURING CELL VOLUME REGULATION IN ASTROCYTES. R.E. Isaacks, A.S. Bender, J.H. Bruce*, and M.D. Norenberg. VA Med. Center and Univ. Miami Sch. Med., Miami, FL 33125.

Our previous studies on *myo*-inositol (MI) uptake in astrocytes under varying media osmolalities suggest that MI may function importantly in volume regulation. The present studies consider the levels of MI in astrocytes exposed to hypo- and hypertonic conditions to determine whether MI may be serving as an osmolyte involving the preservation of astrocyte volume. Primary astrocyte cultures from neonatal rats were incubated in either hypotonic (180 mosm/kg H_2O), isotonic (310 mosm/kg H_2O), or hypertonic (440 mosm/kg H_2O) modified DMEM media containing 40 μM MI. MI levels were measured on extracts of astrocyte cultures by converting to its per(trimethylsilyl) derivative and analyzing by GLC using a glass column packed with 3% OV-17 on 80/100 Chromosorb W(HP) at 190°C column temperature with nitrogen carrier gas flow rate of 90 ml/min. In isotonic media, the level of MI was 30.5 $\pm$ 4.9 μg/mg protein and that level was maintained for 48 hours. When astrocytes were exposed to hypotonic media, the levels of MI were undetectable within 6 hours and remained at that level for 48 hours. Astrocytes in hypertonic media had increased in MI level by 34% within 6 hours which continued to increase to 3-fold by 24 hours. Our results indicate that MI functions as an organic osmolyte in volume regulation of astrocytes.

332.15

HETEROGENEITY IN BRAIN ASTROCYTES AND CULTURED ASTROCYTES REVEALED BY MONOCLONAL ANTIBODIES. Elisabeth Welter, Christine J. Skordeles and Dennis M.D. Landis*. Departments of Neurology and Neurosciences, Case Western Reserve University, Cleveland, OH

We have generated monoclonal antibodies which bind to epitopes present in astrocytes by using a plasmalemmal fraction from cultured rat astrocytes as an immunogen. Many of these antibodies bind to subpopulations in situ or in vitro.

The mAb 4C6 is an IgM which binds to the surface of rare flat cells in secondary cultures of astrocytes; most of these cells do not have GFAP immunoreactivity. In low density cultures, 4C6 may stain a few of a cluster of cells, all of which are GFAP+; this pattern would suggest that clonally related cells are heterogeneous in this aspect of their phenotype. Similar flat cells are stained in cultures enriched for oligodendroglia, but 4C6 does not co-localize with galacto-cerebroside immunoreactivity. The mAb does stain microglia from P2 rat brain and endothelial cells derived from adult brain or aorta.

The mAb 6E9 binds to an intracellular epitope whose distribution resembles that of GFAP. The mAb stains most astrocytes in cerebral cortex, but in the cerebellum it stains only the astrocytes in the granular layer, and not Bergmann glia or astrocytes in the white matter. The mAb 5E6 binds in a complementary pattern in the cerebellum; it stains an intracellular epitope which resembles GFAP in Bergmann processes and in astrocytes in the white matter, but not in the astrocytes of the granular layer.

332.16

MONOCLONAL ANTIBODIES WHICH BIND TO REACTIVE ASTROCYTES. Stephen D. Collins*, Michael B. Bolesta, and Dennis M.D. Landis. Departments of Neurology and Neurosciences, Case Western Reserve University, Cleveland, OH 44106

We have generated monoclonal antibodies (mAb) which bind to epitopes whose expression is changed in the context of the astrocytic reaction to injury.

Millipore filters were inserted into the cerebral cortex of normal adult rats. Ten days later, the filters and adherent cells were removed and used as an immunogen in the generation of mAbs. An epitope bound by the mAb 13A11 is barely detectable in normal brain astrocytes and cultured astrocytes, but its expression is clearly increased after forebrain is injured by a stab wound or implantation of a filter. The mAb 01E4 binds to an intracellular epitope which is distributed very much like GFAP in astrocytes, but is also present in microglia and oligodendroglia; it too is increased after injury. A previously described mAb, 8C10, binds to the surface of cultured astrocytes and oligodendroglia, and the expression of its corresponding epitope is dramatically increased after injury.

Some of the astrocytic changes occurring during the reaction of the brain to injury may involve interactions with microglia. We have found that astrocytes in organotypic cultures of rat hippocampus acquire structural characteristics which resemble those of reactive astrocytes, and may be further modified after stab wounds in the slice. By obtaining slices of hippocampus at various postnatal ages, and by adding cultured microglia, we may be able to determine whether the presence of microglia alters astrocyte differentiation in the slices as it is reflected in mAb binding.

332.17

IN VITRO NUCLEOCYTOPLASMIC LOCALIZATION OF J1-31 ANTIGEN. M.V. Singh, K. Price, R. Bhatnagar, and S.K. Malhotra*, Department of Zoology, University of Alberta, Edmonton, Canada T6G 2E9

A monoclonal antibody, Mab J1-31, was shown to recognize a cytoplasmic, astrocyte specific antigen (J1-31 antigen) in sections of CNS tissue fixed in paraformaldehyde. This antigen is distinct from GFAP and vimentin and is a more intense marker than GFAP for proximal reactive astrocytes located in the immediate vicinity of a destructive CNS lesion (Predy et al., 1988, J. Neurosci. Res. **19**, 397-404; Malhotra et al., 1992, Brain Res. Bull. in press). The reactive antigen was shown to be a 30 kD protein by immunoprecipitation of autopsy samples from a multiple sclerosis patient (Singh et al., 1986, Biosci. Rep. **6**, 73-79). Further studies on cultured astrocytes from neonatal rat brain, glioma cell lines of rat and human origin, cultured human fibroblasts, and biopsies of primary human astrocytomas show that immunostaining with Mab J1-31 is localized in the nucleus as well as in the cytoplasm following methanol fixation. No nuclear staining is detected in sections of normal adult rat CNS or non-CNS tissues. Western blot analyses of C6 and 9L rat glioma cells and human fibroblasts reveal two protein bands at 64 kD and 77 kD in cell homogenates. Nuclei isolated from the glioma cells show the same two bands. Nuclear localization of J1-31 antigen appears to be correlated with cell growth under tissue culture conditions, neoplastic transformation or the stage of cell growth *in vitro*.

Supported by NSERC.

333.1

SYNAPSIN I mRNA IN THE RAT CNS BY IN SITU HYBRIDIZATION. R.H. Melloni, Jr.*, L. M. Hemmendinger, J. E. Hamos, and L. J. DeGennaro. University of Massachusetts Medical Center, Worcester, MA 01655.

Synapsin I is the best characterized member of a family of neuron-specific phosphoproteins thought to be involved in the regulation of neurotransmitter release. Here, we present the first extensive *in situ* hybridization study detailing the regional and cellular distribution of synapsin I mRNA in the developing and adult rat central nervous system. Both the regional distribution and relative levels of synapsin I mRNA established by *in situ* hybridization were confirmed by RNA blot analysis. Our data demonstrates the widespread yet regionally variable expression of synapsin I mRNA throughout the developing and adult rat CNS. In the adult rat brain, the greatest abundance of synapsin I mRNA was found in the large pyramidal neurons of the CA3 and CA4 fields of the hippocampus, and in the mitral and internal granular cell layers of the olfactory bulb. Other areas abundant in synapsin I mRNA were the layer II neurons of the piriform cortex and layer II and V neurons of the entorhinal cortex, the granule cell neurons of the dentate gyrus, the pyramidal neurons of hippocampal fields CA1 and CA2, and the cells of the parasubiculum. In general terms, the patterns of expression of synapsin I mRNAs paralleled those encoding other synaptic terminal-specific proteins, such as synaptophysin, VAMP-2, and SNAP-25. In addition, to determine specifically how synapsin I mRNA levels are related to levels of synapsin I protein, we examined the local distribution patterns of both synapsin I mRNA and protein in the adult rat hippocampus. These data revealed differential levels of synapsin I mRNA and protein within and between defined synaptic circuits of the rat hippocampus.

333.2

THE COMPLETE GENE ENCODING HUMAN B-50/GAP-43

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Previously, we reported the structure of the human B-50/GAP-43 gene - contained in 3 exons - based on the reported human cDNA and on isolated genomic clones for exons 2 and 3, and PCR-derived genomic clones for exon 1 (Schrama et al. 1990, *Soc. Neurosci. Abstr.* 16:154.11; Nielander et al. 1991, *idem* 17:457.14).

We now have isolated the 5' region of the human B-50/GAP-43 gene. Using a PCR-clone as probe, we have screened a Lambda EMBL3 genomic library and have found 10 genomic clones. Thus far, one clone has been analyzed in detail. Restriction analysis showed that it consists of 3 kb sequence upstream of the open reading frame (ORF) and approximately 10 kb of intron 1. There was no overlap with previously isolated genomic clones containing 13 kb intron 1 and the complete exon 2. Comparison of human and rat sequence 5' of the ORF revealed homology of the untranslated region until approximately -1 kb; however, long GT (Z-DNA) and AG repeats (H-DNA) as seen in the rat gene were not present. Computer analysis of the human sequence for potential binding sites of vertebrate-encoded transcription factors (*NAR* 1992;1:3-26) did not reveal any neuron-specific DNA elements. Three TATA box binding protein sequence motifs were present, but none was close to main transcription initiation sites as determined by Northern blotting.

We conclude from these results, that the overall gene structure of human B-50/GAP-43 is similar to that of the rat gene.

333.3

LOCALIZATION OF RNA AT SYNAPSES: IDENTIFICATION OF A SYNAPTOSOMAL GAP-43 RNA-BINDING PROTEIN. *M.E. Chicurel, C. DeFranco, @D.M. Terrian, and H. Potter. Program in Neuroscience, Dept. Neurobiology, Harvard Medical School, Boston, MA; @Dept. Anatomy and Cell Biology, East Carolina School of Medicine, Greenville, NC 27858-4354.

One mechanism that may contribute to both the generation and maintenance of neuronal polarity, and which also provides a potential for synaptic plasticity, is the subcellular compartmentalization of mRNA together with factors involved in translation. This proposal of localized translation is supported by the observation that a specific RNA population is associated with the dendritic component of a vertebrate CNS synapse: the hippocampal mossy fiber (MF)-CA3 synapse. An abundant member of this population is GAP-43 mRNA. We have identified a protein found in a preparation of MF-CA3 synaptosomes that specifically binds the GAP-43 mRNA. The binding site was localized to the 3' untranslated region (UTR) by mobility-shift assay. A pyrimidine-rich tract in the GAP-43 3' UTR is apparently required for protein binding activity. Our preliminary data suggests that this protein may affect translation of GAP-43 mRNA. M.E.C. is a Howard Hughes fellow.

333.4

ANALYSIS OF B-50/GAP-43 TRANSCRIPTION STARTS BY NORTHERN BLOTTING AND GENETIC VARIATION IN H-DNA LENGTH OF THE RAT B-50 (GAP-43) GENE.

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The neural-specific phosphoprotein B-50 (GAP-43) gene is encoded by a single copy gene. The gene includes 3 exons (1,2). In order to gain more insight in the regulatory elements of the B-50 promoter, we have been determining the B-50 transcription starts. Determination of these by conventional techniques such as primer extension, S1 nuclease mapping and RNase protection proved to be very difficult, probably due to the high degree of secondary structure (1-3). Two other techniques were more succesfull: primer extension combined with PCR (PE-PCR) and Northern blotting. With PE-PCR the longest transcripts in 8 day old brain poly(A)$^+$ mRNA are found to extend to -465 upstream of the ORF. Similar data were obtained by others (2). In order to determine the number of B-50 mRNAs with a long 5' UTR, Northern blotting was used. Only a minor portion of the B-50 mRNAs have a long 5' UTR, the majority has a short 5' UTR.

Comparison of the primary structure of the B-50 gene upstream of the ORF with previously published sequences (1,2) showed that the length of H-DNA varied. The H-DNA containing region of the B-50 gene was amplified by PCR on isolated Wistar DNA or commercial Sprague-Dawley DNA (Clontech). The resulting PCR products were subcloned in the TA vector and sequenced with the T7 sequencing method (Pharmacia) and an anti-sense primer very close to the H-DNA. The length of the H-DNA varied between $(AG)_{35}$ and $(AG)_{40}$ in the Wistar DNA and between $(AG)_{36}$ and $(AG)_{42}$ in Sprague-Dawley, whereas analysis of exon 1 genomic clones revealed $(AG)_{25}$ (1), $(AG)_{35}$ (2) and $(AG)_{42}$ (3). The influence of the H-DNA length on the B-50 promoter activity in various stages of EC cell differentiation is currently investigated (Eggen et al., this meeting).

1) Grabczyk et al., *Eur. J. Neurosci.* 2: 822, 1990; 2) Nedivi et al., *J. Neurosci.* 12, 691: 1992; 3) Schrama et al., *Abst. Soc. Neurosci.* 17, 101.4: 1991.

333.5

MOLECULAR CLONIG AND SEQUENCE ANALYSIS OF HUMAN VGF cDNA.

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We have analyzed the sequence of human VGF cDNA. VGF is a protein secreted through the regulated pathway, whose mRNA is rapidly and selectively regulated by NGF in PC12 cell line. VGF expression is confined to neuronal and endocrine tissue (G.Ferri Mol.Brain Res. 13. 1992. 139-143). A cDNA library has been constructed by using mRNA isolated from a human neuroblastoma cell line (N- SK-BE) that expresses VGF mRNA (R. Possenti in press). Analysis of the 3' coding region nucleotide sequence shows a high degree of similarity (90%) with the rat gene. Comparision of the aminoacid sequence predicted by the human VGF cDNA clone and rat VGF sequence reveals 98% of similarity characterized by some conservative point mutations and analogous aminoacid sobstitutions. The strong homology in the 3' region has also been confirmed by the crossreactivity of human protein with polyclonal antibodies raised against the COOH portion of rat protein. Data regarding characterization of monoclonal antibodies produced against human protein will be presented.

Grants: CNR. PF. Invecchiamento. Sigma tau.

333.6

EXPRESSION CLONING OF NOVEL BRAIN-SPECIFIC cDNAs WITH M6 MONCLONAL ANTIBODY.

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M6 is a monoclonal antibody obtained by immunizing a rat with a partially purified membrane fraction of a mouse brain homogenate. The corresponding antigen(s) is a 35 kDa glycoprotein distributed througout the CNS, found primarily on the surface of neuronal cell bodies and their non-myelinated processes, and the choroid plexus and proximal renal tubules. Addition of M6 antibody or its Fab fragments to cultured cerebellar neurons interferes with neurite outgrowth (Lagenaur, et al, 1992). We report here the cloning of mouse brain cDNAs with an M6 antibody probe.

A λgt11 expression library representing adult mouse brain was screened with an M6 antibody recognizing denatured M6 antigen. Two positive clones, 321 and 511 (containing 130 bp and 100 bp inserts respectively), were sequenced, and used as probes for Northern blots. 321 and 511 hybridize to an approx. 4 kb mRNA in brain and kidney, not found in liver or heart. When the library was re-screened with 321, a larger cDNA, 3211, containing a 710 bp insert, was obtained. 3211 contains a segment highly homologous to a trans-membrane domain of proteolipid protein (PLP) exon 3.

333.7

CLONING OF A CDNA ENCODING RAT BRAIN GABA TRANSAMINASE. L.K. Medina-Kauwe*[1], N.J.K. Tillakaratne[2], J.-Y. Wu[4], & A.J. Tobin[1,2,3] [1]Molecular Biology Institute, [2]Department of Biology, [3]Brain Research Institute, University of California Los Angeles, Los Angeles, CA, 90024, [4]Department of Physiology and Cell Biology, University of Kansas, Lawrence, KS, 66045

GABA transaminase (GABA-T) is the catabolic enzyme of the inhibitory neurotransmitter, GABA. We have isolated several cDNA clones encoding rat brain GABA-T by two independent cloning methods. A λZAP rat hippocampal cDNA expression library was screened using an anti-mouse GABA-T antibody (Saito et al., Brain Research 65,287,1974) and a PCR-derived bovine GABA-T cDNA probe (Kwon et al., J Biol Chem 267,7215, 1992). Two of the cDNAs isolated by immunological screening with the anti-GABA-T antibody show high DNA and amino acid sequence identity to porcine heart citrate synthase (Evans et al, Biochemistry 27,4680,1988). However, most of the cDNAs isolated by both methods show a high DNA sequence identity to that reported by Kwon et al. for a bovine GABA-T cDNA. The derived amino acid sequence predicts a putative leader peptide similar to the signal sequences of several proteins targetted to mitochondria. Our cDNAs hybridize strongly to a 6.4 kb RNA, and weakly to a 2.1 kb RNA. We are now in the process of determining whether the protein produced in a bacterial expression system from our cDNAs have GABA-T enzyme activity.

333.8

CLONING OF TWO RELATED ORPHAN RECEPTORS WHICH MAY BE INVOLVED IN DEVELOPMENT. A.J. MacLennan*, D.C. Lado, C.S. Browe, B.R. Davies, and G.P. Shaw. Dept. of Neuroscience, Univ. of Florida, Gainesville, FL 32610.

We have used medium stringency library screening techniques and RACE PCR methods to isolate two rat cDNAs (H218 and rat-edg) that encode putative G-protein coupled receptors (p^{H218} and $p^{rat\text{-}edg}$) which, based on their similarity are likely to be responsive to the same unidentified endogenous ligand(s). Rat-edg is the rat homolog of "edg-1", a gene which has been implicated in endothelial cell differentiation (Hla and Maciag, 1990:JBC, Vol. 265, p. 9308). Our Northern analyses indicate that H218 and rat-edg mRNAs are expressed in brain, several peripheral organs, and PC12, C6, and NIH 3T3 cells. All these tissues except PC12 cells contain a 3.2 kb rat-edg transcript. A 4.9 kb rat-edg transcript was detected only in brain and PC12 cells. In brain, rat-edg mRNA was detected in all regions examined and is most abundant during later stages of postnatal development whereas H218 mRNA is most abundant during embryonic brain development. H218 mRNA is also rapidly increased in RJK88 hamster fibroblasts by the tumor promoter, PMA. In addition, the presumed cytoplasmic C-terminal domain of p^{H218} contains a sequence similar to motif I of the SH2 domain present in phospholipase C type II. Finally, based on overall homology, p^{H218} may be distantly related to TRK.

Supported by NIH grant DA07244 to A.J.M.

333.9

MOLECULAR CLONING AND ANALYSIS OF TWO CEREBELLIN-RELATED GENES CBL-1 AND CBL-2 THAT ARE DIFFERENTIALLY EXPRESSED IN THE RODENT NERVOUS SYSTEM. B. Kavety, J. Hempstead and J.I. Morgan*, Department of Neuroscience, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

To elucidate the molecular mechanisms that govern cell-specific gene expression in the developing nervous system, we have cloned a series of genes whose expression is restricted to cerebellum. One such gene termed cbl-1 encodes a protein, precerebellin, that contains the sequence of cerebellin, a hexadecapeptide previously identified to be highly enriched in cerebellum. The expression of cbl-1 is greatly reduced in certain cerebellar mutant mice that were previously shown to contain reduced levels of cerebellin peptide. Analysis of cbl-1 gene structure reveals the presence of two introns. Furthermore, we have detected a novel type of splicing in the 3' untranslated region of the RNA. Southern analysis suggested the presence of other genes related to cbl-1. A cDNA, termed cbl-2, has been cloned using a PCR strategy from whole brain mRNA. Although this cDNA encodes a highly related polypeptide, that includes a cerebellin-like motif, it appears to be more ubiquitously expressed in the CNS than cbl-1. We are now examining the regulatory sequences of the two genes to determine the element(s) that could account for their differential expression using transgenic mouse technology.

333.10

CHARACTERIZATION OF cDNAs ISOLATED FROM AN IMMORTALIZED MOTOR NEURON HYBRID CELL LINE BY SUBTRACTIVE SCREENING. S.H. Pasternak,* N.R. Cashman, K.E.M. Hastings. Dept of Neurology and Neurosurgery, McGill University, Montreal PQ H3A 2B4

We have developed N18TG2 neuroblastoma X dissociated mouse spinal cord (NSC) hybrid cell lines for the study of the molecular biology of the motor neuron. One of these hybrid cell lines, NSC34-4-6, expresses many motor neuron-like characteristics not found in the parental neuroblastoma, including the ability to extend processes and make stable contact with myotubes in culture, and the expression of choline acetyltransferase, neurofilaments and an S-laminin receptor (J. Neurosci., 1991). A cDNA library of this NSC34 cell line was examined by differential and subtractive screening in order to isolate genes expressed in the hybrid cells but not in the N18 neuroblastoma parent (Neurosci. Abs. '89). Two cDNAs were isolated which correspond to known neural genes: GAP-43 (important in axonal growth cone extension) and chromogranin B (a component of neurosecretory vessicles). Three additional clones were isolated whose sequences are not present in GenBank (release 71). One of these sequences identifies a transcript of 2900 bases on a northern blot which is several fold more abundant in mouse spinal cord than brain or liver, but also expressed at high levels in other tissues (kidney, spleen and lung). A second novel sequence is detectable by PCR at a higher level in spinal cord than brain or liver, but is also present in other mouse tissues. The final cDNA is detectable by PCR primarily in brain and spinal cord. While this expression data suggests that these cloned cDNAs are not motor neuron specific, they may still be expressed in motor neurons. We are currently isolating full length cDNAs of these clones and mapping their cellular expression by *in situ* hybridization.

333.11

NUCLEOTIDE SEQUENCE OF RAT CALRETININ cDNA. **K.I. STRAUSS and D.M. JACOBOWITZ*, Lab. of Clin. Sci., NIMH, Bethesda, MD 20892.**

Calretinin (CR) is a 29 kDa, neuron specific calcium binding protein (CaBP). Previous studies have shown a unique localization of CR throughout the CNS in mammals, birds, reptiles, amphibia, and fish. In order to explore the role of CR in the brain and its regulation at the molecular level, we have cloned and analyzed the rat cDNA coding for CR. An immunoreactive clone was isolated from a rat brain cDNA expression library in lambda gt11. The insert was subcloned into the Eco RI site of the pGEM-4Z transcription vector for further analysis. The identity of the 1.45 kb insert was confirmed by comparison with human CR. The rat cDNA sequence comprised a 54 bp 5' untranslated region, an 816 bp open reading frame (ORF) with start and stop codons, and a 579 bp 3' untranslated region. A polyadenylation signal and 13 adenylate residues were found near the 3' end. The nucleotide and amino acid sequences in the proposed coding region were remarkably homologous to human CR (94.3% and 99.6%, respectively), and chick CR (79% and 87% respectively). An 83% homology was found between human and rat (but not chick) sequences within the 36 bp upstream of the initiation codon, with no other homology in the 3' and 5' untranslated regions. The ORF has 271 codons coding for a putative protein of 31.4 kDa. Only one amino acid differed between rat and human CR. This was a conservative conversion from Ile-235 in human to Met-235 in rat (and chick) CR. Further sequence analysis revealed that, among human, rat, and chick CR coding regions, there was no single position with a different nucleotide in all three species. At the 297 positions where two of the species had identical nucleotides but the third differed, the chick was different 76% of the time, the rat 19% of the time, and the human 5% of the time, supporting the accepted evolutionary distance between birds, lower mammals, and primates. The abundance of CR in the CNS and its evolutionary conservation suggest an important role for this CaBP. The rat CR clone will allow more detailed analyses of CR localization, regulation and function in a variety of experimental manipulations.

333.12

CLONING OF NOVEL CYSTEINE PROTEASES FROM RAT BRAIN USING DEGENERATE OLIGONUCLEOTIDES TO CONSERVED SEQUENCES. Suzana Petanceska and Lakshmi Devi* Department of Pharmacology, New York University Medical Center, NY 10016.

Cysteine proteases are involved in many diverse cellular processes ranging from processing of precursor proteins to intracellular degradation. Since cysteine proteases play a vital role in normal cellular protein metabolism, it is understandable that cysteine proteases have been implicated in a number of disease states including cancer, arthritis, and Alzheimer's disease. In an effort to identify novel cysteine proteases, polymerase chain reaction and primers directed against the catalytic sites of previously cloned cysteine proteases were used. From rat brain mRNA, a 600 basepair band was amplified; cloning and partial sequence analysis of this band resulted in the identification of cathepsins B and L, and five novel sequences. The novel cDNAs contained a number of residues conserved in lysosomal cysteine proteases, including the active site residue His^{159} (papain numbering). In addition, the amino acid homology between the novel sequences and either cathepsins B, L, or H ranged from 63% for PCR27 to 32% for PCR31. Northern blot analysis relvealed that the mRNA for these novel putative cysteine proteases is differentially expressed in brain, lung, liver, kidney, spleen, ileum, and heart. One of the brain-specific putative cysteine proteases is expressed at such low levels that it could be detected only by PCR amplification of the cDNA generated from the brain mRNA. The tissue-specific expression implies highly specific functions for the five novel proteins. This strategy of cloning proteases using PCR amplification of the conserved regions will be useful in the identification of the proteases involved in growth, development and disease states; this was previously limited by the abundance of the mRNA and/or of the tissues.

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333.13

MOLECULAR CLONING AND ANALYSIS OF βHLH-ENCODING cDNA CLONES EXPRESSED IN EMBRYONIC RAT BRAIN. D.I. Lugo*, R. Molinar-Rode, A. Lievano, F. Franco del Amo, and J.I. Morgan. Depts. of Neurosciences and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

One of the goals of developmental neurobiology is to investigate the molecular mechanisms that direct the ontogeny of the nervous system. A strategy for this study is to search for developmental control genes expressed in the early nervous system. An important class of such molecules includes proteins with common DNA-binding and dimerization domains, the basic helix-loop-helix (bHLH) family. Some members of this family are MyoD, involved in myogenic determination and products of the achaete-scute complex of Drosophila which are involved in neural determination. In order to try to identify additional members of this class of molecules that might be implicated in mammalian neurodevelopment we have constructed an embryonic day 10 rat brain library and have begun screening with a 254bp probe spanning the bHLH domain of the recently identified mammalian achaete-scute homologue, MASH-1. This probe was generated by synthesizing specific oligonucleotides and amplifying the bHLH region of MASH-1 from NGF-treated PC12 total RNA by reverse transcriptase coupled to the polymerase chain reaction. Various clones have been isolated using low stringency conditions and preliminary characterization has begun. Sequencing, in-situ hybridization to rat embryonic sections, gel shift assays as well as transfection studies will provide insights as to their potential role during neurodevelopment.

333.14

CLONING AND EXPRESSION OF A RAT GLYCINE TRANSPORTER. B. Borowsky* and B.J. Hoffman. Laboratory of Cell Biology, NIMH, Bethesda, MD 20892.

We have isolated a cDNA clone for a novel Na^+-dependent transporter by screening a rat basophilic leukemia cell (RBL) cDNA library with a degenerate oligonucleotide corresponding to a region conserved between the GABA and NE transporters. Transient expression of this cDNA in CV1 cells confers saturable, Na^+-dependent uptake of glycine (Km= 50-70 uM). This glycine transporter shows significant sequence similarity to other members of the Na^+-dependent transporter family, i.e. GABA, NE, DA, 5HT and betaine. In situ hybridization histochemistry demonstrates the presence of this glycine transporter mRNA in both CNS and peripheral tissues. Glycine is an inhibitory neurotransmitter found predominantly in the lower brainstem and spinal cord. The presence of this transporter in peripheral tissues as well as mast cells suggests that glycine may have important functional roles outside the CNS. Pharmacologic characterization and anatomic distribution of the glycine transporter will be discussed.

333.15

ISOLATION OF cDNAS ENCODING NOVEL MEMBERS OF THE THYROID HORMONE/RETINOIC ACID RECEPTOR SUPERFAMILY FROM RAT HIPPOCAMPUS. Sandra Peña de Ortiz* and Gordon A. Jamieson Jr. Division of Toxicology, Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati Ohio 45267

Steroid hormones, thyroxines, and retinoids regulate gene expression in the hippocampus by activating members of the steroid/thyroid hormone receptor superfamily. Members of this gene superfamily function as nuclear receptors that regulate transcription in a ligand-dependent fashion. We hypothesized that additional ligand molecules exist, which function in the hippocampus by regulating still unidentified nuclear receptors. Hence, we initiated studies to isolate novel members of this gene superfamily from rat hippocampus. To achieve this goal we designed degenerate oligonucleotide primers targeted to the highly conserved zinc finger region of these receptors for use in the Polymerase Chain Reaction (PCR). Rat hippocampal cDNA was prepared and subjected to PCR using the above mentioned primers. We amplified and subsequently cloned the zinc finger regions of four members of the steroid/thyroid hormone receptor superfamily, including the rat glucocorticoid and thyroid hormone receptors. Two of our clones, hippocampal zinc fingers (HZF) 2 and 3, encode novel zinc finger motifs of putative members of the thyroid hormone/retinoic acid receptor superfamily. These novel cDNA clones are 58% to 76% homologous to known nuclear receptors. Analysis of the deduced amino acid sequence for both these clones revealed differences in residues that are conserved among all other known members of the superfamily. Current efforts are focused on using northern blot analysis to determine the tissue specificity of HZF2 and HZF3 expression, *in situ* hybridization to define the distribution of transcripts for these molecules in the brain, and 3'-anchored PCR to isolate the ligand binding region of these receptors so that ligand identification studies can be initiated. *Supported by ONR N00014-90-J-1898 and a NIMH Minority Fellowship.*

333.16

REGULATION OF C-FOS EXPRESSION BY VIP IN PRIMARY CULTURES OF CEREBRAL CORTICAL ASTROCYTES AND NEURONS. L.-L. Martin*[1], S. de Rham[1], C. Rossier[1], T. Bardoscia[1], N. Stella[1,2] and P.J. Magistretti[1]. Institut de Physiologie, Université de Lausanne, Lausanne, Switzerland[1] and INSERM U114, Collège de France, Paris, France[2].

The expression of c-fos was examined in primary cultures of astrocytes and neurons by Northern blot analysis. Cultures were prepared from mouse cerebral cortex; neonates (1-2 days) and 16-17 days embryos were used for astrocytes and neurons respectively. In astrocytes, VIP induced c-fos expression in a concentration-dependent manner, with a significant effect already observed at 0.1 nM; maximal induction occurred at 1 µM. The stimulation of c-fos expression was time-dependent, being detectable at 30 min and maximal between 1 to 3 hours. C-fos mRNA levels returned to control values within 9 hours. Similar effects were observed when VIP was applied as a 10-minute pulse. In contrast the expression of other immediate-early genes such as c-myc, c-jun, c-src, c-Ha-ras and c-mos was not affected by VIP. In view of the induction by VIP of a transcription factor such as c-fos, we have engaged, using a subtractive hybridization approach, in the isolation of cDNA clones whose expression might be enhanced following VIP exposure. To this end we have prepared control and VIP-stimulated astrocyte cDNA libraries with the following characteristics : primary plaque number of $\approx 2 \times 10^6$ (≈ 1% nonrecombinants) and average insert size > 2 kb. The expression of c-fos was also examined in primary neuronal cultures. In this cell type also, VIP 1 µM increased the amount of c-fos transcripts. In addition, glutamate agonists acting at ionotropic, e.g. AMPA and NMDA, or metabotropic receptors, e.g. trans-ACPD, stimulate c-fos expression. However GABA and the cholinergic agonist carbachol were without effect. Supported by FNRS grants 31-32338.91 to JLM and 31-26427.89 to PJM.

333.17

IDENTIFICATION OF IMMEDIATE-EARLY GENES ENCODING NON-NUCLEAR SIGNALING MOLECULES IN THE MOUSE CENTRAL NERVOUS SYSTEM. B. S. Hilbush*, R. Molinar-Rode, M. D. Hayward, and J. I. Morgan. Dept. of Neurosciences, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

Polypeptide growth factors, neurotransmitters, and other ligands elicit a rapid and transient induction of several cellular immediate-early (cIE) genes whose products are thought to mediate long-term phenotypic changes and altered neuronal behavior in target neurons. The class of cIE genes is defined by the property of superinduction in the presence of protein synthesis inhibitors. In general, the cIE class encodes nuclear proteins such as the transcription factors c-fos and c-jun. Since the response to environmental signals may necessitate rapid changes in other components of cellular signaling machinery, the repertoire of inducible genes may not be limited to nuclear proteins. Thus, we have investigated whether the immediate-early response in the nervous system includes inducible genes involved in signal transduction processes. As an initial screen, we utilized reverse transcription coupled to the polymerase chain reaction (RT-PCR) to detect brain transcripts superinduced during cycloheximide treatment. Total RNA was prepared from brains of adult mice treated with or without cycloheximide for 4 hr and 1-2 ug of RNA was used for RT-PCR with PCR primer pairs designed to amplify genes encoding a broad spectrum of signaling molecules. PCR products were identified which were either significantly increased or newly present in response to cycloheximide. The identified products were subsequently subcloned and sequenced. In addition, PCR products were used as cDNA probes for Northern blot analysis to confirm superinduction of specific mRNA by seizure-inducing agents or cycloheximide. Using these approaches, we have identified inducible genes among the superfamilies encoding ligand-gated ion channels and G proteins and synapse-associated proteins. These results suggest that components of signaling processes may constitute part of the immediate-early response in neurons.

333.18

NOVEL ras/rap-RELATED GENE EXPRESSED IN BRAIN AND REGULATED BY SYNAPTIC ACTIVITY. +K. Yamagata and P.F. Worley*. Department of Neuroscience and +Howard Hughes Medical Institute, Johns Hopkins University Sch. of Med., Baltimore, MD 21205.

We are examining the genomic response of neurons to synaptic activity as a means to understand the molecular basis of plasticity. Differential screening of cDNA libraries has been used to identify a previously uncharacterized gene (rasp) that is expressed at high levels in normal adult cortex of the rat and is rapidly inducible in the hippocampus by electroconvulsive seizures. The rasp gene is also inducible in PC12 cells by growth factors (NGF, FGF, EGF) and is expressed in several peripheral tissues. Full length Rasp cDNA (1.1 Kb) encodes a 183 aa protein with 43.3% and 37.7% aa identity with yeast H-Ras 1 and human Rap 2, respectively. Regions of homology include four putative GTP-binding regions and the Ras effector region. A full length bacterial GST-Rasp fusion protein binds GTP. Additionally, Gln 64 of Rasp is homologous to Gln 61 of Ras, a residue that is important for Ras activity and is absent in Rap family members. Our studies identify a novel Ras-like GTP-binding protein that may play a role in the cellular response to neuronal activity.

333.19

TRANSCRIPTIONAL REGULATION OF THE HUMAN NPY GENE DURING DIFFERENTIATION OF SH-SY5Y NEUROBLASTOMA CELLS

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We have examined the transcriptional regulation of the human neuropeptide Y (NPY) gene during differentiation of human SH-SY5Y neuroblastoma cells. Cells were differentiated with the phorbolester 12-O-tetradecanoylphorbol-13-acetate (TPA), in the presence of fetal calf serum and NPY mRNA expression profiles were determined. A sustained strong induction of NPY mRNA expression was detected. The mRNA levels of c-jun and c-fos, that belong to the AP-1 family of transcription factors, were also examined. Both c-jun and c-fos mRNAs were induced with a synchronous and biphasic pattern. An early transient (30-120 min) was followed by a later (>8 hrs) increase in mRNA expression. The induced NPY mRNA expression coincided with the second peak of c-jun and c-fos expression. Nuclear protein extracts were prepared from uninduced and induced SH-SY5Y cells and tested for the ability to bind to an AP-1 DNA element, present in the NPY promoter. Induced AP-1 binding activity was detected following TPA treatment. In contrast, no effects of Sp1 mRNA expression or Sp1 DNA binding activity were detected. Thus it seems clear, that although several Sp1 sites are present in the NPY promoter, the induction of NPY expression in SH-SY5Y cells by TPA does not involve changes in Sp1 activities. Rather, changes in AP-1 activities appear to be important for the induction of NPY expression in this system. In order to functionally correlate these in vitro data, we are performing transient transfections with reporter constructs carrying the NPY promoter AP-1 site.

333.20

PROTEIN KINASE C AS A MEDIATOR OF EXCITATION-ACETYLCHOLINE RECEPTOR GENE INACTIVATION COUPLING IN CHICK SKELETAL MUSCLE C. F. Huang, J. Tong, and J. Schmidt*. Dept. of Biochemistry and Cell Biology, State University of New York at Stony Brook, Stony Brook, NY 11794.

The signaling pathway connecting membrane depolarization and gene activity in skeletal muscle remains largely unknown. We have investigated short-term effects of plasma membrane electrical activity on acetylcholine receptor (AChR) subunit genes. Using transcription elongation (run-on) analysis, we have found that electrical stimulation of denervated chick skeletal muscle in vivo rapidly and selectively inactivates AChR subunit genes.

We have also studied the possible involvement of protein kinase C (PKC) in this response and have made the following observations: (1) Electrical stimulation of skeletal muscle rapidly activates PKC within the nuclear compartment; nuclear enzyme activity which is barely detectable in unstimulated muscle rises by 2 orders of magnitude upon stimulation. (2) Phorbol 12-myristate 13-acetate (PMA), an activator of PKC, blocks AChR subunit gene transcription, in the absence of electrical activity, within minutes after intramuscular application. (3) The activity-triggered gene inactivation is blocked by the protein kinase inhibitor staurosporine or by depletion of PKC resulting from chronic pretreatment of muscle with PMA.

We conclude that PKC is an integral component of the pathway coupling membrane excitation and AChR gene control.

PRESYNAPTIC MECHANISMS V

334.1

TETANUS AND BOTULINUM A NEUROTOXINS: DIFFERENT EFFECTS ON CA++-STIMULATED EXOCYTOSIS. T. Binscheck and H. Bigalke.

(SPON: European Neuroscience Association).

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Tetanus and botulinum A neurotoxins inhibit exocytosis in bovine chromaffin cells by interfering with a process occuring at a late stage in the cascade leading to the fusion of vesicles with the plasma membrane. The intrinsic activity of both toxins can be attributed to their light chain which must be reduced to become toxic. We have investigated the time dependent intracellular activation of the toxins and their interaction with Ca^{++}-dependent processes. The application of the lock-in method allows exocytosis to be monitored on-line by recording the changes in the membrane capacitance which result from the fusion of vesicles with the plasma membrane. Since chromaffin cells lack specific binding sites for both toxins, electroporation was necessary in order to enable the toxins to enter the cells. Two hours after incorporation of BoNtx (6.6nM) stimulation with 1μM Ca^{++} failed to increase the membrane capacitance whereas, in the presence of 100μM Ca^{++}, an increase could still be observed . After 24 hr, however, exocytosis was blocked independently of the Ca^{++}-concentration used. Thus, it is only at early stages, that the target of BoNtx can be modulated by intracellular calcium. In the case of Tetx only the light chain (6.6nM) could block exocytosis within the first two hours, when cells were stimulated with 1μM Ca^{++}. The protoxin (6.6nM), on the other hand, could not inhibit exocytosis before 24 hr. This indicates that Tetx must be activated inside the cell by a time consuming process. *This work was supported by the German Research Council.*

334.2

A NOVEL INSIGHT INTO THE MECHANISM OF ACTION OF BOTULINUM TOXIN IN A CHOLINERGIC NEURONAL CELL CULTURE MODEL. P. Ray*, J.D. Berman, B.G. Schuster and J.K. Lovelace. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC 20067.

Botulinum neurotoxin type A (BoTx) acts by inhibiting the stimulus-induced acetylcholine (ACh) release at peripheral neuromuscular junctions. However, the detailed mechanism of this effect remains elusive. We studied this mechanism using the cholinergic neuronal pheochromocytoma PC12 cell line. Cultured monolayer PC12 cells were differentiated by treatment with 50 ng/ml nerve growth factor for 4 to 5 days to enhance cellular ACh synthesis and release properties. Stimulation of these cells with high K^+ (80 mM) in the perfusion medium caused a marked increase (4-5X) in [^{3}H]ACh release in a Ca^{2+}-dependent manner. K^+-stimulated [^{3}H]ACh release was totally inhibited by pretreatment of cells with a low concentration (< 10 nM) of BoTx for 2 hr. High K^+ also stimulated the release of arachidonic acid ([^{3}H]AA) from the cell membrane, which was inhibited by BoTx (< 10 nM). The toxin concentration-dependence and time course of this stimulated release of [^{3}H]ACh and [^{3}H]AA suggest a possible regulatory role of AA in the stimulus-induced ACh release process and its inhibition by BoTx.

334.3

SYNCHRONIZATION OF QUANTAL INHIBITORY DENDRITIC RESPONSES. H. Korn*, N. Ankri and D.S. Faber+. Institut Pasteur, Paris and +SUNYAB, Buffalo, NY.

Intracellular recordings from the Mauthner (-M) cell's lateral dendrite 300μm distal to the soma were obtained *in vivo* with KCl microelectrodes. As in the soma, spontaneous depolarizing IPSPs (suppressed by strychnine) dominated synaptic noise, but, in contrast, autocorrelations showed periods of regular oscillations in the range of 40 to 60 Hz. In TTX (1μM) bathed preparations, an automatic detection and analysis program revealed two prominent classes of miniatures, with the mean size of the largest being 2 to 4 times that of the first (or minimum quantal unit, mqu), the kinetics of both matching those of quanta recorded in the soma (J. Neurophysiol. 1990, 63,198-222). The mqus occur randomly, whereas the larger components (second class) produce the 40-60 Hz rhythm. Low Ca^{++} high Mg^{++} solutions gave the same initial results and a progressive decrease in the relative number of events comprising the second class. The latter appeared to be composite, i.e. made of synchronized multiples of mqu; desynchronization was manifest by the presence of inflections on their rising phases, incremental units having the same kinetics as in control. Consequently, the first peak of the amplitude distribution appeared in isolation at the end of the recording session. Impulse-independent large events could arise at synapses with extended receptor matrices and/or from multiquantal release at terminal boutons having several closely-spaced active zones. Both hypotheses are consistent with ultrastructural evidence (C. Sur et al., this volume). However the gradual fragmentation of the largest components in conditions of decreased release probability supports the second interpretation.

334.4

AP VELOCITY FROM HH AXON EQUATIONS

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Synchrony of APs at particular positions in neural networks is important for network responses, including synaptic plasticity phenomenae. Since the HH equations are nonlinear, various analyses to calculate velocity of APs in the literature remain as approximations.

To calculate velocity of an AP, V_{AP}, the HH equations were solved numerically for different parameter values. For a 100 μm straight axon, divided into 101 compartments with constant diameter, V_{AP} was calculated. For each i-th compartment, at location X_i, the time, T_i, of maximum AP voltage was calculated. The slope of X_i verus T_i is V_{AP}. Velocity versus the space constant, $\lambda = [R_m r/2\rho]^{1/2}$ is a straight line, for membrane resisitivy, R_m, fiber radius, r, and axoplasmic resistivity, ρ, thus

$$V_{AP}\ \mu m/msec = K\lambda\ cm$$

For example, crustacean constants give K=433 sec^{-1}. This may be viewed as: **Length of the leading bow wave of conductive depolarization determines AP velocity.** This agrees with experimental literature that V_{AP} is proportional to $r^{1/2}$.

This method demonstrates that as an AP propagates over an axon presynaptic varicosity, V_{AP} is decreased to broaden the AP in time. This implies that Ca channels are open longer, hence more neurotransmitter is released.

334.5

RETROGRADE DETERMINATION OF PRESYNAPTIC PLASTICITY: QUANTAL ANALYSIS AT A CENTRAL SYNAPSE. G.W. Davis* and R.K. Murphey. Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01002.

A quantal analysis applied to the sensory synapses of the cricket cercal system supports a presynaptic mechanism for facilitation. EPSPs recorded from the large ventro-medial dendrite of the MGI, at or near the sight of synaptic input, provide enough resolution to discriminate failures from unitary responses. Twin pulses (at 100Hz instantaneous frequency) were delivered to the sensory neuron (SN) to evoke facilitation. The first and second EPSPs were then grouped separately for analysis. The mean quantal content (m), calculated by the failure method, increases in the second EPSP while the quantal size (q) remains constant (N=4).

We have investigated how presynaptic plasticity such as facilitation is determined developmentally by examining a single SN that contacts two target interneurons, MGI and 10-3. Our results show that the terminals of a single, identified SN can express facilitation on MGI, and simultaneously express homosynaptic depression on 10-3. In addition, when numerous identified SNs are examined, all those contacting MGI facilitate while all those contacting 10-3 depress, regardless of the age or amplitude of the synaptic connection. Based on the data indicating that facilitation is pre-synaptic, we hypothesize that the ability of a SN to facilitate is determined locally, at the synapse, by an interaction with the post-synaptic cell. Supported by NSF grant #BNS 90-96180.

334.6

SHORT-TERM MODULATION OF MONOSYNAPTIC TRANSMISSION IN HIPPOCAMPAL CULTURES. S. Mennerick*, D.B. Clifford, and C.F. Zorumski. Depts. of Psychiatry and Neurology, and Program in Neuroscience, Washington Univ. Med. School, St. Louis, MO 63110.

Many chemical synapses display a facilitated response to the second of a pair of short interval stimuli. However, in cultures of postnatal rat hippocampus studied with whole cell recording techniques, the majority of excitatory (glutamatergic) and inhibitory (GABAergic) monosynaptic responses display a marked paired pulse depression (PPD). We have begun to characterize PPD at excitatory synapses.

Stimuli delivered at intervals between 10 msec and 5 sec elicit a PPD that is insensitive to inhibitors of glutamate receptor desensitization and to inhibitors of glutamate uptake. PPD is also not affected by incubation of cells in 50-250 ng/ml pertussis toxin for 12-72 hr. This treatment abolishes presynaptic inhibitory effects of 1μM 2-chloroadenosine (2-CA) and of 10 μM baclofen. This result combined with the finding that PPD occurs in autaptic responses of single excitatory cells on microisland cultures makes a role for modulatory transmitters unlikely.

Treatments that decrease the probability of transmitter release also decrease PPD. Therefore, a low extracellular Ca^{++}:Mg^{++} ratio, baclofen, and 2-CA reduce the first response to a stimulus pair proportionally more than the second response. In some cases decreasing the probability of release can transform PPD into paired pulse facilitation. This result is consistent with a model of transmission in which in which terminals activated during the first of a pair of stimuli become refractory to a second pulse, but terminals not activated by the first stimulus are 'primed' for a response to a second stimulus. Mechanisms of PPD consistent with this model are feedback by glutamate onto presynaptic receptors and rapid depletion of releasable transmitter stores.

334.7

THE EFFECTS OF ADENOSINE, BACLOFEN, AND Cd^{2+} ON MINIATURE EPSCs AND IPSCs IN THE HIPPOCAMPUS REVEAL NOVEL MECHANISM OF PRESYNAPTIC ACTION

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Presynaptic inhibition of neurotransmitter release in the CNS is often thought to be mediated by inhibition of voltage-dependent Ca^{2+} channels in the axon terminal. We have compared the effects of the Ca^{2+} channel antagonist Cd^{2+} and several transmitters that inhibit evoked synaptic transmission with regard to their effects on spontaneous miniature EPSCs and IPSCs (in TTX) using whole-cell voltage-clamp to record from CA3 neurons in hippocampal slice cultures. Cd^{2+} (20-100 uM) blocked evoked EPSCs and IPSCs. Baclofen (10 uM) and adenosine (50 uM) blocked evoked EPSCs and also decreased the frequency of miniature EPSCs, without affecting the distribution of their amplitudes. Baclofen blocked evoked IPSCs, but did not affect miniature IPSC amplitude or frequency. Cd^{2+} had no effect on either the frequency or amplitude of miniature EPSCs or IPSCs. These results demonstrate that baclofen and adenosine do not decrease glutamate release from presynaptic endings by inhibiting axon terminal Ca^{2+} channels, but rather by inhibiting the release process itself. The observation that baclofen reduces the frequency of miniature EPSCs but not IPSCs, and our previous finding that Ba^{2+} blocks the effect of baclofen on IPSPs but not EPSPs (Thompson & Gähwiler, J. Physiol. v451, 1992), allow us to conclude that $GABA_B$ receptors exert distinct actions at excitatory and inhibitory synapses.

334.8

EFFECTS OF PERTUSSIS TOXIN, PHORBOL ESTER, AND BARIUM ON OPIOID PEPTIDE ACTION IN HIPPOCAMPAL SLICE CULTURES.

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Opioid peptides indirectly excite hippocampal pyramidal cells by decreasing GABA release from interneurons. We have investigated the mechanisms underlying this disinhibition in the rat hippocampus *in vitro*. In the presence of CNQX and D-APV (20μM each), stimulation close to intracellularly recorded CA3 cells elicited monosynaptic $GABA_A$ receptor-mediated IPSPs. Under these conditions, application of the μ receptor-preferring enkephalin analogue FK 33-824 (3nM-1μM) produced a dose-dependent, reversible, and naloxone-sensitive decrease in IPSP amplitude. Incubation of cultures with pertussis toxin prevented the reduction of IPSPs by FK 33-824. Stimulation of protein kinase C with phorbol ester strongly diminished the presynaptic effect of FK 33-824. Application of staurosporin slightly enhanced the reduction of IPSPs by FK 33-824 and prevented the effect of phorbol ester on FK 33-824 responses. In contrast, the K^+ channel blocker barium (1mM) did not affect the depression of IPSPs induced by FK 33-824. These results demonstrate that μ receptor-mediated presynaptic inhibition of IPSPs, like that mediated by $GABA_B$ receptors (Thompson & Gähwiler, J. Physiol. v451, 1992), is caused by pertussis toxin-sensitive G proteins which can be inactivated by stimulation of protein kinase C. Unlike $GABA_B$ actions, however, the effects of μ receptors do not result from an increase in a barium-sensitive K^+ conductance.

334.9

PRESYNAPTIC INHIBITION OF EPSPs BY NOREPINEPHRINE IN AREA CA3 OF HIPPOCAMPAL SLICE CULTURES

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Norepinephrine (NE) reduces the frequency of spontaneous epileptiform burst discharges in the disinhibited hippocampus. We investigated the effects of NE on pharmacologically isolated non-NMDA receptor mediated EPSPs which were evoked in CA3 pyramidal cells by mossy fiber or recurrent collateral stimulation in hippocampal slice cultures (Thompson & Gähwiler, J. Physiol. 451, 1992). These EPSPs depended linearly on membrane potential and followed stimulation frequencies up to 10Hz without changing their amplitude or latency, and are therefore likely to represent monosynaptic non-NMDA receptor-mediated responses. NE (5uM) strongly reduced the amplitude of EPSPs without affecting the amplitude of currents induced by application of AMPA, suggesting that NE decreases presynaptic glutamate release. This action was unaffected by the beta receptor antagonist timolol but was blocked by the alpha receptor antagonist phentolamine. Treatment of cultures with pertussis toxin or phorbol esters abolished the effects of NE, while application of the protein kinase inhibitor staurosporin enhanced the effect of NE. Despite the presumed presynaptic action, NE did not affect the frequency or the amplitude distribution of miniature EPSCs recorded in the presence of TTX with the whole-cell patch-clamp technique. Presynaptic inhibition of the EPSP probably underlies the anticonvulsive action of NE in the hippocampus.

334.10

OPPOSITE EFFECTS OF CHOLINOCEPTOR ACTIVATION ON SPONTANEOUS AND EVOKED IPSCs IN HIPPOCAMPAL CA1 PYRAMIDAL CELLS **J.C. Behrends and G. ten Bruggencate, Department of Physiology, Univ. of Munich, Germany.** **Spon.: EBBS**

Among the reported cholinergic actions in the hippocampus is a suppression of evoked inhibitory postsynaptic potentials (IPSPs). This apparent disinhibition by cholinergic drugs contrasts with a simultaneous increase of the frequency of spontaneous IPSPs.

This problem was assessed by recording bicuculline-sensitive spontaneous and stimulus-evoked inhibitory postsynaptic currents (s- and eIPSCs, respectively) in CA1 pyramidal cells (PCs) of guinea pig hippocampal slices. Superfusion of carbachol (1-10 μM) or eserine (10 μM) resulted in a sustained increase in the frequency and amplitudes of sIPSCs, while eIPSCs evoked from the str. radiatum were suppressed. Both effects were antagonized by atropine (1 μM). The facilitatory action on sIPSCs was unaffected by incubation with CNQX and APV (20 μM), while recording in the presence of 0.6 μM TTX yielded no evidence for an action on miniature sIPSCs, suggesting that carbachol as well as released acetylcholine directly excite GABAergic interneurones. IPSCs elicited in the presence of CNQX and APV by stimulation of str. pyramidale were also reduced by carbachol and eserine.

These data suggest that GABAergic interneurones can be excited by cholinoceptor activation in the in vitro hippocampus, resulting in increased tonic inhibition of PCs. The depression of eIPSCs might be explained by interference of tonic discharge with evoked release. The nature of this interaction is currently being investigated.

334.11

ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS REDUCES EPSCs and IPSCs IN NEONATE RAT SYMPATHETIC PREGANGLIONIC NEURONS.

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Whole cell-patch clamp recordings were made from sympathetic preganglionic neurons (SPNs) in transverse spinal cord slices of 10-16 day old rats. Glutamate (L-Glu, 10-300 μM), quisqualate (QA, 0.1-3 μM), kainate (KA, 0.3-10 μM), ibotenate (10-25 μM), L-AP4 (25-100 μM) and the metabotropic receptor agonist trans-ACPD (100-300 μM) attenuated mono-synaptic excitatory (EPSCs) and inhibitory (IPSCs) postsynaptic currents elicited by stimulation of lateral funiculus without causing a significant change of holding current; the reversal potential and decay time constant of EPSCs or IPSCs were also not altered. At higher concentrations, these agonists, except L-AP4, induced an inward current in some SPNs. While reducing the EPSCs, the inward current induced by exogenous L-Glu was not changed by L-AP4 and trans-ACPD. The metabotropic receptor antagonist L-AP3 increased the EPSC amplitude by 20-30%. It is concluded that metabotropic L-Glu receptors are present on excitatory and inhibitory nerve fibers presynaptic to SPNs, and that they may be tonically activated by endogenously released L-Glu or a related excitatory amino acid. (Supported by NS18710).

334.12

PHORBOL ESTERS ENHANCE MONOSYNAPTIC IPSCs IN RAT HIPPOCAMPAL PYRAMIDAL CELLS.

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Activators of protein kinase C (PKC), such as phorbol esters (PEs), enhance excitatory synaptic transmission, but their effects on inhibitory transmission are less clear. Using whole-cell voltage-clamp recording techniques in the rat hippocampal slice preparation we examined the effects of PEs on evoked GABAergic IPSCs. All cells were recorded in the presence of glutamate antagonists CNQX and APV to isolate GABAergic responses. We found that 3 μM phorbol 12,13-diacetate (PDA) significantly enhanced $GABA_A$-mediated synaptic events. 4-α-PDA, an inactive analog which does not activate PKC, had no effect on IPSCs. PDA did not affect iontophoretically applied GABA responses, suggesting that the increase in IPSCs amplitude by PDA was due to a presynaptic action.

PEs also block both pre- and post-synaptic $GABA_B$-mediated effects. Since activation of presynaptic $GABA_B$ receptors inhibits transmitter release, it was possible that the enhanced GABA transmission caused by PDA was due to block of the autoinhibitory $GABA_B$ receptor. We found, however, that paired pulse depression of IPSCs, which is believed to result from presynaptic $GABA_B$ receptor activation, was not blocked by PDA. Our work suggests either that synaptically released GABA doesn't activate presynaptic $GABA_B$ receptors or that $GABA_B$ receptors on presynaptic inhibitory terminals are not affected by PEs, unlike $GABA_B$ receptors previously studied. Furthermore, the increase in GABA release by PDA is not caused by PE-induced depression of the autoinhibitory $GABA_B$ receptor.

334.13

EFFECTS OF $GABA_B$ RECEPTOR ACTIVATION ON SPONTANEOUS SYNAPTIC ACTIVITY OF RAT HIPPOCAMPAL NEURONS IN CULTURE

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Activation of $GABA_B$ receptors inhibits synaptic transmission and decreases neuronal excitability at pre- and postsynaptic sites, presumably by decreasing g_{Ca} and increasing g_K, respectively. We took advantage of the absence of postsynaptic $GABA_B$ receptors in our cultures to compare the effects of presynaptic $GABA_B$ receptor activation on spontaneous excitatory (EPSC) and inhibitory (IPSC) currents generated within small neuronal networks using standard whole-cell voltage-clamp techniques.

Baclofen (BAC, 2-200 μM), a selective $GABA_B$ agonist, blocked spontaneous IPSCs and non-NMDA receptor mediated EPSCs at concentrations that failed to completely block NMDA receptor mediated EPSCs (NMDA-EPSCs). Sustained application of BAC initially reduced the amplitude, duration and frequency of NMDA-EPSCs but was followed by a partial recovery. BAC did not alter responses to exogenous application of NMDA. The proposed $GABA_B$ receptor antagonists, phaclofen (100-300 μM) and 2-OH-saclofen (100 μM) did not consistently block the effects of BAC on NMDA-EPSCs and also acted as partial agonists. The competitive NMDA receptor antagonist, DL-APV (10-30 μM), produced a similar decrease followed by partial recovery of NMDA-EPSCs suggesting that postsynaptic NMDA receptor resensitization during synaptic depression may account for part of the recovery phenomenon. Preliminary paired cell recordings reveal a similar recovery following BAC inhibition of evoked EPSCs raising the possibility of "desensitization" of presynaptic $GABA_B$ receptors in culture.

These results may have important implications regarding the clinical use of baclofen to dampen overexcitation mediated by NMDA receptors. *Supported by the American Epilepsy Society/Milken Family Medical Foundation (KDP) and USPHS grants NS 24927 and NS 24260 (MAD).*

334.14

EFFECTS OF REPETITIVE STIMULATION ON SYNAPTIC TRANSMISSION IN THE CHICK CILIARY GANGLION.

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Four components of stimulation-dependent increases in transmitter release have been observed at peripheral synapses: 2 components of facilitation, augmentation, and potentiation. In this study we investigated whether similar processes are present in the chick ciliary ganglion.

Embryonic (stage 48) chick ciliary ganglia were recorded from using extracellular nerve trunk recording. The preparation was perfused with a low Ca^{++} and/or high Mg^{++} oxygenated saline solution to reduce quantal content (20-23^{0}C).

The preganglionic nerve trunk was stimulated with 1-1000 impulses (20-50 Hz) to condition the nerve terminals. Testing impulses applied after the conditioning trains established that ganglionic transmission was enhanced during tetanic stimulation and returned to control levels following the tetanus with four apparent time constants of decay (~60 ms,~420 ms,~30 s, and ~150 s). These time constants are similar to those reported for other preparations.

Thus, the components of stimulus-dependent increases in release described in other preparations appear to be present in the chick ciliary ganglion. Because of the large size of the calical nerve endings of the ciliary ganglion, it should be possible in the future to record electrical events in the nerve terminal under conditions of increased release. The results of these studies should contribute to a better understanding of the mechanisms underlying stimulation-dependent changes in release.

334.15

INTRACELLULAR MECHANISMS OF FACILITATORY AND INHIBITORY EFFECTS OF L-DOPA ON THE CHOLINERGIC TRANSMISSION IN THE GUINEA-PIG MEISSNER PLEXUS.

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Neurotransmitter-like or neuromodulatory actions of L-DOPA were investigated with intracellular recording from guinea-pig Meissner neurones. L-DOPA at 30 nM augmented fast EPSPs, but not affected cholinergic depolarizations elicited by puff application of acetylcholine (ACh). The augmenting effect of L-DOPA on the fast EPSPs was counteracted by L-DOPA methylester. The fast EPSPs were depressed by 10 μM L-DOPA, but transiently augmented after rinsing the drug. L-DOPA methylester did not affect the inhibitory action on the fast EPSPs, but antagonized the potentiation following the inhibition. The ACh-depolarization was inhibited by 10 μM L-DOPA. Intracellular free calcium concentration ($[Ca^{2+}]_i$) was measured with fura-2-microspectrofluorimetry. A transient increase in the $[Ca^{2+}]_i$, evoked by action potential ($\Delta[Ca^{2+}]_{AP}$) was facilitated by 30 nM L-DOPA, but decreased by the drug at 10 μM.

It is concluded that L-DOPA at low concentration enhances the $\Delta[Ca^{2+}]_{AP}$, increasing the transmitter-release, but at high dose diminishes the $\Delta[Ca^{2+}]_{AP}$, inhibiting the neurotransmission.

334.16

EFFECTS OF SECONDARY MESSENGER ON NERVE TERMINALS OF MOUSE SKELETAL MUSCLE.

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The effects of secondary messenger on the motor nerve activity were studied on mouse triangularis sterni nerve - muscle preparations. The perineural waveforms were recorded with extracellular electrodes placed in the perineural sheaths of motor nerves. Both forskolin, a known activator of adenylate cyclase, and 4-alpha-phorbol-12,13-didecanoate (PD-De), a protein kinase C stimulator, decreased the slow calcium current in the motor nerve terminal. Neither forskolin nor PD-De decreased (a) the sodium current, (b) the fast and slow potassium currents, (c) the calcium activated potassium current and (d) the fast calcium current of the nerve terminal. It is concluded that forskolin and PD-De act on the slow calcium current of the nerve terminal. The secondary messenger may play a functional role on the transmitter releasing process. (Supported by grants, NSC-79-0412-B-002-113 and NSC-81-0412-B002-27, from National Science Council, Taipei, Taiwan, R.O.C.).

334.17

DIFFERENTIAL EFFECTS ON MEMBRANE POLARIZATION BY THE κ_1-OPIOID RECEPTOR AGONIST U-50,488 IN PRESYNAPTIC CALYX NERVE TERMINALS OF THE CHICK CILIARY GANGLION. G.H. Fletcher* and V.A. Chiappinelli, Dept. of Pharm. and Physiol. Sci., St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

This study examined the electrophysiological effects of κ-opioids, since previous work in this laboratory has shown that opioid peptides acting at mu and delta receptors are active on presynaptic calyx nerve terminals of chick ciliary ganglion. The actions of the κ_1-opioid receptor agonist U-50,488 on calyciform nerve terminals were investigated in vitro by intracellular recording. Bath application of U-50,488 (30-300 μM) elicited dose-dependent membrane hyperpolarization (2-12 mV) with an increase in input resistance (R_{in}). However, at the highest concentration examined (1 mM), there was first a membrane hyperpolarization followed by a slow depolarization (2-10 mV), that was associated with a further rise in R_{in}. The hyperpolarizing response elicited by 300 μM U-50,488 was completely blocked by 3 mM Cs^+, a known blocker of a cationic inward rectifier in this terminal region (Fletcher and Chiappinelli, Brain Res. 575:103-112, 1992). This resulted in the unmasking of a depolarizing action of U-50,488 at this lower concentration, which was subsequently inhibited by the K^+ channel blocker Ba^{2+} (1 mM). These dual effects of U-50,488 were antagonized by the κ_1-opioid receptor antagonist nor-binaltorphimine (10-30 μM), indicating that the drug-induced effects are likely to represent specific actions linked to the activation of opioid receptors. Supported by NIH grant EY06564 to V.A.C.

334.18

ENKEPHALINS PRODUCE DIFFERENT EFFECTS IN CELL BODIES AND NERVE TERMINALS OF CHICK EDINGER-WESTPHAL NEURONS. V.A. Chiappinelli*, K.M. Wolf, C. Feng and L.L. McMahon, Dept. Pharm. Phys. Sci., Saint Louis Univ. Sch. Med., St. Louis MO 63104

Within a single neuron, receptors may be located near the cell soma or some distance away in the axon terminals. Since the roles of these two classes of receptors are distinct, we asked whether the effect of activation of receptors differs depending on their location within a neuron. Neurons located in the lateral division of the avian Edinger-Westphal nucleus (EWL) were used to test this point. These cells innervate ciliary ganglion neurons via large, calyciform nerve endings, many of which contain the peptides leucine-enkephalin (LENK) and substance P (SP; Reiner et al., Vis. Neurosci. 6:451, 1991). Presynaptic receptors for LENK and SP are also found on these terminals (Dryer and Chiappinelli, Brain Res. 336:190, 1985). Intracellular current clamp recordings from EWL somas were obtained in brain slices using methods described in Sorenson and Chiappinelli (Neuron 5:307, 1990), while EWL nerve terminals were impaled in intact ciliary ganglia in vitro. Pressure ejection of LENK depolarized and decreased input resistance in 81% (17/21) of EWL nerve terminals, due in part to activation of a sodium current. In sharp contrast, similar exposure to LENK hyperpolarized 88% (15/17) of EWL somas by up to 25 mV. This was associated with a decrease in input resistance, indicating that LENK activated a potassium channel in EWL cell bodies as it does in certain other central neurons (North et al., PNAS 84:5487, 1987). We conclude that LENK-sensitive receptors on EWL somas activate different ion channels than LENK-sensitive receptors on EWL terminals in the ciliary ganglia. Supported by NIH EY06564 to V.A.C.

334.19

USE-DEPENDENT ACTIONS OF NICOTINIC BLOCKERS IN BULLFROG SYMPATHETIC GANGLIA: ARE THEY PRESYNAPTIC? W Shen* and JP Horn Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

We have examined the blockade of fast cholinergic EPSPs in bullfrog sympathetic ganglia. Surprisingly, different nicotinic receptor antagonists all produced use-dependent block during repetitive stimulation at low frequencies that are physiologically realistic.

Compound nicotinic EPSPs and resultant action potentials (APs) in sympathetic B neurons were recorded extracellularly from rami of lumbar ganglia. In the absence of drugs, postsynaptic responses followed repetitive stimulation without decrement for hundreds of impulses at frequencies as high as 20 Hz. At doses that partially antagonize nicotinic transmission, structurally unrelated drugs (eg. 30 μM d-tubocurarine, 30 μM mecamylamine, 30 μM nicotine, 0.1-0.3 μM neuronal bungarotoxin, and 2 analogs of lophotoxin) all produced a similar use-dependent block of compound fast EPSPs and APs. This block occured at frequencies as low as 0.5-1 Hz. It was progressive during the first 7-9 responses in a train and thereafter persisted at a plateau ranging between 20-80% of the first response. The rate of decrement was faster during 10 Hz stimulation. Postsynaptic response amplitude recovered when the preparation was allowed to rest for 10 seconds. It seems unlikely that all the drugs used are open channel blockers. Instead we propose that use-dependent block reflects presynaptic nicotinic receptors which may normally act to facilitate Ach release in sympathetic ganglia. Preliminary intracellular recordings indicate that the properties of use-dependent block are similar at the single cell level. Supported by NIH grants NS21065 and NS01427.

POTASSIUM CHANNELS: PHARMACOLOGY AND EFFECTS OF TRANSMITTERS

335.1

BLOCKADE BY PEPTIDIC TOXINS OF MK1 POTASSIUM CURRENTS IN CHO CELLS. D.G. Owen, B. Robertson*, A. Hall[@] & J.A. Scott, Wyeth Research (UK), Huntercombe Lane Sth., Berks. SL6 0PH, UK and [@]Dept. Biochemistry, Imperial College, Imperial College Rd, South Kensington, London SW7 2AY, UK.

Toxin I and other dendrotoxin (Dtx) homologues are potent blockers of voltage-activated K^+ currents in cultured rat sensory neurones (Owen et al, 1990) and α-Dtx and δ-Dtx, in particular, block different components of K^+ current which may reflect distinct K^+ channel subtypes (Hall et al, 1991). In the present study we have examined the properties of a number of Dtx homologues versus a single voltage-activated K^+ channel type identified in mouse brain, MK1 (Tempel et al, 1988).

MK1 was expressed stably in chinese hamster ovary (CHO) cells and sub-cultured according to standard tissue culture methods. Whole-cell electrophysiological recordings were made 0-3 days following replating of cells, using an AxoPatch-1D or AxoClamp-2A voltage-clamp amplifier, patch electrodes (2-8Mohm) and standard filling and bathing solutions. Cells were held at between -60mV and -100mV and outward currents which were elicited with depolarizing voltage steps activated within 3ms and decayed only slightly ($\leq$ 10%) during a 500ms period. The threshold for activation was *ca* -40mV. No corresponding voltage-activated K^+ currents were observed in cells transfected with control vector (lacking MK1-sequence). α-Dtx (10nM), δ-Dtx (10nM) and Toxin I (100nM), inhibited 52%, 85% and 90%, respectively, of the total outward current recorded at +60mV. Mast cell degranulating peptide (MCDP) also blocked a proportion of the current (23% at 1μM). Whereas block by α-Dtx and δ-Dtx was more pronounced at depolarized potentials, Toxin I showed little voltage-dependence. Interestingly, the maximal degree of block of MK1 by δ-Dtx, in particular, was much higher than that observed for the native K^+ currents in DRG cells (*ca* 30%).

Tempel et al (1988). Nature, **332**, 837-9.

Owen et al (1990). *Soc. Neurosci. Abstr.*, **16**, 156.8.

Hall et al (1991).*10th World Congress on Animal, Plant & Microbial Toxins*, Abstr. 371.

335.2

THE SEIZURE-INDUCING ANESTHETIC ENFLURANE REDUCES TRANSIENT OUTWARD CURRENT IN CULTURED RAT HIPPOCAMPAL NEURONS M.V. Jones* Dept. Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637

The volatile anesthetic enflurane (ENF) is known to induce seizure-like activity in the cortical EEG[1], and synchronous spike firing in hippocampal pyramidal neurons *in vitro*.[2] The mechanism of this effect is unknown, however many convulsant agents, such as 4-aminopyridine, are able to reduce transient outward currents (TOC), and induce repetitive firing.[3] I have studied the action of ENF and halothane (HAL) on neuronal TOC. Hippocampal neurons from day 20 embryonic rats were dissociated and grown in culture. Whole-cell recordings were made at 25°C using an intracellular solution based on K gluconate, and perfused with saline containing 50mM TEA, 5 mM CsCl, and 0.2 μM TTX. Depolarizing commands from hyperpolarized potentials (-120 to -40 mV) evoked TOCs, defined as the current remaining after all currents not steady state inactivated at -40 mV were subtracted. TOCs show half-activation near -20 mV, half-inactivation near -80 mV, and decay as a sum of exponentials. The bathing medium was sampled repeatedly during each experiment for anesthetic measurement by gas chromatography.[4] ENF (0.1 - 1.2 mM) produced up to a 40% reversible depression of TOC amplitude, and increased the overall rate of inactivation in all ten neurons studied. Higher concentrations of ENF produced larger effects. When the current in the presence of ENF is subtracted from the control current, the difference current is itself a TOC which decays as a single exponential function (τ = 34 $\pm$ 3 ms (S.E.M.)). HAL (n=4) also modulated TOCs, but to a lesser degree. ENF appears to produce about twice the effect of HAL, at concentrations that are equieffective at producing clinical anesthesia. This effect may contribute to the ability of ENF to induce seizure activity, and may explain why HAL does not share this property. Supported by training grant 5-T32-GM-07151 and by RO1-GM45129 to N.L. Harrison. 1) Prog. Drug Res. 26: 225, 2) Br. J. Anaesth. 62: 301, 3) Neurosci. Lett. 64: 299, 4) J. Physiol. 449: 279.

335.3

DENDROTOXIN AND MAST CELL DEGRANULATING PEPTIDE: POTASSIUM CHANNEL TOXINS INDUCE NEURONAL DAMAGE IN THE RAT IN VIVO. C. Mourre[1], M. Lazdunski[2], H.E. King*[1], L.E. Jarrard[1]. [1]Dept. Psychol., Washington and Lee Univ., Lexington, VA 24450, and [2]CNRS-IPMC, Sophia-Antipolis, F-06650 Valbonne, France.

Dendrotoxin (DTX, 200 pmol) and mast cell degranulating peptide (MCD, 200 pmol) were injected unilaterally into the ventricles. Patterns of cell loss and neuronal degeneration were studied after 48 h survival times using cresyl violet and Fink-Heimer silver staining procedures.

Following injections of DTX, all rats were observed to display similar seizures but neuronal damage was found in only 30% of the subjects. The pattern of damage was bilateral and included argyrophilic cells and degeneration in numerous structures especially hippocampus (CA1 and CA3 cell fields), septum, amygdala, thalamus, hypothalamus and entorhinal cortex. In several subjects additional damage was noted in neocortex, cerebellum and the colliculi.

The behavioral pattern of seizures following injections of MCD differed from that found with DTX and the neuronal damage was more limited but included hippocampus (CA1 and CA3 cell fields), ventral subiculum, septum, amygdala, and nucleus accumbens.

Although the amounts of DTX and MCD injected were near the lethal dose, the potassium blockers induced different patterns of seizure activity and neuronal damage.

335.4

A-CHANNEL BLOCK BY α-DENDROTOXIN IS REVERSED BY A BLOCKER OF DELAYED RECTIFIER K CHANNELS. R.S. Rogowski, B.K. Krueger* & M.P. Blaustein, Physiol. Dept., U. of Maryland Med. Sch., Baltimore, MD 21201.

Toxins Ts_{K2} and Ts_{K4}, from the scorpion, *Tityus serrulatus*, selectively block delayed rectifier K (DRK) channels in synaptosomes (IC_{50}'s = 60 and 7 nM, respectively; *Mol. Pharmacol.* 40:932, 1991). In contrast, α-dendrotoxin (α-DaTX) selectively blocks A-type K channels, but not DRK channels (*loc. cit.* 34:152, 1988). We studied interactions between the *Tityus* toxins and α-DaTX in binding and ^{86}Rb efflux experiments. Ts_{K4} displaced ^{125}I-α-DaTX from synaptic membranes, SM (IC_{50} = 1 nM), but 600 nM Ts_{K2} did not affect ^{125}I-α-DaTX binding. Scatchard analysis showed that maximum binding of ^{125}I-α-DaTX, 2.8 pmol/mg SM protein, was reduced to 1.9 and 1.6 pmol/mg protein by, respectively, 2 and 4 nM Ts_{K4}. The apparent affinity of ^{125}I-α-DaTX for its receptor (K_D = 0.8 nM) was decreased by 2 and 4 nM Ts_{K4}: K_D increased to 1.4 and 2.5 nM, respectively. These data suggest an un-competitive, allosteric interaction between Ts_{K4} and α-DaTX at the ^{125}I-α-DaTX receptor. Ts_{K2} and Ts_{K4} both selectively blocked the non-inactivating (DRK channel) component of K-stimulated ^{86}Rb efflux from synaptosomes; their effects were not additive. α-DaTX (IC_{50} = 90 nM) inhibited only the rapidly-inactivating (A-channel) component of ^{86}Rb efflux. The effects of Ts_{K2} and α-DaTX on the ^{86}Rb efflux were additive, as expected for two toxins that block different K channels. In the presence of 600 nM α-DaTX and 80 nM Ts_{K4}, however, only the DRK channel component was blocked. Thus, Ts_{K4} relieves the block of A-channels by α-DaTX.

335.5

Block by capsaicin of potassium currents in pituitary melanotrophs. S.J. Kehl*. Dept. of Physiology, U.B.C., Vancouver, B.C., V6T 1Z3.

Capsaicin blocks K currents in dorsal root ganglion neurones and vertebrate axons. I report here that capsaicin also affects both the fast ($I_K(f)$) and the slow ($I_K(s)$) voltage-gated K currents of rat melanotrophs. Whole-cell currents were recorded at room temperature by using conventional patch clamp techniques. In acutely dissociated cells 50 μM external capsaicin decreased the amplitude and increased the rate of decay of $I_K(f)$ and $I_K(s)$. The onset of the block was fast and in most cells was completely reversible. In cultured melanotrophs expressing only $I_K(s)$ capsaicin had identical effects. In addition to being concentration dependent, the actions of capsaicin were voltage-dependent. For example, compared to the current evoked at 20 mV the steady-state current at -10 mV was reduced to a smaller extent and "inactivation" was less evident. The relaxation rate of current tails in 50 μM capsaicin was well-fitted by the sum of two exponentials, one being faster the other slower than the single exponential fitted to control responses. The activation rate of $I_K(s)$ was not altered. The simplest interpretation of these data is that capsaicin acts as an open channel blocker.

335.6

EFFECT OF 4-AMINOPYRIDINE ON BOVINE CHROMAFFIN CELL MEMBRANES. L. Kapural^, I. Bekavac^, J.M. Trifaro§ and M.I. Glavinovic^^+Γ. Departments of Anesthesia+, Anesthesia Research^^ and Physiology^Γ McGill University, Montreal, P.Q., Canada H3G 1Y6 and Department of Pharmacology§, University of Ottawa, Ottawa, Ont., Canada K1H 8M5.

Ever since the early findings in squid axon (Yeh et al. Biophys. J. 16, 77-81, 1976) it has been well established that 4-aminopyridine (4-AP) blocks potassium currents. 4-AP is now known to block fast inactivating (I_A) potassium current. Such actions of 4-AP usually occur at concentrations that are comparatively high (0.1-1mM). 4-AP is also known to augment quantal release at squid synapse (Llinas et al., Biophys. J., 16, 83-86, 1976) and at neuromuscular junction (Lundh & Thesleff, J. Pharmacol., 42, 411-12, 1977) but at micromolar concentrations. If as speculated augmentation of release occurs because of blockade of potassium conductance this raises the question of whether otherwise the same channels have different 4-AP sensitivity at secretory cells or whether these are altogether different potassium channels.

Experiments were done on excised inside out membrane patches from bovine chromaffin cells. The microelectrodes were filled with Locke solution. The internal solution nominally had 0.1 - 0.3 μM free calcium. Experiments were performed at room temperature (20-23°C). 4-AP at low concentrations (10-100 μM) reduced outward current by blocking a small conductance channel which judging by I-V curve was permeable mainly to potassium. Outward current was further reduced due to the shift in the current baseline that appears to be essentially voltage independent and whose variance increased. The baseline changes could be due to activation of transport mechanism (or a pump) and/or due to the effect on probably several very small channels.

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335.7

MICROMOLAR ZINC MODULATES K^+ CHANNEL GATING

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Zn^{2+} ions are present in many regions of the brain, and are released from the mossy fibers into the CA3 region of the hippocampus on stimulation. We have studied the effects of Zn^{2+} on a variety of K^+ currents. Transient outward currents (TOC) were recorded in cultured (e20) rat hippocampal neurons and acutely isolated guinea pig CA1 neurons. The K^+ channels RK1, HK1 and HK2 (rKv1.1, hKv1.4 and hKv1.5), cloned from rat and human tissue and present in brain, were studied in mouse L cells stably transfected with the appropriate cDNA. Whole-cell recordings were made from single neurons at 20°C, using intracellular solutions based on 145mM K gluconate and continuous extracellular perfusion with HEPES-buffered saline containing 5mM K^+, 0.2mM Ca^{2+}, 20mM TEA, 5mM Cs^+ and 500nM TTX. Depolarizing commands from -120mV elicited transient outward currents (TOCs) in both types of neuron. Recordings from L cells were performed without ion channel blockers. Zn^{2+} solutions were applied by bath, or from a rapid perfusion system. All effects of Zn^{2+} were reversible. In cultured (e20) rat hippocampal neurons and guinea pig isolated hippocampal neurons, Zn^{2+} (2-200μM) shifted the activation and inactivation curves for the TOC to the right by a maximum of 30-50mV; this concentration-dependent modulation of gating behavior was modeled using a single binding site model, giving a K_D for Zn^{2+} of approximately 15μM. Zn^{2+} did not alter the reversal potential for tail currents of the TOC (-88mV), but did slow activation of the current. Similar effects were observed in L cells expressing the HK1 channel, a rapidly inactivating K^+ channel. In L cells expressing the non-inactivating delayed rectifier-type channels RK1 and HK2, a similar modulation of gating was observed, with activation slowed and the activation curve shifted to the right, consistent with a K_D for Zn^{2+} of approximately 20μM. We conclude that some of the physiological effects of Zn^{2+} may result from modulation of K^+ channel gating.

335.8

A NEW INTERPRETATION OF THE MECHANISM OF BLOCKADE OF THE DELAYED RECTIFIER POTASSIUM ION CHANNEL IN NERVE AXON MEMBRANE BY INTRACELLULAR TETRAETHYLAMMONIUM IONS. J.R. Clay* Lab of Biophysics, NIH, Bethesda, MD 20892.

K^+ channels in nerve are blocked in a voltage-dependent manner when TEA^+ is applied intracellularly. The original TEA^+ results from squid axons appeared to suggest that the channel gates had to open before blockade could occur. A re-examination of this issue in squid axons has revealed that the effect of TEA^+ is consistent with an alternative interpretation in which TEA^+ can bind to its blocking site within the channel equally well when the channel gates are either closed or open. Blockade by other ions does appear to depend upon the state of the gating mechanism. For example, the channel must be open for blockade to occur by either butyltriethylammonium (C_4) or pentyltriethylammonium (C_5), but not with propyltriethylammonium (C_3) or TEA^+ (C_2). The electrical distance of blockade for all of these ions is ~0.3 from the inner membrane surface. These observations suggest that the "head" of the C_n ion cannot reach the blocking site with $n > 3$ unless the gates are open thereby allowing the "tail" of the molecule to enter the permeation pathway. In contrast, TEA^+ and C_3 can reach the blocking site independent of gating, because these ions do not have a "tail" sufficiently long to interfere with gating.

335.9

DIVALENT CATIONS SELECTIVELY ALTER THE VOLTAGE DEPENDENCE OF INACTIVATION OF A-CURRENTS IN CHICK AUTONOMIC NEURONS. Mary E. Wisgirda* and Stuart E. Dryer, *Program in Neuroscience, Department of Biological Science, Florida State University, Tallahassee, FL 32306.*

A-currents were studied in acutely dissociated chick autonomic neurons. Parasympathetic neurons from the ciliary ganglion were obtained from E10-E14 embryos, and neurons from the lumbar sympathetic chain were obtained from E16-E19 embryos. Standard whole-cell patch clamp recording methods were used. Switching from salines containing 4 mM Mg^{2+} to salines containing 4 mM Ca^{2+} caused a large positive shift in the steady-state inactivation curve, but had no effect upon the voltage dependence or kinetics of activation, deactivation, the rate at which channels became inactivated or the rate at which channels recovered from inactivation. This effect saturated with increasing concentrations of Ca^{2+}. Other group IIA divalent cations were less effective than Ca^{2+}, in the order $Ca^{2+}>Ba^{2+}>Mg^{2+}=Sr^{2+}$. Application of 4mM Mg^{2+} partially antagonized the effects of 4mM Ca^{2+}. The results suggest the existence of a specific divalent cation binding site accessible from the external face of the membrane that selectively affects inactivation.

This work was supported by NIH Grant NS-27013.

335.10

GLIBENCLAMIDE-SENSITIVE HYPERPOLARIZARIZATION BY CROMAKALIM, MIMICKED BY METABOLIC INHIBITION, IS ABSENT IN ONE OF TWO SUBSTRAINS OF N1E-115 CELLS.
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Miles Inst. Preclin. Pharmacol., West Haven, CT 06516.

A specific subset of K-channels (K_{ATP}) defined pharmacologically by sulfonylurea inhibitors such as glibenclamide (GLB), and by K-channel agonists including cromakalim (CRK), is known to be present in mammalian neurons. We now report the presence of GLB-inhibitable, CRK-induced hyperpolarization in only one of two substrains of the clonal neuroblastoma line, N1E-115. In original N1E-115 cells depolarized in the continued presence of 55 mM K^+, CRK produced a dose-dependent and complete repolarization, with similar potency to that seen in hippocampal neurons (ED_{50}=300 μM). Furthermore, with single cell measurements using a fluorescent membrane potential dye, only this strain, responding to CRK and GLB, also responded to acute metabolic inhibition by oligomycin and 2-deoxy-D-glucose application. The repolarization produced by oligo/2DG in depolarized cells was rapid (within 10 min) and complete. Finally, the most potent block of maximal CRK response was achieved with GLB only when applied before the agonist, suggesting the possibility of a state-dependence to GLB block. In conclusion, absence of a hyperpolarizing response to both CRK and metabolic challenge in a substrain of N1E-115 (MIPP-N1E) cells strongly supports the K_{ATP} channel as mediator of this CRK action in original N1E-115 cells.

335.11

CHARACTERIZATION OF K^+ CHANNELS ACTIVATED BY CROMAKALIM IN INSIDE-OUT PATCHES FROM CULTURED RAT HIPPOCAMPAL NEURONS. D.M. Politi* and M.A. Rogawski. Neuronal Excitability Section, Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

We have previously reported that cromakalim activates a glyburide-sensitive K^+ channel in cell-attached recordings from cultured embryonic rat hippocampal neurons (single channel conductance, 16–34 pS). In the present study, we investigated the pharmacological properties of cromakalim-activated single channel currents in inside-out excised patches. In these recordings, the cromakalim-activated K^+ channels had a unitary conductance between 23 and 50 pS in physiological solution and a maximal conductance of 60–70 pS in symmetrical K^+. The channels showed a moderate degree of outward rectification. In several patches these channels were inhibited by application of 3 mM ATP-K_2 to the cytoplasmic side of the membrane. These data, and the observation that cromakalim and energy depleting conditions activate the same glyburide-sensitive channels, indicate that the K^+ channels opened by cromakalim in hippocampal neurons are likely to be of the ATP-sensitive type.

335.12

MODULATION BY ADENOSINE OF THE CROMAKALIM-INDUCED CURRENT IN GUINEA PIG VENTRICULAR MYOCYTES. John R. McCullough* and Mary L. Conder. Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton NJ 08543-4000.

Although adenosine (ADEN) alone has relatively few electrophysiological actions in mammalian ventricle, it has recently been reported to activate ventricular ATP-sensitive K^+ channels (KATP). The potassium channel opener (KCO), cromakalim (CROM), is also known to open KATP in cardiac cells. The effects of ADEN on the CROM-induced current in cardiac cells are not known. We used whole-cell patch clamping techniques to study the interaction of ADEN and CROM in ventricular myocytes isolated from adult guinea pigs (350g). Steady state whole-cell K^+ currents were elicited by slow voltage ramps (6-10 mV/sec from -130 to +50 mV) from a holding potential of -80 mV and drug effects were quantified by comparing the outward K^+ current at +20 mV (IK). ADEN alone (50 μM) had no significant effect on IK: 0.48±0.07 nA in control vs 0.46±0.13 nA in ADEN (n=13). In 9 cells, a threshold concentration of CROM (30 μM) caused a small, but significant increase in IK (0.42±0.05 nA, control vs 1.08±0.27 nA, CROM [$p<0.05$]) and addition of 50 μM ADEN further increased IK (1.76±0.46 nA, $p<0.03$). Similar effects were seen with the addition of 10 μM ADEN to 30 μM CROM. The possible G-protein involvement in ADEN enhancement of CROM effects was studied by using intracellular GTP-γS and A-protomer of pertussis toxin (PT). GTP-γS (100 μM) enhanced the IK effects of 30 μM CROM (1.3±0.18 nA control vs 3.3±0.85 nA, CROM, $p<0.05$), while PT (0.2 μg/ml) inhibited ADEN enhancement of CROM. These results suggest that in cardiac ventricular cells ADEN may modulate the actions of CROM on IK through a G-protein mediated mechanism. Since ADEN is released in myocardial ischemia, ADEN may enhance the ability of low concentrations of KCO to open KATP.

335.13

DRK1 K+ CHANNEL TRANSCRIPTS AND POLYPEPTIDES ARE EXPRESSED IN PC12 CELLS AND ARE REGULATED BY NGF. N. Sharma , A. K. Kleinklaus, N. V. Marrion, G. D'Arcangelo, S. Halegoua, and J. S. Trimmer*, Departments of Pharmacology, Neurobiology & Behavior, and Biochemistry & Cell Biology, SUNY, Stony Brook, NY 11794.

Changes in neuronal membrane excitability occur with development and plasticity due to differences in the levels of activity of specific ion channels. We have shown that, in rat brain, the delayed rectifier K+ channel isoform drk1 ($K_v2.1$) is restricted to neurons, and that levels of drk1 polypeptide expression in brain exhibit distinct temporal and spatial patterns. Thus differential expression of drk1 may be involved in determining excitability changes in neurons. We have investigated the expression of drk1 in the rat pheochromocytoma cell line PC12, which has been used as a model system to study changes in neuronal phenotype upon neuronal growth factor treatment. PC12 cells express endogenous drk1 polypeptide at levels similar to those found in adult rat brain neurons. In response to 24 hours of NGF treatment, levels of drk1 polypeptide increase 4-5-fold. This increase is maintained up to 144 hours of NGF treatment. An increase in drk1 mRNA levels is also seen upon NGF treatment. Immunofluorescent localization of the drk1 polypeptide in these cells reveals plasma membrane-associated staining of the cell bodies in both untreated and NGF-treated PC12 cells. Intense staining is observed in the growth cones of NGF-differentiated PC12 cells. Thus drk1 is expressed at high levels in the plasma membrane of both untreated and NGF-treated PC12 cells and may be a component of the delayed rectifier-like K+ currents found in these cells.

335.14

M CHANNEL MODULATION BY MUSCARINE IS AFFECTED BY INTRACELLULAR FREE Ca^{2+} AND A LIPOXYGENASE INHIBITOR
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Muscarinic modulation of the M-current (I_M) features a suppression followed by a transient overrecovery upon removal of agonist. We reported (Biophys J. 59:78a, 1991) that $[Ca^{2+}]_i$ modulates I_M in bullfrog sympathetic ganglion neurons. Increasing $[Ca^{2+}]_i$ from zero to 80 nM by intracellular perfusion doubled the size of I_M. The arachidonic acid (AA) pathway is involved in the Ca^{2+} modulation of I_M (Soc Neurosc Abs. 17(1):67, 1991). We studied the possible relations between $[Ca^{2+}]_i$ and muscarine responses, and the role of the AA pathway in overrecovery.

The experiments were performed in dissociated bullfrog ganglion B cells using whole cell recording and intracellular perfusion. Ringer contained 0.5 mM EGTA, 2.0 mM Mn^{2+} and no added Ca^{2+}. To keep the concentration of free BAPTA constant, internal solutions of zero and 80 nM Ca^{2+} were prepared with 11.4 mM and 20 mM BAPTA respectively.

Muscarine (1 μM) inhibited most of I_M (69±9% blockade, n=6) at 80 nM $[Ca^{2+}]_i$, while it displayed much less effect (29±12% blockade, n=8) at zero $[Ca^{2+}]_i$. At zero $[Ca^{2+}]_i$, little overrecovery occurred. At 80 nM $[Ca^{2+}]_i$, I_M transiently increased by 38% (n=21) when muscarine was removed. Overrecovery diminished with repeated application of muscarine. In intracellular perfusion experiments performed on a single cell starting with zero $[Ca^{2+}]_i$, larger overrecovery was observed from a second application of muscarine after raising $[Ca^{2+}]_i$ to 80 nM (n=11). The lipoxygenase inhibitor, NDGA (1 μM), blocked overrecovery (1.06±0.04 fold increase, n=4) with little effect on suppression of I_M. Indomethacin (10 μM), a cyclooxygenase inhibitor, neither affected overrecovery (1.47±0.07 fold increase, n=3) nor I_M suppression.

The data suggest that a minimum level of $[Ca^{2+}]_i$ is required for optimal suppression of I_M by muscarine and for overrecovery. The lipoxygenase pathway may be involved in the transient enhancement of I_M.

335.15

METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION INHIBITS POTASSIUM CONDUCTANCES IN NEURONS OF THE BASOLATERAL AMYGDALA. M.D. Womble*, K. Rusin and H.C. Moises. Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109.

The activation of muscarinic cholinergic receptors or metabotropic glutamate receptors on hippocampal pyramidal neurons produces membrane depolarization and a loss of the slow afterhyperpolarization (AHP). We have previously shown in neurons of the basolateral amygdala (BLA) that muscarinic agonists have a similar effect, partially due to the inhibition of the M-current (I_M) and the slowly-decaying AHP current (I_{AHP}). Therefore, we examined I_M and I_{AHP} in BLA pyramidal neurons of rat ventral forebrain slices for possible modulation by *trans*-ACPD, a metabotropic glutamate receptor agonist. Bath application of ACPD (20-100 μM) de-polarized the cell, decreased spike frequency accommodation and reduced the slow AHP. The M-current was identified in single-electrode voltage-clamp as a slow inward current relaxation during a 1 s voltage step to -55 mV from a holding potential of -40 mV. Application of ACPD reduced I_M amplitude to 33 $\pm$ 7.5% of control ($\pm$S.E.M.; n=12). The decay of I_{AHP} was examined following a 900 ms step depolarization from a holding potential of -60 mV. The amplitude of this current was reduced to 43.6 $\pm$ 12.2% of control (n=12) following ACPD application. The inhibitory effect of ACPD on I_M and I_{AHP} was reversed by washing and was not prevented by APV (50 μM), CNQX (20 μM) or atropine (2 μM). Since the metabotropic glutamate receptor subtype has been shown to be linked to stimulation of phosphoinositide (PI) hydrolysis, these findings suggest that the inhibition of I_M and I_{AHP} in BLA pyramidal neurones may be mediated by an increased rate of PI turnover. (Supported by DA03365 & AG10667.)

335.16

TWO MUSCARINE SENSITIVE POTASSIUM CURRENTS DIFFERENTIALLY INHIBITED BY BARIUM AND CESIUM. J.S. Coggan*, D.L. Kreulen. Dept. of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ

Muscarine evoked currents were studied under voltage clamp conditions in guinea pig celiac ganglion neurons in primary culture. Muscarine was applied for a short duration (1.5 sec) and responses were analyzed by time course, amplitude and voltage dependency. Cells were also tested for the presence of M-current, identified by its kinetics, voltage dependency and sensitivity to muscarinic agonists. No correlation was found between the magnitude of M-current and the amplitude of the response to muscarine.

Inward current responses to muscarine were produced by the inhibition of two potassium currents: the M-current and a voltage-independent K+ current. In most cells, the net response to muscarine was due to inhibition of both currents. Cesium and barium were used to test whether these two currents could be separated by differential sensitivity to potassium channel blockers. The cations were added to the bath medium as the chloride salts, each at 2.0 mM.

M-current was blocked to a greater degree by both barium and cesium (93% and 56%, respectively) than was the net response to muscarine (60% and 18% , respectively). In cells with no M-current, cesium had no effect on response to muscarine. These data indicate that M- current and the muscarine sensitive leak current may be mediated by different channels. HL-27781, Lilly Research Labs.

335.17

MUSCARINIC MODULATION OF VOLTAGE-GATED POTASSIUM CURRENTS IN ACUTELY-DISSOCIATED RAT NEOCORTICAL NEURONS. R.C. Foehring. Department of Anatomy and Neurobiology, University of Tennessee, Memphis, Memphis, TN 38103-4901.

Acetylcholine, acting at neocortical muscarinic receptors, has been implicated as important in learning, memory and several other forms of plasticity. Deficits in these processes accompanies the loss of cholinergic neurons in disease states such as Alzheimer's disease, but little is known regarding the biophysical mechanisms of action of muscarinic agonists on neocortical neurons.

This study examined the modulation of voltage-gated K+ currents by muscarinic agonists in acutely-dissociated neurons from rat sensorimotor or visual cortex and human temporal neocortex. There are three components to the voltage-gated K+ currents in these cells: non-inactivating (I_K), rapidly inactivating (I_A) and slowly-inactivating. Muscarine (50nM-100 μM; n=22 cells) or Carbachol (500 -1 mM; n=17) reduced, in a dose-dependent manner, the amplitude of the slowly-inactivating current observed during a 200 ms test pulse to -10 mV. This reduction resulted from a 3-10 mV hyperpolarizing shift in the steady-state inactivation curves. Activation voltage-dependence was unaltered. In 17 of 22 cells, muscarine also increased the current amplitude during the early part of the test command. This was due to an increase in the contribution of I_A. All of these effects were readily reversible. Supported by NINDS grant NS27180 and EPICARE, Memphis, TN.

335.18

SUBSTANCE-P MODULATES SEPARATE SET OF POTASSIUM CURRENTS IN BULLFROG PRIMARY AFFERENT NEURONS. T. Tokimasa*, M. Ishimatsu & T. Akasu. Dept. Physiol., Kurume Univ. Sch. Med., 67 Asahi-machi, Kurume 830, Japan.

A slowly inactivating delayed rectifier potassium current (I_K) and a non-inactivating M-type potassium current (I_M) were recorded from dissociated bullfrog dorsal root ganglion cells as described previously (Tokimasa *et al.* J. Physiol., **435**: 585-604, 1991). The pipette solution contained ATP (5 mM). Substance-P (SP, 100 nM) was added to the superfusate for 1-2 min every 25 min. SP facilitated by up to 70 % the I_K activation at the onset of step commands from -120 mV to potentials less negative than -60 mV. However, this was associated with a marked reduction in the amplitude for the 'non-zero' steady-state I_K (facilitation of inactivation), an action being responsible for a membrane depolarization in unclamped cells. These actions of SP quickly desensitized by about 80 % in about 2 min. Control I_K was regained within 5 min when the bath application of SP was discontinued. Substitution with ATP-γ-S in place of 5 mM-ATP in the pipette solution rendered the SP actions on I_K irreversible. GTP-γ-S (30-100 μM in the presence of ATP) gave essentially the same results as with ATP-γ-S. The actions of SP on I_K were attenuated to less than 30 % by pre-incubating the cells with pertussis toxin (PTX, 500 ng ml^{-1}) for 24 h at 22 °C. These observations suggest that the receptor occupancy by SP results in the activation of a class of GTP-binding proteins leading to the facilitation of both the activation and inactivation of I_K and that desensitization of the SP-induced responses may require a hydrolyzable form of ATP inside the cells. I_M was also inhibited by SP to less than 30 %. In terms of desensitization and its sensitivity to ATP-γ-S and GTP-γ-S, the actions of SP on I_M were quite similar to that for I_K. However, pre-incubation of the cells with PTX (500 ng ml^{-1}, 24 h, 22 °C) did not significantly attenuate the SP-induced inhibition of I_M implying that another class of GTP-binding proteins is involved for I_M.

335.19

PROSTAGLANDIN E_2 MODULATES I_K IN SUBCULTURED RAT TAIL ARTERY VASCULAR SMOOTH MUSCLE CELLS J. Ren*, E. Karpinski and C.G. Benishin.
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Arachidonic acid has been shown to modulate potassium (K) channels in vascular smooth muscle cells (VSMC). The role of prostaglandin E_2 (PGE_2), a metabolite of arachidonic acid, in the modulation of K channels has not been demonstrated. In this study, vascular smooth muscle cells were isolated by collegenase-elastase dissociation using the tail artery of 12-14 week male Wistar-Kyoto (WKY) rats. The cells were cultured in DMEM with antibiotics and supplemented with 10% fetal bovine serum. Subcultured cells (passages 8-12) were used for these studies. The whole-cell version of the patch clamp technique was used to measure potassium channel currents in these cells. Recordings were made using low intracellular Ca^{2+} (high pipette EGTA) solution to minimize the activation of the Ca^{2+}-activated K channels. From a holding potential of -80 mv, test pulses to more positive potentials generated a voltage-dependent, delayed rectifier K current (I_K). The current was activated at -40 to -30 mv and showed almost no inactivation. This current was inhibited by approximately 50% (test potential +30 mv) with 10 mM TEA. PGE_2 (1-100 μM) suppressed the outward K current. This suppression was concentration dependent. The kinetics of the current remained the same before and after PGE_2 and there was no shift in the activation voltage. This result suggests that PGE_2 modulates K channels (I_K) in subcultured rat tail artery vascular smooth muscle cells.

335.20

FURTHER STUDIES OF THE BLOCKING ACTION OF TRH ON A PERSISTENT K^+ CURRENT OF RAT MOTONEURONES. N. Fisher[1] and A. Nistri[2]. [1] Queen Mary and Westfield College, London E1 4NS, U.K. and [2] Lab. Biophysics, SISSA, Via Beirut 4, 34014 Trieste, Italy.

In spinal motoneurones a K^+ current active at rest ($I_{K(T)}$) is depressed by TRH in a voltage-sensitive fashion, thus producing an inward (depolarizing) current associated with a fall in cell conductance. Using single electrode voltage clamp the properties of this effect of TRH were investigated on neonatal rat motoneurones *in vitro* held at resting membrane potential (around -75 mV) and bathed in tetrodotoxin-containing medium. In the presence of TRH (1 μM) hyperpolarizing test pulses elicited Cs^+-insensitive inward currents displaying time-dependent outward relaxations which were enhanced or depressed by depolarizing or hyperpolarizing prepulses, respectively. The steadystate inward current induced by TRH was attenuated by high concentrations (1.5 mM) of Ba^{2+}, an effect seemingly independent of Ca^{2+} channel activity as it was maintained after application of Cd^{2+} (0.2-0.5 mM). Carbachol (50 μM) produced an inward current and a cell conductance decrease but did not prevent the effects of TRH. Substance P (30 μM) mimicked the action of TRH as it also produced a Ba^{2+}-sensitive inward current (with a conductance fall), although its potency was several fold smaller than that of TRH. Two pharmacologically inactive analogues of TRH, its deamidated form and histidylprolinediketopiperazine were found to be ineffective. These data support a selective and potent action of TRH in modulating $I_{K(T)}$ of spinal motoneurones. Supported by Takeda-Cyanamid.

335.21

AN ANTI-$G_{\alpha q}$ ANTIBODY BLOCKS THE PLC-DEPENDENT BRADYKININ RESPONSE IN NG108-15 CELLS. F. Belardetti *, S. Gutowski and P. Sternweis Dept. Pharmacology, Univ. TX Southwestern Med. Ctr. Dallas, Dallas, TX 75235.

Specific transmitter receptors, through activation of a pertussis toxin (PTX)-insensitive G protein, stimulate PIP_2 breakdown and generation of IP_3 and DAG in central neurons. By triggering Ca^{++} release in the cytoplasm and activating PKC, respectively, these two messengers regulate a variety of effector molecules, including ion channels. We have examined the role of a recently discovered PTX-insensitive G protein, G_q (Science, 251, 804), in coupling the bradykinin (BK) receptor to this cascade by using the NG108-15 neuroblastoma x glioma cell line and an antibody raised against the common C-terminal peptide of $G_{\alpha q}$ and its homolog, $G_{\alpha 11}$ (JBC 266, 20519). Differentiated cells were voltage-clamped at -40 mV using large patch pipettes (<1 M Ω) filled with internal medium (IM, mM): 120 KCl, 20 HEPES, 0.1 $MgCl_2$, 2 ATP and 0.1 GTP. Under these conditions, external application of BK (10 μM) transiently activated a Ca^{++}-dependent outward K^+ current. When IM plus the antibody (9:1) was perfused inside the pipette for 20-30 min using a fine tubing placed near its mouth, the BK response averaged 246 ± 256 pA (n=6, mean ±SD). If instead preimmune IgG was perfused in cells of approximately similar size, the response to BK was 550 ± 493 pA (n=6). These data indicate that G_q and/or its homologs mediate the modulatory action of a neurotransmitter on a K^+ channel.

335.22

PROPERTIES OF THREE DISTINCT VOLTAGE-ACTIVATED K^+ CURRENTS OF MURINE HIPPOCAMPAL NEURONS. X.Y. Li, J.J. Choi*, and J.J. McArdle. Departments of Pharmacology & Toxicology and Anesthesiology, New Jersey Medical School (UMDNJ), Newark, NJ 07103-2714.

Using the whole cell configuration of the patch voltage-clamp technique, two transient K^+ currents were distinguished from the delayed rectifier current (IK) of hippocampal neurons enzymatically isolated from adult mice. The time constant of inactivation was 55 ± 16 ms and 560 ± 150 ms for IAf and IAs, respectively. While 4-aminopyridine (4-AP, 5 mM) depressed the peak amplitude of IAf by 50%, neither TEA (10 mM) nor the chemical phosphatase 2,3-butanedione monoxime (BDM, 10 mM) altered this parameter. In contrast, the amplitude of IAs was reduced to 50% and 60% of control, respectively, by TEA and BDM. A pre-conditioning hyperpolarizing voltage step to -120 mV from a holding potential of -50 mV enhanced the amplitude of IAs. The magnitude of this enhancement was directly proportional to the duration of the pre-pulse and suppressed by both TEA and BDM, but not by 4-AP. Similar voltage-clamp protocols only slightly enhanced or even decreased IAf. Likewise, non-inactivating IK, which accounted for 10-30% of the total whole cell K^+ current, was unaffected by varying membrane potential from -120 to -50 mV. While TEA inhibited IK, both 4-AP and BDM were without effect. The sensitivity of these K^+ currents to the external Ca^{+2} concentration was IAs>IAf=IK. These data suggest that three pharmacologically distinct K^+ channels contribute to the whole cell current of adult hippocampal neurons. NIAAA grant AA08025 supported this work.

ION CHANNEL MODULATION II

336.1

DIRECT MEASUREMENT OF CYTOPLASMIC Ca BUFFERING IN SNAIL NEURONS SUGGESTS CO-LOCALIZATION OF Ca AND CAN CHANNELS. L.D. Partridge*, T.H. Müller, D. Swandulla. Max Planck Institute for Biophysical Chemistry, Göttingen, Germany & Dept. of Physiology, University of New Mexico, Albuquerque, NM 87131.

Calcium ions play an important role in coupling electrical activity in neurons to the activation of Ca-activated channels such as the CAN channel. The number Ca ions available to activate CAN channels depends on the diffusion of Ca from open Ca channels and on the cytoplasmic Ca buffering capacity. We measured the Ca binding capacity of snail neurons so that we could estimate Ca availability for CAN channel activation.

The large burster neuron of *Helix pomatia* was voltage clamped with a two-electrode voltage clamp and I_{Ca} was measured in response to depolarizing voltage clamp pulses. Fura-2 was iontophoresed into the cell and simultaneous fluorescent images were obtained. 380/360 ratio measurements were used to determine $[Ca]_i$ while $[fura\text{-}2]_i$ was determined from the 360 signal. The fraction of incoming Ca bound to fura-2 was calculated along with the fura-2 buffering capacity of the cell at various times during the iontophoresis. A double reciprocal plot was used to determine the endogenous buffering capacity of the cytoplasm, which was found to be approximately 500. Thus only about one in 500 Ca ions entering the burster neuron are free to activate Ca-activated channels. Comparing model calculations of CAN current activation with experimentally observed CAN currents suggests that the actual distance between Ca and CAN channels is significantly less than is expected from a uniform distribution of both channel types. Hence Ca and CAN channels are likely to be co-localized.

336.2

PATCH CLAMP STUDY OF Ca-ACTIVATED NONSELECTIVE CATION CHANNELS IN CHICK EMBRYO DORSAL ROOT GANGLION CELLS. S. Razani-Boroujerdi*, L.D. Partridge. Dept. of Physiology, Univ. of New Mexico, Albuquerque, NM 87131

Calcium-activated nonselective cation (CAN) channels have been studied in a number of cell types. In neurons, these channels are frequently associated with bursting and secretory activity. We have used inside-out membrane patches to study Ca activation of CAN channels in isolated sensory neurons of 10 to 14 day old chick embryos. The CAN channel in these neurons has a unit conductance of about 30 pS, is slightly voltage sensitive, and is rapidly activated by cytoplasmic free Ca^{++}. Calcium activation of this channel was studied by perfusing the cytoplasmic surface of inside-out patches with buffered Ca^{++} solutions. Free $[Ca^{++}]$ between 10^{-11} and 10^{-3} M was prepared from Ca^{++}/EGTA or Ca^{++}/HEDTA solutions calculated for the ionic strength, pH, and temperature. Free Ca^{++} was determined using fura-2 and fluorescent microscopy. The K_D for Ca^{++} activation of the CAN channel was between 0.1 and 1.0 μM and the Hill coefficient was not significantly different from one.

336.3

OPIOID PEPTIDE MODULATION OF CALCIUM-ACTIVATED POTASSIUM CURRENT IN ADRENAL CHROMAFFIN CELLS. W. A. Twitchell* and S. G. Rane. Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.

Adrenal chromaffin cells have opioid peptide receptors, they co-secrete opioids and catecholamines, and there is a peptide component to their preganglionic input. We are investigating opioid peptide effects on chromaffin cell potassium (K) and calcium (Ca) currents in order to assess the physiologic role of these peptides in the adrenal gland, and to develop a cellular model for opioid receptor action on neuronal cells. Adult bovine chromaffin cells were isolated by standard techniques and cultured on collagen-coated substrate at a starting purity of >90%. To isolate Ca-activated K currents in whole-cell (WC) and outside-out patch (OP) configurations the pipette solution had 150 mM K and 1 μM free Ca, and the bath had cadmium to block voltage-gated Ca current. Cells (or patches) were held at 0 mV to inactivate voltage-gated Na current and a delayed-rectifier type K current. Voltage ramps to 100 mV evoked WC currents or large-conductance, single channel currents (OP) with the voltage-dependence characteristic of maxi-K type Ca-activated K channels. The general opioid receptor agonist Leu-enkephalin (10 μM) increased WC Ca-activated K current throughout the voltage ramp by 95±9% (n=19 cells). The μ receptor agonist DAGO (10 nM) also increased Ca-activated K current (82±18%, n=4) while the κ receptor agonist DYNA 1-13 (30 nM) was ineffective (n=5). Activity of large-conductance (100 pS) K channels in OPs was potentiated by bath applied Leu-enk (5/5 patches) or DAGO (3/3 patches), suggestive of tight coupling between receptor and channel. To insure that opioid potentiation of WC Ca-activated K current was not an indirect consequence of up-modulation of Ca current, we tested Leu-enk on WC Ba currents (10 mM Ba in the bath, 150 mM Cs, 2 mM BAPTA in the pipette). Leu-enk (10 μM) inhibited Ba currents by 18±2 % (n= 4 cells), an effect which would be expected to suppress rather than potentiate Ca-activated K currents. DAGO was ineffective against Ba currents. δ receptor agonists have not yet been tested on either K or Ca currents.

336.4

DIFFERENTIAL MODULATION OF TTX-SENSITIVE AND TTX-INSENSITIVE Na^+ CURRENTS BY PROTEIN KINASE C IN SPINAL CORD ASTROCYTES

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Cultures of rat spinal cord astrocytes contain two astrocyte types which are distinguishable by their morphology as stellate, process bearing, or flat, "pancake," cells. Both astrocyte types are capable of expressing high densities of voltage-activated Na^+ channels (~8/μm^2). Stellate astrocytes, like most cultured neurons, express exclusively TTX sensitive currents (Kd~5nM), whereas pancake cells express TTX insensitive sodium currents (Kd~1000nM). We have found that these currents also differ in their response to activation of protein kinase C (PKC) by phorbol 12-myristate 13-acetate (PMA). TTX sensitive sodium currents, expressed in stellate astrocytes, showed a marked (up to 55%) decrease in peak current in response to a 5-10 minute exposure to 1μM PMA. In contrast, TTX insensitive sodium currents, expressed in pancake cells, increased up to 30% upon exposure to 1μM PMA. Both PMA effects could be blocked by pretreatment with the PKC inhibitor H7 suggesting that the effect is mediated by activation of PKC. Analysis of current kinetics (τ_m, τ_h) suggests that the observed changes are due to alterations in inactivation (τ_h), while in both types of responses, activation (τ_m), steady-state inactivation (h_∞) curves and the current voltage relationship remain unchanged in PMA.

336.5

ACTIVITY OF CYCLIC-AMP DEPENDENT SODIUM CHANNELS IN *Pleurobranchaea californica* PEDAL GANGLION IS ELEVATED WITH INTERNAL CALCIUM. L.C. Sudlow* & R. Gillette. Dept. of Physiology and Biophysics, U. of Ill., Urbana Il 61801.

Cyclic-AMP-activated sodium currents ($I_{(Na,cAMP)}$) underly neuromodulator induced bursting in the neurons of *P. californica* (Green & Gillette, 1983, Nature 306:784; 1988, J.Neurophys. 59:248). Using inside-out patches from serotonergic pedal neurons of *P. californica* (Sudlow & Gillette, 1991, Soc.Nsci.Abs 17:1390) we have characterized the interactions of cAMP and Ca^{++}_i on $I_{(Na,cAMP)}$ at the single channel level. cAMP (1 mM) activated an inward current of -2.08 ± 0.19 pA (mean ± sem, n=8) with a 22 pS conductance. As the saline contained no ATP, phosphorylation does not appear to be required for activity. The channels exhibited a percent open time (Po) of 2.84 ± 0.45% and a mean open time (MOT) of 2.46 ± 0.46 msec. The I-V relationship demonstrated a decrease in current at hyperpolarizing potentials and an increase at depolarizing potentials. When Ca^{++}_i and cAMP were applied Po increased in a $[Ca^{++}]_i$-dependent manner but there was no change in the amplitude of the openings. Po (13.9%) and MOT (3.57 msec) were maximal in 200 nM Ca^{++}_i with cAMP. Ca^{++}_i entering during bursting augments the activation of $I_{(Na,cAMP)}$ by cAMP. From the I-V curve, depolarization causes an elevation in $I_{(Na,cAMP)}$. This positive feedback contributes to the excitation during bursting. This research was supported by NIH RO1 NS26838 to RG.

336.6

ROLE FOR PROTEIN KINASE C IN MUSCARINIC MODULATION OF SINGLE CALCIUM CHANNEL CURRENTS IN INSULIN-SECRETING HIT-T15 CELLS. J.A. Love*, N.W. Richards, C. Owyang, and D.C. Dawson. Depts. of Internal Medicine and Physiology, Univ. of Michigan Med. Center, Ann Arbor, MI 48109

Muscarinic agonists potentiate glucose-stimulated insulin secretion and protein kinase C (PKC) appears to play an important role in this effect. We have demonstrated (Neurosci. Abst. 17:62, 1991) that muscarinic agonists increase the activity of single, L-type, voltage-dependent Ca^{++} channels, independent of changes in membrane potential, in cell-attached patches from HIT-T15 cells. We used phorbol esters and DAG analogs to test the role of PKC in channel activation. Acute exposure (5-40 min) to the phorbol ester PMA (200 nM) increased fractional open-time (.048 vs .120) in 5/5 cells while the inactive compound 4 α-phorbol had no effect (.064 vs .075) in 3/3 cells. Similarly, the DAG analog DC_{10} (5 μg/ml) significantly increased fractional open-time (.040 vs .086) in 5/7 cells. Exposure to bethanechol (100 μM) resulted in an significantly increased fractional open-time (.030 vs .101) in 6/6 cells. However, in cells chronically exposed to PMA (200 nM; 20-32 hrs) to deplete PKC activity, bethanechol caused a small increase in fractional open-time (.061 vs .076) in only 3/7 cells. Conversely, in 4/5 cells chronically exposed to the inactive compound 4α-phorbol (200 nM; 20-32 hrs), bethanechol caused increases in fractional open-time (.038 vs .120) comparable to those seen in untreated cells. These results suggest that PKC plays an important role in mediating muscarinic stimulation of Ca^{++} channel activity in HIT-T15 cells. Supported by the NIH.

336.7

Comparison of the Action of two Protein Kinase C Activators on Dihydropyridine-sensitive Ca^{2+} Channels in Neonatal Rat Ventricular Myocytes

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It has been reported that Ca^{2+} channels can be modulated by protein kinase C (PKC) which phosphorylates Ca^{2+} channel proteins. Two PKC activators, 4β-phorbol 12-myristate 13-acetate (PMA) and 1-oleoyl-2-acetyl-rac-glycerol (OAG), were used to investigate the effect of PKC activators on Ca^{2+} channel currents in neonatal rat ventricular cells. The whole cell version of the patch clamp technique was used. From a holding potential of -40 mV, test pulses to more positive potentials generate L type Ca^{2+} channel currents (20 mM Ba^{2+} was used as the charger carrier). The current was activated at -20 mV and the peak current occurred at +10 mV. PMA (200 nM) or OAG (60 μM) significantly increased the L type Ca^{2+} channel current in cardiac myocytes. However, the effect of PMA on the L type current reached its peak at 3-5 min after addition of the drug to the bath and then gradually decreased. Fifteen min after the addition of PMA, the current decreased below the control level. When OAG was used, the current reached its peak at 5-10 min and then was maintained at this level for 15 min. The results obtained show that the two PKC activators increase Ca^{2+} channel currents uniquely with respect to time. An explanation of these results which are consistent with other reports is that PMA down-regulates PKC whereas OAG persistently activates PKC. In addition, the Ca^{2+} channels modulated by PMA or OAG were sensitive to nifedipine. Nifedipine (10 μM) completely blocked the L type Ca^{2+} channel current which included both the enhanced current induced by OAG or PMA and the current generated by depolarization, indicating that PKC-activated channels were also DHP-sensitive channels. In summary, the results from these experiments suggest that PMA and OAG, PKC activators, activate DHP-sensitive L type Ca^{2+} channel current in neonatal rat ventricular cells in unique temporal patterns.

336.8

CYCLIC GMP REGULATES CALCIUM CHANNELS IN NEURONS OF RABBIT VESICAL PARASYMPATHETIC GANGLIA. T. Akasu*[1], T. Nishimura[1] & J. Krier[2]. [1]Dept. Physiol., Kurume Univ. Sch. Med., Kurume 830, Japan & [2]Dept. Physiol. Mich. State Univ. East Lansing, MI 48824, USA.

The effects of dibutyryl guanosine 3',5'-cyclic monophosphate (db-cGMP) were studied, in vitro, on calcium channels of neurons in rabbit vesical parasympathetic ganglia (VPG), using intracellular and single-electrode voltage-clamp recordings. Db-cGMP (100 μM) produced a biphasic response, initial depolarization associated with a decrease in membrane input resistance followed by hyperpolarization with increased input resistance. Db-cGMP increased the amplitude and duration of spontaneous hyperpolarization. Under voltage-clamp recording, db-cGMP (0.01-1 mM) caused a concentration dependent, transient inward current followed by a long-lasting outward current. Membrane conductance was increased and decreased during the inward and outward currents, respectively. The inward current produced by db-cGMP was depressed in nominally Ca^{2+}-free solution, by Co^{2+} (1 mM) and nicardipine (10 μM) but not by ω-conotoxin (2 μM). The mean reversal potentials of the inward current was +42 mV and -20 mV in the presence and absence of Ca^{2+} in the external solution, respectively. The inward current was not altered by lowering $[Na^+]_o$, raising $[K^+]_o$ or by $[Cs^+]_i$. Ca^{2+}-insensitive component of the inward current was increased by lowering $[Cl^-]_o$ and blocked by a stilbene derivative, SITS. Voltage-dependent high-threshold (ω-conotoxin sensitive) Ca^{2+} currents were depressed during the db-cGMP-induced inward current and facilitated during the outward current. IBMX potentiated the effects of db-cGMP on Ca^{2+} channels. CGMP (1 mM) was less potent than db-cGMP (1 mM) in causing both inward and outward currents or modulation of Ca^{2+} currents. GTP, GDP, GMP, guanosine, 8-bromo-cAMP and forskolin had no effect on the membrane currents. It is concluded that intracellular cGMP not only activates resting Ca^{2+} and Cl^- channels but also modulates voltage-dependent Ca^{2+} channels in VPG neurons.

336.9

SHORT-TERM INCREASES IN TRANSIENT Ca CURRENTS IN RAT THALAMIC CELLS FOLLOWING CORTICAL INJURY.

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Changes in voltage-dependent ionic conductances in cerebral neurons following cortical injury have not been extensively investigated. Such alterations might have a role in mediating neuropathologic processes such as retrograde degeneration. Unilateral ablations of somatosensory cortex were employed to produce axotomy and deafferentation of thalamic relay neurons (RNs). We isolated neurons from ventrobasal complexes both ipsi- and contralateral to the lesion and recorded Ca currents using whole-cell voltage-clamp. One day after cortical ablation, transient currents (I_T), but not L-type currents, increased significantly in RNs ipsi- (I_T = -293 pA, n=10) but not contralateral (I_T = -174 pA, n=10) to the site of cortical lesion. Three days post lesion, I_T amplitudes were -103 pA (n=6) for ipsi- and -233 pA (n=6) for contralateral RNs. The early increases in I_T were not accompanied by changes in either kinetics or voltage dependence of activation and inactivation. These findings suggest that cortical lesions can alter I_T through changes in the number of available channels or the channel unit conductance. We speculate that this selective early increase in I_T may have a role in the development of thalamic injury following cortical lesions. (Supported by NIH grants NS06477 and a Pimley postdoctoral fellowship to JMC.)

336.10

EFFECTS OF ADENOSINE ON VOLTAGE-DEPENDENT CALCIUM CURRENTS IN MOUSE SPINAL NEURONS. M. Mynlieff* and K.G. Beam. Dept. of Physiology, Colorado State University, Fort Collins, CO 80523

Adenosine has been shown to reduce evoked release of acetylcholine from the mammalian neuromuscular junction, an effect mediated by A1 receptors and abolished by pertussis toxin treatment. We have previously shown that adenosine (40 μM) decreased the transient high-voltage activated (HVA) calcium current (28.9 ± 3.7%, n = 9), the sustained HVA calcium current (23.2 ± 2.7%, n = 9) and T-current (25.0 ± 10.4%, n = 7) in identified mouse motoneurons. Adenosine did not inhibit dihydropyridine potentiated tail current indicating that the inhibition of the sustained HVA current is not due to an inhibition of L type channels. Currently we are using whole-cell voltage clamp recording from large (> 15 μm in diameter) embryonic mouse spinal neurons to study the pharmacology of the adenosine effect. The A1 agonist, N^6-cyclohexyladenosine (CHA) inhibited the transient HVA current (Kd ≈ 50 nM, maximal effect = 38.1% inhibition). The non-selective agonist, 5'-N-ethylcarboxamide adenosine (NECA) inhibited the transient HVA current (Kd ≈ 31 nM, max. eff. = 47.8%) and the sustained HVA current (Kd ≈ 43 nM, max. eff. = 34.6%). In contrast to CHA, NECA also showed variable inhibition of the T-current. The A1 antagonist 8-cyclopentyltheophylline (CPT, 100nM) blocked the effect of 250 nM NECA on the HVA currents but not the effect on T-current. These data suggest that the inhibition of HVA current is mediated by A1 receptors and the inhibition of T-current may be mediated by A2 receptors. Since the inhibition of neuromuscular transmission has been shown to be mediated by the A1 receptor, it is likely that this effect is produced by a reduction in the non-L type HVA calcium current. Supported by NIH grant NS-26416 to KGB and NIH fellowship NS-08769 to MM.

336.11

COMPARISON BETWEEN THE EFFECTS ON Ca CURRENT OF AN ADENOSINE ANALOGUE AND THE GENERAL ANAESTHETIC PROPOFOL IN CHICK-EMBRYO SENSORY NEURONS. M.Ottolia, V.Magnelli, M.Nobile, C.Usai
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Adenosine and its analogue are known to interact with some general anaesthetics. We studied Ca current in dorsal root ganglion (DRG) neurons from 10÷12 day old chick embryos, treated with the adenosine analogue R-PIA (10 μM) and the general anaesthetic Propofol (300 μM). R-PIA reduced by 50% low-voltage activated (LVA) calcium current in a reversible manner. R-PIA also affected the ω-conotoxin sensitive component of the high-voltage activated (HVA) Ca^{++} current (70% reduction). In cells where the HVA current was almost completely insensitive to this toxin, R-PIA was also ineffective. Both Propofol and R-PIA blocked part of the HVA current,but, when applied together, the resulting current block was the linear sum of the separate reductions.Reciprocal potentiation was not observed.In neurones where Propofol blocked completely the calcium current R-PIA did not produce effects. These results suggest that Propofol interacts mainly with type L channels, while R-PIA acts on type N Ca channels.

336.12

INHIBITORY AND EXCITATORY EFFECTS OF ADENOSINE ON CA1 NEURONES IN RAT HIPPOCAMPUS *IN VITRO*. H. Li* & J.L. Henry Depts of Physiol. & Psychiat., McGill Univ. Montreal, Quebec
Adenosine participates in synaptic and metabolic functions in the CNS. Inhibitory actions are brought about by a presynaptic inhibition of transmitter release and through a postsynaptic increase in a K^+ conductance. Recently, we have found in *in vivo* experiments that a postsynaptic inhibitory effect is blocked by the K_{ATP} channel blocker, glibenclamide (*Synapse*, in press). Experiments were done to explore this possibility *in vitro*. Results from 64 cells in the CA1 hippocampal region indicated that adenosine and adenosine 5'-monophosphate(AMP) added to the perfusion fluid at 100 μM evoked a hyperpolarization (range 3-15 mV) associated with a 30% reduction in input resistance (range 5-50%; n=32), and blocked EPSPs and IPSPs evoked by stimulation of the stratum radiatum and alveus, respectively. In addition to this well-known hyperpolarization, adenosine and AMP also produced a delayed depolarization associated with an increase in spontaneous spikes in 50 % of the cells (n=32); recovery occurred over 30 min. For comparison, baclofen was tested as it is also thought to be coupled to a K^+ channel; 100 μM induced a hyperpolarization similar to that induced by the purines but the delayed depolarization was not observed. When only 1 μM of adenosine or AMP was given no measurable effect occurred on resting membrane potential or input resistance but there was a delayed increase in spontaneous action potentials, suggesting a dose-dependent separation of the inhibitory and excitatory effects. Glibenclamide prevented purine-induced hyperpolarization but did not block the inhibition of PSPs or the delayed depolarization. Our results indicate that the modulation of synaptic transmission in the hippocampus by adenosine involves inhibitory effects mediated via at least 2 or 3 mechanisms, as well as excitatory effects. (Support: Canadian MRC)

336.13

THE GENERAL ANAESTHETIC PROPOFOL AFFECTS Ca^{++} CURRENT IN CHICK EMBRYO SENSORY NEURONS.
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The effects of the general anaesthetic Propofol on calcium current were studied, in dorsal root ganglion neurons from 10÷12 day old chick embryos. Propofol (300 μM) reversibly reduced both low-voltage activated Ca current (LVA) by 80% and high-voltage activated Ca current (HVA) by 20÷70%. Moreover the anaesthetic modified HVA current inactivation kinetics: with Propofol in the external solution the inactivation time constant τ_h was approximately the 50% of the control (Propofol: $\tau_h \approx 55$ ms; control: $\tau_h \approx 120$ ms). To discriminate between HVA current components we used ω-conotoxin (5 μM) that selectively blocks N type Ca channels. We found that the effects of propofol were stronger on ω-conotoxin insensitive currents. This result suggests that propofol interacts preferentially with ω-conotoxin insensitive "L" type Ca channels. In cell attached patches, Propofol added to the external solution had no effects on channel activity, indicating that the block is mediated by a tight drug-channel interaction.

336.14

NOVEL MODULATION OF VOLTAGE-DEPENDENT SODIUM CHANNELS BY G-PROTEINS IN SENSORY NEURONS. R.Y.K. Pun* and R. C. Gesteland. Dept. of Physiol. & Biophy., and Dept. of Anat. & Cell Biol., Univ. of Cincinnati, OH 45267.
There is considerable evidence that voltage-dependent ion channels in excitable membranes are modulated by G-proteins and phosphorylation. We report here a novel action in which the availability of Na^+ channels in frog olfactory neurons (ORNs) is enhanced by GTP and GTP-γ-S. The steady-state inactivation of Na^+ currents (I_{Na}) was shifted ~20 mV towards positive membrane potentials by both GTP and GTP-γ-S, whereas the activation was not significantly shifted. Our results indicate that activation of G-proteins, and/or phosphorylation of the Na^+ channels in frog ORNs can lower the threshold for spike generation by increasing the availability of Na^+ channels. This is in contrast to the findings in the heart where G-proteins reduce Na^+ channels availability. Experiments were performed on freshly isolated ORNs using the whole-cell voltage clamp technique. The effects of the nucleotides (100 μM) were evaluated by inclusion in the pipet-solution. With pipet solution alone, the $V_{1/2}$ for I_{Na} inactivation was -80.2 $\pm$ 3.8 mV (n=8; mean $\pm$ s.e.m). GTP-γ-S and GTP both shifted the $V_{1/2}$ to more positive potentials (GTP-γ-S, -61.5 $\pm$ 3 mV, n=9; GTP, -61.7 $\pm$ 3.1 mV, n=10; t-test, $p<0.01$ in both cases). The $V_{1/2}$ for I_{Na} activation was not shifted significantly (control, -46.8 $\pm$ 2.9 mV, GTP-γ-S, 41.1 $\pm$ 2.4; n=6). Interestingly, the negative shift in I_{Na} inactivation with time was also delayed and reduced when GTP or GTP-γ-S was present in the pipet. (Supported by NIH Grants DC 00352 and DC 00347)

336.15

GTP ANALOGUES MAINTAIN $GABA_A$ RECEPTOR FUNCTION IN DORSAL ROOT GANGLION CELLS. D. J. Bradshaw* and M. A. Simmons Dept. of Pharmacology, Marshall University, Huntington, WV 25755-9310.
Preliminary data from our lab has agreed with previous reports that the hydrolysis of ATP might be necessary for the $GABA_A$ receptor to maintain function with repeated agonist exposure. The objective of this study was to evaluate a possible role for GTP on $GABA_A$ receptor function. Individual bullfrog dorsal root ganglion (DRG) cells were voltage clamped at -70 mV using whole cell recording. GABA (100 μM) was applied extracellularly via superfusion. ATP, UDP, and GTP analogues were applied intracellularly via the recording electrode. The GABA-activated inward current was recorded in the presence of tetrodotoxin and cesium. Results are given as the mean percentage of current remaining at 10 and 25 minutes, respectively, postimpalement. When the recording electrode contained no ATP or GTP the response to GABA exhibited run-down to 79% at 10 minutes and 4% at 25 minutes. Inclusion of GTP-γ-S or GMP-PNP (5 mM) protected against this run-down. With GTP-γ-S the responses remaining were 118% and 78% at 10 and 25 minutes, respectively. With GMP-PNP the responses that remained were 108% and 70%. Intracellular application of ATP (1.5 mM) gave similar results. Inclusion of UDP (10 mM) with ATP, to inhibit transphosphorylation, revealed 2 types of neurons. Cells with large initial responses to GABA (mean of -2.86 nA) gave stable responses of 108% and 102%. Cells that had smaller intitial currents (-0.16 nA) ran down to 49% and 23%. Our results show that GTP may regulate the function of the $GABA_A$ receptor by a mechanism that is distinct from the regulation by ATP.

336.16

PERTUSSIS TOXIN SENSITIVE G-PROTEIN(S) MEDIATE MULTIPLE ACTIONS OF FMRFAMIDE IN AN IDENTIFIED *HELISOMA* NEURON. F.H. Bahls* and P.G. Haydon. Department of Zoology and Genetics, Iowa State University, Ames, IA, 50011.
FMRFamide produces a presynaptic inhibition of transmitter release in neuron B5 of *Helisoma trivolvis* through multiple pathways. These include a reduction in a voltage sensitive calcium current, a reduction in the responsiveness of secretory machinery to changes in internal calcium and an increase in a potassium current. It has previously been demonstrated that the actions of FMRFamide on calcium current and secretory machinery are mediated through a pertussis toxin (PTX) sensitive G-protein(s). To test for the involvement of a similar G-protein in mediating activation of the potassium current, we injected the A protomer of PTX into acutely isolated B5 somata. PTX injection reduced the magnitude of the FMRF-amide-activated potassium current at 0 mV from 1.68 $\pm$ 0.66 nA (n=4, heat-inactivated PTX) to 0.06 $\pm$ 0.24 nA (n=4) suggesting that a PTX-sensitive G-protein is also involved in mediating the action of FMRFamide on the potassium current.
Arachidonic acid metabolites have been shown to be required for the activation of the potassium current. FMRFamide's activation of potassium current is reduced to 10 $\pm$ 7% of control by the phospholipase A_2 inhibitor BPB. However, in the presence of 10μM BPB, FMRFamide's reduction of calcium current remained at 87 $\pm$ 28% (n=7) of control, suggesting that arachidonic acid is not involved in the modulation of calcium current. However, the time constant of the effect of FMRFamide on calcium current is 1.82 $\pm$ 0.56 sec suggesting that other, as yet undetermined second messengers are involved in the reduction of calcium current by FMRFamide.

336.17

5-HT ACTIVATES MULTIPLE SUBSTATES OF INWARDLY RECTIFYING K^+ CHANNELS IN RAT DORSAL RAPHE NEURONS. N.J.Penington,* J.S.Kelly and A.P.Fox Univ.Chicago,Chicago IL,60637; Univ.Edinburgh U.K.

Single inwardly rectifying K^+ channel currents were recorded from acutely isolated adult serotonergic dorsal raphe (DR) neurons using the cell-attached patch (C/A) and outside-out (O/O) configuration. Four equally spaced conductance levels were observed in both modes of recording, with conductance levels averaging 11, 21, 30, and 40 pS but a range of larger conductance levels (50-120 pS) were also seen less frequently. The single channel I-V relation was linear in the range -20 mV to -100mV. The different conductance levels showed direct transitions between each other and apparent direct closures from the higher conductance state. This suggested that they resulted from substates of a single channel rather than several different channels. When the neurotransmitter 5-Hydroxytryptamine (5-HT) was added to the bath, during recording in the C/A configuration, the channels in the patch showed no change in their probability of opening indicating a lack of an easily diffusible second messenger. The single K^+ channel activity however was increased by an average of 670% by the addition of 5-HT to the bath when recording channel activity in the O/O configuration. In high $[K^+]_o$ some single channel activity was always present even in the absence of any ligand. All K^+ channel subconductance levels increased in activity, but in 15 cells the level that increased most was the 30 followed by the 40 pS conductance level. These findings lend further support to the interpretation that the conductance levels may be substates of one another, and that the 30 and 40 pS levels are favored in the presence of 5-HT. If the different conductance levels represented separate channels, the effect of 5-HT would not be expected to be observed on so many conductance levels.

336.18

5-HT_3 CHANNEL ACTIVATION BY SEROTONIN IS ANTAGONIZED BY COCAINE IN NEURONS FROM RAT NODOSE GANGLION. P. Fan, S. Visentin and F.F. Weight*. Lab. Molecular & Cellular Neurobiology, National Institute on Alcohol Abuse & Alcoholism, Rockville, MD 20852.

Behavioral effects related to cocaine administration have been reported to be altered by 5-HT_3 antagonists (*Pharmac. Ther.* 47: 181-202, 1990). We studied the effect of cocaine on serotonin activation of 5-HT_3 channels in neurons freshly dissociated from rat nodose ganglia using the whole-cell patch-clamp technique. Serotonin activated a fast inward current with a concentration-response curve from 0.1 to 100 μM (EC_{50}=4.55 μM; Hill coefficient=1.33). Cocaine produced a concentration-dependent reduction of peak serotonin-activated current amplitude (IC_{50}=0.99 μM, Hill coefficient =1.15 with 1 μM serotonin). Increasing concentrations of serotonin produced a parallel shift to the right of the cocaine concentration-response curve (IC_{50}=4.9 μM, Hill coefficient=1.26 with 10 μM serotonin). Schild plots revealed an average pA_2=5.4 and K_B=3.8 μM. The results are consistent with competitive antagonism of serotonin activation of neuronal 5-HT_3 channels by cocaine. This effect is in a concentration range that occurs in plasma during cocaine abuse.

336.19

NEUROTRANSMITTER MODULATION OF CALCIUM CURRENTS RECORDED IN RAT SUBSTANTIA NIGRA ZONA COMPACTA NEURONS *IN VITRO*. M.S. Washburn* and L.T. Meltzer Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI. 48105.

Modulation of calcium currents (I_{Ca}s)by various neurotransmitter agents was investigated using whole-cell recordings from substantia nigra zona comapcta (SNZC) dopaminergic neurons *in vitro*. Such cells were identified electrophysiologically in current-clamp recordings by their broad action potentials, regular pacemaker firing and strong anomalous rectification. We earlier presented evidence for the existence of I_{Ca}s which appeared to represent the T, N and L classes of I_{Ca}s (Soc. Neurosci. Abstr, 1991). In the present study, GABA (10-30 μM) reversibly reduced the inward currents evoked by stepping from a holding potential of -80 or -40 mV to a comand potential of 0 mV. These effects appeared to be mediated via activation of $GABA_B$ receptors since they occurred in the presence of the $GABA_A$ antagonist bicuculline methiodide (30 μM), were mimicked by the $GABA_B$ agonist baclofen (3-30 μM) and were reversed by the $GABA_B$ antagonist 2-hydroxysaclofen (100-200 μM). Baclofen (10 μM) reduced the high-threshold currents evoked from -40 or -80 mV by approximately 20% and did not affect the voltage-dependence of activation of the currents, although the activation of the currents evoked from -40 mV was slowed in some cells. The transient low-threshold calcium current evoked by stepping from -95 mV to between -60 and -35 mV was not reduced by GABA or baclofen. These results provide additional physiological significance for the GABAergic innervation of the SNZC from the striatum and substantia nigra pars reticulata. Furthermore, the results suggest that GABA, in addition to hyperpolarizing SNZC neurons, may modulate the activity of these cells by changing their calcium permeability.

A number of other agents were tested for their ability to modulate I_{Ca}s in SNZC cells. The D2 dopamine agonist quinpirole, serotonin, noradrenaline, the metabotropic glutamate receptor agonist trans-ACPD and the cholinergic agonists oxotremorine-M and carbachol (each at a concentration of 10-30 μM) had no consistent effect on either the high- or low-threshold I_{Ca}s recorded in SNZC dopaminergic neurons.

336.20

FMRF-AMIDE MODULATES MULTIPLE IONIC CONDUCTANCES IN THE LEECH RETZIUS CELL. C.L. Sahley*[1], I.A. Strong[1], and A. Kleinhaus[2]. [1]Dept. of Biological Sciences, Purdue University, W. Lafayette, IN 47907 and [2]Dept. of Cell Biology and Anatomy, New York Medical College, Valhalla, NY 10595.

The Retzius cell (R) is a serotonergic, multifunction neuron which is crucial for the control and integration of vital behaviors. We have previously reported that the endogenous transmitters serotonin (5-HT) and FMRF-NH2 (FMRF) modulate the excitability of the R cell in opposite directions (Acosta et al., J. Exp. Biol., 1989; Kleinhaus & Sahley, Soc Neurosci Abs., 1989). 5-HT inhibits the R cell and decreases the action potential (AP). FMRF transiently depolarized the R cell, broadened its AP and transformed its firing pattern from a "beating" to a "bursting" rhythm. We now present voltage-clamp evidence that FMRF's effects on the R cell can be explained by the modulation of multiple ionic conductances. In the negative potential region (-110 to -30 mV) FMRF transiently induced a voltage-independent inward current, (I_{ns}), a nonspecific or divalent cation current. This current is unaffected by altering Na_0, K_0 (.3 to 30 mM) and Cl_0 (20 to 140 mM) and Cl_I and can be observed in solutions with normal Ca^{+2} or Ba^{+2} (1 to 8 mM). This current may underlie the FMRF-induced depolarization. In addition, FMRF also increased the amplitude of voltage activated Ca^{+2} currents and decreased the amplitude of I_A without altering their voltage dependencies. We suggest that these effects account for FMRF's effect on AP duration and increased firing rate. (Whitehall CS, AK; R01 MH 44789 CS)

336.21

INOSITOL TRISPHOSPHATE-MEDIATED CURRENTS IN XENOPUS OOCYTES REFLECT SPATIAL AND TEMPORAL DERIVATIVES OF INTRACELLULAR CALCIUM I. Parker* and Y. Yao. Lab. Cellular and Molecular Neurobiology, Dept. Psychobiology, University of California, Irvine, CA 92717.

Inositol 1,4,5-trisphosphate ($InsP_3$) functions as a second messenger in Xenopus oocytes to liberate Ca^{2+} from intracellular stores, and thereby activate a Ca^{2+}-dependent membrane Cl^- conductance. However, signals recorded by fluorescent indicators reveal that elevations of intracellular free Ca^{2+} are more prolonged than the corresponding membrane current responses. To resolve this discrepancy, we have imaged intracellular free Ca^{2+} (using fluo-3) while simultaneously recording membrane currents evoked by agonist application and photorelease of $InsP_3$ from a caged precursor. The results show that the time course of the current corresponds well with the temporal and spatial derivatives of intracellular free Ca^{2+} (ie. the rate of rise of Ca^{2+} concentration with time and across the oocyte surface). The basis of this effect remains to be determined, but probably does not involve inactivation or desensitization of the Cl^- channels, since little depression is seen with paired photorelease of Ca^{2+} from caged Ca^{2+}. Whatever the mechanism, the Cl^- conductance appears to act as a high-pass filter, so that spikes or oscillations of Ca^{2+} are transduced as electrical signals, while steady signals are blocked. This property is consistent with the notion (Berridge et al., Phil. Trans. R. Soc. Lond. B320, 325, 1988) that information transmitted by the $InsP_3/Ca^{2+}$ pathway may be encoded digitally as the frequency of Ca^{2+} spiking.

Supported by grant GM39831.

337.1

INTERACTIONS OF DITHIOLS WITH REDUCED OR ARSENYLATED NICOTINIC RECEPTORS AND RECEPTOR PEPTIDES. R.H. Loring,* Y.M. Dou, W. Lane, C. Rossant, and E. Hawrot‡. Dept. of Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115 & ‡Dept. of Molecular & Biochemical Pharmacology, Brown Univ., Providence, RI 02912.

We have shown that reduced Torpedo nicotinic receptors arsenylated with p-aminophenylarsinoxide (APA) 1) cannot be reoxidized with dithiobisnitrobenzoate (DTNB), 2) do not bind ^{125}I-α-bungarotoxin (BGT), and 3) are protected against irreversible bromoacetylcholine (BAC) alkylation (Loring, et al., Mol. Brain Res., in press). Effects 1 & 2 are reversed by the antiarsenical dithiol, dimercapto-propanesulfonate (DMPS). Curiously, DMPS by itself protects reduced receptors against irreversible alkylation by BAC, suggesting that DMPS oxidizes reduced nicotinic receptors. We therefore investigated whether similar dithiols either oxidize or remove APA from various subtypes of reduced or arsenylated nicotinic receptors. In solution, APA forms stable complexes with DMPS, dimercaptosuccinate (DMSA), dimercaptopropanol (BAL), and a reduced peptide of Torpedo receptor (α182-197), but does not react with with dithiothreitol (DTT) or mercaptoethanesulfonate (MESA). APA (10 μM) plus 300 μM DTT readily arsenylates immunoimmobilized Torpedo receptors. BAL and DMPS both remove APA from arsenylated Torpedo receptors and protect reduced receptors against alkylation by BAC, while DMSA and MESA do not. Preliminary studies suggest that DMPS removes APA from an arsenylated Torpedo peptide (α181-198), and that BAL and DMPS reverse the effects of arsenylation on ^{125}I-BGT binding sites in chick retina, and on ^{3}H-cytisine binding sites immunoprecipitated from chick brain using mAb299. Thus, APA may be used with DTT to trap a variety of reduced nicotinic receptor subtypes in an arsenylated state, and BAL or DMPS may be used to reverse the effects of APA and to reoxidize the receptors.
(Supported by NS22472 and the Smokeless Tobacco Research Council).

337.2

REDOX EFFECTS OF NEREISTOXIN ON NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs) OF CHICK CILIARY GANGLION. Y. Xie*, L-H. Tang‡, E. Aizenman‡ & R.H. Loring. Dept. of Pharmaceutical Sciences, Northeastern University, Boston, MA 02115 & ‡Dept. of Physiology, University of Pittsburgh, Pittsburgh, PA 15261

We have shown that nereistoxin (NTX, 4-N,N-dimethylamino-1,2-dithiolane) blocks nicotinic function in chick retina by reducing nAChRs. Here we examine NTX effects on nAChRs in chick ciliary ganglia. Extracellular recordings reveal that NTX (IC_{50}≈10 μM) inhibits nAChR-mediated synaptic transmission in a long-lasting manner that is not reversible upon washing. The oxidizing agent dithiobisnitrobenzoic acid (DTNB, 1 mM) completely reverses NTX's actions. NTX-treated ganglia are irreversibly alkylated by bromoacetylcholine (BAC, 100 μM, with 2 μM neostigmine). We find similar results in preliminary studies using the whole-cell patch-clamp recording technique. 100 μM NTX substantially blocks inward currents in ciliary ganglion cells induced by the nicotinic agonist DMPP (100 μM, 1 sec). DTNB (500 μM, 1 min) completely reverses NTX's inhibition. These studies suggest that NTX reduces nAChRs of chick ciliary ganglia and thus inhibits nicotinic function. In intact chick ciliary ganglia, 30 μM DMPP only weakly protects nAChRs against NTX reduction (23% of control) but substantially protects nAChRs against reduction by dithiothreitol (2 mM; 86% of control). We find that NTX is a potent and selective reducing agent for nAChRs and has no effect on NMDA receptors. We hypothesized that NTX might diffuse across the cell membrane to be reduced to dihydronereistoxin, which could reduce the disulfide bond near the agonist binding site of nAChRs. However, low pH (4-6) decreases NTX potency only 10-15 fold, whereas high pH (8-10) slightly enhanced NTX inhibition (≈2 fold). Thus, protonized NTX (NTX pK_a=7.2) still blocks nAChRs. The precise mechanism of NTX's actions remains to be determined.
(Supported by NS22472, DA04975, and the Smokeless Tobacco Research Council).

337.3

A DISULPHIDE BOND IS INVOLVED IN THE AGONIST BINDING SITE OF NICOTINIC ACETYLCHOLINE RECEPTORS IN THE INSECT NERVOUS SYSTEM. D.Bai, C.A Leech and D.B.Sattelle*. AFRC Laboratory of Molecular Signalling, Department of Zoology, Downing Street, Cambridge, CB2 3EJ, UK.

Insect nicotinic acetylcholine receptors can be functionally expressed in *Xenopus* oocytes following injection of mRNA encoding an α-subunit alone. These studies indicate that the agonist binding site is located on the α-subunit and sequencing of these subunits has shown the presence of conserved cysteine residues which could form an extracellular disulphide bond. Compounds which reduce disulphide bonds, such as dithiothreitol, inhibit the nicotine-induced depolarization of cockroach motoneurone D_f by 55%. This effect can be prevented by high concentrations of agonist suggesting that occupancy of the ligand binding site masks the disulphide bond against reduction. Here we show that the nicotine-induced depolarization in cells with reduced receptors can be restored to control values by treatment with dithionitrobenzoate, which reoxidizes the disulphide bond. These reversible effects on the agonist-induced depolarization suggests an important role of disulphide bonds in the extracellular ligand binding site of α-subunits from insect nervous system nicotinic acetylcholine receptors.

337.4

NEW ACETYLCHOLINE RECEPTOR LIGANDS FROM *CONUS* VENOMS. L. J. Cruz*, C. Hopkins, J. Torres, J. Dykert, C. Miller, D. Yoshikami, B. M. Olivera, and J. Rivier. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112, Marine Science Inst. U. P. Diliman, Philippines, and Clayton Foundation Laboratories for Peptide Biology, Salk Institute, La Jolla, CA 92037.

The venoms of fish-hunting *Conus* species have provided a number of ligands for ion channels and receptors. Among these are 6 homologous α-conotoxins (13 - 15 amino acids long with 2 disulfide bonds) which inhibit nicotinic acetylcholine receptors and compete with the binding of α-bungarotoxin. Recently 3 new conotoxins paralytic to fish and acting on nAChR have been isolated, sequenced and synthesized. Two of these are structurally very different from the known α-conotoxins; one represents a novel class of nAChR ligands. The 23-amino acid peptide from *C. purpurascens* (conotoxin PIII) reversibly decreases the peak of the endplate current and accelerates the decay of the epc. The ability of conotoxin PIII to affect the kinetics of the epc distinguishes it from most other AChR antagonists and suggests a use-dependent block of the AChR. The other peptide from *C. purpurascens* (αA-conotoxin PI) has 25 amino acid residues and 3 disulfide bonds, and blocks postsynaptic responses to iontophoretically applied carbamylcholine and competes with the binding of α-bungarotoxin. Thus, it behaves as an α-type toxin although its structure is very different from that of known α-conotoxins. AChR ligands from *Conus* species with different feeding preferences are currently being characterized.

337.5

NICOTINIC BINDING IN RAT BRAIN MEMBRANES ENHANCED BY CLASSICAL NEUROLEPTICS A.J. Dwork* and D. Liu, Neuropathology, Columbia U. and N. Y. State Psychiatric Inst. New York, NY 10032

Various phenothiazines behave as noncompetitive antagonists of peripheral nicotinic acetylcholine receptors (NACHR). Antagonism of central NACHR by neuroleptic drugs could contribute to the effects of these drugs *in vivo*, and differences among drugs might account in part for different clinical profiles. As a first step in evaluating this possibility, we examined the effects of various neuroleptics on the specific binding of [^{3}H](-)nicotine to rat brain membranes, assuming that noncompetitive antagonists would enhance desensitization and hence increase the affinity of NACHR for agonist. Binding was assayed by filtration, with nonspecific binding determined in the presence of 100 μM carbamylcholine. The assays were performed on ice, neuroleptics were shielded from light, and alcohol was removed from the radioligand before use. Under these conditions, specific binding of 1-2 nm [^{3}H](-)nicotine was enhanced 15-45% by either 100 μM thioridazine, 0.01-100 μM haloperidol, or 100 μM-1 mM fluphenazine. Neither clozapine nor (-)sulpiride produced any enhancement at concentrations up to 1 mM, and (-)sulpiride inhibited binding in a dose-dependent manner with IC_{50}=0.1-1.0 mM. Saturation studies demonstrated a decrease in Kd for (-)nicotine of ~15% in the presence of 100 μM thioridazine or fluphenazine, and of ~30% in the presence of 100 μM haloperidol, suggesting that these drugs do cause a conformational change in brain NACHR consistent with desensitization. Fluphenazine also produced an apparent increase in Bmax (~25%), which could perhaps be explained by exposure of previously sequestered receptors via an effect of the drug on membranes.

Supported by the Smokeless Tobacco Research Council.

337.6

DEFINITION OF A NOVEL AGONIST BINDING SITE ON NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs). T. Tano[1,2*], E.F.R. Pereira[1,2], A. Maelicke[3] and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD, USA, 21201; [2]Lab. Mol. Pharmacol. II, IBCCF, RJ, Brazil, 21944; [3]Inst. Physiol. Chem., Johannes-Gutenberg Univ. of Med. Sch., Duesbergweg, D-6500 Mainz, Germany.

Recent studies have demonstrated that physostigmine (Phy) activates nAChRs from several sources (*Mol.Pharmacol.*, 28:527, 1985; *Eur.J.Biochem.*, 200:671, 1991; *Soc.Neurosci.Abs.*, 17:233.15, 1991). However, the agonist action of Phy observed in *Torpedo* vesicles, cell-attached patches obtained from single fibers of frog interosseal muscle and outside-out patches excised from cultured fetal rat hippocampal neurons were not blocked by competitive nicotinic antagonists. On the other hand, benzoquinonium (BZQ), a competitive and non-competitive antagonist at nAChRs, blocked the agonist action of Phy in all these preparations. Here, using outside-out patches obtained from fetal rat hippocampal neurons grown in culture for 15-25 days, we showed that BZQ (1 μM) also activated single channel currents in the presence of APV (50 μM), atropine (1 μM) and TTX (0.3 μM), which resembled those evoked by Phy and had conductances of about 28 and 42 pS. Furthermore, photolabeling studies carried out in *Torpedo* membranes revealed that the binding site of Phy on the nAChR was located just a few positions in front of the disulfide-bridged loop, Cys-128-Cys-142. Most of the labeling appeared on the Lys-125. This residue (and its nearest neighbors) is conserved in most nAChR α-polypeptides, but is absent in non-α polypeptides (*TiBS*, 13:199, 1988; *TiPS*, 11:485, 1990). Clearly, the area around and including Lys-125 is within the N-terminal hydrophilic domain of the α-subunit, a region well suited for the binding of extracellular ligands. The Phy site thus appears to be located adjacent to, but separate from the ACh binding site on the extracellular side of the nAChR. These results raised the question whether there is an endogenous agonist for this binding site, and if so, what the physiological relevance of such a finding would be. Support: U.S. Army Med. Res. & Devel. Comm. DAMD 17-88-C-8119 & USPHS NS25296. CNPq Fellow (EFRP).

337.7

AGONIST SENSITIVITY OF THE NICOTINIC ACETYLCHOLINE RECEPTORS (nAChR) OF FETAL RAT HIPPOCAMPAL NEURONS IN CULTURE. M. Alkondon* and E.X. Albuquerque. Dept. Pharmacol. Exp. Ther., Univ. Maryland, Sch. Medicine, Baltimore, MD 21201.

In the present study, we used the whole cell patch clamp technique to characterize further the subtype/s of nAChR present on cultured fetal rat hippocampal neurons. Several nicotinic agonists such as (+)Anatoxin-a (AnTX) (1-50 μM), cytisine (10-300 μM), DMPP (10-600 μM), (-)nicotine (10-300 μM), acetylcholine (ACh) (10-600 μM) and carbachol (10-1200 μM) evoked whole cell currents, whose peak amplitude increased in a concentration-dependent manner. Among these agonists, AnTX showed the highest potency while carbachol showed the least. Increasing the concentration of each of the agonists shortened the rise time and also accelerated the decay phase of the whole cell currents which followed the peak during a brief agonist pulse of 1-2 sec. *Hill plot* analysis gave slope value of more than one, indicating the possible involvement of two agonist binding sites. The EC50 values determined from these plots showed some variation among different neurons (2-3 fold). Also, the measured peak amplitude of the whole cell currents activated by higher concentrations of the agonists was surprisingly larger than that predicted from the double reciprocal plot using lower range of agonist concentrations. These results suggest that there may be more than one subtype of nAChR on the fetal rat hippocampal neurons grown in culture. Support: U.S. Army Med. Res. & Devel. Comm. Contr. DAMD-17-88-C-8119 and USPHS NS25296.

337.8

AGE RELATED CHANGES IN RAT BRAIN NEURONAL NICOTINIC RECEPTORS D.J. Anderson*, J.L. Raszkiewicz, and S.P. Arneric, Neuroscience, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064.

Regionally selective loss of neuronal nicotinic receptors (nAChRs) labeled by [^{3}H](-)nicotine ([^{3}H]NIC) has been correlated with age- and disease- associated dementias. Recently nicotine has been shown to improve performance of learning and memory tasks in aged rodents, monkeys and humans. This study sought to determine: 1) whether similar age and regionally selective losses could be detected with the new nicotinic ligand, [^{3}H]cytisine ([^{3}H]CYT); and 2) whether the relative affinities of 8 reference compounds were altered by age or region. Washed membranes were prepared from the frontoparietal cortex (CX), striatum (STR), hippocampus (HPC), and brain stem (BS) of male Sprague-Dawley rats which were 3-4 months or 27 months of age. Brain areas were pooled from groups of three animals. Maximal binding was determined with 4 nM [^{3}H]CYT and 6 nM [^{3}H]NIC using 10 μM (-)NIC to determine nonspecific binding. K_i values for reference compounds were determined from concentration-inhibition curves using 1.2 nM [^{3}H]CYT. Aged animals showed a similar reduction of receptor density in all brain areas investigated. The decreases in [^{3}H]CYT and [^{3}H]NIC binding were 24% and 29% in STR, 23% and 25% in CX, 21% and 20% in HPC, and 26% and 24% in BS respectively. The rank order potencies of reference compounds against [^{3}H]CYT were: Cyt> anatoxin-A> (-)NIC> lobeline= MCC> DMPP> (+)NIC> DHβE. No differences in rank order or affinity were seen between the young and aged animals for each of the brain regions, although regional differences in affinity do exist. The age-related loss of nAChRs independent of a change in the affinity of ligands for the receptor supports the concept that the therapeutic potential of nicotinic agents will be maintained with age.

337.9

CHRONIC NMDA RECEPTOR ACTIVATION DOWN-REGULATES NEURONAL NICOTINIC RECEPTOR IN CULTURED CEREBELLAR GRANULE CELLS. M. Didier, S.A. Berman AND S. Bursztajn*. Mailman Res. Ctr, McLean Hosp/ Harvard Med. Sch. Belmont, MA 02178.

Nicotinic acetylcholine receptors (nAchRs), as other calcium permeable channel-receptors, may play a crucial role during neuronal development. We have characterize nAchRs in developing cerebellar granule cells in primary culture which require high concentration of K+ or NMDA to survive and differentiate. L-[3H] nicotine binding experiments revealed the presence of a single class of saturable and specific binding sites, having a high affinity for nicotine (2 nM). The nicotinic binding site expression followed a transient developmental pattern with a peak during the synaptogenesis period in high K+ culture condition (Bmax in order of 200 fmole/ mg protein). Nicotine-dependent 45Ca2+ uptake was d-tubocurarine-sensitive and shared a similar developmental pattern (i.e. a maximum around 10-14 days in culture). In contrast, chronic application of NMDA decreased by 50 % the total number of nicotine binding sites with no change in the affinity for nicotine and correlated with a reduction in nicotine-activated 45Ca2+ uptake. Sensitivity to NMDA receptor antagonists as well as to removal of glutamate by pyruvate transaminase treatment suggest a glutamatertgic component in the nicotine-stimulated 45Ca2+ influx. Preliminary results using monoclonal antibodies, revealed the presence of alpha4 and beta2 subunits in most of neurons. Non-neuronal cells did not express nAchRs as shown by [3H]nicotine binding and immunocytochemistry. The time-dependent specific nAchRs expression and the potential association between nAchRs and the NMDA receptor activation suggest that nAchRs may regulate the glutamatergic activity during synaptogenesis. Moreover, the NMDA receptor could modulate the nAchRs expression in the developing central nervous system. Further experiments, using molecular probes, are in progress to determine the nAchR subtypes expressed and the potential change in their expression by chronic NMDA treatment.

337.10

MODULATORY ROLE OF NICOTINE ON NMDA-INDUCED INCREASES IN cGMP LEVELS IN RAT CEREBELLAR SLICES. Pamala Adams, Hong Cheng, Kathy Kohlhaas, Stephen P. Arneric and James P. Sullivan*. Neuroscience, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL, 60064-3500

Accumulating evidence suggests that pharmacological similarities exist between nicotinic receptors and the glutamate receptor specific for N-methyl-D-Aspartate (NMDA). The nicotinic agonists (-) nicotine and lobeline partially inhibit NMDA-induced responses in cultures rat cortical neurons and displace [^{3}H] MK-801 binding in washed membranes (Aizenman et. al., Brain Res. 551: 355, 1991). The present study investigates the effect of nicotine on NMDA-induced increases in cGMP levels in rat cerebellar granule slices.

Slices (0.4 mm) were prepared from 8-10-day-old Sprague-Dawley rat cerebella and incubated at 37° for 60 min. in Krebs-Henseleit buffer. The slices were subsequently exposed to NMDA for 3 min. and cGMP levels determined by radioimmunoassay as described previously (Steiner et. al., J. Biol. Chem. 247: 1106, 1972). Addition of NMDA (100 μM) caused cGMP levels in the slices to rise from < 1 to about 110 pmol/mg protein ($n=5$; $p<0.05$). Preincubation of the slices with nicotine (100 μM) for 5 min. completely inhibited this response ($n=4$; $p<0.05$). The inhibition was half-maximal at about 1 μM. No changes in cGMP levels were observed when nicotine (100-1000 μM) was administered on its own. These results suggest a role for nicotine in modulating the increases in cGMP levels elicited by activation of NMDA receptors.

337.11

CARDIOVASCULAR RESPONSES TO NICOTINE IN THE PARAVENTRICULAR NUCLEUS OF THE RAT. A.A. Houdi*, R.T. Dowell, M. Welch and J.N. Diana. Tobacco and Health Research Institute and College of Pharmacy and Dept. of Physiology, University of Kentucky, Lexington, KY 40546.

Although the central effects of nicotine in mediating sympathoadrenal and cardiovascular responses are well documented, the precise mechanisms and the brain sites involved in mediating these central effects of nicotine remain undefined. The paraventricular nucleus (PVN) has been suggested as site of integration for autonomic and endocrine cardiovascular responses. In this study, we investigated the role of the PVN in nicotine -induced alteration in mean blood pressure (MAP), heart rate (HR) and peripheral vascular resistance in conscious, freely moving rats. Bilateral administration of nicotine (4.5ug/0.1ul/site) into the PVN produced dose dependent rapid, and marked increase in MAP (+28 mmHg) and a mild decrease in HR (-13 bpm). Nicotine into the PVN also produced a significant parallel increase in mesentric vascular resistance (+100% of control) but had no appreciable effect on either iliac or renal vascular resistances. On the other hand, intracerebroventricular (icv) administration of nicotine (19ug/rat) produced a lesser increase in MAP and a more pronounced bradycardia when compared to PVN administration.Also, icv administered nicotine, increased both mesentric and iliac vascular resistances (+60% and +40% of control, respectively). Intra-arterial (i.a.) administration of nicotine (0.1mg/kg) produced a lesser increase in MAP and a marked decrease in HR compared to either icv or PVN administration. Nicotine, administered i.a., also increased mesentric and renal vascular resistances (+60% and +50% of control respectively) and an initial decrease followed by an increase in iliac vascular resistance. This effect of nicotine was dose dependent. Saline treatments of rats had no significant effects on cardiovascular parameters.These data suggest that PVN nicotinic receptors play a major role in central sympathoadrenal activation by nicotine, while i.a. and icv administration of nicotine activates both sympathetic and parasympathetic nervous system components.

337.12

NICOTINIC CHOLINERGIC RECEPTORS IN CEREBRAL MICROVESSELS ISOLATED FROM HUMAN AND PIG BRAIN. P. Homayoun*, P.J. Whitehouse and R.N. Kalaria. Depts. of Neurology & Neurosciences, C.W.R.U. School of Medicine, Cleveland, OH 44106

Current evidence suggests that the cerebral microvasculature is directly influenced by cholinergic nerve input and certain cholinergic agents. In this study we measured nicotinic cholinergic receptors associated with the brain microvasculature using [^{3}H]acetylcholine (ACh) and [^{3}H]nicotine as ligands in cerebral microvessels (CMV) isolated from postmortem human and pig brains. Specific binding sites for ACh (displaced by carbachol and in presence of 1 nM atropine) were higher in CMV (71 ±17 and 22 ±7 fmol/mg protein for human and pig, respectively) compared to cerebral cortex (27 ±4 and 16 ±2; n=3-6; at about Kd conc.) irrespective of species. Similar results were obtained with [^{3}H]nicotine binding. In contrast, [^{3}H]pirenzipine and [^{3}H]QNB binding, indices for muscarinic receptors, wre low (about 10-20% of cortex) in CMV compared to the cerebral cortex in both species. These results and other studies on the localization of receptor mRNAs suggest that CMV contain nicotinic receptors which may be involved in the modulation of the cerebral microcirculation by cholinergic neurons.

337.13

MUSCARINIC AGONIST OXOTREMORINE-M EVOKES NICOTINIC RESPONSE IN CELIAC GANGLION NEURONS. S.R. Knoper, J.S. Coggan, H. Xian, D.L. Kreulen and R. Gruener*. Depts. of Pharmacol., Physiol. and Internal Med., Univ. of AZ, Tucson, AZ.

Oxotremorine-M (Oxo-M, RBI, Inc.) is commonly used as a selective muscarinic agonist (Birdsall et. al., 1978). In guinea pig celiac ganglion neurons in primary culture Oxo-M elicits fast inward current transients, in addition to slow muscarinic transients, under whole cell voltage clamp which were similar to nicotinic responses evoked by acetylcholine (ACh) and 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP). The potency for Oxo-M (EC_{50} = 300 μM) was less than that for ACh (EC_{50} = 50 μM). The reversal potentials of Oxo-M and ACh responses were near 0 mV. Neither response showed rectification. Reduction in $[Na+]_0$ shifted the reversal potential toward hyperpolarized potentials. The nicotinic receptor antagonist d-tubocurarine (dTC, 30 μM) blocked the inward current response to Oxo-M. In contrast the muscarinic antagonist atropine (1.2 μM) did not attenuate the response. Another selective muscarinic agonist, oxotremorine (sesquifumarate salt), did not produce any nicotinic response. HL-27781, Lilly Research Labs.

Birdsall, N.J.M., Burgen, A.S.V., Hulme,E.C. The binding of agonists to brain muscarinic receptors. Mol. Pharmacol. 14, 723-36 (1978).

EXCITATORY AMINO ACIDS: ANATOMY AND PHYSIOLOGY II

338.1

METABOTROPIC EXCITATORY AMINO ACID RECEPTOR ACTIVATION TRIGGERS RESPONSIVENESS OF PURKINJE CELLS TO THE TROPHIC ACTION OF NGF H. Mount,* C.F. Dreyfus, & I.B. Black, Dept. Neuroscience & Cell Biology, Robert Wood Johnson Medical School, UMDNJ, Piscataway, NJ 08854.

We have previously reported that trophic factors and neurotransmitters in concert regulate survival of cultured rat cerebellar Purkinje cells (Cohen-Cory et al. *J. Neurosci.* 11, 462, 1991). In particular, excitatory amino acid (EAA) transmitters and nerve growth factor (NGF) increased survival, whereas neither alone was effective. In the present studies, we sought to identify molecular mechanisms through which EAAs participate in the trophic interaction.

Initially, we characterized the potential role of ionotropic EAA receptors by employing the antagonists MK-801, D-APV and DNQX. Each increased cell number, suggesting that endogenous ionotropic activity decreased survival. To determine whether metabotropic EAA receptor stimulation modulates survival, the metabotropic agonist ACPD ([1S,3R]-1-aminocyclo-pentane-1,3-dicarboxylic acid, 1 μM) was tested. ACPD alone had no effect on survival. However, simultaneous exposure to ACPD and NGF increased Purkinje number. Moreover, this increase in survival was blocked by the selective metabotropic antagonist, L-AP3 (L(+)-2-amino-3-phosphono-propionic acid, 1 μM). Use of L-AP3, in the absence of added agonist, reduced cell number, suggesting that endogenous metabotropic stimulation is normally necessary for survival. In sum, these studies reveal a novel mechanism whereby an excitatory neurotransmitter shapes neural development by simultaneous neurotoxic and neurotrophic actions that are respectively mediated by ionotropic and metabotropic receptors.

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338.2

NMDA EXPOSURE REDUCES INTRACELLULAR pH_i IN CULTURED HIPPOCAMPAL NEURONS. K.M. Raley-Susman†, R. R. Kopito††, and R. M. Sapolsky††. † Dept. of Biology, Vassar College, Poughkeepsie NY 12601; †† Dept. of Biological Sciences, Stanford University, Stanford CA 94305.

Previous work has demonstrated transient changes in pH associated with exposure to GABA and glutamate . Further, changes in extracellular pH have profound effects on NMDA receptor-mediated currents (Traynelis and Cull-Candy, Nature 345:347. 1990). Thus, pH changes appear to play an important modulatory role in neuronal function. In this study, we analyzed the changes in intracellular pH (pH_i) in cultured hippocampal neurons in response to bath application of NMDA, under constant extracellular pH conditions . Briefly, cultured hippocampal neurons, days 10-12 in culture, were loaded with the pH indicator dye, BCECF. Intracellular pH was determined as the change in the ratio of emission at 499 and 439 nm. Cells were superfused with Ringer's solution (pH 7.4), buffered with HCO_3/CO_2. Following measurement of basal intracellular pH for 1-2 min, cells were exposed to Ringer's containing different concentrations of NMDA (10, 100, 500, 1000 μM) for 1 min. Solutions were switched back to normal Ringer's. The average basal pH_i of the neurons tested was 7.195 ± 0.045 (n= 42 neurons). Superfusion with 500 μM NMDA produced an acidification of intracellular pH (average ΔpH_i -0.164 ± 0.019 pH units). In most cases, the acidification was transient. The acidification occurred over a wide range of NMDA concentrations, and occurred in the presence and the absence of HCO_3. Intriguingly, the effect of NMDA on intracellular pH exhibited a refractory period of several minutes. These results suggest that NMDA exposure can have a substantial effect on intracellular pH, which could have profound effects on cellular metabolism and neuronal function.

338.3

Glutamate receptor channels on embryonic *Drosophila* myotubes: Ion permeation

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The ion permeation mechanism of the glutamate receptor channel in embryonic *Drosophila* myotubes was studied using the patch clamp technique in the inside-out configuration. The channel was found cation-selective. When the Na^+ concentration was 140mM on both sides of the patch membrane the current-voltage relation was straight between -100 and +100mV and the single channel conductance was 159pS. When the Na^+ concentration was reduced symmetrically the I-V curve remained straight but the slope declined. The conductance-activity relation for Na^+ was described by a hyperbolic curve. The maximal conductance was 196pS and the half-saturating activity was 40mM. In the bi-ionic condition the reversal potential was measured and K^+, Rb^+, Cs^+ and NH_4^+ were found to have similar permeabilities as Na^+ but Li^+ had a smaller permeability. Divalent cations, Ca^{2+} and Mg^{2+}, permeated the channel but reduced the single channel conductance. A model based on the Eyring rate theory with two binding sites was used to fit these data.

338.4

1S,3R-ACPD POTENTIATES AMPA- AND SUPPRESSES MUSCIMOL-CURRENTS IN THE NUCLEUS OF THE TRACTUS SOLITARIUS (NTS). Steven R. Glaum* & Richard J. Miller, Dept. Pharm. Phys. Sci. The University of Chicago, 947 E. 58th, Chicago, IL 60637.

The NTS is the principle central nucleus for the coordination of cardiovascular, respiratory and visceral autonomic reflexes. We recently reported that the metabotropic glutamate (GLU) receptor agonist 1S,3R-ACPD inhibited EPSCs and IPSCs evoked from the region of the tractus solitarius (TS) and also produced a direct postsynaptic excitatory effect mediated by the reduction of a K^+ current (Glaum & Miller, JNS, in press). The latter effect, mimicked by high frequency stimulation of the TS, was occluded in the presence of 1S,3R-ACPD, suggesting endogenous GLU can activate ACPD receptors. We have now examined the effects of 1S,3R-ACPD (2-100μM) on postsynaptic currents evoked by pressure-ejection of the GLU agonist AMPA (I_{AMPA}) and the GABA-A agonist muscimol (I_{MUSC}) onto neurons located in the dorsomedial subdivision of the NTS adjacent to the area postrema in transverse brainstem slices. We also compared the effects of 1S,3R-ACPD and high frequency TS stimulation on I_{MUSC}.

Whole-cell patch recordings of visually-identified cells were made with K-gluconate electrodes in the presence of TTX (0.5μM) and either DNQX (10μM)/D-AP5 (50μM) or bicuculline (10μM). 1S,3R-ACPD reversibly reduced the amplitude of I_{MUSC} by 10-80% (n=17). The effect did not desensitize with repeat exposure to 1S,3R-ACPD and was not affected when cells were dialyzed with GTP-γ-S (400μM, n=4). In the absence of TTX, 50Hz stimulation of the TS also suppressed I_{MUSC}, an effect occluded by 1S,3R-ACPD (100μM). In contrast, I_{AMPA} was potentiated in the presence of 1S,3R-ACPD by 113-428% (n=19) in a reversible, non-desensitizing, GTP-γ-S-insensitive (n=3) manner. These effects are discussed in reference to the role of GLU and GABA at baroreceptor afferent synapses. Supported by DA-02575, DA-02121 & MH40165.

338.5

ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS DECREASES SYNAPTIC INHIBITION IN HIPPOCAMPUS BY REDUCING EXCITATION OF INHIBITORY INTERNEURONS. Manisha A. Desai* and P. Jeffrey Conn. Dept. of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322.

We have previously shown that the selective metabotropic glutamate receptor agonist *trans*-1-amino-1,3-cyclopentanedicarboxylic acid (*t*-ACPD) decreases synaptic inhibition in hippocampal area CA1. We now report a series of experiments aimed at determining the mechanism of *t*-ACPD-induced disinhibition. Our data support the hypothesis that *t*-ACPD decreases inhibitory postsynaptic potentials (IPSPs) by decreasing synaptic excitation of inhibitory interneurons. This is based largely on the finding that the active isomer of *t*-ACPD, 1S,3R-ACPD does not decrease monosynaptic IPSPs elicited by stimulation of inhibitory interneurons in the presence of glutamate receptor antagonists nor does it decrease spontaneous IPSPs recorded in pyramidal cells. We also found that 1S,3R-ACPD reduces both feedforward and recurrent inhibition. Furthermore, 1S,3R-ACPD decreases both the fast (GABAa) and slow (GABAb) components of the IPSP. The GABAa component of the IPSP is mediated by interneurons at the stratum oriens/alveus border and basket cells. In contrast, GABAb IPSPs are mediated by interneurons in the stratum lacunosum/moleculare. Thus, 1S,3R-ACPD is likely to reduce synaptic excitation of both of these groups of interneurons.

338.6

PROTECTION FROM NMDA-INDUCED DELAYED INJURY IN THE HIPPOCAMPAL SLICE WITH BRIEF POST-NMDA MAGNESIUM. R.A.Wallis* and K.L.Panizzon. Neuronal Injury Laboratory, Dept. of Neurology, UCLA Sch. of Med., Los Angeles, CA 90024.

Delayed neuronal injury induced by NMDA receptor activation appears to exacerbate neuronal damage seen with ischemia and trauma. We investigated the role of delayed opening of the NMDA receptor associated channel in this injury, using the hippocampal slice as an in vitro model of delayed injury. With this preparation, exposure to 10 μM NMDA for a mean of 5.3 ± 0.6 mins., produced rapid loss of the orthodromic CA1 population spike (PS). This potential returned with perfusion of standard artificial CSF. However, the orthodromic CA1 PS could be elicited thereafter for only a mean 12.4 ± 1.6 hrs., compared to paired, unmedicated slices, in which a PS could be evoked for a mean of 22.6 ± 4.0 hrs. With final loss of the orthodromic CA1 PS, the antidromic CA1 PS was lost as well, suggesting that delayed PS loss was due to neuronal cell injury, rather than effects of synaptic depression. High magnesium treatment, given after NMDA exposure, protected against NMDA-induced delayed PS loss. With perfusion of artificial CSF containing 10 mM magnesium for 35 mins. after NMDA exposure, the orthodromic CA1 PS could be evoked for 20.3 ± 2.1 hrs. In paired slices receiving NMDA exposure only, this potential could be evoked for only 13.8 ± 1.0 hrs. These data suggest that brief NMDA exposure induces a persistent open state of the NMDA receptor associated channel, which may be an important component in the evolution of NMDA-induced delayed neuronal injury. They additionally indicate that blockade of these channels after NMDA receptor activation can protect against the later development of delayed injury.

338.7

DEVELOPMENTAL CHANGE OF NMDA RECEPTOR-MEDIATED EXCITATORY POSTSYNAPTIC CURRENTS IN RAT VISUAL CORTICAL NEURONS. G. Carmignoto* and S. Vicini FGIN, Georgetown Univ., Wash. DC

Following the hypothesis that in the visual cortex NMDA receptors partecipate in experience-dependent modifications of synaptic strength, we investigated whether a change of the functional properties of NMDA receptors involved in synaptic transmission is one of the cause for the age-dependent decline of visual cortical plasticity . NMDA receptor function was studied in coronal slices prepared from rats of various age. Whole-cell patch-clamp recordings were used to study spontaneous and evoked post-synaptic currents (EPSCs). Pure NMDA-mediated EPSCs were recorded from layer IV neurons in response to stimuli applied to either the white matter or various intracortical locations in the presence of 10 µM NBQX and 10 µM bicuculline methiodide. These EPSCs recorded at a holding potential 50 mV positive to the reversal potential (4 ± 2 mV, mean ± SD, n=20) were best described by a two component exponential decay with a fast (30-70 msec) and a slow (200-300 msec) time constant. The relative contribution to the EPSC amplitude of the slow component changes dramatically with age, decreasing from 100 % (n=14) at day 9 to less than 20% (n=11) at day 35. The time course of spontaneous NMDA receptor mediated EPSCs matched that of evoked currents. This decrease was significantly delayed in dark reared rats and it was completetely prevented in animals whose neuronal cortical activity was totally blocked by daily intracortical TTX injections (0.5 mM in 2 µl citrate buffer). These results indicate that the age-dependent decline of visual cortical plasticity is associated with a change in synaptic NMDA receptor function. Current experiments are exploring in outside-out patches excised from layer IV visual cortical neurons of developing rats the intrinsic kinetic properties of NMDA receptor-channels which may underlie this change. Supported by NIH grant PO1 NS 28130.

338.8

GLUTAMATE-ACTIVATED CURRENTS WITH SLOWER DESENSITIZATION RATES IN OUTSIDE-OUT PATCHES FROM SPINY VS. ASPINY HIPPOCAMPAL HILAR NEURONS C.T. Livsey*+ and S. Vicini FGIN and +Dept. of Pharmacology, Georgetown Univ., Washington., DC 20007

The desensitization rate of non-NMDA glutamate receptors was investigated in outside-out membrane patches obtained from morphologically-identified spiny "mossy cells" (SMCs) and aspiny hilar interneurons (AHIs) in young rat hippocampal slices. The fast (less than 0.2 msec on rate) application of a 1 mM pulse of L-glutamate for 100 msec in the presence of TTX (1 µM) and MK801 (10 µM) onto patches produced large (30-1000 pA mean amplitude) glutamate-activated currents (GACs) that decayed exponentially despite the continued presence of agonist. These desensitization rates differed markedly between patches obtained from the two cell types, 8.0 ± 1.9 ms (mean ± SD) in 15 SMCs compared to 2.2 ± 0.41 ms in 8 AHIs. Spontaneous excitatory postsynaptic current (sEPSC) decay time constants in the same neurons averaged 8.2 ± 2.8 ms and 2.7 ± 0.71 ms, respectively. Current to voltage relationships of the GACs did not differ between SMCs and AHIs, with comparable reversal potentials and no evidence of outward rectification. An increase of the L-glutamate concentration to 5 mM increased GAC amplitudes but did not affect desensitization rates. Brief (< 2 ms) pulses of 1 mM glutamate produced rapidly decaying (2.3 ± 0.3 ms, n=7) GACs that were indistinguishable between the two neuronal classes. The difference in desensitization rates between SMCs and AHIs might demonstrate a functional correlate to the substantial heterogeneity in the molecular structure of glutamate receptor subunits. Supported by NIH grant PO1 NS 28130.

338.9

CORTICOSTEROID ACTIONS ON SYNAPTIC TRANSMISSION IN THE RAT CA1 HIPPOCAMPAL AREA. M. Joëls and E.R. de Kloet*. Exp. Zoology, Univ. Amsterdam and *Leiden Univ., The Netherlands.

The rat adrenal hormone corticosterone enters the brain and binds to mineralocorticoid (MRs) and glucocorticoid receptors (GRs). We studied the effect of MR- and GR-occupation on intracellularly recorded EPSPs, f(ast) IPSPs and l(ate) IPSPs evoked in hippocampal CA1 neurons of adrenalectomized (ADX) rats by stimulation of the Schaffer collaterals. We used maximal stimulus intensity, which evoked a large EPSP at the Erev for the fIPSP but was just subthreshold for an action potential, and an intensity that evoked a half-maximal EPSP. Recording was performed at Erev for the fIPSP (ca. -73mV) and 7mV depolarised from this level (suprathreshold for induction of action potentials). We compared synaptic responses before, during steroid application (20 min.) and up to one hour after steroid treatment. In another set of experiments we compared synaptic responses before steroid application with responses in other neurons obtained 1-4hrs after steroid treatment. In control recordings from ADX rats resting membrane potential, resistance, accomodation and afterhyperpolarization (AHP), EPSP- and fIPSP-amplitude did not change over 80 min.; however, the number of action potential evoked by maximal synaptic stimulation at Erev+7mV decreased. Occupation of MR prevented this decrease. GR-activation reduced both the number of action potentials and the EPSP-amplitude. Regardless of the steroid-treatment lIPSPs diminished in time. The steroid-actions on synaptic transmission do not seem to be long-lasting since 1-4 hrs after application EPSP-, fIPSP- and lIPSP-amplitudes were no longer different from the controls. By contrast, GR-mediated increases of the AHP-amplitude (confirming previous findings) predominantly occur after such a long delay. We conclude that corticosteroids have a short-term effect on synaptic transmission in the CA1 area such that MR-occupation maintains the excitability while GR-activation decreases EPSP-amplitude and excitability.

338.10

VOLTAGE DEPENDENCE OF SPONTANEOUS EXCITATORY CURRENTS DURING POSTNATAL DEVELOPMENT. E.C. Burgard* and J.J. Hablitz. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL. 35294.

Spontaneous and evoked EPSCs were recorded in neocortical slices from 3-12 day old rat pups using whole cell patch clamp techniques. Spontaneous EPSCs in layer II-III neurons were rare on postnatal day 3 and gradually increased in frequency over the next 9 days. In normal extracellular saline, the 10-90% rise time, area, and decay time constant of spontaneous EPSCs increased with depolarization and decreased upon hyperpolarization from RMP (-65 mV). EPSC amplitude decreased with depolarization and increased with hyperpolarization. The NMDA receptor antagonist APV (10µM) decreased these parameters only at depolarized membrane potentials. Removal of extracellular magnesium reversibly increased these parameters in an APV-sensitive manner. Evoked EPSCs, at negative holding potentials, were much larger and longer than spontaneous EPSCs. Evoked EPSCs displayed a biphasic decay time constant characterized by a late APV-sensitive component that was not observed in spontaneous EPSCs. All EPSCs were blocked by CNQX (5µM).

These results indicate that NMDA receptors are spontaneously activated and display magnesium sensitivity early in postnatal development. The contribution of NMDA receptors to evoked EPSCs appears to be much greater than to spontaneous EPSCs. (NS22373)

338.11

GLUTAMATE-INDUCED INHIBITION OF PAIRED PULSE FACILITATION OF MONOSYNAPTIC EXCITATORY POSTSYNAPTIC POTENTIALS AND CURRENTS IN SPINAL MOTONEURONS. F. Nakamura, M. Kuno*, H. Gotani and S. Matsuura. Dept. of Physiol., Osaka City Univ. Med. Sch., Abeno-ku, Osaka 545, Japan.

Excitatory amino acids are distributed throughout the central nervous system and might modulate neuronal activity via both pre- and postsynaptic mechanisms. We examined glutamate-induced changes in the paired pulse facilitation of monosynaptic excitatory postsynaptic potentials evoked by stimulation of the lateral column fibers (LC-EPSPs) on lumbar motoneurons in the frog spinal cord. The spinal cord was isolated under anesthesia with pentobarbitone and arterially perfused with oxygenated Ringer. Glutamate (1 mM) depolarized cells with either increase or decrease in EPSP amplitude. In most cells tested, the paired pulse facilitatin was reduced by glutamate when the EPSP amplitude either increased or decreased. The glutamate-induced facilitation was seen in both the presence and absence of Mg^{2+} and was not affected by the concomitant application of glutamate and antagonists for non-NMDA and NMDA receptors, 6-cyano-7-nitro-quinoxalinediones (CNQX) and 2-amino-5-phosphonovalerate (APV). Glutamate reduced the facilitation of excitatory postsynaptic currents (EPSCs) recorded at a constant membrane potential under voltage- clamp, independently of changes in the EPSC amplitude and the input conductance. These results suggest that glutamate modulates release of excitatory transmitters via mechanisms insensitive to Mg^{2+}, CNQX and APV.

338.12

VASOPRESSIN MODULATES GLUTAMATE ACTION IN THE MEDIAL SEPTUM AND DIAGONAL BAND OF BROCA OF THE RAT.
Frank J.C.M. van Eerdenburg* and Quentin J. Pittman. Neuroscience Research Group, University of Calgary, Calgary, T2N 4N1, Canada.

Vasopressin (AVP) injected in the ventral septal area reduces fever caused by bacterial endotoxin or prostaglandin E. AVP can also initiate motor disturbances and convulsive activity in this area. Intracellular recordings were carried out with K-Acetate/biocytin filled electrodes in coronal brain slices of the diagonal band of Broca (DBB) and medial septum (MS) of the rat. AVP (1 μM) reduced or blocked the depolarizing effect of glutamate in 22 out of 55 neurons studied. This effect of AVP appeared to be (time)dose dependent and in some cells voltage dependent (the modulatory effect of AVP disappeared when the cell was hyperpolarized by injecting (-)current). The effect could be blocked by a V1 receptor antagonist. In most of the cells in which the depolarization by glutamate was modulated by AVP, AVP itself had no visible effect on the cell's electrophysiological characteristics. In 17 out of 69 cells studied AVP (1μM) depolarized the cell. An effect also present when a V2 agonist, dDAVP (1μM), was used and was most prominent the first and second time AVP was applied, after which the effect diminished and disappeared. Since the DBB and MS are of importance for cortical input, this study indicates that AVP may modulate cortical functions.

338.13

ACTIVATION OF NMDA RECEPTORS BY GLUTAMATE & ASPARTATE
B. Edmonds* & D. Colquhoun. UCL, Gower St., London, WC1E6BT, UK

We have investigated the dependence of the decay of NMDA receptor currents on the apparent affinity of the agonist used to activate the receptor. Concentration jumps of 0.1 s duration (exchange time < 0.2 ms) were made with 3 μM glycine (GLY) plus either aspartate (ASP, 0.1 mM) or the higher apparent affinity agonist glutamate (GLU, 0.02 mM) on outside-out patches from hippocampal granule cells. Relaxations after agonist removal were fitted with either 1 or 2 exponentials. For ASP the time constant (τ) of the fast component was 41±6 ms (n=5) whereas GLU gave a τ of 74±9 ms (n=8). Unlike ASP, GLU currents usually required 2 exponentials for good fits. These data support the idea that slow relaxations with GLU are partly due to the high apparent affinity of GLU for the receptor.

The single channel basis for different relaxation rates was investigated using 0.1 μM GLY and either GLU (0.03 μM) or ASP (0.15 μM). Open- and closed-time distributions were fitted with 2 and 5 components, respectively. For GLU (n=3) the mean τs (ms) (and relative areas (%)) for gaps were: τ_1=.06±.00 (41±.12); τ_2=.76±.12 (21±2.3); τ_3=13.1±.25 (9.1±.99); τ_4=367±47 (5.2±.98); τ_5=4996±2135 (24±1.9). Gaps for ASP (n=4): τ_1=.06±.01 (40±2.7); τ_2=.62±.08 (20±2.0); τ_3=6.9±3.4 (4.7±1.5); τ_4=127±19 (3.7±.7); τ_5=4896±1579 (32±3.0). Open times for GLU: τ_1=.34±.02 (34±3.5); τ_2=3.2±.27 (67±3.5). Open times for ASP: τ_1=.36±.05 (36±6.0); τ_2=2.5±.23 (64±6.0). Gap components 3 and 4 were shorter and had smaller relative areas for ASP than for GLU, which may contribute to the faster relaxations with ASP.

PEPTIDES: ANATOMICAL LOCALIZATION—HUMAN AND PRIMATE

339.1

CALCITONIN GENE-RELATED PEPTIDE IMMUNOREACTIVITY IN VISCERAL SENSORY PATHWAYS IN THE HUMAN BRAIN. S. de Lacalle* and C. B. Saper. Departments of Pharmacological and Physiological Sciences and Neurology, University of Chicago, USA.

It has been proposed that calcitonin gene-related peptide (CGRP) may serve as a neuromodulator in visceral sensory pathways, particularly in the parabrachial projections to the thalamus, amygdala and cortex in the rat (Yasui et al., J Comp Neurol 290:487,1989; 308:293,1991). We have examined the distribution of CGRP-like immunoreactive (CGRP-ir) innervation of several areas of the human brain involved in the transmission of visceral sensory information. Two populations of CGRP-ir cells were present: one in the parabrachial complex (PB), including the homologues of the external medial and external lateral subnuclei; and the second in the posterior intralaminar thalamic complex, including the subparafascicular, lateral subparafascicular, posterior intralaminar, and peripeduncular nuclei. In addition, scattered CGRP-ir cells were found in the periventricular hypothalamus. CGRP-ir terminals were found in projection areas of the external medial PB, including the ventroposterior parvicellular nucleus of the thalamus, the principal visceral sensory relay nucleus and the insular cortex; in external lateral PB targets, including the central nucleus of the amygdala and the bed nucleus of the stria terminalis; and in the terminal field of the posterior intralaminar thalamic complex, including the amygdaloatriatal transition region and the insular cortex. These CGRP-ir pathways are nearly identical to those that have been defined in the rat, suggesting that CGRP may code chemically for ascending visceral sensory pathways in the human brain as well.

339.2

LOCALIZATION OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) IMMUNOREACTIVITY FOLLOWING HUMAN SPINAL CORD INJURY. R. Melinek* and V.R. Holets The Miami Project and Depts of Neurological Surgery and Cell Biology and Anatomy, University of Miami, Miami, Fl 33136

Changes that occur in CGRP immunoreactivity following injury to the human spinal cord were examined in spinal cords from 3 female patients (aged 37-69 years) at 1, 8 and 13 years after injury. Two of the patients had cervical injuries (C5 and C7) and one had a high thoracic injury (T2). CGRP immunoreactivity above (C1-C3) and below (lumbar in two cases, thoracic in the third) the level of injury was compared to age- and sex-matched normal human spinal cord. Fifty to 100 μm vibratome sections were cut and processed for immunostaining using the avidin-biotin immunoperoxidase method. Whereas motoneurons above the lesion were CGRP-immunoreactive, below the lesion they were not. By contrast, both above and below the lesion, primary afferent fibers in the dorsal horn (lamina I-IV) and in lamina X showed intense CGRP-like immunoreactivity. The results corroborate those of animal studies, and suggest that CGRP levels in motoneurons may be regulated, in part, by supraspinal afferent input. (Supported by The Miami Project to Cure Paralysis, The Daniel Heumann Fund for Spinal Cord Injury, and the American Heart Association, Florida Affiliate)

339.3

ATRIAL NATRIURETIC PEPTIDE-LIKE IMMUNOREACTIVITY (ANP-LIR) IN THE HUMAN BRAIN. J.C. McKenzie, N.E.J. Berman, L.Y. Compton, W.-L. Liu and R.M.Klein*. Dept. of Anatomy, Howard Univ. Col. Med., Washington, DC 20059 and Dept. of Anatomy and Cell Biology, Univ. Kansas Med. Ctr., Kansas City, KS 66160.

Atrial natriuretic peptide is a potent vasorelaxant which has been identified in Types I & II astrocytes and neurons in the canine brain and has been implicated in control of brain fluid and electrolyte homeostasis (McKenzie, 1992). To determine whether a similar system might exist in human brain, we stained sections of striate cortex, cerebellum and hypothalamus from 3 hr postmortem human brain using antibodies against proANP (Thibault) and ANP IV (Inagami). The proANP antibody stained fibrous astrocytes (type II) in white matter of striate cortex and protoplasmic astrocytes in Layer I and infragranular cortical layers. The type II astroglia possessed long thin processes which often appeared beaded. Many of these processes closely approached cerebral blood vessels. The ANP IV antibody, which is directed against the C-terminus of the peptide, stained both types I and II astroglia in cerebral cortex as well as modified type I astroglia forming part of the glia limitans. This antibody also stained type I astrocytes in the hypothalamus and scattered Bergmann glia of the cerebellum, as well as isolated pyramidal neurons in the striate cortex. These results suggest that ANP may play a role in control of cerebral blood flow or maintenance of the blood-brain barrier in human brain. The difference in the population of cells stained by the two antibodies implies a difference in processing of ANP among the cell types. Supported by AHA-Kansas, MH38399 and HL45241.

339.4

PEPTIDERGIC NEURONS IN HUMAN HIPPOCAMPUS. R.E. Powers* and W.S. Young, III. UAB Brain Resource Program, University of Alabama at Birmingham, Birmingham, Alabama 35294 and NIMH, Bethesda, MD 20892.

The distribution of peptidergic neurons in human hippocampus was mapped using hybridization histochemistry in sequential sections of fresh frozen hippocampus from six normal human subjects. Hybridization histochemistry was performed with ^{35}S-labeled oligodeoxynucleotide probes for somatostatin (SS), substance P (SP), leucine enkephalin (LEU), dynorphin (DYN), neuropeptide Y (NPY), and cholecystokinin (CCK). Neuronal labeling was mapped at 3 levels, rostral at the pes hippocampus, mid-hippocampus at the level of the lateral geniculate, and posterior hippocampus at at the level of the splenium of the corpus callosum.

Large numbers of granule cells expressed DYN. Many neurons in the polymorphic layer of the dentate gyrus contained SS, NPY, SP, CCK and DYN transcripts. Pyramidal neurons were infrequently labeled with any probe. Bipolar and basket shaped neurons in the stratum oriens were labeled with SS, SP, NPY, CCK and DYN probes. The distribution of labeled neurons for all peptides were relatively constant throughout the rostrocaudal extent of the hippocampus. SS, NPY, LEU and SP probes labeled similar populations of neurons in the dentate and CA_4.

This study demonstrates and extensive network of peptidergic neurons in human hippocampus that is similar in all levels. These studies are in general agreement with mapping using immunocytochemistry and the distribution is similar to that seen in rodents and non-human primates.

339.5

PEPTIDERGIC INNERVATION OF THE HUMAN SUPRA-TEMPORAL PLANE. P. LaRussa*, R.E. Powers, G. Zhang and X. Tang. Department of Psychiatry and UAB Brain Resource Program, University of Alabama at Birmingham, Birmingham, Alabama 35294.

The distribution of peptidergic neurons and fibers was examined in supratemporal planes (STP) from six normal human subjects including superior temporal gyrus, Heschl's gyrus and the planum temporale.

Multiple levels of acrolien fixed right and left STP were sequentially sectioned and immunostained in the coronal, sagittal and axial plane using antisera to somatostatin (SS) substance P (SP), neuropeptide Y (NPY) and neurotensin (NT). Representative sections from each region were mapped using standard cytoarchitectonics definitions. A variable plexus of NPY fibers was seen in most regions of the STP however few fibers were stained with antisera to NT. A variable number of SS immunostained neurons were present throughout the STP and few neurons were stained with antisera to NT. A modest number of NPY immunostained neurons were present in the cortex of STG in HG and PT however many medium and small sized NPY neurons were present in the subcortical white matter. A continuous lamina of SP immunostained neurons resembling double tufted cells was present in layer 3 of posterior STG, HG and rostral portions of the PT and a smaller number were present in layers 5 and 6. Few SP immunoreactive neurons were present in the white matter. These data support substance P and neuropeptide Y as important transmitters in human auditory cortex.

339.6

LOCALIZATION OF CORTICOTROPIN-RELEASING FACTOR, GALANIN AND NEUROPEPTIDE Y IN THE HUMAN LOCUS COERULEUS. *M.C. Austin*, J.A. Weikel, P.M. Rice, J.J. Mann and V. Arango.* Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

Studies report that the locus coeruleus (LC) of the rat receives a substantial corticotropin-releasing factor (CRF) innervation, and that CRF has an excitatory influence on LC neurons. CRF-immunoreactive (IR) fibers are also found in the LC of non-human primates, but there is no documentation of a CRF input to the human LC. The significance of demonstrating such an input lies in the fact that CRF is hypersecreted and norepinephrine neurotransmission is altered in depressed patients. Immunocytochemical studies report that galanin and neuropeptide Y (NPY) are found in LC neurons of the rat and human. Using immunocytochemistry or *in situ* hybridization, we sought to determine whether the human LC receives a CRF input and to examine gene expression for galanin and NPY in the human LC. Postmortem human brain tissue was obtained at autopsy, the brainstem dissected and either frozen or immersed in formalin. All cases had negative toxicological screens and no known neurologic or psychiatric disorders. Coronal sections of the LC were processed for *in situ* hybridization or immunocytochemistry. Dense CRF-IR fibers were found predominantly in the dorsal LC and the medial and lateral aspects of the central gray of the pons. The density of CRF fibers was less in the ventral LC. CRF-IR fibers contained numerous large varicosities and appeared to envelop some LC perikarya. Galanin and NPY mRNA were localized to a minority of LC neurons. Both galanin and NPY mRNA were found in pigmented as well as non-pigmented cells. This study confirms the presence of CRF-IR terminals in the human LC providing an anatomical basis for CRF modulation of LC activity and that galanin and NPY are co-transmitters in the human LC. The localization of these neuropeptides in the human noradrenergic LC suggests a role for these peptides in the pathophysiology of depression. (Supported by MH40210, MH46745)

339.7

THE DISTRIBUTION OF CHOLECYSTOKININ AND NEUROTENSIN IN THE NUCLEUS ACCUMBENS OF MACAQUE MONKEY. K.Ikemoto*, M. Narita, T. Maeda, M. Hayashi, and K. Satoh. Dept. of Psychiatry and Anatomy, Shiga University of Medical Science, Otsu 520-21, Primate Research Center, Kyoto University, Japan.

Using avidin-biotin peroxidase immunohistochemical technique the distribution of cholecystokinin (CCK) and neurotensin was investigated in the nucleus accumbens of macaca fuscata. The monkeys were deeply anesthesized and perfused with mixed aldehydes. In the rostral portion of the nucleus accumbens, neurotensin containing-cell bodies were triangular or fusiform in shape, and they were observed in its lateral part. At the mid-level of the nucleus, a similar type of neurotensin-containing cell bodies was observed in the mid-lateral part, and at the caudal level of the nucleus, immunoreactive cells, fusiform in shape, were found in its medial part. Neurotensin-containing fibers were densely distributed in the medial half of the nucleus. CCK-containing fibers were observed in the medial-dorsal part of the nucleus accumbens. The distribution of these two neuropeptides was apparently different, suggesting that they might have different roles in the nucleus accumbens.

339.8

SUBSTANCE P/NEUROKININ A (SP/NKA) mRNA EXPRESSION IN THE PRIMATE ENTERIC NERVOUS SYSTEM (ENS). K. Anderson* and C. Sternini. CURE, Department of Medicine, Brain Research Institute, UCLA and VAWC at Wadsworth, Los Angeles, CA 90073.

The mRNAs encoding for the tachykinin peptides, SP, NKA and neurokinin B (NKB) are generated by separate genes, the preprotachykinin (PPT) I, encoding for SP and NKA, and the PPT II, encoding for NKB, which are differentially expressed in the central nervous system. We investigated the expression of tachykinin-encoding mRNAs in the ENS of primates by means of *in situ* hybridization histochemistry with ^{35}S-labeled RNA probes complementary to SP/NKA or NKB mRNA. Fixed sections or whole mounts of different regions of the gastrointestinal tract of the *Macaca* and *Cebus* monkeys were used. SP/NKA-encoding mRNA is expressed in numerous ganglion cells of the myenteric and submucous plexuses with the following density: stomach, duodenum, and ileum > colon > > jejunum. By contrast, specific signal could not be detected with the NKB antisense RNA probe. Hybridization signal was not observed in tissue hybridized with sense RNA probes. We can conclude that: 1) the PPT I gene, encoding for SP, NKA and the posttranslational products, neuropeptide K and γ, is transcribed in enteric neurons of primates, and 2) the NKB-encoding PPT II gene is either not transcribed or transcribed at very low levels in the ENS.

Supported by NIH grants DK38752 & 40469. We thank Dr. J.E. Krause for the PPT cDNAs.

339.9

High levels of enkephalin mRNA are found in the striatum and limbic-associated regions of the monkey telencephalon. W.X.Lu* and S.N. Haber, Dept. of Neurobiology and Anatomy, Univ. of Rochester, Rochester, N.Y. 14642

Using a free floating method for *in situ* hybridization (Brain Res, in press), we studied the distribution of mRNA for preproenkephalin (ENK) in the monkey forebrain. The free-floating sections, stored in cryoprotectant solution, were rinsed in PBS, and directly hybridised at 37°C for 20 hours in a tube with ENK oligonucleotide probe labeled with ^{35}S-dATP and terminal deoxynucleotidyl transferase(10 x 10^6 cpm/ml). After post-hybridization wash in 0.5 x SSC at 60 °C, the sections were mounted and processed for autoradiography. The results from the autoradiograms reveal high levels of specific hybridization to mRNA for ENK in the monkey striatum with low background levels. The distribution of specific label is patchy, with clusters of silver grains separated by areas containing less label. Within a given small area, densely labeled individual cells are found among cells with little or no specific labeling. At the macroscopic level, within a general region of the striatum, there are patches of specific hybridization intermingled with areas containing low background, less-specific hybridization, forming a mosaic-like pattern. Adjacent sections stained for ENK immunohistochemistry and processed for *in situ* hybridization show a high correlation between patches of dense immunoreactivity and high specific ENK mRNA hybridization. Specific hybridization to mRNA of ENK is evident throughout the cortical mantal, concentrated in layers 2 and 3. High levels are found in the orbitofrontal cortex, agranular insular cortex, entorhinal cortex, the dentate gyrus, and parts of the amygdala. Supported by NIMH MH 45573 7 NIH NS22511

339.10

IDENTIFICATION OF TWO GANGLIONATED PLEXUSES IN THE HUMAN GALLBLADDER (GB). R. De Giorgio*, J.E. Parodi, T. Zittel, R. Kleinman, F.C. Brunicardi, V.L.W. Go, J.M. Becker and C. Sternini. CURE, Depts. of Medicine and Surgery, BRI, UCLA & VAMC-Wadsworth, Los Angeles, CA 90073; and Dept. of Surgery, Harvard Medical School, Brigham & Women's Hospital, Boston, MA 02115.

The neurochemical organization of the human GB innervation is still undefined. We studied the tissue targets and the chemical coding of neurons of human GBs obtained from cholecystectomized patients and donors. Tissue sections were processed for immunohistochemistry with calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY), tachykinin (TK), and vasoactive intestinal polypeptide (VIP) antibodies. Two major ganglionated plexuses were identified by peptide immunoreactivities (IRs): the innermost, in the lamina propria; the outermost, in the fibromuscular layer. In both plexuses, VIP-IR was detected in almost all neurons. NPY- and TK-IRs were found in numerous ganglion cells (NPY > TK). Many VIP-IR neurons co-expressed NPY-, and some TK-IR, suggesting that VIP-, NPY- and TK-IRs colocalized in a subpopulation of neurons. CGRP-IR was restricted to axons which often contained TK-IR. The densities of IR nerves distributed to the mucosa and fibromuscular layer was: VIP≥NPY > TK > > CGRP. In conclusion: 1) the human GB has two ganglionated plexuses and is supplied by a dense peptidergic neural network; 2) distinct populations of neurons can be identified by their phenotypical expression.

Supported by NIH grant DK 38752 (CS); SKB Fellowship (RDG).

339.11

INCIDENCE OF NEUROHYPOPHYSEAL HORMONES IN NEUROENDOCRINE AND NON-NEUROENDOCRINE LUNG TUMORS *A.S. Friedmann*, V. A. Memoli, and W.G. North, Depts of Physiology and Pathology, Dartmouth Medical School, Lebanon, NH 03756 USA*

Previous studies performed in this laboratory have shown that the majority of patients diagnosed with small cell lung carcinoma have elevated plasma levels of vasopressin (VP), oxytocin (OT), and their associated human neurophysins (VP-HNP, OT-HNP), while levels of these substances in patients diagnosed with non-neuroendocrine lung tumors are almost all normal. To further investigate the expression of neurohypophyseal peptides by lung tumors, the incidence of VP-HNP in 44 neuroendocrine tumors was determined immunohistochemically using rabbit polyclonal antibodies specific for this neurophysin. Twenty-two non-neuroendocrine tumors were also examined for the presence of VP, VP-HNP, OT, and OT-HNP using mouse monoclonal and rabbit polyclonal antibodies specific for these substances. Widespread VP-HNP immunoreactivity was demonstrated in 38 of the 44 neuroendocrine tumors. In the 22 non-neuroendocrine tumors, positive staining for VP-HNP was limited to one squamous cell tumor and one adenocarcinoma. Very intense staining was also seen in healthy bronchial glands adjacent to the neurophysin-containing adenocarcinoma. Immunoreactivity for VP, OT, and OT-HNP could not be detected in any of the non-neuroendocrine tumors. These data indicate that the high incidence of neurohypophyseal peptide production in lung carcinomas is specific to neuroendocrine tumors and could be of value in tumor diagnosis.

ONTOGENY OF SEROTONIN RECEPTORS

340.1

SEROTONIN HYPERSENSITIVITY IN ADULT RAT NEOSTRIATUM AFTER NEONATAL 6-OHDA TREATMENT: AN IONTOPHORETIC STUDY. M. El Mansari, A. Ferron* and L. Descarries. Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, Montréal, Québec, CANADA H3C 3J7.

Destruction of nigro-striatal dopamine neurons by cerebroventricular administration of 6-OHDA in newborn rat (3-day-old) results in a serotonin (5-HT) hyperinnervation of the rostral neostriatum in the adult, accompanied by an increased number of 5-HT1B and 5-HT2 receptors (Radja et al., *this volume*). To determine the functional consequences of these changes, the effects of iontophoretically applied 5-HT and two 5-HT agonists, mCPP (5-HT1B exclusive) and DOI (5-HT2 selective), were examined in the rostral neostriatum of neonatally lesioned rats anesthetized with urethane. Neuronal responsiveness was quantitatively assessed by means of the IT50 index, which measures the amount of drug required to obtain a 50% depression of firing rate. In controls, the firing rate of most spontaneously active units was reduced by all three agents, with comparable mean IT50 values for 5-HT (1800 nC), mCPP (1780 nC) and DOI (1470 nC). Three months after the lesion, the sensitivity to 5-HT and its two agonists was significantly increased compared to control (5-HT, 915 nC; mCPP, 960 nC; DOI, 900 nC; $p < 0.001$; t test). These results indicate that the 5-HT hyperinnervation and up-regulation of 5-HT receptors is also associated with an hypersensitivity to this transmitter. Thus, even though microdialysis experiments in this model have shown that the basal levels of extracellular 5-HT remain similar to control because of increased reuptake (Jackson D. and Abercrombie E.D., *J. Neurochem.* 58:890, 1992), this same concentration of 5-HT could have greater functional effects. (Supported by a H.H. Jasper Fellowship and MRC grant MT-3544).

340.2

SEROTONIN 5-HT1 AND 5-HT2 RECEPTORS IN ADULT RAT BRAIN AFTER NEONATAL 6-OHDA TREATMENT: AN AUTORADIOGRAPHIC STUDY. F. Radja*, T.A. Reader, K. M. Dewar and L. Descarries, Centre de recherche en sciences neurologiques (Départements de physiologie, psychiatrie et pathologie), Université de Montréal, Montréal, Qué., CANADA.

Neonatal destruction of nigro-striatal dopamine (DA) neurons by cerebroventricular injection of 6-OHDA results in a serotonin (5-HT) hyperinnervation of the rostral neostriatum (NS) in adult rat. Quantitative ligand binding autoradiography was used to evaluate the density of 5-HT1 and 5-HT2 receptors in the forebrain and midbrain, 3 or 6 months after the lesion. 5-HT1A, 5-HT1B, 5-HT1nonAB and 5-HT2 sites were labeled with [3H]8-OH-DPAT, [125I]cyanopindolol, [3H]5-HT and [125I]DOI, respectively. Three months after the lesion, the density of 5-HT1B and 5-HT1nonAB receptors was increased by almost 40% throughout the NS, and by 50% in the substantia nigra (SN). 5-HT1B receptors were also elevated (30%) in the globus pallidus. Binding to 5-HT2 receptors was considerably increased (60%), though this was restricted to the rostral NS only. In contrast, 5-HT1A binding sites, measured after 6 months, were unchanged in all regions studied. These data indicate that the 5-HT hyperinnervation of the rostral NS following neonatal nigro-striatal DA lesion is accompanied by an up-regulation of 5-HT1B and 5-HT1nonAB receptors throughout the NS and in some of its territories of projection (SN and globus pallidus), which suggests an effect of the DA denervation. In SN, the elevation of these receptors is consistent with their predominant axon terminal localization on afferent fibers. The even greater elevation of 5-HT2 receptors in the rostral NS remains to be explained; its distribution suggests a causal link with the 5-HT hyperinnervation. (Supported by the FRSQ, and MRC grants MT-6967 and MT-3544).

340.3

EXPRESSION OF SEROTONIN 5-HT$_{1C}$ and 5-HT$_2$ RECEPTORS IN THE DEVELOPING RAT CENTRAL NERVOUS SYSTEM. R. P. Hellendall*, U. Schambra, J. P. Liu, and J. M. Lauder. Dept. of Cell. Biol. and Anat., Univ. of N. Carolina, Chapel Hill, NC 27599.

Previous work has provided evidence for serotonin (5-HT) as a differentiation signal in the developing CNS. To analyze the putative sites of action of 5-HT during neurogenesis, we have studied the expression of mRNAs for two 5-HT receptors (5-HT$_{1C}$, 5-HT$_2$) by evaluating *in situ* hybridization in the prenatal rat brain. At E14, signal for 5-HT$_{1C}$ was found in the choroid plexus and in marginal/intermediate (m/i) zones of the midbrain, brainstem, and spinal cord including catecholaminergic and 5-HT cell groups. By E18-21 many forebrain regions displayed label, including the hippocampus (CA1), in addition to more intense signal in midbrain, brainstem, and spinal cord. Expression in the choroid plexus appeared to peak between E16-E18 although considerable hybridization signal remained at E21. 5-HT$_2$ transcripts were first apparent at E14 in the ventricular and m/i zones of the midbrain and in the intermediate zones of a number of other areas extending through the rostral-caudal axis of the brain. As with 5-HT$_{1C}$ mRNA, signal increased over rostral and brainstem areas at late gestational ages with significant labeling appearing in the olfactory bulb, cerebellum, cortical plate and subplate, dentate gyrus, noradrenergic and 5-HT raphe brainstem nuclei. These observations are consistent with previous studies in the adult. In addition, both the 5-HT$_{1C}$ and 5-HT$_2$ receptor transcripts were also present over the meninges at E16 which has not been previously reported and may represent a transient expression of these receptors. The pattern of expression in the embryonic rat brain indicates receptors for 5-HT are often present along pathways and at target sites for 5-HT axons prior to synaptogenesis. This observation, in conjunction with our work indicating 5-HT can act as a differentiation signal for target neurons, suggests prenatal 5-HT receptors are positioned to mediate this effect.

340.4

PRENATAL COCAINE EXPOSURE ALTERS HIPPOCAMPAL SEROTONIN, 5-HT$_{1A}$ RECEPTOR, AND S-100β IMMUNOREACTIVITY IN THE NEONATAL RAT.

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We have previously found that cocaine delays the maturation of the serotonin (5-HT) innervation of the hippocampus. Recent work in our laboratory has shown that serotonin modulates the release of S-100β, a cortical and serotonergic trophic factor, by activating 5HT$_{1A}$ receptors. In this study, we immunocytochemically examined the hippocampal distribution of 5-HT fibers, 5-HT$_{1A}$ receptors, and S-100β in neonatal rats prenatally exposed to cocaine. Timed pregnant dams were injected subcutaneously with either cocaine (40 mg/kg/3 cc) or saline from gestational day 13 to parturition. Pups representing a minimum of three dams were randomly selected on postnatal day 7 and processed for immunocytochemistry. In cocaine-treated animals, 5-HT immunoreactive fibers were decreased as previously described (Akbari et al., 1992), while increased 5-HT$_{1A}$ immunoreactivity was seen in the hilus of the dentate gyrus and in the stratum lacunosum-moleculare of the CA fields of the hippocampus. As predicted, S-100β immunoreactivity was lowest in regions of highest 5-HT fiber depletion. These results support the hypothesis that 5-HT functions as a developmental signal by interacting with the 5-HT$_{1A}$ receptor and increasing S-100β levels. Our findings demonstrate that the delayed maturation of the 5-HT innervation of the hippocampus results in decreased levels of the trophic molecule S-100β, which could cause a retardation of dendritic and axonal arborizations of hippocampal neurons. This may represent a molecular mechanism for the neurobehavioral abnormalities associated with prenatal cocaine abuse. Supported by NIDA contract 271-90-7403.

340.5

PLASTICITY AND ONTOGENY OF [^{3}H]PAROXETINE BINDING SITES. M.R. Pranzatelli* and J.M. Martens. Departments of Neurology, Pediatrics, Pharmacology, The George Washington University, Washington, DC 20010.

5,7-Dihydroxytryptamine (5,7-DHT) injected i.p. in neonatal rats increases 5-HT in brainstem while depleting it in cortex, hippocampus, and spinal cord. To study the mechanism of regional differences, we measured the 5-HT transporter site using [^{3}H]paroxetine. Regional differences in B_{MAX} of vehicle-injected rats were significant: brainstem, diencephalon > striatum, cortex, spinal cord > hippocampus, cerebellum. Regional ontogeny also differed: at day 7, B_{MAX} was 39% of adult levels in cortex compared to 63% in brainstem. Thirty days after 100 mg/kg 5,7-DHT i.p., B_{MAX} was increased in brainstem (+67%) and diencephalon (+136%), but decreased in cortex (-59%), hippocampus (-94%), spinal cord (-99%), striatum (-41%), and cerebellum (-37%). K_D was unaltered. Changes in B_{MAX} correlated with the 5-HT concentration we reported previously using HPLC. The threshold dose for B_{MAX} effects was 50 mg/kg and 200 mg/kg was lethal. Binding site changes were apparent one week after 5,7-DHT lesions, but increases in diencephalon and brainstem were not maximal until 3 weeks, whereas percent decrease in cortical sites did not change over time. Lesion effects on [^{3}H]paroxetine site ontogeny were region-dependent: cortical sites increased with age but spinal sites did not. The regional, dose, and time effects of neonatal 5,7-DHT lesions made by i.p. injection on [^{3}H]paroxetine binding sites are evidence for brainstem 5-HT hyperinnervation (sprouting) as a cause of increased 5-HT concentrations.

340.6

EVIDENCE FOR CRITICAL PERIODS OF 5-HT$_{1a}$ RECEPTOR INFLUENCE ON BRAIN DEVELOPMENT X. Zhang*, E.C. Azmitia, P.M. Whitaker-Azmitia Dept. of Psychiatry, State University of New York, Stony Brook, New York 11794-8101

5HT$_{1a}$ receptors play an important role in regulating brain development. The current study examined the effects of 1 mg/kg 8-OH-DPAT given at various postnatal ages on subsequent development of rats. Treatment times were PD 3-10, PD 10-17 and PD 15-22.

Animals treated PD 3-10 gained weight more rapidly than controls and maintained a higher weight until PD 17 (28.8g vs. 33.4 $p<.05$). Incisor eruption and eye-opening took place one day earlier in 8-OH-DPAT animals. Conversely, animals treated PD 10-17 showed eye-opening one day later and gained weight more slowly. These animals were still significantly lighter at PD 40 (139.8 g vs. 114.6 $p<.01$). Animals treated from PD 15-22 were also significantly lighter at PD 40 (132.3 g vs. 113.8 $p<.04$).

At PD 25, animals treated from PD 3-10 were significantly more anxious than controls, based on time spent in the dark (61.6% vs. 85.6 $p<.01$), but no other treatment time produced an effect. At PD 24, animals treated from 10-17 (but no other time) showed greater rates of spontaneous alternation (40.7% vs. 70.9 % $p<.001$). None of the treatment groups showed changes in open field activity, catalepsy or olfaction.

340.7

LOCALIZATION OF 5-HT$_{1a}$ RECEPTORS ON ASTROGLIAL CELLS OF ADULT RAT BRAIN P.M. Whitaker-Azmitia*, C. Clarke, and E. Azmitia Dept. of Psychiatry, State University of New York, Stony Brook, New York 11794-8101

Astroglial cells in primary culture have 5-HT$_{1a}$ receptors which are linked to the release of the neuronal growth factor, S-100B. Since S-100B, and 5-HT$_{1a}$ receptors have been implicated in both Down's Syndrome and Alzheimer's Disease, it is important to know if 5-HT$_{1a}$ receptors do in fact still occur in astrocytes in the mature brain where they could continue to regulate S-100B. However, to date, the presence of this receptor on astroglial cells of an intact adult animal has not been shown. In the current study, we have used double-immunolabelling to show the presence of 5-HT$_{1a}$ receptors on astroglial cells in adult rat brain. Astroglial cells were stained using a monoclonal anti-GFAP (1/400) and a peroxidase-conjugated secondary anti-body (visualized with DAB). 5-HT$_{1a}$ receptors were stained with an anti-5-HT$_{1a}$ (1/5,000) and an alkaline-phosphatase ABC kit (visualized with Vector Labs alkaline-phosphatase substrate III).

Frequency of double-labelled cells was highest in the hippocampus and almost completely absent from the striatum. 5-HT$_{1a}$ labelling was apparent in the somal cyto-plasm (but not the nucleus) and along the processes especially the distal ends. Within brain regions, 5-HT$_{1a}$ staining was unevenly distributed with some heavily labelled astrocytes immediately adjacent to unlabelled astrocytes.

340.8

Functional role of the 2nd extracellular loop of the 5-HT$_{1A}$ receptor using an antipeptide antibody in primary neuronal culture. Efrain C. Azmitia*, Xiao Ping Hou[1], Xi Gu[1] and Patricia-Whitaker-Azmitia[2] Department of Biology; New York University, NY,NY 10002 and SUNY at Stony Brook, NY 11794

The 5-HT$_{1A}$ receptor has been cloned and shown to be a seven transmembrane spanning molecule. An antipeptide antibody was raised against a specific segment of the 2nd extracellular loop of the receptor (Azmitia et al, 1992). The antibody and the peptide itself were tested in a serum-free midbrain raphe tissue culture system to determine if the neuronotrophic actions of the 5-HT$_{1A}$ receptor mediated by activation of S-100ß could be altered. Application of ipsapirone, a 5-HT$_{1A}$ agonist, and S-100ß were able to stimulate the uptake development of serotonergic neurons. Spiroxatrine, a non-selective 5-HT$_{1A}$ antagonist, and antibodies against S-100ß, inhibited the 5-HT uptake maturation. Serum with antibodies raised against the 2nd external loop, but not against GFAP or the 5-HT$_{1A}$ 3rd cytoplasmic loop, was inhibitory at a dilution of 1/25,000. Purification of the antibody immunoglobulin by protein A Sepharose column separation showed significant inhibition of the 5-HT uptake development at a protein concentration of 80 pg/ml. The inhibition produced by the purified and crude antibody could be reversed by addition of excess 5-HT$_{1A}$ agonist. The results suggest that the 2nd cytoplasmic loop may be active in regulating the expression of 5-HT$_{1A}$ receptor.

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340.9

SEROTONERGIC FIBERS ARE NOT DEVELOPED IN THE HIPPOCAMPUS AND NEOCORTEX IN THE S-100β RETARDED MUTANT MOUSE (POLYDACTYLY NAGOYA).

S.Ueda*, X.F.Gu and E.C.Azmitia. Dept. of Biology, New York University, NY, NY 10003.

The homozygote (Pdn/Pdn) of this mutant mouse shows several brain abnormalities, and a significant decrease of S-100β (Naruse et al. Dev. Brain Res.51(2)1990). In order to clarify the effects of the retarded production of S-100β on the development of monoaminergic neuron system, immunocytochemical studies of TH, 5-HT, S-100β and GFAP, and HPLC study for 5-HT and 5-HIAA of Pdn/Pdn mouse were examined, the results were compared with those of wild type (+/+) mouse. In both animals, S-100β positive cells and serotonergic fibers were widely distributed throughout the brains. However, hippocampus and caudo-dorsal cortex of Pdn/Pdn mouse were markedly reduced in S-100β positive cells and serotonergic fibers. Abnormal distribution of GFAP positive fibers was also observed in the neocortex and hippocampus of the Pdn/Pdn brain. No difference were seen in the TH fibers distribution. In the HPLC study, the content of 5-HT and 5-HIAA of hippocampus and cortex of Pdn/Pdn mouse was lower than that of +/+ mouse. These results suggest that S-100β is a serotonergic growth factor, and this mutant mouse is a useful model to study neuro-glial neurotrophic interactions.

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340.10

5-HT, 5-HT$_{1A}$ Receptor, and S-100ß Immunocytochemical Distribution In The Monkey and Rat Spinal Cord.

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Three previously described antipeptide antibodies directed against different regions of the rat 5-HT$_{1A}$ receptor (the N-terminus loop, the 2nd extracellular loop, and the 3rd cytoplasmic loop [Azmitia et al., 1992]) were used to immunocytochemically characterize the distribution of the 5-HT$_{1A}$ receptor in the spinal cord of adult monkey (Macaca f.) and its developmental expression in rat spinal cord. Commercial antibodies against 5-HT and S-100ß were used on adjacent sections to compare the 5-HT fiber and S-100ß cellular distribution to that of the 5-HT$_{1A}$ receptor. 5-HT$_{1A}$ immunoreactive (IR) cells (neurons and astrocytes) and processes were observed to be heavily labelled in the superficial lamina of the dorsal horn, in the region of the central grey, and in the ventral horn of the spinal cord. In adults, we found 5-HT$_{1A}$-IR and S-100-IR in ventral horn motor neurons, but not in the neonatal rat. 5-HT$_{1A}$-IR cells were also seen in the Descending Motor Root of V and in the Supraspinal nucleus of the lower medulla. In all regions, 5-HT$_{1A}$ stained cells are localized to the same areas where dense 5-HT fiber innervation and intense S-100ß cellular labelling was observed. Our results using 5-HT$_{1A}$ receptor antibodies correlate with previous autoradiographic studies, and suggest co-localization with S-100ß. This supports the notion that 5-HT may play a role, through the 5-HT$_{1A}$ receptor in regulating S-100ß, a protein shown to have neuronotrophic properties on motor and serotonergic neurons.

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340.11

DEVELOPMENT OF BRAINSTEM AND HYPOTHALAMIC SEROTONIN IMMUNOREACTIVE SYSTEMS IN THE HAMSTER.

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The appearance of serotonin immunoreactive (5-HT-IR) cells and fibers in the brainstem and basal forebrain has been studied from embryonic day 8 to post-natal day 21. On **E8**, a relatively small bilateral group of very densely IR neurons is present in the most ventral part of the rhombencephalon just caudal to the mesencephalic flexure (MF). A few 5-HT-IR ascending and descending fibers of the raphe nuclei are also seen near the cells. **E11-E12:** A dramatic increase in 5-HT-IR neurons occurs between the MF and pontine flexure, where cells are located in both ventral and dorsal parts. **E11:** a dense bundle of 5-HT-IR axons ascends laterally over the curvature of the MF. **E13:** the bundle reaches the posterior hypothalamus and bifurcates with one group ascending rostrally through the dorsal preoptic area. The second group ascends through the ventro-lateral hypothalamus. The hypothalamic area adjacent to the suprachiasmatic nucleus (SCN) and optic chiasm has significant 5-HT-IR. The bilateral group of 5-HT-IR neurons in the ventral medulla is large by E13, and a much smaller set is also found more dorsally. From **E13 to E15**, terminal arborization is occurring and aggregations of 5-HT-IR neurons becomes looser, but changes in the distri-bution of 5-HT-IR cells and fibers within the brain stem and hypothalamus appear to be minimal.

Neurogenesis in the hamster SCN occurs between E10-E13 (Davis et al., Brain Res. 1990, 519, 192-199) and the major ascending 5-HT pathway is adjacent to the SCN as early as E13. Nevertheless, no 5-HT-IR is seen within the SCN prenatally. The 5-HT-IR fibers appear in the SCN by **postnatal day 3-5**, largely occupying the periphery of the nucleus, and achieve the adult pattern by **postnatal day 10**. Supported by NS22168.

SECOND MESSENGERS V

341.1

THE INFLUENCE OF INSULIN ON MODULATION OF ION CURRENTS IN THE BAG CELL NEURONS OF *APLYSIA*.

E.A. Jonas, R.J. Knox, D.H. Solomon, J.H. Schwartz*, and L.K. Kaczmarek. Dept of Pharmacology, Yale University School of Medicine.

Receptors, such as the insulin receptor, which stimulate tyrosine kinase activity, play an important role in cell growth. There have been few studies, however, on the actions of such pathways on the excitability of neurons. An insulin receptor is expressed in the bag cell neurons of *Aplysia* (see Solomon *et. al.*, Neurosci. abst., 1992). These cells control a sequence of reproductive behaviors in this animal. We have now investigated the effects of insulin on the regulation of ionic conductances in the bag cell neurons. By using a single electrode voltage clamp technique, we have demonstrated that a twenty four hour treatment with 5μM insulin induces a two fold increase in the voltage-dependent calcium current in the bag cell neurons as compared to untreated controls. In addition, insulin also causes an acute increase (over the course of twenty minutes to an hour) in action potential amplitude and a decrease in action potential duration. These acute effects were seen in 65% of cells from animals examined during the summer months, when the animals are most likely to reproduce. The acute changes are associated with an increase in both the voltage-dependent calcium current and an increase in the delayed rectifier potassium current. Our studies suggest that insulin may play a role in the regulation of both short and long term changes in excitability in the bag cell neurons.

341.2

A TYROSINE KINASE RECEPTOR FOR APLYSIA INSULIN-LIKE PEPTIDE(S) MODULATES EXCITABILITY IN *APLYSIA* BAG CELL NEURONS.

D.H. Solomon*, E.A. Jonas, R.J. Knox, A.M. Elste, L.K. Kaczmarek and J.H. Schwartz. Center for Neurobiology & Behavior, Columbia University, New York, NY 10032.

While well characterized receptor-coupled second messenger pathways produce ongoing modulation of neuronal activity, tyrosine kinases are typically implicated in critical choices of survival or differentiation of developing neurons. But how receptor tyrosine kinases modulate synaptic transmission is not yet understood. In the marine mollusc, *Aplysia*, we have characterized a tyrosine kinase receptor for a putative molluscan insulin, which is a possible invertebrate neurotransmitter. Using a *vertebrate* insulin-binding domain antibody, we found an M_r 130,000 protein that is predominately localized to bag cell neurons and which is similar in size to the *Drosophila* insulin receptor α subunit. We cloned the cDNA encoding this protein from a bag cell neuron library by a degenerate PCR strategy. Sequencing revealed a cDNA with an overall inferred amino acid sequence identity of 42% to the human insulin receptor and 93% sequence identity to the *Drosophila* receptor kinase domain. The tetrabasic α/β subunit cleavage site is conserved as are three of the four tyrosine autophosphorylation sites. Immunoprecipitation with an insulin receptor-tyrosine kinase domain monoclonal antibody and kinase assays demonstrate that, in bag cell neurons, high nanomolar concentrations of vertebrate insulin stimulate insulin-specific tyrosine autophosphorylation. Immunocytochemistry shows that the abundance of the bag cell receptor is regulated during *Aplysia* reproductive development: in sexually immature animals we find strong immunoreactivity in the cell bodies whereas in large mature animals very little receptor is seen. In addition, insulin applied to bag cell neurons in culture results in an increase in evoked action-potential amplitude, suggesting that the peptide also produces a tyrosine kinase-mediated change in the excitability of these cells. In addition to their role in growth and metabolism, endogenous *Aplysia* insulin-like peptides may function to modulate synaptic transmission controlling reproductive behaviors through tyrosine protein phosphorylation.

341.3

LONG-TERM MEMORY IN *APLYSIA*: MOLECULAR SIGNALS FOR THE UBIQUITIN-MEDIATED PROTEOLYSIS OF REGULATORY SUBUNITS OF THE cAMP-DEPENDENT PROTEIN KINASE. A. N. Hegde*, D. Chain and J.H. Schwartz, Cent. for Neurobiol. & Behav., Columbia Univ., NY, NY 10032

Behavioral sensitization of defensive reflexes and the underlying presynaptic facilitation of sensory-to-motor neuron synapses lasts for several min (short-term) or days (long-term). Short-term facilitation has been explained by cAMP-dependent modulation of ion channel function. Long-term (LT) facilitation requires additional molecular processes including new protein synthesis. A key molecular event is persistent activation of the cAMP-dependent protein kinase (PKA). Greenberg et al (*Nature* **329:** 65, 1987) showed that regulatory (R) subunits are lost 24 h after LT training; this could account for the persistent activation of PKA at basal levels of cAMP that occurs in LT facilitation (Sweatt & Kandel, *Nature* **339:** 51, 1989). Despite dependence on new protein synthesis, loss of R results from proteolysis rather than diminished synthesis (Bergold et al, *PNAS* **87:** 3788, 1990; *Neuron* **8:** 387,1992). Loss of R is tissue-specific: in muscle, R is not downregulated at high levels of intracellular cAMP. Earlier we established that neuronal and embryonic R subunits are degraded by ubiquitin-mediated proteolysis and postulated that factors targeting R for degradation, which are limiting in naive neurons, are among the new proteins synthesized. To test this hypothesis we compared degradation of R subunits from neurons and muscle. We find that muscle R (M1) can readily be degraded *in vitro* through the ubiquitin pathway. Evidence from molecular cloning indicates that M1 is encoded by the same gene as N4, a major neuronal isoform and a homolog of vertebrate R_I. In addition, vertebrate R_I and R_{II} can be degraded by the same mechanism. We also find that free ubiquitin is present at very low concentrations in muscle. The signals for loss of Rs may be a feature of tissues that exhibit plasticity rather than tissue-specific structural specialization of R subunits. Ubiquitin itself and components of the ubiquitin proteolytic pathway might be among the new proteins made in sensory neurons during LT sensitization.

341.4

THE CA^{2+}-ACTIVATED PROTEIN KINASE C (PKC), APL I , IS PERSISTENTLY STIMULATED DURING LONG TERM SENSITIZATION IN *APLYSIA*. W. S. Sossin*, R. Diaz-Arrastia, T. Sacktor, and J. H. Schwartz. Center for Neurobiology & Behavior, Columbia University, New York, NY 10032.

Apl I, a Ca^{2+}-activated PKC, and Apl II, a Ca^{2+}-independent PKC, are present in the nervous system of the marine mollusk *Aplysia* (Kruger et al, *J. Neurosci.* 11:2303-2313, 1991). 5-HT, a transmitter that causes presynaptic facilitation in *Aplysia*, stimulates only a Ca^{2+}-activated form (Sossin & Schwartz, *J. Neurosci.* **12**:1160-1168, 1992). But surprisingly, translocation of Apl I is not detected under these conditions. To investigate the possibility that other isoforms of PKC are translocated after application of 5-HT, we separated PKCs on hydroxyapatite and examined the resulting fractions for kinase activity, reaction with antibodies to PKCs, and autophosphorylation. The results indicate that Apl I and Apl II are the only two major isoforms of PKC in the *Aplysia* nervous system. Thus, the activation caused by 5-HT must be due to stimulation of Apl I, perhaps by increasing the specific activity of an already membrane-associated form. Long-term sensitization training also only stimulates a Ca^{2+}-activated isoform, presumably Apl I, and this stimulation is present 2 h after the last sensitizing stimuli. A Ca^{2+}-independent PKC activity though, presumably Apl II, is stimulated during the transition between short- and long-term memory, suggesting that it too has a role in the induction of long-term sensitization. The presence of only two isoforms makes *Aplysia* advantageous for understanding the specific roles of Ca^{2+}-activated and Ca^{2+}-independent PKCs in the physiological control of neuronal function.

341.5

THE ROLE OF PHOSPHOLIPASE A_2-ACTIVATING PROTEINS IN NEURONAL SIGNAL TRANSDUCTION. R. Diaz-Arrastia*, A.M. Elste, and J.H. Schwartz, Dept. of Neurology and Center for Neurobiol. & Behav., Columbia Univ., New York, NY 10032

Lipoxygenase metabolites of arachidonic acid (AA) are second messengers in *Aplysia* neurons (Piomelli et al, *Nature* **328**:38, 1987). Evidence has been presented that these metabolites are produced from AA released by receptor-mediated activation of a phospholipase-A_2 (PLA_2). Calignano et al (*Mol Brain Res* **9**:347, 1991) described a M_r 30,000 protein that stimulates PLA_2 in the nervous system of *Aplysia*. A protein with similar activity, phospholipase A_2-activating protein (PLAP), has been cloned from murine smooth muscle (Clark et al, *PNAS* **88**:5418, 1991). We used molecular probes from smooth muscle PLAP to demonstrate that a similar (but not identical) protein is present in vertebrate brain. Northern blots reveal that PLAP cDNA hybridizes to mRNA from vertebrate brain. By immunoblotting we find that anti-PLAP antibodies recognize a M_r 45,000 protein that is abundant in brain, and subcellular fractionation reveals that the PLAP antigen is enriched in synaptosomal and microsomal fractions. Immunocytochemistry indicates that the PLAP antigen is present in many (but not all) neurons throughout the vertebrate brain. We are currently studying how the production of second messengers through the AA pathway is regulated by PLA_2 and the role of PLAP in this process.

341.6

G PROTEIN α-SUBUNITS EXHIBIT A NEURON-SPECIFIC LOCALIZATION IN THE CENTRAL NERVOUS SYSTEM OF THE POND SNAIL *LYMNAEA STAGNALIS*. H van Heerikhuizen*, W. Weidemann, J.C. Knol, E.R. van Kesteren, R.J. Planta and E. Vreugdenhil, Department of Biochemistry and Molecular Biology, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.

G protein mediated signal transduction pathways play an important role in neuronal communication. Aiming at the elucidation of such pathways we have chosen the central nervous system (CNS) of the pond snail *Lymnaea stagnalis* as a model. This CNS contains a relatively small number of neurons (~ 15,000) many of which can easily be identified, are of giant size (up to 0.4 mm) and have been extensively and multidisciplinary studied.

We have cloned cDNAs encoding G protein-coupled receptors, G protein α-subunits and G protein effector molecules. Here we report on the cloning of four subtypes of G protein α-subunits, $G\alpha_o$, $G\alpha_i$, $G\alpha_s$ and $G\alpha_q$. Using immunocytochemistry and molecular biological techniques we have studied the neuron-specific expression of $G\alpha_o$, $G\alpha_i$ and $G\alpha_s$. Surprisingly expression is mainly found in axon tracts rather than in cell bodies, suggesting that signal transduction mediated by these G protein α-subunits is primarily taking place at the axonal level. A number of well-characterized, identified cell groups have been found to (co-)express specific G protein α-subunits. E.g., $G\alpha_o$, $G\alpha_i$ and $G\alpha_s$ are expressed in a subset of the growth-controlling light green cells , whereas $G\alpha_i$ and $G\alpha_s$ are expressed in the caudodorsal cells which are involved in egg-laying and egg-laying behaviour.

Benefiting from the large size of these neurons, we are currently performing micro-injections with plasmids expressing G protein α-subunits, or synthetic oligonucleotides antisense to G protein α-subunit cDNAs. This way we can either up or down regulate a specific G protein α-subunit subtype and study the effect of such a perturbation on ligand-stimulated signal transduction in these neurons.

341.7

ACETYLCHOLINE AND PROCTOLIN DO NOT STIMULATE cAMP PRODUCTION IN THE LOBSTER CARDIAC GANGLION, YET THEIR PHYSIOLOGICAL ACTIONS ARE MIMICKED BY CYCLIC NUCLEOTIDES. H. Hashemzadeh-Gargari, D. G. Winder, and J. Freschi . Neurology Department and Neuroscience Program, Emory University, Atlanta, GA 30322.

The neuromodulators acetylcholine (ACh) and proctolin cause a slow depolarization by activating a voltage-dependent Na^+ current in motoneurons of the lobster cardiac ganglion (Freschi & Livengood, *J. Neurophysiol.* 62: 984, 1989; Freschi, *Neurosci. Lett.* 106: 188, 1989). To characterize the biochemical second messengers that couple the receptors to the ion channels, we conducted electrophysiological and biochemical experiments. When applied by superfusion, phosphodiesterase inhibitors, lipid-soluble analogues of cAMP and cGMP, and the adenylate cyclase stimulator forskolin mimicked ACh and proctolin by producing a voltage-dependent Na^+ current in voltage-clamped motoneurons. However, by biochemical assay we found that neither methacholine nor proctolin caused an increase of cAMP production above baseline control. In these same experiments, serotonin, which had previously been shown to stimulate cAMP production in the cardiac ganglion (Lemos & Berlind, *J. Exp. Biol.* 90: 307, 1981), increased cAMP production an average of 800% above control. Furthermore, serotonin did not evoke an inward Na^+ current. Various nonspecific inhibitors of protein kinase did not block the effects of the neuromodulators. In conclusion, our present results suggest that the activation of a voltage-dependent Na^+ conductance by ACh and proctolin does not occur through a cAMP-dependent mechanism. It remains possible that other cyclic nucleotides, such as cGMP, serve as second messengers for either neuromodulator. We further hypothesize that the stimulated second messenger may interact with the ion channel without activating protein kinase.

341.8

IP_3-ACTIVATED ION CHANNELS IN THE PLASMA MEMBRANE OF OLFACTORY RECEPTOR NEURONS. D.A. Fadool* and B.W. Ache. Whitney Lab. Depts. of Zool. & Neuroscience, Univ. of Florida, St. Augustine, FL 32086.

An antibody directed to the 19 C-terminal residues of a cDNA clone of a mammalian cerebellar IP_3 receptor (kindly supplied by Dr. P. DeCamilli) immunolabelled the outer dendrites of lobster olfactory receptor neurons (ORNs) *in situ*. The IP_3 receptor antibody (αIP_3-R), which recognized a band >200 kDa in membrane preparations of mouse brain, also recognized a band of similar m.w. in membrane preparations of the outer dendrites of lobster ORNs and 36h cultured lobster ORNs. A second band ≈127 kDa was also labelled in the dendrites. Excitatory odors evoke a transient inward current in voltage-clamped cultured ORNs. Introducing αIP_3-R into cultured ORNs through the patch pipette selectively increased odor-evoked inward current an average of 427±48% (n=5). Odors applied to cultured ORNs evoked channel activity in 5 of 19 cell-attached patches. IP_3 applied to the inside face of cell-free patches of membrane activated two types of channels in 55 of 68 patches (73.7±5.7 pS (n=12) and 30.0±1.6 pS (n=16)). Both channels had mean open times best fit by a double exponential; t_1 =0.45±0.07 msec and t_2 =5.47 ±1.6 msec. The Pr_{open} was both pH, IP_3 and calcium concentration dependent, and for the larger channel, was independent of voltage between -90 mV and 60 mV. Neither channel inactivated under continuous stimulation and both were completely and reversibly blocked by 10 μm ruthenium red or 2.5 μm heparin. In one cell it was possible to calibrate an inside-out patch of membrane to IP_3 and then "cram" it into a second cell. Applying an excitatory odor to the bath subsequently activated a channel with properties identical to the one in the control calibration. Collectively, the data suggest that IP_3 directly activates at least one type of ion channel in the plasma membrane of lobster ORNs, which is the effector of the excitatory transduction cascade in these cells.

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341.9

EFFECTS OF EXTRACELLULAR ATP ON NEURONAL Ca^{2+} HOMEOSTASIS. M.L. Koenig*C.A. Fincke, and M.A. DeCoster. Div. Neuropsych., Walter Reed Army Inst. Research, Washington, DC 20307-5100.

Although there are numerous reports in the literature indicating that extracellular ATP can influence Ca^{2+} homeostasis in different cell types (review: Biochim Biophys Acta **1134**, 31-45; 1992), relatively little has been published on potential effects in primary neurons or neuronal cell lines. Since substantial quantities of ATP can be co-released from nerve terminals along with classical neurotransmitters, and because ATP may even be the primary neurotransmitter in some neurons, we have investigated the possibility that micromolar concentrations of ATP can alter intracellular Ca^{2+} ([Ca]) dynamics in cultures of rat forebrain neurons and NG108-15 neuroblastoma-glioma hybrid cells.

Neurons taken from the forebrains of fetal rat pups (embryonic day 15) and differentiated NG108-15 cells were plated onto glass coverslip chamber slides and loaded with the Ca^{2+}-sensitive dye indo-1 (2 μM; 60 min; 37°C). $[Ca]_i$ was measured confocally in single, identified cells (typical field = 120 μm^2;5-10 cells) using an ACAS 570 interactive laser cytometer (Meridian; Okemos, MI).

Fetal neurons were generally not responsive to ATP at concentrations up to 200 μM. In a representative experiment, 100 μM ATP induced an immediate, but small and transient increase in $[Ca]_i$ in only 3 of 10 neurons. In contrast, differentiated NG108-15 cells were very sensitive to treatment with extracellular ATP showing a rapid (2-3s) rise in $[Ca]_i$ when exposed to 10 μM ATP followed by a prolonged (60-100s) return to the pre-peak baseline. $[Ca]_i$ increased in all cells by 6-7 fold in the presence of extracellular Ca^{2+} ($[Ca]_o$), but by only 4-6 fold in it's absence. The data suggest that purinergic receptors are more common and abundant on NG108-15 cells than they are on fetal neurons and that at least part of the the ATP-induced increase in $[Ca]_i$ in NG108-15 cells is due to an influx of $[Ca]_o$.

341.10

OBSERVATION OF NEURONAL INTRACELLULAR CALCIUM OSCILLATIONS USING A REAL-TIME LASER SCANNING CONFOCAL MICROSCOPE. M.A. DECOSTER*, B.J. DAVIS, M.L. KOENIG AND F.C. TORTELLA. Dept. Med. Neurosci., Walter Reed Army Inst. Res., Washington, D.C. 20307-5100.

The 10^4-fold concentration difference between extracellular ($[Ca]_o$) and intracellular ($[Ca]_i$) calcium requires that neurons be able to buffer changes in $[Ca]_i$ or face a disruption of normal physiology and potentially, cell death. Using a real-time laser scanning confocal microscope (Insight System, Meridian Instruments, Inc.), we have monitored intracellular calcium $[Ca]_i$ oscillations in primary rat cortical neurons loaded with the fluorescent calcium dye Fluo-3. Using ionomycin to maximize and EGTA to minimize $[Ca]_i$, we calculated basal concentrations of neuronal free calcium to average 125 nM. Basal $[Ca]_i$ oscillations were only observed in neurons that had been in culture for greater than a week, suggesting that older cultures may be more sensitive to excitatory stimuli. Often, the $[Ca]_i$ oscillations observed were synchronized between multiple neurons simultaneously analyzed in a given field. A sustained increase in $[Ca]_i$ was established in mature neurons (>7 days) when treated with toxic concentrations of glutamate (> 40 μM). In contrast, younger neurons (<7 days) were less sensitive to glutamate. When mature neurons were monitored in calcium-free medium, basal $[Ca]_i$ oscillations were absent or appeared less robust than oscillations observed for cells in calcium-containing medium. Addition of calcium-containing medium to neurons in calcium-free medium elicited an immediate increase in neuronal $[Ca]_i$, but this higher $[Ca]_i$ was eventually buffered by the neurons. In conclusion, monitoring the $[Ca]_i$ dynamics of young and mature neuronal cultures using a real-time laser scanning confocal microscope provides information about the relative ability of these neurons to buffer endogenous and exogenous changes in calcium.

341.11

MEASUREMENT OF SEROTONIN STIMULATED CALCIUM RELEASE USING BIOLUMINESCENT AEQUORIN EXPRESSED IN HUMAN CELLS. Y. Sheu*, L. Kricka, D. Pritchett, Children's Seashore House; Depts. of Ped. and Pharm., Path. Lab. Med., HUP., U. of Penn.; Phila., PA 19104

Aequorin, a bioluminescent protein from the jellyfish, consists of apoaequorin, coelenterazine, and bound oxygen. It emits light upon binding calcium. We investigated whether aequorin expressed in mammalian cells is an appropriate indicator of calcium release in response to neurotransmitters. The 5-HT2 receptors, a member of G protein coupled receptor family, stimulate phosphoinositide hydrolysis upon interacting with agonists and release intracellular calcium. The coding region of aequorin cDNA, prepared from mRNA of *Aequorea victoria*, was amplified by PCR and cloned into the eukaryotic expression vector pRK7. Serotonin 5-HT2 receptor cDNA was cloned into a similar vector. Two days after human embryonic kidney 293 cells were co-transfected with aequorin cDNA and 5-HT2 receptor cDNA, aequorin was reconstituted by incubating with coelenterazine in the reaction solution. These cells luminesced upon serotonin treatment. The response was saturable and dose dependent, and it was inhibited by serotonin antagonists. Human 293 cells stably expressing aequorin seem to suffer no harmful effects due to long term expression of aequorin. This method proves to be a convenient and reliable way to access intracellular calcium concentration change in mammalian cells. This technique thus broadens the range of experiments in which calcium can be measured.

341.12

REGULATION OF CYTOSOLIC FREE CALCIUM IN FURA-2 LOADED DOG TRACHEAL SMOOTH MUSCLE CELLS. C.M.Yang and W.M.Hu*. Depart. of Pharmacol. Chang Gung Medical College, Taiwan, R.O.C.

In order to analyze the factors regulating agonist-stimulated Ca^{+2} mobilization, cytosolic free $[Ca^{+2}]$ ($[Ca^{+2}]_i$) was measured directly in fura-2 loaded canine tracheal smooth muscle cells (TSMCs). Stimulation of muscarinic receptors (mAChRs) by carbachol produced a dose-dependent rise in $[Ca^{+2}]_i$. In the presence of external Ca^{+2}, the initial transient $[Ca^{+2}]_i$ rapidly occurred and followed by a sustained elevation. The sustained elevation was dependent on the external Ca^{+2}. Removal of Ca^{+2} by the addition of EGTA caused a rapid decline in $[Ca^{+2}]_i$ to baseline. In the absence of external Ca^{+2}, only an initial $[Ca^{+2}]_i$ was seen which then ddeclined to baseline; the sustained elevation could be evoked by addition of 1.8 mM Ca^{+2} in the continued presence of carbachol. mAChR occupation by carbachol was required to maintain the elevated level of $[Ca^{+2}]_i$; addition of muscarinic anatgonist atropine, caused $[Ca^{+2}]_i$ to decline to base line. The Ca^{+2}-channel blockers, diltiazem and verapamil, decreased both the initial and sustained elevation of $[Ca^{+2}]_i$ in response to carbachol. These results demonstrate that the initial detectable increase in $[Ca^{+2}]_i$ stimulated by carbachol is due to the release of Ca^{+2} from the internal stores, whereas the contribution of external Ca^{+2} occurs later and at least partially involves a diltiazem- and verapamil- sensitive process. (supported by NSC81-0412-B182-4 and CMRP-340).

341.13

THAPSIGARGIN-INDUCED CA^{2+} INFLUX IS INDEPENDENT OF DEPLETION OF THE IP_3-SENSITIVE CA^{2+} STORE IN NG108-15 CELLS. T.M. Lo* and S.A. Thayer. Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN55455.

Bradykinin (BK)-induced intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) transients were studied in single NG108-15 cells with indo-1-based microfluorimetry. 1 min exposure to BK (30 nM) completely depleted the Inositol 1,4,5-triphosphate (IP_3)-sensitive store which required extracellular Ca^{2+} to refill. Depletion of the store with BK did not activate Ca^{2+}, Ba^{2+} or Mn^{2+} influx, suggesting that capacitative Ca^{2+} entry is not present in these cells. However, Ca^{2+} influx could be elicited by either prolonged exposure to BK, or treatment with the microsomal Ca^{2+}-ATPase inhibitor, thapsigargin (TG). BK-induced Ca^{2+} influx was sustained as long as the agonist was present. TG (10 nM) mobilized Ca^{2+} over the course of 3-5 min, completely depleting the IP_3-sensitive Ca^{2+} store. In contrast, removing extracellular Ca^{2+} for 10 min only partially depleted the store, suggesting that Ca^{2+} leaks from the store at a relatively slow rate. Thus, TG releases Ca^{2+} from the store in addition to preventing uptake. TG also elicited a sustained and irreversible Ca^{2+} or Ba^{2+} influx, in contrast to depletion of the store with BK. These data suggest that depletion of the IP_3-sensitive Ca^{2+} store and induction of Ca^{2+} influx by TG are mediated by different mechanisms. We question the validity of using TG-induced Ca^{2+} influx as a measure of capacitative Ca^{2+} entry.

341.14

MITOCHONDRIA BUFFER PHYSIOLOGICAL CALCIUM LOADS IN RAT DRG NEURONS. J.L. Werth* and S.A. Thayer, Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN 55455.

Intracellular calcium concentration ($[Ca^{2+}]_i$) was measured in single rat dorsal root ganglion (DRG) neurons grown in primary culture. Superfusion with 50 mM K^+ increased $[Ca^{2+}]_i$ from 97 ± 12 to 1199 ± 108 nM. Recovery to basal $[Ca^{2+}]_i$ consisted of three distinct phases; a rapid initial phase followed by a plateau (528 ± 53 nM) and a slow phase. When stimulated in the presence of the mitochondrial uncoupler CCCP (1 μM) the plateau was absent and recovery consisted of a single slow phase. The slow phase, studied in the presence of CCCP, was not affected by removal of extracellular Na^+, indicating that Na/Ca exchange is not responsible for this phase. During the plateau phase, intracellular pH (pH_i) decreased from 7.13 to 6.97. This decrease in pH_i was dependent on extracellular Ca^{2+}, suggesting that during the plateau Ca^{2+} was cycled by the mitochondria. Ca^{2+} cycling effectively uncoupled electron transport from ATP synthesis and resulted in a H^+ accumulation in the cytoplasm. Ten action potentials (10 Hz), elicited by electrical field stimulation, increased $[Ca^{2+}]_i$ to 412 ± 26 nM. Recovery to basal $[Ca^{2+}]_i$ was described well by a single exponential corresponding to the slow, Na^+ -independent phase (tau=7-17 seconds). A plateau in the recovery became apparent as the train was lengthened to 25-30 action potentials. Return to basal $[Ca^{2+}]_i$ consisted of a rapid initial phase (tau $\leq$ 2 seconds), a plateau and a slow phase (tau =8-50 seconds). 1 μM CCCP eliminated the plateau and the recovery was fit well by a single exponential. The time constant of recovery from small $[Ca^{2+}]_i$ loads was unchanged by CCCP. We conclude that large Ca^{2+} loads are initially buffered by fast mitochondrial sequestration, Ca^{2+} is then cycled by mitochondria until a slow, Na^+ -independent process, presumably a Ca^{2+} ATPase, removes the Ca^{2+} from the cytoplasm. Mitochondria do not contribute to buffering of small $[Ca^{2+}]_i$ transients. A train of 25-30 action potentials introduces a Ca^{2+} load of sufficient magnitude to recruit mitochondrial Ca^{2+} buffering.

341.15

INHIBITION OF GUANYLATE CYCLASE BY METHYLENE BLUE INCREASES [Ca2+]i IN MOUSE CORTICAL ASTROCYTES. J.A. Edwards* and H.S. White. Anticonvulsant Drug Development Program, Department of Pharmacology & Toxicology, University of Utah, Salt Lake City, UT 84108.

The role of astrocytes in the etiology of seizure disorders has only recently begun to be investigated. Type I mouse cortical astrocytes are known to both synthesize and release nitric oxide (Murphy et al., 1990), a molecular messenger which increases intracellular cGMP through activation of soluble guanylate cyclase (GC). De Sarro, et al. (1991) have shown that nitric oxide plays a role in excitatory amino acid (EAA)-induced seizure activity since N-monomethyl-L-arginine (an inhibitor of nitric oxide synthesis) and methylene blue (MB, an inhibitor of GC) were both shown to block EAA-induced seizures in rats. Since seizure activity is partially calcium dependent, we examined the effects of MB on the intracellular calcium concentration in type I mouse cortical astrocytes using the fluorescent probe indo-1. The results obtained to date demonstrate that MB increases the intracellular calcium concentration in a biphasic manner. Inhibition of GC by perfusion of 300 nM, 1 μM, and 3 μM MB for 10 min. increased [Ca2+]i by 26, 132, and 66%, respectively. In contrast, increasing [cGMP]i (50 μM 8-bromo-cGMP, 10 min.) decreased [Ca2+]i by 13%. Perfusion with a combination of 8-bromo-cGMP (50 μM) and MB (1 μM) attenuated the increase in [Ca2+]i induced by MB alone. These data suggest that cGMP may be involved in the regulation of [Ca2+]i in mouse cortical astrocytes. Future studies will examine further the effects of nitric oxide, guanylate cyclase, and cGMP on [Ca2+]i in mouse cortical astrocytes and the role of these second messengers in the initiation and propagation of seizure acitivity. (Supported by NIH grants 2-RO1-NS22200 and GM07579).

341.16

EFFECTS OF CAFFEINE ON $[Ca^{2+}]_{in}$ SIGNALS AND SECRETION IN PORCINE ADRENAL CHROMAFFIN CELLS. Y. Xu and E.J. Forsberg.* Dept. of Physiology, Univ. Wisconsin, Madison, WI 53706.

A possible involvement of Ca^{2+}-induced Ca^{2+} release (CICR) in muscarinic agonist-induced, extracellular Ca^{2+}-independent, secretion in porcine adrenal chromaffin cells was examined. Caffeine produced rapid increases in $[Ca^{2+}]_{in}$ in a ryanodine-sensitive manner. In addition, caffeine pretreatment eliminated methacholine- (a selective muscarinic agonist) and thapsigargin- (thapsigargin depletes $Ins(1,4,5)P_3$-sensitive Ca^{2+} pools) induced increases in $[Ca^{2+}]_{in}$ as well as methacholine-induced secretion when Ca^{2+}-free solutions were used. However, other results suggest that CICR does not play a major role in methacholine-induced increases in $[Ca^{2+}]_{in}$ but rather indicate that $Ins(1,4,5)P_3$ and caffeine activate Ca^{2+} release from the same Ca^{2+} pool, or that distinct Ca^{2+} pools, if they exist, can rapidly equilibrate. Caffeine was also observed to block nicotinic responses but not K^+-induced responses, suggesting that caffeine blocks nicotinic receptor-channels. We also observed that caffeine consistently decreased $[Ca^{2+}]_{in}$ when $[Ca^{2+}]_{in}$ had been raised by either methacholine, nicotine or K^+ - an effect which may have been due to the inhibition of either adenosine receptors or cyclic nucleotide phosphodiesterases since aminophylline, 3-isobutyl-1-methylxanthine and caffeine, all of which share these inhibitory activities, were all found to reduce elevated $[Ca^{2+}]_{in}$.

341.17

PHARMACOLOGICAL CHARACTERIZATION OF IP_3-DEPENDENT CA^{2+}-REGULATED CA^{2+} RELEASE. E.A. Finch[1,3], J.L. Hill[3], and S.M. Goldin[2,3]. [1]Program in Neuroscience and [2]Biol. Chem. Dept., Harvard Med. Sch., Boston, MA; [3]Cambridge NeuroScience, Inc., Cambridge, MA.

We have previously reported that extravesicular Ca^{2+} acts as a coagonist with IP_3 to rapidly potentiate and more slowly inactivate IP_3-induced $^{45}Ca^{2+}$ release (Finch et al., Science 252:443, 1991) and that ATP further potentiates this Ca^{2+}-regulated IP_3-dependent Ca^{2+} release. These findings suggest functional homology with the structurally homologous ryanodine receptor of sarcoplasmic reticulum. The IP_3-dependent Ca^{2+}-regulated Ca^{2+} release activity was highly enriched in vesicles derived from cerebellum which also expressed high levels of IP_3 receptor, as demonstrated by use of IP_3 receptor antibodies. We have examined the effects of various pharmacological agents known to act on components of Ca^{2+} stores on the subsecond kinetics of both IP_3-induced and Ca^{2+}-induced Ca^{2+} release.

The microsomal vesicle preparation used for these studies contained both IP_3-sensitive and ryanodine-sensitive Ca^{2+} stores. Thapsigargin (2 μM), which inhibits microsomal Ca^{2+}-ATPase, inhibited up to 80% of ATP-dependent Ca^{2+} uptake. Vesicles preincubated with submaximal concentrations of thapsigargin prior to Ca^{2+} loading exhibited diminished IP_3- and Ca^{2+}-induced Ca^{2+} release. The regulation of the remaining IP_3-mediated Ca^{2+} release by Ca^{2+} and the kinetics of Ca^{2+} release were unaffected by the diminished Ca^{2+} content of the vesicles.

Exposure of microsomal vesicles to ryanodine (10μM), caffeine (10mM), or ruthenium red (10μM) prior to and during Ca^{2+} loading did not alter the Ca^{2+} release pattern elicited by subsequent superfusion with IP_3. However, simultaneous exposure of vesicles to IP_3 and either caffeine or ruthenium red inhibited IP_3-mediated Ca^{2+} release. These results further support the hypothesis of functional homology between IP_3 and ryanodine receptors and demonstrate that pharmacological agents such as caffeine and ruthenium red which were thought to be selective for Ca^{2+}-induced Ca^{2+} release inhibit IP_3-induced Ca^{2+} release as well.

341.18

PURIFICATION AND CALCIUM-BINDING ACTIVITY OF CALRETININ EXPRESSED IN *E. COLI* .

J.H. Rogers, W-T. Cheung and D.E. Richards (SPON: Brain Research Association). Physiological Lab., University of Cambridge, Cambridge CB2 3EG, England.

Calretinin is a neuronal cytosolic calcium-binding protein of the EF-hand family, with 60% homology to calbindin-D28k. The chick calretinin cDNA sequence was reconstructed in a M13 vector and then transferred into an expression plasmid, so that the calretinin gene was under a T7 promoter and inducible by IPTG. This was expressed in E. coli and produced immunoreactive calretinin, which in two-dimensional electrophoresis had the same size (29 kDa) and pI (4.8–5.0) as chick retinal calretinin. The bacterially expressed calretinin was purified with successive ammonium sulfate precipitation, DEAE chromatography, hydroxyapatite chromatography, G-75 chromatography, and Mono-Q chromatography. About 1.0–1.5 mg of pure calretinin was obtained per litre of bacterial culture, representing 0.5–1.5% of the initial protein. Calcium-binding activity of purified calretinin was measured by equilibrium dialysis in Ca.EGTA mixtures with ^{45}Ca, in 100 mM NaCl, 20 mM PIPES pH 7.0. It displayed a K_d of 0.3 μM.

341.19

IMMUNOREACTIVITY FOR CALCIUM-BINDING PROTEINS IN THE CHICK EDINGER WESTPHAL NUCLEUS. J.T. Fujii* and Z. Lucaj. Dept. of Anatomy and Cell Biology. Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Antibodies against parvalbumin and calbindin were used to screen Edinger Westphal nuclei of newly hatched chicks to determine whether immunoreactivity for either protein is preferentially associated with a particular subpopulation of Edinger Westphal neurons. Primary antibodies were obtained from Dr. M. Celio or Sigma and were visualized using biotin-avidin HRP. In some experiments, sections were also stained for neuron specific enolase (BioGenex).

Light to moderate immunoreactivity for parvalbumin was observed in scattered neurons throughout the Edinger Westphal nucleus. The most heavily stained neurons were often found localized in the lateral and central parts of the nucleus. Consistently in some sections of each brainstem, the most heavily stained neurons were clustered along the lateral edge of the nucleus. The medial parts of the nucleus, particularly at the more rostral levels, had fewer and more lightly stained positive neurons. This pattern of parvalbumin immunoreactivity corresponds to the distribution of rapidly firing neurons in the Edinger Westphal nucleus and is in agreement with previous work which associates parvalbumin with neurons firing at high frequency.

Despite the presence of robust staining in our positive controls, no immunoreactivity for calbindin was observed in the Edinger Westphal nucleus.

This work was funded by NSF BNS-8719391.

341.20

GLUCOSE-6-PHOSPHATE MODULATES REGIONALLY SPECIFIC ENDOPLASMIC RETICULUM CALCIUM POOLS IN RAT BRAIN. *A. Verma*, D.J. Hirsch and S.H. Snyder. Johns Hopkins University Sch. of Med., Dept. of Neurosci, Baltimore, MD 21205.*

Glucose-6-phosphate (G6P) is a major intermediary metabolite with several important functions. Recently energy dependent $^{45}Ca^{2+}$ uptake has been shown to be enhanced by mM amounts of G6P in microsomal preparations from these tissues. Using phosphate or oxalate supported, Mg-ATP dependent $^{45}Ca^{2+}$ uptake into rat brain microsomes and fresh frozen sections, G6P was found to dose-dependently reduce net $^{45}Ca^{2+}$ accumulation. 15% of the rat brain Ca^{2+} pools are affected by G6P at maximally effective doses of 5-10 mM. Sensitivity to G6P is specific as other sugar analogs including glucose-1-phosphate, glucose-1,6-diphosphate, galactose-phosphate, glucose-6-sulfate and 2-deoxy glucose-6-phosphate were without effect. Effects of G6P were reversed by heparin and were additive with effects of inostiol 1,4,5-trisphosphate and caffeine. Regional analyses using microsomal preparations or fresh frozen sections evaluated by autoradiography revealed selective enrichment of G6P sensitive Ca^{2+} pools in the brain stem, spinal cord, deep cerebellar nuclei, white matter and hippocampus. These patterns do not resemble any previously localized Ca^{2+} pools and suggest a new role for G6P in CNS physiology. Relationship of the G6P sensitive Ca^{2+} pool to

342.1

EFFECTS OF BENZODIAZEPINE LIGANDS ON FREEZING BEHAVIOR AND REGIONAL EEG ACTIVITY IN RHESUS MONKEYS. S.E. Shelton*, R.J. Kekstas, R.J. Davidson, and N.H. Kalin. Departments of Psychiatry and Psychology, Univ. of Wisconsin, Madison, WI 53792.

Many species "freeze" as an adaptive response to threatening situations. The equivalent of freezing behavior in humans may be the extreme behavioral inhibition exhibited by pathologically shy children. In addition, an animal's duration of freezing is believed to reflect its degree of fearfulness.

We previously established that presenting the profile of a face to rhesus monkeys elicits freezing behavior. To examine the brain regions that mediate freezing, we implanted electrodes on the left and right frontal parietal cortical surfaces of two young rhesus monkeys. The monkeys were exposed to a human facial profile and EEG was monitored by radiotelemetry. Because we have found that benzodiazepines modulate freezing and affect frontal EEG asymmetry, we also examined the effects of benzodiazepine ligands on regional EEG. One subject exhibited selective freezing behavior in response to the challenge; the other did not. The animal that froze also had the more pronounced EEG changes, characterized by relative activation of the right frontal cortex. This is consistent with the right frontal activation seen in humans and monkeys in relation to negative affective states. We compared the effects of diazepam and β-carboline on EEG asymmetry and found that the latter produced greater relative right-sided activation than the former in both frontal and parietal cortical regions. The effects of diazepam are consistent with our earlier study measuring EEG with scalp electrodes in manually restrained monkeys. The data suggest that the therapeutic actions of benzodiazepines may be due to the modulation of the balance of activation between select regions of the two cerebral hemispheres.

342.2

MASKING OF THE CHLORDIAZEPOXIDE (CDP) CUE BY CAFFEINE (CAF) IN A THREE-CHOICE CDP-SAL-CAF DRUG DISCRIMINATION TASK. F.A. Holloway*, K.L. Goulden, & D.V. Gauvin. Univ. Oklahoma Hlth. Sci. Cntr., Dept of Psychiatry & Behavioral Sciences, Oklahoma City, OK USA 73190.

We have reported the blockade of the cuing properties of CDP (5 mg/kg) by CAF in rats trained in a two-choice drug discrimination (DD) task (see Peirce, Gauvin, & Holloway, this meeting). To further explore the nature of this interaction, we trained 12 rats in a three-choice DD task using 3 mg/kg CDP, SAL, and 50 mg/kg CAF as training stimuli.

Both CDP and CAF produced dose- and drug-dependent increases in responding on the injection-appropriate lever. Interaction tests were conducted with CAF and CDP. We have previously shown that high CAF doses produced pentylenetetrazole (PTZ)-appropriate responding in rats trained in a 3-choice CDP-SAL-PTZ DD task (Gauvin & Holloway, *Beh. Pharmac.*, 2, 417-428, 1991) suggesting that these CAF doses were anxiogenic. We predicted that anxiogenic doses of CAF would block the DD (i.e. anxiolytic) properties of CDP. High doses of CAF in combination with CDP engendered saline- or default-appropriate responding. These results suggest that the interoceptive stimuli produced by both training stimuli may lie in overlapping orthogonal dimensions.

342.3

MASKING OF THE CHLORDIAZEPOXIDE DISCRIMINATIVE STIMULUS BY BUSPIRONE AND CAFFEINE. **J.M. Peirce*, D.V. Gauvin, & F.A. Holloway. Univ. of Okla. Hlth. Sci. Cntr., Dept. Psychiat. & Behav. Sci., Okla. City, OK USA 73190-3000.**

Twelve rats were trained in a drug discrimination (DD) task after injections of either chlordiazepoxide (CDP; 5 mg/kg, i.p.) or saline (SAL) in a two-lever food-motivated operant task.

We compared the interaction between CDP and: 1) flumazenil, 2) BUSPIRONE (BUSP) and 3) CAFFEINE (CAF). Co-administration of CAF shifted the CDP DD function to the right. These results are similar to those previously reported by Ator & Griffiths (*Eur J Pharm*, 237, 393-403, 1986) with both baboon and rat subjects.

Because of the disruptive effects of CDP-BUSP combinations, interaction tests had to be conducted with 3 mg/kg CDP, a dose which, by itself, engendered >90% CDP-appropriate responding. Like CAF, BUSP attenuated the DD properties of 3CDP, and shifted the DD function to the right.

The shifts in the CDP DD functions by both CAF and BUSP were paralleled by a downward shift in the rate functions. These drug interactions are contrasted by those produced by CDP-flumazenil tests. The data are similar to previous reports of "perceptual masking" of drug stimuli (Gauvin & Young, *Drug Dev. Res.*, 16, 151-162, 1989).

342.4

STUDIES ON THE DEVELOPMENT OF BEHAVIORAL TOLERANCE TO MIDAZOLAM IN RATS. J.S. Fu and N.W. Pedigo, Jr.*, Departments of Pharmacology and Anesthesiology, Univ. of Kentucky Medical Center, Lexington, KY 40536.

The pharmacological tolerance to benzodiazepines has been extensively investigated, but few studies have assessed the tolerance to ultra-short acting benzodiazepines. Development of behavioral tolerance to midazolam (MDZ) was assessed in Fischer 344 rats trained to respond for food presentation under a fixed-ratio 30 schedule (FR30). The dose-response curve for rate-suppressing actions of MDZ (0.1-3.2 mg/kg) was established in experimentally naive rats, with an ED_{50} of 0.53 ± 0.04 mg/kg. At the highest dose (3.2 mg/kg, sc), MDZ almost completely inhibited response rate. These rats were divided into two groups ($n=6$ each) to study the chronic effects of MDZ on scheduled behavior: group I animals were administered MDZ (3.2 mg/kg/day) 5 min before the FR30 session, while subjects in group II were administrated MDZ immediately after the session. Following six weeks chronic treatment, the rate-suppressing effects of a challenge dose of MDZ (3.2 mg/kg) were significantly impaired in group I animals compared to naive rats (26% *vs* 90% reduction from baseline, respectively, $p<.05$). In group II, the same dose of MDZ produced moderate tolerance, with a 50% decrease in baseline response rate on the FR30 schedule. Based on these studies, we conclude that chronic MDZ induced both pharmacological and behavioral tolerance.

342.5

DIFFERENTIAL EFFECTS OF CARBAMAZEPINE AND PK11195 ON CONFLICT BEHAVIOR IN THE RAT. T.J. Hill*, and R.L. Commissaris. Dept. Pharmaceut. Sci., Wayne State Univ., Detroit, MI 48202.

The present study examined the effects of the anti-seizure agent carbamazepine (CBZ) and the peripheral benzodiazepine antagonist PK11195 on behavior in the conditioned suppression of drinking (CSD) conflict task. In daily 10-min sessions, water-restricted female rats drank from a tube which was occasionally electrified (0.25-0.5 mA shocks signaled by a tone). Acute challenges with CBZ or PK11195 were conducted at weekly intervals using a cross-over design. When examined following 10, 20 or 60-min pretreatment (preTx) intervals, CBZ exerted modest and dose-related anti-conflict effects at doses from 5-20 mg/kg; higher doses significantly suppressed both punished and unpunished responding (water intake). In contrast, whether tested using a 10, 20 or 60-min pre-Tx, PK11195 failed to increase punished responding at doses of 5-40 mg/kg. In addition, combination studies revealed that there was no evidence for additivity between 10 mg/kg CBZ and 10 mg/kg PK11195. The present findings are not consistent with the hypothesis that CBZ exerts its anticonflict effects via antagonist actions at peripheral BZ receptors. The mechanism underlying the anti-conflict actions of CBZ remains undetermined. (Supported in part by USPHS MH47181).

342.6

NON-SEDATIVE DOSE LORAZEPAM DISSOCIATES THE NEUROCHEMICAL AND BEHAVIORAL CORRELATES OF FEAR CONDITIONING IN THE RAT. A.M. Rasmusson*, L.E. Goldstein, B.S. Bunney, and R.H. Roth. Child Study Center and Depts. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

We have recently rigorously characterized a model of fear conditioning in the rat. The studies are carried out during the rat's dark (active) phase. On Day 1, the rat is placed in a novel environment for 30 minutes (habituation phase) and then randomly presented ten tones (CS) paired with ten 0.4 mA footshocks (US) over 30 minutes (conditioning phase). On Day 2, the rat is reexposed to the environment and presented ten tones over 30 minutes (extinction phase). The animals are then sacrificed and their brains dissected for tissue analysis of dopamine (DA), serotonin (5HT), and metabolite levels by HPLC-ED. Trunk blood is collected for serum corticosterone measurement. Animal behaviors are remotely coded during the habituation, conditioning, and extinction periods for locomotion, grooming, freezing and ultrasonic vocalizations. Previous studies using this model have shown that increases in plasma serum corticosterone, DA and 5HT turnover in the prefrontal cortex (PFC), ultrasonic vocalizations, and freezing behavior are under strong CS control; the latter four components of the conditioned fear response (CFR) are also highly correlated. A non-sedative dose of lorazepam (0.5 mg/kg, IP), given 20 minutes prior to testing, completely blocks increases in PFC DA & 5HT turnover, but does not affect the neurohumoral and behavioral components of the CFR. Ondansetron (1.0 mg/kg IP) had no discernable effect on any of the components of the CFR. R016-6028, a partial benzodiazepine agonist,is currently under study. Supported in part by USPHS grants MH14092 and MH25842, and Tourette's Society of America.

342.7

EFFECTS OF ORAL LORAZEPAM AND ALPRAZOLAM ON FR RESPONDING IN RATS. M.J. Kallman*, H.B. Ayvasik, and S. Allen. Depts. of Psychology and Pharmacology, University of Mississippi, University, MS 38677.

The oral effects of two benzodiazepines, lorazepam (LZ) and alprazolam (AP), were compared on fixed ratio (FR)-30 responding. Twenty, male S-D rats were trained to respond on an FR-30 schedule for a sweetened milk reinforcer. Once responding stabilized a dose response and time course were determined for each drug (N=10 rats/drug) when the drug was given by oral gavage. A dose of 3.0 mg/kg LZ produced a significant reduction in lever responses and a reduction in lever contact time. The peak effect for a 3.0 mg/kg dose of LZ was observed at 180 min. after gavage. Onset of activity for AP was more rapid than observed for LZ since the timepoint of peak effect was at 15 min. following gavage. AP produced significant increases in rate of responding at low doses and decreases in rate and increases in duration of responses at higher doses (10-15 mg/kg). Both benzodiazepines alter FR responding when given orally but the less potent LZ has a more rapid onset of effect.

This research was supported by DA-05253.

342.8

MITOCHONDRIAL BENZODIAZEPINE RECEPTORS AND RAPID TOLERANCE TO THE SEDATIVE EFFECTS OF BENZODIAZEPINES IN RATS. C. Ambrosio, R. Reggio and M. Massotti*. Lab. of Pharmacology, Istituto Superiore di Sanità, 00161, Roma, Italy.

In studying tolerance to benzodiazepines (BZ), it is important to take into account that along with sedation these drugs also induce signs of weak behavioural stimulation. These can be clearly observed in electroencephalographic (EEG) studies. In the EEG pattern, the sedative effects of BZ is associated with high voltage, low frequency waves (synchronization), and the stimulatory effect is associated with >14Hz, low voltage waves (β-like activity).

The i.v. administration once a day for 5 days of diazepam (10 mg/kg) flunitrazepam (2.5 mg/kg), clonazepam (2.5 mg/kg), zolpidem (3 mg/kg), alpidem (10 mg/kg) and Cl 218,872 (10 mg mg/kg) suggests that these drugs can be classified into three distinct groups on the basis of their ability to change both the EEG pattern with respect to the 1st day of injection and the density of mitochondrial BZ binding sites in rat brain cortex: a) with diazepam and flunitrazepam, changes in the EEG periods of synchronization (decrease) and β-like activity (increase) are associated with changes in the Bmax values of 3H-PK 11195 (increase) and 3H-Ro 5-4864 (decrease); b) with alpidem, a reduction in the periods of EEG synchronization, without changes in β-like activity, is associated with a reduction in both 3H-PK 11195 and 3H-Ro 5-4864 Bmax values; c) with zolpidem, clonazepam and Cl 218,872 no change in the EEG or binding parameters are observed.

These data suggest that mitochondrial BZ site can play a role in the central adaptive changes underlying tolerance to the BZ.

342.9

EFFECTS OF POST-TRAINING ADMINISTRATION OF THE M_2 ANTAGONIST METHOCTRAMINE ON SPATIAL MEMORY. M.J. Stillman*,[1] B. Shukitt-Hale,[1] R.M. Kong, A. Levy,[2] and H.R. Lieberman. Military Performance and Neuroscience Division, United States Army Research Institute of Environmental Medicine, Natick, MA 01760-5007, [1]GEO-CENTERS, INC., Newton Centre, MA 02159, and [2]IIBR, Ness Ziona, ISRAEL.

Muscarinic autoreceptors in the hippocampus appear to be of the M_2 receptor subtype, since both *in vitro* and *in vivo* investigations have shown increased acetylcholine (ACh) release following administration of M_2 antagonists. Therefore, M_2 antagonists could improve memory and performance by potentiating cholinergic function. One M_2 antagonist, AF-DX 116, appears to improve spatial memory. The purpose of this study was to examine the effect of post-training administration of the M_2 antagonist methoctramine on a spatial memory task. Seven male Fischer rats were trained to criteria on an eight-arm radial maze task, using a within-subjects design. On test days, rats were removed from the maze after their first four choices and then injected with methoctramine (0.15, 0.30, or 0.60 mg/kg, s.c.) or vehicle in a randomized order. Retention was then tested after a four-hour delay. Methoctramine had no effect on: percent correct in the next four choices; the number of trials needed to obtain the remaining four pellets; or the latency to obtain the remaining pellets.

342.10

EFFECTS OF REPEATED ADMINISTRATION OF HORSE SERUM BUTYRYLCHOLINESTERASE ON BEHAVIOR, BLOOD-ENZYME LEVELS AND ANTIBODY PRODUCTION IN RATS. R.F. Genovese*, X-C.M. Lu, M.K. Gentry, R. Larrison and B.P. Doctor, Divisions of Neuropsychiatry and Biochemistry, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Administration of butyrylcholinesterase (BChE) improves outcome from subsequent exposure to organophosphorus (OP) agents. We examined the effects of repeated administration of purified horse serum BChE. Rats (n=10) were trained to lever-press for food under a variable interval 18 s (VI18) schedule of reinforcement. The schedule produced relatively fast and constant response rates throughout daily 30-min sessions. BChE (500 U) was injected IP prior to the start of 5 sessions over a 15-day period. Blood was sampled before the first injection, and on the day following each injection. Blood BChE activity increased following injections 1-3 but returned to near control levels following injections 4-5. Moreover, production of IgG and IgM serum antibodies correlated with the reduction in blood BChE activity. Response rates under the VI18 schedule were unaffected throughout the period. Thus, purified horse serum BChE may be advantageous for extended prophylactic OP therapy since behavioral performance was not disrupted. Long term efficacy from repeated administration, however, may be limited, since 1) increases in blood-enzyme levels were not maintained, and 2) immunoreactive effects from repeated administration are unknown.

342.11

TARGET SITES FOR THE STEROID ANESTHETIC 3α-5α-PREGNANOLONE ARE PRESENT IN MIDBRAIN-HINDBRAIN OF MICE. J.G. Thalhammer*, C. Bukusoglu, W.M. Mok and N.R. Krieger. Anesthesia Res. Lab. Harvard Med. School and Brigham & Women's Hospital, Boston, MA 02115.

The steroid 3α-hydroxy-5α-pregnan-20-one (3α), a metabolite of progesterone, mediates the loss of the righting response (LRR) in mice by its actions at a receptor target site in brain (J. Neurochem. 57,1296, 1991). To localize this receptor by pharmacological methods, we removed all brain structures that are not required for the righting behavior. Midbrain decerebrate mice with brainstem and cerebellum intact are fully able to right themselves (Klin. Wochenschrift 9,404, 1923). Here we report that 3α is still able to induce LRR in decerebrate mice.

Male albino mice (CD-1, 20 g) were anesthetized and the forebrain was removed by gentle suction with the guidance of a dissection microscope. After mice were completely recovered, 3α was injected (i.v.) in a cremaphor (20%) saline (80%) vehicle and LRR was monitored. For a group of 10 decerebrate mice (which were fully able to right themselves), a dose of 1.5 mg/kg 3α resulted in LRR within 40 sec for 100% of the mice. This result is essentially comparable to that previously observed with intact mice although LRR is achieved more rapidly. The same dose administered to intact mice resulted in LRR within 9 min for 80% of those tested (n = 5).

This is the first evidence that functional target sites for 3α and the neuroanatomical circuits that mediate steroid responsive LRR are present in the midbrain-hindbrain. The behavior did not reveal any involvement of the spinal cord although a possible involvement cannot be fully ruled out. Other related or identical receptors may also be present in the forebrain but are not essential to the behavior studied. (Supported by NIH grant GM 42672)

342.12

THE POTENT, RAPID AND BEHAVIOR-SPECIFIC ACTIONS OF ANDROGENS. D. Domek, I. Niekrasz, A. Garnica and T. Seale*. Dept. Pediatrics, U. Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK 73104

Abuse of testosterone derivatives and the finding that non-anabolic steroids have behavioral and neurochemical effects underscore the need for study of the acute and chronic behavioral actions of androgens. We have identified anxiolytic-like effects in mice after the acute administration of testosterone (T) and dihydrotestosterone (DHT) as measured in a novel murine assay (Toubas et al. 1990 Pharmacol. Biochem. Behav. 35:121) sensitive to benzodiazepine and serotonin anxiolytics. Behavior was assessed in male 10 week BALB/cBK mice (n=10/dose) 30 min. after IP injection of T and DHT. Large, dose dependent reductions in aversive behavior were observed without changes in locomotor activity. The estimated ED50 values for T and DHT were 0.032 and 0.06 mg/kg respectively (110 and 210 nmoles/kg). Mouse T production is about 0.16 mg/kg/d (560 nmoles). Effects of T and DHT were compared to those of estradiol (E) and dexamethasone (DEX). Both E and DEX elicited similar behavioral changes but were much less potent. ED50 values for E and DEX respectively were 0.3 and 0.25 mg/kg (1100 and 637 nmole/kg). Classic androgen mediated events require changes in gene expression taking hours to days. The rapid, paradigm-specific behavioral changes we found upon androgen administration and the high potency of these androgens compared to the non-androgenic steroids suggest the presence of androgen binding sites in the brain that modulate non-sexual behaviors.

342.13

EFFECTS OF THE CCK ANTAGONISTS CI-988 AND MK-329 ON THE DISCRIMINATIVE-STIMULUS EFFECTS OF COCAINE. B.W. Massey*, K.E. Vanover, and W.L. Woolverton. The Drug Abuse Research Center, The University of Chicago, Chicago IL 60637.

Cholecystokinin (CCK) and dopamine (DA) have been shown to be colocalized in neurons in the mesolimbic DA pathway and there is evidence that CCK modulates DA neurotransmission. Antagonists for both CCK A and CCK B receptors have been developed and produce opposite effects on DA neurotransmission. Since blockade of DA reuptake into mesolimbic DA neurons is involved in at least some of the behavioral effects of cocaine, compounds that act on CCK receptors may alter those behavioral effects of cocaine. Rats (N=7) and rhesus monkeys were trained in a two-lever drug discrimination paradigm to discriminate cocaine (rats: 8.0 mg/kg, i.p.; monkeys: 0.2 or 0.4 mg/kg, i.m.; 10 min pre-session) from saline rats (rats: 1.0 ml/kg, i.p.; monkeys: 0.1 ml/kg, i.m.; 10 min pre-session). Lever pressing was maintained by food (all rats, two monkeys) or shock avoidance (one monkey). In rats, the CCK B antagonist CI-988 (1.0-32 mg/kg), the CCK A antagonist MK-329 (0.5-2.0 mg/kg) or their vehicles were administered i.p., 30 min prior to cumulative cocaine dose-response functions. In monkeys, CI- 988 was administered i.m., 30, 60 or 120 min pre-session in combination with the training dose of cocaine. In both species, the percentage of responses emitted on the drug lever (% DL) varied between approximately 100% following the training dose of cocaine and 0% following saline. Intermediate doses of cocaine occasioned intermediate % DL. CI-988 had no effect on either % DL or rate of lever pressing in either rats or monkeys. Although there was a tendency for the cocaine dose-response function to shift to the right in combination with MK-329 in rats, this trend did not achieve statistical significance. Higher MK-329 doses are now being tested. These results suggest that blockade of CCK receptors does not alter the DS effects of cocaine (Supported by NIDA grants DA-00250, DA-05951 and DA-00161).

342.14

UNIQUE BEHAVIORAL PROFILES OF CCK ANTAGONISTS IN RATS. J.G. Wettstein*[1], A. Grouhel[1], B. Earley[2] and J.L. Junien.[1] [1]Institut de Recherche Jouveinal, 94265 Fresnes, France and [2]University College, Galway, Ireland.

The non-peptide, benzodiazepine-derived CCK-A and CCK-B receptor antagonists devazepide and L-365260, respectively, were studied alone and with other drugs in conditioned and unconditioned behavioral procedures in rats. The individual effects of the CCK antagonists were usually determined over a range of doses covering 2 - 4 log units (e.g., 0.01 - 10.0 mg/kg). Devazepide and L-365260 had little or no effect under the following three operant schedules: VI/FR conflict, FI-2 (food presentation) and FR-10 (food presentation). Neither drug markedly altered the behavioral effects of amphetamine nor the β-carboline FG 7142 under the VI/FR schedule; L-365260 did not alter the rate-decreasing effects of the dopamine agonists [-]apomorphine and quinpirole under the FI schedule; and both CCK antagonists potentiated the rate-decreasing effects of the dopamine antagonists SKF-83566 and raclopride under the FR schedule. In a conditioned avoidance procedure, however, L-365260 did not alter behavior itself and apparently did not influence the actions of SKF-83566 and raclopride. The CCK antagonists had limited effects on general behavioral activity as measured by locomotor and open field testing. Lastly, although devazepide and L-365260 had some antianxiety-like effects in the elevated plus maze procedure, these actions were neither particularly consistent nor dose-related. Together, the results show that two prototype CCK antagonists from this chemical series possess profiles of action that do not resemble those of known drugs with which one can make direct comparisons.

RECEPTOR MODULATION, UP AND DOWN REGULATION II

343.1

FUNCTIONAL SUBSENSITIVITY OF α_2-ADRENERGIC HETERORECEPTORS OR 5-HT_{1C} RECEPTORS MEDIATING CLONIDINE-INDUCED GROWTH HORMONE RELEASE FOLLOWING CHRONIC GLUCOCORTICOID AND 5-HT UPTAKE INHIBITING ANTIDEPRESSANT TREATMENTS. Charanjit S. Aulakh, James L. Hill, S. N. Pradhan*, and Dennis L. Murphy. Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

We have recently demonstrated that clonidine stimulates growth hormone secretion by activation of α_2-adrenergic heteroreceptors present on 5-HT nerve terminals which in turn, enhance 5-HT activity via stimulation of post synaptic 5-HT_{1C} receptors to promote growth hormone releasing factor (Aulakh et al., in press). In the present study, we investigated the effects of chronic treatment with hydrocortisone (25 mg/kg/day), fluoxetine (2.5 mg/kg/day), clomipramine (5-mg/kg/day), imipramine (5 mg/kg/day), clorgyline (1 mg/kg/day), m-chlorophenylpiperazine (m-CPP, 2.5 mg/kg/day), 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, 2.5 mg/kg/day) and 8-OHDPAT (0.25 mg/kg/day) on clonidine-induced increases in growth hormone levels in male Wistar rats.

Chronic treatment with clorgyline, m-CPP, DOI and 8-OHDPAT did not have any significant effect but chronic treatment with hydrocortisone, fluoxetine, clomipramine and imipramine significantly attenuated clonidine-induced increases in growth hormone levels. These findings suggest development of functional subsensitivity of either α_2-heteroreceptors or 5-HT_{1C} receptors or both following chronic treatment with 5-HT uptake inhibitors and glucocorticoids.

343.2

MOLECULAR MECHANISMS OF CHRONIC MORPHINE UPREGULATION OF THE cAMP SYSTEM IN THE LOCUS COERULEUS. K.L. Widnell, C.M. Bergson, J.A. Clark and E.J. Nestler*. Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale School of Medicine, New Haven, CT 06508.

We have previously shown that chronic opiate administration increases levels of Gi/Go ADP-ribosylation and of adenylate cyclase and cAMP-dependent protein kinase (PKA) activities in the rat locus coeruleus (LC) (see *J. Neurosci.* 12:2439, 1992). This upregulation of the cAMP system has been shown to mediate aspects of opiate tolerance, dependence and withdrawal exhibited by these neurons (*Eur. J. Pharmacol.*, 211:47,1992). In the current study, we addressed the mechanisms underlying the previous changes in ADP ribosylation and enzyme activities. We found that chronic morphine increases levels of immunoreactivity in the LC of Goα, Giα1, Giα2, PKA catalytic subunit and PKA regulatory subunit II (RII), but has no effect on Gsα, Gß, or RI. Northern blot analysis is underway to assess whether these protein changes are associated with alterations in mRNA levels. We previously showed an upregulation of tyrosine hydroxylase (TH) protein and mRNA levels in the LC after chronic morphine treatment (*J. Neurosci.*, 10:2649, 1990). We are currently studying morphine regulation of TH transcription in the LC using a transgenic mouse line (kindly provided by D. Chikaraishi at Tufts). The transgene contains 4.8 kb of upstream DNA from the rat TH gene positioned 5' to the bacterial chloramphenicol acetyltransferase (CAT) reporter gene. This transgenic mouse could provide a model system for studies of the molecular mechanisms of opiate action.

343.3

DOWN-REGULATION OF OPIOID RECEPTOR BINDING IN NEUROBLASTOMA CELLS BY DELTORPHIN C AND DERMORPHIN. L.M.J. Attila*, L.H. Lazarus, and S. Salvadori†. LMIN/NIEHS, RTP, NC 27709 and †Univ. of Ferrara, I-4400 Ferrara, Italy.

There is clear evidence that δ opioid receptors are down-regulated in NG108-15 neuroblastoma glioma cells by long-term opioid agonist treatment. The effects of agonists on μ opioid receptors in clonal cell lines is not yet clarified: this may be due to lack of specific opioid agonists in these experiments. In this study we used frog skin heptapeptides, deltorphin C (DEL-C) and dermorphin (DM), to induce down-regulation of opioid δ and μ receptors, respectively. Receptor binding was assessed with [^{3}H]DPDPE (δ) and [^{3}H]DAGO (μ). NG108-15, SK-N-SH or SH-SY5Y cells were treated for 24 h with nM concentrations of DEL-C or DM, washed and the cells allowed to recover in fresh media for 0 to 5 days. Cells were treated with pertussis toxin (PTX; 0.1-100 ng/ml) or cholera toxin A (CTX-A; 10 ng/ml) for 24 h either before or after the opioid peptide treatment.

Down-regulation of δ binding was found both after DEL-C and DM treatment, the latter being 100-fold less potent. PTX dose-dependently down-regulated δ binding 10-80%, and was additive with DEL-C. Recovery of down-regulation of δ binding took several days when induced by PTX but only 1-2 days when induced by DEL-C or DM which was, however, prevented by PTX. CTX-A treatment alone tended to up-regulate δ binding but did not change the effects of DEL-C or DM. Down-regulation of μ binding was also found after Del-C or DM treatment. The recovery of μ binding after washout of the opioid agonist was, however, much slower than δ binding. PTX pretreatment similarly caused an additive down-regulation of μ binding or, when given after the opioid, prevented recovery. CTX-A pretreatment seemed to potentiate the opioid-induced down-regulation of μ binding. In conclusion, δ receptor turnover seems to be faster and more readily modified by agonist treatment than μ receptors and differs in its response to CTX-A.

343.4

DIFFERENTIAL REGULATION OF β1-ADRENERGIC RECEPTOR (β1AR) mRNA AND LIGAND BINDING BY ANTIDEPRESSANTS AND NOREPINEPHRINE (NE) DEPLETION. M. O. Butler*, K. Hosoda and R.S. Duman. Depts. of Psychiatry and Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06508.

The density of β1AR binding sites is decreased by chronic antidepressant treatments while levels of β1AR binding are increased by administration of agents which deplete NE. The present study was carried out to examine the influence of these treatments on expression of β1AR mRNA measured by RNase protection assay. Chronic ECS treatment (10-14d) decreased levels of both β1AR mRNA and ligand binding in frontal cortex. β1AR ligand binding was also decreased by imipramine (IMI) treatment (3-28d) but expression of β1AR mRNA was regulated in a biphasic manner; IMI treatment for 7-14d increased while treatment for 17-28d decreased β1AR mRNA levels. In contrast, NE depletion had an effect on β1AR mRNA opposite to that of IMI; 7d after 6-hydroxydopamine (6-OHDA) treatment levels of β1AR mRNA were decreased but 14 d after neurotoxin treatment levels of β1AR mRNA returned to control. Taken together, the results demonstrate differential regulation of β1AR mRNA by antidepressant and 6-OHDA treatments and a complex relationship between β1AR mRNA expression and synaptic levels of NE. These results will be discussed with regard to the mechanisms that regulate β1AR mRNA expression, which have been elucidated in C6 glioma cells (see Hosoda et al, this volume).

343.5

"LOCUS COERULEUS-LIKE" CELL LINE: A MODEL SYSTEM FOR STUDIES OF OPIATE AND ANTIDEPRESSANT DRUG ACTION. R.S. Duman*, R.Z. Terwilliger, and E.J. Nestler. Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

In recent years, we have been studying the molecular mechanisms underlying the actions of a number of psychotropic drugs and stress in the locus coeruleus (LC) in vivo. In an effort to establish an in vitro model system for further characterization of the in vivo findings, we have carried out preliminary studies on an "LC-like" cell line derived from a brainstem tumor of a transgenic mouse in which the transgene consisted of a 773 bp fragment of the tyrosine hydroxylase (TH) promotor fused to the SV-40 T antigen (Suri, Fung, and Chikaraishi, *Soc Neurosci Abs* 17, 528, 1991). The cell line expresses TH and synthesizes norepinephrine but not epinephrine; it also expresses neurofilaments and neuron-specific enolase but not glial fibrillary acidic protein. Membranes prepared from the cells display high levels of CRF- and VIP-stimulation of adenylate cyclase, and of α_2-adrenergic, opiate-, and NPY-inhibition of the enzyme, receptor responses expected in LC. Treatment of the cells with CRF, forskolin, or cyclic AMP analogues causes the cells to extend neuritic processes within 2 hr. In addition, CRF or forskolin treatments down regulate CRF- but not VIP-stimulated adenylate cyclase. Treatment of the cells with morphine for 5 days increases levels of cyclic AMP-dependent protein kinase and TH as seen in the LC in vivo. These studies support the use of this cell line to investigate the molecular regulation of LC neuronal function by opiate, CRF, and antidepressant treatments.

343.6

β1-ADRENERGIC RECEPTOR (β1AR) DOWN REGULATION IN C6 GLIOMA CELLS: EVIDENCE FOR INHIBITION OF β1AR GENE TRANSCRIPTION BY A TRANSIENT REPRESSOR. K. Hosoda*, G. Feussner¥, P.H. Fishman¥, and R.S. Duman. Depts. of Psychiatry and Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06508 and ¥National Institutes of Neurological Disorders and Stroke, NIH, Bethesda, MD 20892.

To elucidate the molecular mechanisms underlying regulation of β1AR expression in brain and other tissues, we are studying β1AR in C6 glioma as a model cell system. Exposure of cells to isoproterenol (ISO) or forskolin for 1hr increased levels of β1AR mRNA by approximately 70% while longer treatment (2-7 hr) resulted in a time dependent 50% decrease in levels of β1AR mRNA. Levels of βAR ligand binding were decreased by ISO and forskolin treatments (1-7 hr). The half-life of β1AR mRNA, determined in the presence of actinomycin D, was 104 min and was not significantly influenced by either ISO or forskolin treatments, indicating no change in mRNA stability. However, ISO treatment (2 hr) did decrease by 40% the rate of β1AR transcription, measured by in vivo labeling of nascent mRNA with thiouridine. Addition of exotoxin A, a protein synthesis inhibitor, blocked the down regulation of β1AR mRNA; in fact, in the presence of exotoxin A, ISO or forskolin treatment (2-7 hr) increased levels of β1AR mRNA by up to 400%. The results demonstrate that down regulation of β1AR mRNA occurs via decreased transcription rate and suggest that this decrease may be dependent on the synthesis of a transient repressor protein.

343.7

DIFFERENTIAL REGULATION OF ß-ADRENERGIC RECEPTORS (ß-ARs) AND ß-AR mRNAS BY DESIPRAMINE AND CARBAMAZEPINE IN CULTURED C6 GLIOMA CELLS G.Chen, C.Hough, H.Manji*, D-M.Chuang, and W.Z.Potter, Exp Ther Branch and Biol.Psych. Branch, NIMH, Bethesda, MD 20892

Chronic treatment with the tricyclic antidepressant desipramine (DMI) causes downregulation and desensitization of ß-adrenergic receptor *in vivo* (animals) and *in vitro* (cultured cells). We recently found that DMI treatmant (10 µM for 6 days) induced ß-AR downregulation and desensitization in C6 glioma cells was accompanied by a significant decrease in the steady-state level of ß-AR mRNAs, which was time (10 µM for 0-7 day) and dose (0-25 µM for 6 days) dependent . Carbamazepine (CARB), an anticonvulsant, has a similar structure to DMI, but in contrast to DMI, CARB is a clinical effective antimanic agent. Exposure of C6 glioma cells to CARB resulted in a time (50 µM for 6 days) and dose (0-100 µM for 6 days) dependent increase in the number of ß-AR. CARB exposure also signicantly altered the steady-state level of ß-AR mRNAs. The mechanism(s) by which these two tricyclic agents differentially regulate the levels of ß-AR mRNAs is currently under investigation

343.8

DESIPRAMINE DESENSITIZES BETA-ADRENERGIC SIGNAL TRANSDUCTION WITH IMPAIRED RECOVERY IN OLD AND SUPERSENSITIVITY IN MATURE RATS. G. Rajakumar*, M.M. Koller, K. Horvath and P.J. Scarpace, GRECC, VA Medical Center, and University of Florida, Gainesville, Florida 32608

We assessed ß-adrenergic receptors (ßAR) in the cerebral cortex and ßAR and isoproterenol dose resopnse stimulation of adenylate cyclase activity (AC) in the parotid gland (P) in control, 28 d desipramine (DMI) (10 mg/kg) and following a 2 d and 14 d washout (WO) period in 6-, 12- and 24-month old female F344 rats. ßAR density decreased in cortex (73 ± 5 fmol/mg, 6 mo; 63 ± 2, 12 mo; 52 ± 3, 24 mo; $p < 0.001$) but was unchanged with age in P (54 ± 5, 55 ± 2, 49 ± 4). DMI down regulated ßAR in cortex (45 ± 3, $p < 0.005$) and P (32 ± 1, $p < 0.001$). In cortex, there was full recovery by 2 d WO in 6 mo (79 ± 3), upregulation at 14 d WO in 12 mo (100 ± 7), and there was no recovery in 24 mo (39 ± 2, $p < 0.05$). In P there was full recovery by 2 d at all ages with an upregulation in 12 mo (82 ± 9, $p < 0.002$). In P maximum isoproterenol (32 µM)-stimulated AC was unchanged with age but decreased with DMI treatment (57 ± 5.8 pmol cAMP/min/mg, control; 22 ± 5, DMI, $p < 0.001$). There was full recovery by 2 d in 6 mo (51 ± 6) and by 14 d WO in 24 mo (54 ± 10). In 12 mo there was a supersensitivity of AC (93 ± 15) that corresponded to the receptor upregulation. These data indicate that DMI desensitizes ßARs in peripheral tissue similar to the well known effects on brain tissue. There is an unexplained upregulation of ßAR and supersensitivity of AC in the 12 mo rats after 14 d WO and impaired recovery of AC in the 24 mo. Supported by the Dept. of Vet. Affairs, PHS grant DE 08845 and Univ. Zurich, Switzerland.

343.9

LONG-TERM EFFECTS OF PIRENZEPINE ON REPRESENTATIONAL MEMORY AND MUSCARINIC RECEPTORS. W.S. Messer, Jr.* and M. Bohnett. Department of Medicinal & Biological Chemistry, College of Pharmacy, Univ. of Toledo, Toledo, OH 43606.

Previous studies have documented a role for M_1 muscarinic receptors in representational memory function. Intrahippocampal injections of pirenzepine initially result in memory impairments, while repeated treatments are less effective and lead to increases in muscarinic receptor binding. The long-term behavioral and neurochemical effects of pirenzepine were examined following intrahippocampal injections. Autoradiographic methods were utilized to localize muscarinic receptors.

In behavioral studies, intrahippocampal injections of pirenzepine (70 µg per hippocampus) initially impaired memory function (71 ± 1.7 %). By the third session, performances were significantly above chance levels (83 ± 0.0 %). In autoradiographic studies, the binding of 2.0 nM [^{3}H]-(R)-QNB to rat brain sections provided an estimate of maximal levels of muscarinic receptor binding. [^{3}H]-(R)-QNB binding increased in several brain areas after four days (15 - 53 %) and two weeks (11 - 56 %) of treatment relative to control animals.

The time-course of behavioral and biochemical effects suggest that long-term pirenzepine injections increase the number of muscarinic receptors, which is associated with improved performance on the representational memory task. Supported by NS 25765 and NS 01493.

343.10

REGIONAL CHANGES IN RAT CENTRAL NICOTINIC ACETYLCHOLINE RECEPTORS FOLLOWING NOSE-ONLY EXPOSURE TO MAINSTREAM CIGARETTE SMOKE. S.L. Yates*[1], K.G. Fernandes[2], E.N. Fluhler[2], P.M. Lippiello[2]. [1]Duke University Medical Center, Integrated Toxicology Program, Durham, NC 27710 and [2]RJR-NABISCO, Pharmacology Division, Winston-Salem, NC 27102.

Smokers are reported to have a higher density of nicotinic acetylcholine receptors (nAChR) than non-smokers. Whether this increased receptor density is a response to smoking or a result of genetic variability is not known. While chronic nicotine infusion of rats and mice results in the up regulation of central nAChR, changes in receptor density in response to nicotine exposure *via* cigarette smoke have not previously been studied in animals. Furthermore, a causal relationship between cigarette smoking and changes in nAChR density in humans has not been established. In this study male Sprague-Dawley rats were nose-only exposed 1-hr/day, 5-days/week, for 13-weeks to mainstream cigarette smoke, which consisted of the following (Mean±SD): 0.64±0.03 mg/L wet total particulate material, 773±53 ppm CO, and 37±2 µg/L nicotine. ^{3}H-Nicotine binding was measured in various brain regions of smoke-exposed and sham-exposed animals. In smoke exposed animals there was a trend for nAChR up regulation (approximately 25%) in the cortex and striatum, while there was no apparent change in receptor density in the midbrain, hippocampus, and cerebellum. Smoke exposure did not alter the affinity of the receptor for nicotine in these brain regions. These results suggest that smoke exposure can alter nAChR density in rats, and may be relevant to understanding the basis of receptor up regulation in human smokers.

343.11

DIFFERENTIAL EFFECTS OF OLFACTORY BULBECTOMY ON GABA RECEPTORS IN THE RAT BRAIN. T. Dennis*, V. Beauchemin and N. Lavoie, Neurobiological Psychiatry Unit, Department of Psychiatry, McGill University, Montréal, Quebéc, Canada, H3A 1A1.

Olfactory bulbectomy (OBX) is generally recognized as a useful model for the study of the neurobiology of depression and the mechanisms of action of antidepressant treatments. Following OBX, rats display antidepressant-reversible behavioral and biochemical changes. Although $GABA_B$ binding densities are decreased in frontal cortex 14 days after OBX (*Lloyd & Pichat, Br.J.Pharmacol. 87:36P, 1985*), the time-course of these changes have not been determined. The present study investigated the effects of OBX on $GABA_A$, $GABA_B$ and benzodiazepine (BZD) binding parameters 7, 14, 21 and 28 days after bilateral OBX.

Male Sprague-Dawley rats were anesthetized and mounted in a stereotaxic frame. The olfactory bulbs were exposed, sectioned and aspirated. Animals were housed in groups of 4 until sacrifice. Cerebral membranes were incubated with [^{3}H]GABA, in the presence of baclofen or isoguvacine, and [^{3}H]flunitrazepam to define $GABA_A$, $GABA_B$ and BZD receptors, respectively. $GABA_A$ and BZD receptor densities in frontal cortex were increased 7, 14, 21 and 28 days after OBX. In hippocampus, $GABA_A$ receptor densities increased after 7 days but decreased thereafter. No change in BZD Bmax values were observed in hippocampus, while, in hypothalamus, a transient increase was observed 7 days post-lesion. $GABA_B$ receptor densities were unaltered in hippocampus and hypothalamus up to 28 days following OBX, but were decreased from 14 days onward in frontal cortex. Increased $GABA_B$ binding densities in cerebellum were accompanied by decreases in affinity.

These changes in $GABA_A$ and $GABA_B$ binding parameters are likely to result in alterations of GABAergic neurotransmission, which might underlie the behavioral deficits elicited by OBX. *Supported by MRCC and FRSQ.*

343.12

BLOCKADE OF KINASE AND PHOSPHATASE ACTIVITY PREVENTS CORTICAL AMINO ACID RECEPTOR REGULATION. B.A. Pasqualotto*, R.A. Lanius, and C. Shaw. Departments of Ophthalmology, Neuroscience, and Physiology, University of British Columbia, Vancouver, Canada.

Using an in vitro adult rat cortical slice preparation we have examined the effects of increases in cell electrical activity or agonist stimulation in relation to the action of phosphorylating and dephosphorylating enzymes on an AMPA receptor population using the antagonist [^{3}H]-CNQX. Increases in electrical activity induced by the administration of veratridine and glutamate (V+G) result in a decrease in the number of AMPA receptors in adult cortex; conversely, the inhibitory $GABA_A$ receptor population is increased by this treatment. Both receptor populations decrease following treatment with their respective agonists, quisqualate and muscimol (Shaw & Lanius, Soc. Neurosci. Abstr. 1992). Increases in both AMPA and $GABA_A$ receptors were seen following treatment with alkaline phosphatase. Decreases were observed following protein kinase administration (Lanius & Shaw, Soc. Neurosi. Abstr. 1992). For AMPA receptors the down-regulation induced by V+G, agonist, or kinase could be blocked by protein kinase inhibitor. Up-regulation induced by alkaline phosphatase could be blocked by phosphatase inhibitors such as sodium vanadate or phenylarsine oxide. These results suggest that the down-regulation of AMPA receptors by increases in cell electrical activity and by agonist are dependant on the activity of phophorylating enzymes while the up-regulation of these receptors is dependant on the activity of dephosphorylating enzymes.

343.13

EXPOSURE TO WAR IS ASSOCIATED WITH DECREASED PLATELET BENZODIAZEPINE RECEPTORS IN HUMANS. M. Gavish*, N. Laor, L. Karp, E. Dagan, A. Reiss and R. Weizman. Dept. of Pharmacol., Rappaport Fac. of Med., Technion, Haifa, Israel.

Mitochondrial benzodiazepine receptors (MBR) play a major role in steroidogenesis and are sensitive to stress and hormonal changes. Stress affects MBR bidirectionally, i.e., acute stress up-regulates MBR, while repeated stress down-regulates these receptors. To further explore the possible link between stress and MBR, we determined the pharmacodynamic characteristics of platelet MBR in 11 Israeli civilians 1 day prior to the Gulf war, during the war (30 days after initiation of the missile attacks), and 4 weeks after cessation of the war. Hamilton anxiety rating scale (HAM-A) and Beck depression inventory (BDI) scores were significantly higher before and during the war when compared to the postwar scores. The density of platelet MBR before the war was significantly reduced (-22%, $p<0.05$) when compared to postwar values. Values during the war did not differ significantly from the postwar values. The decrease in HAM-A scores (postwar values minus prewar values) correlated significantly with the increase in MBR density ($r = -0.62$, $p<0.05$). A significant correlation was also obtained when comparing the changes between post- and during-war levels [postwar values minus during-war values ($r = -0.70$, $p<0.05$)]. No such correlation was demonstrated with BDI. It is concluded that sustained stress of war is associated with reduction in MBR density.

343.14

CROSS-TOLERANCE OF LORAZEPAM AND ALPRAZOLAM: PRESERVATION OF SPECIFIC REGIONAL EFFECTS ON BENZODIAZEPINE BINDING. L.G. Miller*, J.J. Byrnes, D.J. Greenblatt, R.I. Shader. Dept. of Pharmacology and Experimental Therapeutics and Neuroscience Program, Tufts Univ. School of Medicine, Boston, MA 02111.

Clinical and behavioral data support cross-tolerance among some benzodiazepines, but evidence is limited concerning the most commonly used benzodiazepine alprazolam. We administered alprazolam (ALP) and lorazepam (LRZ), 2 mg/kg/d, to mice for 1-14 days using subcutaneous osmotic pumps. Some mice were treated with ALP for 7 d followed by LRZ for 7 d, or vice-versa. Pentylenetetrazole-induced seizure threshhold was determined, and benzodiazepine binding was evalued in 5 brain regions at 1 and 14 d. Mice became tolerant to both ALP and LRZ after 7 d. Discontinuation of either drug after 7 d led to a decrease in threshhold at 11 d. Substitution of LRZ for ALP or vice-versa maintained tolerance in both cases. Tolerance was similar in ALP 7d/LRZ 7 d or the reverse combination and either ALP or LRZ for 14 d. Benzodiazepine receptor binding in cortex was reduced at 14 d compared to 1 d in cortex and hippocampus after lorazepam, but only in cortex after alprazolam. Substitution of LRZ for ALP produced results similar to 14 d of LRZ, and vice-versa. These results indicate complete behavioral cross-tolerance between lorazepam and alprazolam. Benzodiazepine receptor downregulation occurs in cortex and hippocampus with lorazepam, but in cortex alone with alprazolam.

343.15

ALTERATIONS IN HIPPOCAMPAL GLUTAMATERGIC BINDING SITES FOLLOWING LESIONS OF THE RAT MEDIAL SEPTUM. F.M. Inglis, J.A.D.M. Tonnaer† & J. McCulloch (SPON: Brain Research Association) Wellcome Surgical Institute, University of Glasgow, U.K. and †Organon Laboratories Ltd, Oss, Netherlands

Using quantitative in vitro autoradiography, we investigated the glutamatergic response to cholinergic deafferentation of the hippocampus.

Lesions of the medial septum were produced in rats by intracerebral injection of ibotenate at stereotaxically defined coordinates. Three weeks post-lesion, binding to glutamate receptor subtypes in the hippocampal and septal regions was imaged with [3H]-kainate, [3H]-AMPA and NMDA-displaceable [3H]-glutamate. Muscarinic binding sites were measured using [3H]-QNB.

Following septal lesions, [3H]-QNB binding was reduced significantly in the subiculum, and in the medial and lateral septum compared to sham-operated controls. In other hippocampal regions, [3H]-QNB binding was unaltered. Following septal lesions, [^{3}H]-AMPA binding to AMPA receptors was significantly reduced in the stratum pyramidale of CA1-3, in the CA1 stratum radiatum, and in the CA2 stratum launosum moleculare. [3H]-AMPA binding was also reduced in the deep layers of the medial entorhinal area, the superficial layers of the lateral entorhinal area and in the medial septum. No significant alterations were measured in the dentate gyrus. In contrast to reductions in [3H]-AMPA binding, [3H]-kainate and NMDA-displaceable [^{3}H]-glutamate binding to kainate and NMDA receptors remained unaltered in the hippocampal and septal regions following medial septal lesions. These results demonstrate that in addition to cholinergic modifications, glutamatergic receptor subtypes are altered differentially following cholinergic denervation of the hippocampus.

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343.16

SEIZURE-INDUCED CHANGES IN GluR2 AND GluR3 mRNA EXPRESSION SUGGEST DISTINCT REGULATORY MECHANISMS. §S Gold*, §G. Lynch, and †C. Gall. §Dept. of Psychobiology and †Dept. of Anatomy & Neurobiology, Univ. of California, Irvine CA 92717.

We recently demonstrated that limbic seizures decrease the expression of mRNA encoding the GluR1 subtype of non-NMDA glutamate receptor (Gall et al., 1990); 30 hrs after the placement of a seizure-producing lesion, GluR1 mRNA was reduced by 80% and 48% in the dentate gyrus granule cells and superficial neocortex, respectively. We now report seizure-induced changes in mRNA levels for the GluR2 and GluR3 receptor subtypes. A 10 hr episode of recurrent limbic seizures was induced by electrolytic lesion of the dentate gyrus hilus and mRNA levels were evaluated by in situ hybridization using ^{35}S-labeled cRNA probes. In animals killed 30 hours post-lesion, hybridization to GluR2 mRNA was decreased by 45% in stratum granulosum and by 70% in superficial neocortex (layers II/III). At this time point, GluR3 mRNA was also decreased in superficial neocortex (54%) and piriform cortex (63%) but, in contrast to the other subtypes, GluR3 mRNA was *increased* to 133% of control levels in stratum granulosum. These opposing changes at the mRNA level for GluR2 and GluR3 in stratum granulosum suggest the possibility that receptor properties change in the granule cells following seizures. Hollman et al. (1991) have demonstrated differences in calcium permeability dependent upon the proportionate expression of the GluR1, GluR2, and GluR3 mRNA's. Thus, the post-seizure change in the ratio of GluR2 to GluR3 mRNA described here may alter Ca^{++}-mediated post-synaptic processes in the dentate gyrus. Supported by a FAW from NSF and NS26748 to C.G.

343.17

MODIFICATION OF THE ω6 POLYUNSATURATED FATTY-ACID COMPOSITION OF RAT ASTROCYTES IN PRIMARY CULTURE: EFFECTS ON BASAL AND RECEPTOR-MEDIATED cAMP FORMATION AND UPTAKE OF ADENOSINE AND GLUTAMATE

M.G. Murphy* and Z. Byczko. Dept. of Physiology & Biophysics, Dalhousie University, Halifax, Nova Scotia Canada B3H 4H7

Receptor and transport functions were examined in primary cultures of rat astrocytes whose membrane polyunsaturated-fatty-acid (PUFA) profiles were dramatically modified by 3-weeks' exposure to delipidated serum ± linoleic acid. Enrichment in ω6 PUFA (20:4, 22:4, 22:5) did not alter basal levels of cAMP, or cAMP formation stimulated by forskolin, histamine, isoproterenol, NECA or 5-HT. However, dopamine (DA, 10^{-4} M)-stimulated cAMP synthesis was significantly lower in PUFA-enriched than in PUFA-deficient cells (620 ± 30 and 410 ± 20 pmol/mg protein, respectively). The cells did not contain inhibitory DA receptors. [^{3}H]glutamate uptake into the astrocytes was not affected by ω6 PUFA enrichment, however, that of [^{3}H]adenosine was reduced relative to control values. These findings indicate that ω6 PUFA affect membrane processes in a very selective manner, and suggest that they may play a role in directing events that occur during early development. (Supported by the Canadian MRC)

343.18

PRETREATMENT OF CORTICAL CULTURES WITH BDNF SIGNIFICANTLY REDUCED THE EFFECTS OF ACUTE BDNF ON THE STIMULATION OF PHOSPHATIDYLINOSITOL HYDROLYSIS.

D.R. Kaplan+, H.R. Widmer, F. Hefti, F. Ohsawa, T. Denton* and B. Knüsel. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089 and +NCI-Cancer Research and Developmental Center, Frederik, MD 21701.

The hydrolysis of phosphatidyl 4,5-bisphosphate (PI) produces two potent intracellular second messenger molecules. Recently we reported that the neurotrophin, brain-derived neurotrophic factor (BDNF), stimulates PI-hydrolysis in primary cultures of fetal brain neurons within 15 min. We also observed that BDNF rapidly stimulates the phosphorylation of phospholipase-Cγ1 (PLC) by activating *trk*-type protein kinase receptors. The action of BDNF seems to be neuron specific since the relative effect of BDNF on PI hydrolysis in cortical cultures treated with cytosine arabinoside (which resulted in glial cell-free cultures) was significantly increased compared to untreated cultures. Moreover, we reported earlier that no stimulation was observed in preparations of non-neuronal cultures. We now present evidence that long-term pretreatment with BDNF for 4 days essentially abolishes the PI response to acute stimulation with the same factor (104%, compared to 132% without pretreatment). The stimulation due to addition of bFGF was not affected (119% and 122%). These results suggest specific downregulation of a BDNF responsive PI mechanism. Pretreatment resulted in slightly increased protein (+7%) and lipid levels (+4%). No effect was observed on the number of GFAP positive cells and on GFAP protein level. We conclude from our data that signal transduction of BDNF in rat brain cultures involves PI hydrolysis specific for this factor. Other results show that PLC is stimulated by activation of *trk*-type receptor proteins.

343.19

CHARACTERIZATION OF TRANSACTIVATION PROPERTIES AND FUNCTIONAL DOMAINS OF THE MINERALOCORTICOID RECEPTOR.

R. Rupprecht, J. L. Arriza, D. Spengler, J. M. H. M. Reul, R. M. Evans, F. Holsboer* and K. Damm. Max-Planck-Institute of Psychiatry, Clinical Institute, Dept. of Neuroendocrinology, Munich, Germany and Gene Expression Lab., The Salk Institute, La Jolla, CA 92037.

The human mineralocorticoid (hMR) and glucocorticoid receptor (hGR) mediate biological responses to adrenal corticosteroids and synthetic ligands. In transient transfection studies employing a human neuroblastoma cell line, a mouse mammary tumour virus luciferase fusion gene was used to monitor the trans-activation properties of both receptors. The hMR was more sensitive for cortisol than the hGR. However, the hMR stimulation of this promoter was only 2-4% of the maximum level obtained with the hGR. To examine the molecular basis of this differential response, a series of hMR/hGR hybrid receptors were created and their properties determined. These experiments demonstrated that the hMR amino terminus is lacking the strong trans-activation function present in the equivalent hGR domain. In addition various reporter constructs revealed that the amino termini of hGR and hMR differentially promote or inhibit synergistic trans-activation functions of the DNA- and ligand binding domain of both receptors.

343.20

IMMUNOCYTOCHEMICAL DETECTION OF NEURONAL ESTROGEN RECEPTORS AFTER TIMED ESTROGEN ADMINISTRATION AND WITH DIFFERENT ANTI-RECEPTOR ANTIBODIES.

A. A. Gerall* and L. Givon. Dept. of Psychology, Tulane Univ., New Orleans, LA 70118.

Endogenous or exogenous estrogen reduces estrogen receptor (ER) immunoreactivity (ir) when detected with the anti-ER monoclonal antibody H222spγ. This lessening of ER-ir could be due to estrogen's changing the receptor's conformation so that H222's binding site is obscured or to its forming an estrogen-ER complex that down-regulates ER expression. Estrogen could alter its receptor's conformation within 10 min after administration, but over an hour is required for it to instigate down-regulation. Ovariectomized Swiss-Webster mice were injected i.m. with 4 μg estradiol-17β (E_2) or oil at either 10 min, 1, 4, 12, 24, or 48 hr before perfusion. Brain sections were processed immunocytochemically with H222spγ (Courtesy of Abbott Labs.). No ER-ir was obtained in the 10 min and 1 hr E_2-mice. ER-ir began to appear in the 4 hr E_2-mice and increased gradually to oil-control levels by 48 hr.

Secondly, ER-ir obtained with H222, whose epitope is in the ER's steroid binding domain, was compared with that detected with the anti-ER polyclonal antibody, ER-715 (National Hormone and Pituitary Program and NIDDK), whose epitope is in the ER's hinge region. Alternate serial brain sections from ovariectomized mice injected with oil or E_2 10 min or 1 hr before perfusion were processed with H222 or ER-715. Both antibodies yielded similar distribution and density of neuronal ER-ir in oil-mice and no ER-ir in the 10 min and 1 hr E_2-mice. Neither conformational nor down-regulation processes alone account for the obtained results.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION: OTHER

344.1

CHRONIC FREE TESTOSTERONE (T) IN FEMALE AND FEMINIZED MALE RATS DURING 15-30 DAYS OF AGE: MASCULINIZATION OF POSITIVE FEEDBACK REGULATION OF FSH AND LH.

S.D. GALE*, AND G.J. BLOCH, Dep't of Psychology, Brigham Young U., Provo,UT,84602.

T implants during postnatal days 15-30 can permanently defeminize endocrine and behavioral function in female rats, as well as defeminize and masculinize reproductive function in neonatally gonadectomized (NeoGxd) males (Gale et al., Neurosci. 90,91). To determine whether this chronic T-treatment affects positive feedback regulation of LH and FSH, female and NeoGxd (feminized) male rats were given estradiol benzoate at 75 days of age, followed by 2mg of progesterone 48 hours later. Radioimmunoassay indicated significantly reduced plasma LH and FSH levels in T-implanted female rats when compared to controls (blank implants). Similarly, plasma LH levels were reduced in T-implanted, neonatally gonadectomized males. These results indicate that in addition to previously reported permanent effects on behavior and estrus cycles, chronic T during 15-30 days of age, a period well beyond the classical critical period of 6-10 days, also masculinizes the hypothalamic-pituitary regulation of gonadotropins. NIH HD-27334.

344.2

SEX STEROIDS AND GALANIN-IMMUNOREACTIVE (GAL-I) CELLS IN THE MEDIAL PREOPTIC AREA (MPOA): CELL SIZE AND MALE-SPECIFIC PLEXUS.

C.B. Eckersell, R. Mills, and G.J. Bloch*. Dep't of Psychology, Brigham Young Univ., Provo, UT, 84602.

GAL-I cells are localized within sexually dimorphic components of the MPOA, are dependent on gonadal steroids, and a high percentage accumulate estrogen (Eckersell,Neurosci,91;Bloch,Brain Res, in press). We measured the size of GAL-I cells in the central medial preoptic nucleus (MPNc) in gonadectomized (Gxd) males & females; Gxd, testosterone males (T males); Gxd, estrogen males & females (E males, E females) and intact males. Diameters were largest in the intact and T males, smaller in E males & E females, and smallest in Gxd rats. In addition, a highly dense area of GAL-I cell bodies with interdigitating processes was defined as the MPOA "GAL-I plexus." Plexus volume was greatest in E, T and intact males, smaller in the Gxd male, but was vanishingly small within the Gxd or E female. The density of cells and processes within the plexus was greatest in the T, E and intact male, less in the Gxd male and least in the Gxd female; total cell number was distributed similarly. These results suggest a male-specific role of gonadal steroid-sensitive GAL cells within the MPN. NIH HD-27334

344.3

SEX DIFFERENCE IN THE EFFECT OF MATING ON *C-FOS* IMMUNOREACTIVITY IN LHRH NEURONS OF THE FERRET. G.M. Lambert*, B.S. Rubin and M.J. Baum. Dept. of Biology, Boston University, Boston, MA 02215 and Dept. of Anatomy, Tufts University, Boston, MA 02111.

Several studies suggest that LHRH output from basal hypothalamic neurons and the consequent pituitary secretion of LH rises in female, but not in male ferrets after mating. At the rostral, medial, and caudal levels of the preoptic-hypothalamic continuum a significantly higher percentage of LHRH-immunoreactive (IR) neurons was co-labelled with nuclear FOS-IR in mated than in unpaired females. No such effect occurred in males, suggesting that the sex dimorphism in mating-induced LH secretion reflects a selective activation of LHRH neurons in the female ferret forebrain. Significantly more FOS-IR neurons (not co-labelled with LHRH) were detected in the bed nucleus of the stria terminalis (BNST), the medial preoptic area (mPOA) and the medial amygdala (MA) of mated versus unpaired females. By contrast, mating significantly augmented FOS-IR only in the MA of male ferrets. The heightened responsiveness of LHRH neurons to mating in females may depend on sensory inputs from a limbic circuit which includes the MA, BNST, and mPOA. (supported by MH89012;HD21094 and MH00392)

344.4

IMMUNOCYTOCHEMICAL COLOCALIZATION OF DOPAMINE BETA-HYDROXYLASE AND LUTEINIZING HORMONE RELEASING HORMONE NEURONAL ELEMENTS BY ELECTRON MICROSCOPY IN THE ROSTRAL FOREBRAIN OF THE FEMALE C57BL/6J MOUSE. **M.M.Miller* and L. Zhu. Departments of Obstetrics and Gynecology, Experimental Medicine and Anatomy, McGill University, Montreal, Quebec.**

Dopamine beta-hydroxylase (DBH), a marker for the noradrenergic (NA) system, is synthesized in a subpopulation of midbrain neurons projecting to preoptic area (POA) where luteinizing hormone releasing hormone (LHRH) perikarya are located. Both NA and LHRH are important to reproductive function. The present ultrastructural study was designed to determine the frequency with which NA neuronal elements interact with LHRH structures. Sections from four normally cycling proestrous female C57BL/6J mice (5 mo old) were evaluated using polyclonal antibodies and 3,3',5,5'-tetramethylbenzidine to identify LHRH, and 3 3' diaminobenzidine to identify DBH neuronal elements. Neuronal elements from randomly selected ultrathin tissue sections were counted according to category and corrected for surface area analysed. Categories included neurons, dendrites, axons, and terminals. When frequency of immunolabeling of each type of immunolabeled neuronal element was assessed, DBH was found in 5.0±0.42% of terminals and 0.06±0.01% of axons. A small population of POA perikarya contained LHRH (0.89±0.19%); 1.19±0.11% of all recipient dendrites were LHRH immunopositive. Among double labeled structures only one DBH terminal was seen to contact the LHRH perikarya while a 8.16±0.4% of all LHRH dendrites were impinged upon by DBH terminals. These data suggest that direct NA interactions with LHRH neurons may form an important component in the neural network which modulates gonadotropin secretion. Supported by NIH R01 AG7795 (MMM).

344.5

AN ANALYSIS OF TEMPORAL CHANGES IN TYROSINE HYDROXYLASE (TH) mRNA LEVELS IN A1 AND LOCUS COERULEUS (LC) NEURONS IN PROESTROUS, DIESTROUS AND ANDROGEN-STERILIZED RATS (ASR). J-J.Liaw*, J-R.He and C.A.Barraclough. Sch. Med., Univ. Maryland, Baltimore, MD 21201.

TH is the rate limiting enzyme for the synthesis of norepinephrine (NE) and we showed that increases in TH mRNA levels in A1 and LC neurons can serve as an index of increased activity within these cells. These noradrenergic neurons innervate the hypothalamus and NE secretion increases concomitant with preovulatory LH surges in proestrous but not diestrous and ASR which do not have LH surges. ASR received 1.25 mg of testosterone propionate at 5 days of age and both cyclic and sterile rats were used for study at ~100 days of age. Rats were sacrificed at various hours throughout the day and the brains were frozen-sectioned and processed for *in situ* hybridization histochemistry using a synthetic 48 base mer specific to TH mRNA. Levels of TH mRNA were measured by quantitative image analysis methods. Plasma LH in proestrous rats began to rise about 1500 h, and peaked between 16-1700 h. In similar proestrous rats, A1 TH mRNA levels increased significantly between 1645-1715 h and were still elevated between 20-2030 h compared to 0930-1030 h. No changes in A1 message levels occurred at any hour in diestrous or ASR. TH mRNA in LC did not change in any of the groups of animals studied.

These observations suggest that the increased secretion of hypothalamic NE during proestrous afternoon is due, in part, to increased activity within A1 neurons not solely to modulation of NE release by local inhibitory neurons within the hypothalamus. Supported by NIH Grant HD-02138.

344.6

TYROSINE HYDROXYLASE (TH) mRNA IN LOCUS COERULEUS OF DJUNGARIAN HAMSTERS IS REDUCED UNDER LONG DAY VS. SHORT DAY CONDITIONS. T.Porkka-Heiskanen*, J.H.Urban, M.H.Vitaterna, F.W.Turek, J.E.Levine. Department of Neurobiology and Physiology, Northwestern University, Evanston,IL 60208.

Previous work has demonstrated that in hamsters kept under short day (SD) norepinephrine turnover is decreased in median eminence but increased in anterior hypothalamic area, while dopamine turnover is decreased in both these areas. In the mediobasal hypothalamus, more complex, time dependent changes in cathecholamine turnover was observed (Steger et al, Biol Reprod 44,76-82,1991). In the present study we assesed the biosynthetic capacity of cathecholaminergic neurons under short day and long day (LD) conditions. Ten male Djungarian hamsters were moved to SD at the age of 3 weeks. 4 weeks later, after gonadal regression, 5 hamsters were moved to LD and 5 animals stayed under SD; both groups were sacrificed 3 weeks later. TH mRNA levels were measured using in situ hybridization. Sections through the brainstem were labelled using an oligonucleotide probe for TH. Computer analysis of the hybridization intensity revealed increases in tyrosine hydroxylase mRNA in hamsters kept under short day (80.8 ± 5.8 in SD vs. 69.4 ± 3.6 in LD, mean and std.dev. respectively, $p<0.01$). This result suggests that biosynthesis of tyrosine hydroxylase is increased in SD. We are continuing studies to determine possible changes in time-and region dependent patterns in cathecholamine synthesis in different cathecholaminergic nuclei in hamsters kept under different photoperiodic conditions.

(NIH HD 20677, HD 00879 & HD 21921)

344.7

DOPAMINERGIC BLOCKADE REVEALS A SEXUALLY-DIVERSE ROLE FOR OXYTOCIN (OXY) IN THE REGULATION OF PULSATILE PROLACTIN (PRL) SECRETION. F. J. López and J. Bartolomé*. Reprod. Neuroendocr. Section, LMIN. N.I.E.H.S. RTP, NC 27709.

Pulsatile PRL secretion is primarily controlled, at the hypothalamic level, by a dopaminergic inhibitory tone. As previously shown by our group, the effects of some hypothalamic peptides with PRL releasing activity are masked by this prevailing inhibitory tone. In the present studies, the role of OXY on pulsatile PRL secretion is evaluated in females during the afternoon of estrus and male rats. Animals were assigned to one of four groups: control, oxytocin antagonist (OXYa), domperidone (DOM) and DOM + OXYa. They were bled at 3 min intervals for 3 hours and PRL levels evaluated for pulsatility using the algorithm Detect. In male rats, OXYa did not modify any pulsatility parameter under basal condi-tions; however, under a complete dopaminergic blockade the antagonists increased quantitative parameters of pulsatile PRL secretion. Qualitative parameters were not affected by OXYa, although DOM treatment significantly increased pulse frequency. In female rats, however, OXY appears to exhibit a stimulatory role on pulsatile PRL secretion. Quantitative parameters of pulsatile PRL secretion were reduced by OXYa administration. This effect was also apparent under a complete dopaminergic blockade. As in the case of male rats, qualitative parameters were not affected by OXY, nevertheless, DOM induced a marked elevation in pulse frequency. This data indicate a differential role of OXY in the regulation of pulsatile PRL secretion in male and female rats during estrus. Whereas in male rats OXY appears to play an inhibitory role which is evident after a complete dopaminergic blockade, in female rats endogenous OXY stimulates pulsatile PRL secretion.

344.8

SEASONAL REGULATION OF PROLACTIN SECRETION MAY INVOLVE MULTIPLE CONTROL MECHANISMS. L.L. Badura*, A. Lee, S. A. Zinn, and J.D. Salamone. Depts. Physiology & Neurobiology, Animal Science, and Psychology, University of Connecticut, Storrs, CT 06269.

In many mammals, exposure to short or long photoperiods decreases or increases PRL secretion, respectively. Knife cuts, that disrupt the neural photoperiodic pathway, do not prevent the effects of short-days on PRL release, and induce a decline in PRL in animals exposed to long days. In this study, DA turnover (DOPAC/DA) was measured in the region of TIDA cell bodies (ARC) and terminals (ME), and in the caudate region (CAUD), in animals exposed to long or short days, and that received knife cuts or sham surgery. In addition, a subset of these animals was infused with PRL for two days before sacrifice to determine the response of TIDA neurons to the positive feedback effects of PRL. DA and DOPAC levels for the tissues were determined via HPLC.

No differences in DA turnover across groups were detected for the CAUD. In the ARC, knife cuts, which lowered PRL levels, and PRL infusion resulted in the lowest levels of turnover; differences between PRL levels for short- and long-day animals were not mirrored by a similar effect upon turnover. In the ME, PRL levels were correlated with turnover for short day-housed animals. There were no differences between long-day sham and knife cut animals, indicating that the cuts did not disrupt DA activity in the ME, and suggesting the existence of a second regulatory system which may be photoperiod-sensitive. In addition, infusion of PRL increased DA turnover in the ME, but not ARC, of both knife cut and sham groups. PRL and photoperiod feedback effects differ for the cell body and terminal regions; thus the modulation of the TIDA system may involve multiple mechanisms, .

344.9

ULTRASTRUCTURAL CHANGES IN RAT HYPOTHALAMIC ARCUATE NUCLEUS FOLLOWING DIAMINOCHLOROTRIAZINE FEEDING. M. K. Tennant[1], W. G. Jerome[2], J. C. Eldridge[1] and D. K. Sundberg*[1]. Depts of Physiology and Pharmacology[1], and Pathology[2], Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1083.

Administration of triazine herbicides to female rats was recently shown to increase the proportion of estrus days during estrous cycles, leading to an increase in circulating estradiol (Eldridge *et al., Biol Reprod 44 (Suppl 1)*:133, 1991). Since estradiol has been shown to increase hypothalamic damage (Brawer *et al., Endocrinology,* 1978 and Schipper *et al., Biol Reprod, 1981*), the possibility exists that triazine compounds could also induce hypothalamic damage due to their effects on estrous cycles. To test this hypothesis electron microscopy was used to assess hypothalamic gliosis, namely astrocyte and microglia granular content in the arcuate nucleus in animals chronically fed triazine. 20 week-old female Sprague-Dawley rats were fed 1000ppm Diaminochlorotriazine (DACT), the principal triazine metabolite in rats. DACT treatment resulted in a 4% increase in the granular content of astrocytes by 32 weeks of age (12 weeks of treatment) and a significant 36% increase by 48 weeks of age (28 weeks of treatment) compared to age-matched vehicle fed controls. In addition, the total number of microglia was 41% higher after 12 weeks of treatment and 17% higher after 28 weeks of treatment. Despite the increase in microglia number, granular content did not appear affected by treatment when assessed as number of microglia with five or more inclusion granules. Our results suggest that DACT treatment is able to induce hypothalamic damage in female rats, as seen by degeneration in the arcuate nucleus. Since DACT alone has no intrinsic estrogenic activity, the treatment-associated pathology was likely caused by increased estrogen secretion resulting from a greater number of days in estrus.

344.10

CASTRATION AND PROLACTIN · BOTH INCREASE THE TURNOVER RATE OF DOPAMINE IN THE MEDIAN EMINENCE OF THE MALE RAT. M. Selmanoff, S.-K. Park, D.A. Strouse and J.-R. He*, Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201-1559.

Central catecholaminergic neurons projecting to specific hypothalamic structures are involved in stimulating and inhibiting the LHRH-containing neurosecretory neurons. We investigated the effects of orchidectomy and hyperprolactinemia on the tuberoinfundibular dopaminergic (TIDA) neurons 0, 2 and 6 days postcastration and in 2 day castrates receiving physiological testosterone (T) replacement. Adult male rats were administered purified ovine prolactin (oPRL), or its diluent, polyvinylpyrrolidone (PVP), every 12h (2,400µg/sc injection) for 0, 2 or 6 days, beginning at time 0. Dopamine (DA) turnover was estimated by the α-methyl-p-tyrosine method. In control rats, median eminence DA turnover increased at 2 days, and then further, at 6 days postcastration. Testosterone replacement reversed this increase in 2 day rats. Exposure to oPRL increased DA turnover compared with PVP controls at both 2 and 6 days. Autoregulatory feedback suppression of oPRL on endogenous rat PRL secretion was observed at both 2 (7.1 ± 1.4 vs 2.9 ± 0.1 ng/ml rPRL-RP-3) and 6 (7.7 ± 0.8 vs 3.3 ± 0.2) days postcastration. These findings demonstrate, for the first time, that autofeedback regulation of rPRL secretion persists through at least 6 days of oPRL exposure, and is temporally associated with markedly increased turnover in the TIDA neurons. The TIDA neurons respond predictably to sex steroid manipulation but apparently are not involved in the postcastration LH rise as direct inhibitory afferents to LHRH neurons. (Supported by NIH grant HD21351).

NEUROENDOCRINE REGULATION: OTHER

345.1

Increase of Tryptophan Hydroxylase Enzyme by Dexamethasone in Adrenalectomized Rat Midbrain Using Western Blots and Immunocytochemistry with Antipeptide Antibodies. Baolang Liao* and Efrain C. Azmitia. Dept. of Biology, New York University, Washington Square East, New York, N.Y. 10003

We and others have previously demonstrated that adrenal steroids can regulate the activity of tryptophan hydroxylase activity after stress. This increase in activity has been proposed to consist of de novo protein synthesis because it was blocked by protein synthesis inhibitors and shown not to be due to an allosteric increase produced by phosphorylation of the enzyme. Recently, we have succeeded in raising antibodies against two unique amino acid sequences of the tryptophan hydroxylase molecule which show no homology to the other aromatic hydroxylase enzymes. The peptide domains selected were near the N-terminus (aa 66-91) and the C-terminus (aa 412-443). A single principle band was seen (approximate M.WT. 49,000) in Western analysis with WH-412 and WH-66 for the midbrain raphe area. In Western analysis from tissue obtained from adrenalectomized (adx) rats treated with dexamethasone (dex) or vehicle, an increase after dex was seen in the WH-412 labeled band after 12-96 hr. of exposure, but not after only 4 hours. The percentage change seen by densitometry measurement showed a 30-50% increase. The immunocytochemical staining with WH-66 at dilution upto 1/10,000 showed specific cellular staining in all the serotonergic raphe nuclei. Comparison of the WH-immunoreactive cells in the dorsal and median raphe nuclei showed a marked increase in staining after 48 hr. exposure to dexamethasone. These findings indicate that glucocorticoids secreted during stress increase the amount of tryptophan hydroxylase enzyme protein in the serotonergic neurons of the rat brain. This increase in protein amount can explain the long-term increases in serotonin synthesis after exposure to stress, and provides important insights into the role of translation of tryptophan hydroxylase in serotonergic cell body in neuroendocrine homeostasis. Supported by NSF 88-12892.

345.2

NEUROPEPTIDE-Y(NPY), WHICH DECREASES THYROID BLOOD FLOW(TBF), IS NOT A PRIMARY MEDIATOR OF THE ACUTE TBF RESPONSE TO SYMPATHETIC NERVE STIMULATION. M. Dey, M. Michalkiewicz, L. Huffman and G.A. Hedge. Dept. of Physiology, West Virginia University Health Sciences Center North, Morgantown, WV 26506.

It has been suggested that TBF is regulated by both sympathetic and parasympathetic nerves. The purpose of our experiments was to study the role of NPY in the sympathetic neural control of TBF. Sympathetic nerve fibers to the thyroid contain both norepinephrine(NE) and NPY. Therefore, NE(15 nmol; iv bolus) and NPY(12 or 1.7 nmol/kg BW; iv infusion; 4 min) were administered to anesthetized male rats(250-300g)either alone or together, with or without an α adrenergic receptor blocker (phentolamine; 10 mg/kg BW; iv bolus). Experiments were also performed in which the cervical sympathetic trunks(CST) were stimulated(30 Hz, 10 V; 0.5msec; 2 min) with or without phentolamine. TBF was monitored continuously by laser Doppler flowmetry. Results are expressed either as TBF or thyroid vascular conductance(TVC). NE or NPY at both doses decreased TVC relative to that in control, saline-infused, rats($p<0.05$). No potentiation of the NE effect by NPY was observed when NE(iv bolus) was injected 2 min after a high or low dose of NPY. However, the effect of a second dose of NE, injected 15 min after the end of the low dose of NPY, was prolonged compared to the effect of a second dose of NE in saline-infused rats. Phentolamine blocked the effect of NE, but not that of NPY. Stimulation of the CST decreased TBF ($p<0.01$ *vs.* sham) and this effect was completely blocked by phentolamine. These results suggest that NPY does not take part in the mediation of the acute TBF response during sympathetic nerve stimulation. However, NPY may prevent adrenergic desensitization following prolonged sympathetic activity. This is suggested by the observation that the TBF effect of a second dose of NE is prolonged in rats pretreated with NPY.(Supp. by NSF DCB-8904470).

345.3

THYROID FUNCTION RUNS IN PHASE WITH REPRODUCTION AND PINEAL ACTIVITY IN THE FREE-LIVING INDIAN PARAKEET. **T. N. Krishnaprasadan and Vibhakar C. Kotak* Sardar Patel University, Vallabh Vidyanagar 388 120 Gujarat, India.**

Seasonal changes in the ultrastructure of thyroid, and circulating levels of T3 & T4 were examined in relation to reproductive cycles and pineal function in the free-living, sub-tropical Indian Rose Ring Parakeet. These birds peak reproduction during January-March when pituitary LH, spermatogenic activity, and androgen turnover are highest and reproductive behaviors are fully expressed. Moderate synthetic changes in the thyroid and elevation in T4 during the prebreeding months of November and December is related to drop in ambient temperature. Elaboration of microvilli, colloid turnover, and increases in appearance of well defined Golgi, RER, mitochondria and T3 & T4 occurred during the breeding months of January to March that may augment greater androgen turnover, and possibly rules out any inhibitory influence of androgens over thyroid. This active thyroid profile also ran in phase with pro-reproductive pineal function. Significant ($P<0.05$) drop in T3 & T4 levels after March occurred during long-day length, post-breeding months of summer when thyroid synthetic and secretory activity was minimal. Low levels of thyroid hormones during the post-breeding phase may, via long loop, sensitize the hypothalamus to the negative feedback of androgen eventually to reduce pituitary LH output, and may also be responsible for low androgen clearance. In sum, pituitary, thyroid, pineal and testis endocrine functions run hand in hand in this winter breeding species.

345.4

EFFECT OF PHOTOPERIOD ON FAT CELL RECEPTORS AND ADENYLATE CYCLASE IN DJUNGARIAN HAMSTERS. J.G. Mercer, C.B. Lawrence and P.J. Morgan. The Rowett Research Institute, Bucksburn, Aberdeen, U.K. AB2 9SB.

The Djungarian hamster, *Phodopus sungorus campbelli*, presents a useful model for the study of the effect of photoperiod on food intake and body weight regulation. Adult male hamsters respond to short photoperiod (8:16 h light:dark) with dramatic reductions in body weight followed by decreased voluntary food intake; body weight reductions of 20% are typical following 8 weeks exposure. Reductions in the size of adipose tissue pads in excess of 50% contribute to these weight changes, although the net lipolysis exhibited by animals housed under short photoperiods is non-uniformly distributed among subcutaneous and abdominal fat pads. In other biological systems, such as the Zucker rat, such differences in adiposity are associated with modulated sympathetic tone, and at the level of the adipocyte by differences in receptor levels and in the activation of adenylate cyclase by stimulatory ligands (Strassheim et al., Biochem. J. 276:197, 1991). We have adopted a similar approach to the mechanisms underlying short-photoperiod induced fat mobilisation. β-adrenergic and A_1-adenosine receptors have been characterised by binding incubations with ^{125}I-iodocyanopindolol and ^{3}H-PIA, respectively. The β-adrenergic and A_1-adenosine receptor profiles of animals with well established photoperiod-induced weight loss did not differ significantly from those of long-day housed controls. The effect of photoperiod on the ability of lipolytic ligands to stimulate adipocyte adenylate cyclase is being investigated.

345.5

UNILATERAL BUT NOT BILATERAL OLFACTORY BULBECTOMY INHIBITS BODY WEIGHT GAIN IN GOLDEN HAMSTERS. D.R. Pieper*, K.D. Johnson and C.A. Lobocki. Department of Physiology, Providence Hospital, Southfield, MI 48037.

The relation of the olfactory bulbs and photoperiod to the regulation of body weight was studied in male golden hamsters.

METHODS: The animals underwent sham operation (SH), bilateral olfactory bulbectomy (BX) or unilateral bulbectomy (UBX). They were left on long photoperiod (LD 14:10) for 5 weeks and were transferred to short photoperiod (LD 10:14) for 11 weeks.

RESULTS: The UBX hamsters gained less weight on long or short photoperiod than the SH group, while the BX hamsters gained at least as much weight as the SH group. Final body weights: SH 144 ± 2.7; BX 151 ± 4.0; UBX 104 ± 4.8 (mean ± SE); $p < 0.001$

SUMMARY: Thus we report the novel finding that unilateral, but not bilateral olfactory bulbectomy, reduces body weight gain in male golden hamsters.

345.6

The Effect of Abdominal Sympathectomy on Bone Remodeling in Rats During Simulated Weightlessness. H.W. Burden, C. M. Manring, G. Price and D.M.Terrian*. Dept. of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858.

Adult female Sprague Dawley rats were subjected to lumbar sympathectomy (retroperitoneal approach) or sham surgery. One week later, sympathectomized and sham operated rats were fitted in a back harness and the animals suspended at an angle of 30° head-down tilt (day 0). The harness allowed full ambulation via the forelimbs while the hindlimbs were completely nonweight bearing. A control group of sham operated animals were allowed to ambulate normally (gravity control). On day 14, tibias were removed for histomorphometric analysis. Osteoclasts in the proximal tibial metaphysis were visualized with a tartrate-resistant acid phosphatase procedure and the area and perimeter of metaphyseal trabeculae were determined using a point counting method with a Merz grid. The area of metaphyseal trabeculae was significantly reduced in suspended sham-operated animals ($18.7 \mp 1.2\ \mu^2$ sham-suspended vs $31.9 \mp 1.2\ \mu^2$ gravity control, $p<0.05$). Hindlimb suspension also significantly ($p<0.05$) reduced the trabecular perimeter length. Sympathectomy significantly reversed the effect of simulated weightlessness on the area ($24.7 \mp 12\mu^2, p<0.05$), but not perimeter length, of the trabeculae. Lastly, sympathectomy significantly ($p<0.05$) increased the number of osteoclasts along the perimeter of tracebulae of suspended rats. These data indicate that the sympathetic innervation to bone actively participates in the bone remodeling that occurs in this model of simulated weightlessness. Supported by NASA Grant No. NAGW-2927.

345.7

BRAIN STEM AFFERENTS TO THE DIAGONAL BAND OF BROCA (DBB) IN THE RAT: RETROGRADE TRACING WITH RHODAMINE LATEX MICROSPHERES (RLM). V.V. Senatorov*, R.M. Buijs and L.P. Renaud. *Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Ontario, Canada, K1Y 4E9.*

Previous electrophysiological studies in the rat have revealed that an abrupt rise in arterial pressure prompts a transient and selective cessation of activity in hypothalamic vasopressin (VP)-secreting magnocellular cells. That this response is attenuated after 6-hydroxydopamine lesions in the DBB suggests participation of ascending catecholaminergic afferents. Earlier studies using retrograde tracers that diffuse from the injection site have indeed indicated that DBB is likely to receive innervation from brainstem areas containing catecholaminergic cells. We now report that discrete DBB injections of a non-diffusible retrograde tracer RLM also label cells in brainstem areas containing catecholaminergic neurons. In pentobarbital anaesthetized Long-Evans rats, a picospritzer was used to inject RLM (0.03-0.20 μL) stereotaxically into the left horizontal limb of the DBB. After 7 days animals were reanaesthetized and sacrificed by transcardial perfusion with 3% paraformaldehyde. Brains were sectioned at 50 μm in the coronal plane by vibratome. In addition to other regions, retrogradely labeled neurons were found among the following catecholaminergic cell groups: nucleus tractus solitarius (NTS); rostral and caudal ventrolateral medulla (VLM); and the locus coeruleus (LC). In NTS and VLM, labeled cells were observed bilaterally; in LC labeled cells were generally ipsilateral to the injection site. The utilization of this tracer offers a more precise definition of DBB afferents. Moreover, confirmation that at least some of the labeled cells in these brainstem sites are noradrenergic in nature should provide an anatomical basis for the inference that specific noradrenergic neurons participate in a neural network that transmits information from peripheral baroreceptors to VP neurons. Supported by MRC & IBRO/UNESCO.

345.8

ELECTROTONIC COUPLING BETWEEN NEUROSECRETORY CELLS IN THE CRAYFISH EYE-STALK. Alvarado-Alvarez, R., García, U*. and Aréchiga, H. Dept. Fisiología, Biofísica y Neurociencias, CINVESTAV-IPN, Apdo. Postal 14-740, 07000 México, D.F.

Electrotonic coupling is a common feature in neurosecretory systems. It has been described among rat magnocellular neurons and bag cells of Aplysia. In the X organ-sinus gland system, of crustaceans dye-coupling and gap-junctions have been described, but no electrophysiological evidences of electrical coupling have so far been documented (Aréchiga et al, 1985). The purpose of this work is to study such coupling by using both dye injection and electrophysiological recordings. By using conventional intracellular recordings (tip resistance 30-60 MΩ) pairs of neurons from the most superficial layer of X organ (about 30 neurons) were simultaneously impaled. We estimated that only 30% of them were coupled. When pairs of coupled neurons were found, Lucifer yellow was injected only in one neuron with a low tip resistance micropipette (about 2 MΩ), in all the cases more than one neuron was stained. Mean values of input resistance from coupled and uncoupled neurons were 24 + 16 MΩ(n=46) and 58 + 18 MΩ(n=53) respectively. Voltage changes induced by hyperpolarizing current injection in the cell body decreased about 24% when recorded in the other coupled cell body. Action potentials evoked by depolarizing current injection showed peak time latencies about 300 μs, the coupling was non-rectifying. Three cases were found with indirectly electrical coupling, it means uncoupled neurons but synchronized presumably by a third, not recorded cell. From dye injection experiments we not only found pairs of stained neurons, but also groups of three or more neurons. Dye injections suggest that the site of coupling could be more or less 100 μm away from the soma.

345.9

SECRETION OF THIOPHOSPHORYLATED AND SULFATED CHROMAFFIN VESICLE MATRIX PROTEINS. J.C. Brooks* and M.H. Brooks. Marquette Univ. Sch. of Dent., Milwaukee, WI 53233.

Incubation of chromaffin cells with either [^{35}S]thiophosphate or $^{35}SO_4$ resulted in radiolabelling of the same set of proteins; these were isolated along with chromaffin vesicles and were released upon vesicle lysis. We have now studied the ability of the cells to synthesize and release these proteins in an effort to understand their role in chromaffin vesicle function.

Thiophosphorylated or sulfated proteins were released upon nicotine stimulation of chromaffin cells, collected and identified by SDS-PAGE and fluorography. Radiolabel was removed from both thiophosphorylated and sulfated proteins by chondroitinase ABC treatment, suggesting that they are part of the proteoglycan component of chromaffin vesicle matrix proteins. The data also suggested that the cells might interchangeably use sulfate or thiophosphate in sulfation reactions. However, the two compounds appear to be independently inserted into matrix proteins. Pretreatment of chromaffin cells with DCNP (2,6-dichloro-4-nitrophenol), an inhibitor of protein sulfation by PAPS (3'-phosphoadenosine 5'-phosphosulfate), resulted in the apparent loss of label from normally labeled proteins. However, all of the label was present in a band that moved near the front in gels and was released upon stimulation of the cells. The data suggest that thiophosphate is incorporated into a small molecular weight component of a larger proteoglycan molecule and that sulfation may be necessary for attachment of the small component to the larger component. Supported by NIH Grant 1 A15 NS23101-01A2.

345.10

MORPHOLOGICAL AND BIOCHEMICAL ALTERATIONS OF THE CEREBRAL CORTEX AND BRAIN STEM IN ADULTS ATHYMIC MICE. **G.G. Ortíz., I.E. Velázquez-Brizuela, C. Beas-Zárate, M. Cervantes. I. de la Rosa A.* Y A. Feria-Velasco. Lab. Exp. Neuropath., Centro Inv. Biomed. Occte. I.M.S.S., Lab. Neurochem. Univ. de Guadalajara., Guadalajara, JAL., Universidad Autónoma de Guadalajara and Unidad Biomed. Centro Médico Siglo XXI., I.M.S.S. México D.F.**

The athymic mouse is a mutant with thymus hypoplasia and for this reason serious deficiencies in its cellular immune system are present. It also presents alterations in its neuroendocrine system due to a decrease in its hormones (LH, FSH, T3, T4, etc). In this report a morphological (ligth miscroscopy) and biochemical (DNA, RNA, and protein quantification) study of the cerebral cortex, cerebellum and brain stem of adult athymic mice (60 and 90 postnatal days) was performed. The sensory-motor of the cerebral cortex showed a packaging in layers V-VI with loss of its boundaries. The cerebellum did not show structural alterations (under this systems of analysis), and the brain stem displayed degeneration in the superior and inferior olivary nuclei and in a trajectory of neurons that cross the medial vestibular and intercalated nuclei and localized between the hypoglossal nucleus and dorsal motor nucleus of the vagus. At 60 postnatal days low levels of RNA and proteins were found, and at 90 postnatal days DNA, RNA, and proteins were found to be significantly less in all studied areas.

Conclusion: The athymic mouse presents degeneration and cellular death as a result of a secondary effect of low levels of trophic hormones that it normally displays during its development,

345.11

RUTA CHALEPENSIS ACTION ON INTESTINAL SMOOTH MUSCLE OF FEMALE GUINEA PIG. X. García*, L. Cartas-Heredia and E. Gijón. Department of Physiology, Sch. of Med. UNAM. Ap. P. 70-250, México D.F., 04510. MEXICO.

Previous studies have shown different responses between male and female guinea pigs to the autocholinergic action of rue alcoholic extracts(1). Rue action is studied in isometric recordings of intestinal segments in vitro of female guinea pig, during different phases of estral cycle determined by vaginal smear. Tissues showed different sensitivity to the same dose of acetylcholine or histamine (0.1µg/ml) depending of the estral cycle phase. Rue causes biphasic responses, where relaxation of the smooth muscle predominates when the tissue comes from an animal in estro, while during other cycle phases there is a contraction-relaxation response. Rue blocks contraction responses induced by acetylcholine or histamine in dose dependent form. This effect reverts after washing the preparation. These results suggest that the anticholinergic or antihistaminergic effect is not modified during the estral cycle, while that rue direct action on tonus and basal activity of smooth muscle is modified by hormonal levels.

1. Molina O., Borges M., Reyes J., Huerta M., Chavez M., and García X. Proc. West. Pharmacol. Soc. 34:205-208, 1991.

345.12

ALCOHOLIC EXTRACT OF RUTA CHALEPENSIS' ACTION ON SPONTANEOUS MIOMETRIAL ACTIVITY OF FEMALE GUINEA PIG. E. Gijón*, L. Cartas-Heredia and X. García. Department of Physiology, Sch. of Med., UNAM, A.P. 70250, 04510 México, D.F. MEXICO

Rue is an european plant that has been used in traditional medicine from infusions or alcoholic extracts for the treatment of menstrual disorders, histeria, parasitic infections, muscular pain, and as an abortive. Alcoholic extract of Ruta Chalepensis' action is studied on isometric mechanical activity from uterine segments of female guinea pig on different stages of the estral cycle, determined by vaginal smear. Uterine sensibility to acetylcholine or histamine 0.1 µg/ml was tested before and after adding rue alcoholic extract to the bath. First, it was observed that basal sensibility to acetylcholine or histamine is modified during the estral cycle, being higher during proestro. Secondly, rue increases tonus and spontaneous activity in all phases of estral cycle, showing higher effects during estro. Third, rue inhibits in dose dependent form acetylcholine or histamine contraction. The increase in basal activity and inhibitory effect of rue, revert after washing the preparations. These results suggest that rue exerts an effect on sarcolema and an increase of intracellular calcium.

NEUROENDOCRINE REGULATION: OSMOTIC REGULATION II

346.1

ANGIOTENSIN II DEPOLARIZES NEURONS IN THE SUBFORNICAL ORGAN OF RAT *IN VITRO*. K. Inenaga, T. Nagatomo, H. Kannan and H. Yamashita* Dept. of Physiol., Univ. Occupational & Environmental Health, Sch. Med., Kitakyushu 807, Japan.

Angiotensin II (AII) excites the subfornical organ (SFO) neurons. To clarify excitatory mechanisms of AII on the SFO neurons, intracellular recordings were made in SFO neurons of rat brain slice preparations. The slices were made from adult male Wistar rats after decapitation. Bath application of AII at 1µM depolarized the neurons with increase of firing rate in normal and TTX-containing media. The non-peptidergic AII-receptor antagonist Losartan potassium at 100 nM suppressed the depolarizing effects. The AII responses might be induced in two ways. First the depolarization was accompanied with increase of membrane resistance, and the amplitude of the depolarization decreased with increase of resting membrane potentials, suggesting blockade of potassium channels by AII. Second the response was accompanied with apparent decrease of membrane resistance and the amplitude of the depolarization was increased with increase of the resting membrane potentials, suggesting that different channels other than potassium channels are involved in the latter response. These suggest that AII directly depolarized neurons in the SFO through some channels containing potassium channels.

346.2

RELAXIN EXCITES SUBFORNICAL ORGAN NEURONES ANTIDROMICALLY IDENTIFIED AS PROJECTING TO THE SUPRAOPTIC NUCLEUS BUT NOT THOSE PROJECTING TO THE MEDIAL SEPTUM. B.C. Wilson and A.J.S. Summerlee* Dept. of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada, N1G-2W1.

Previous work has indicated that relaxin (RXN) acts at the subfornical organ (SFO) to affect the release of oxytocin (OT) and vasopressin (VP). In addition, RXN has been shown to bind to the SFO. Therefore, experiments were done to determine if exogenous RXN affected the extracellular electrical activity of two populations of SFO neurones.

Extracellular electrical recordings were made from SFO neurones antidromically identified from either the medial septum (MS)(n=6) or the right supraoptic nucleus (rSON)(n=7) in urethane-anaesthetized female rats (n=20). Control recordings were taken from neurones located in parietal association cerebral cortex (n=7). Five minutes after stable recording, rats were treated with saline (SAL; 0.1 ml i.v.) as a vehicle control and then five minutes later with VP (5.5 mU in 0.1 ml SAL i.v.) to control for nonspecific cardiovascular effects of RXN-induced VP release. Fifteen minutes later, rats were treated with RXN (5µg in 0.1 ml SAL i.v.) after which a further 10 minutes of recording was completed. Firing frequency (Hz) was calculated for each neurone in five time bins (bin length= 1min) in the pretreatment period and after each successive treatment. Mean frequencies in each time bin were calculated for each group of neurones and compared using ANOVA and Student's "t" test.

RXN i.v. significantly ($P<0.05$) elevated mean firing frequency of SFO neurones antidromically identified as projecting to the rSON 3 minutes post-treatment. However, RXN had no effect on the extracellular electrical activity of MS-projecting neurones or cortical control neurones. This suggests that RXN is affecting release of OT and VP through a monosynaptic pathway linking the SFO and the magnocellular nuclei. Supported by NSERC Canada.

346.3

ACTIVATION OF cFOS IN PVN OXYTOCIN NEURONS EXPOSED TO COCAINE AND DOPAMINE. L.J. Sim* and M. Morris. Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, N.C. 27157.

A hypothalamic tissue culture system has been developed to evaluate the effects of cocaine and related agents on specific neurons of the paraventricular nucleus (PVN). Dissociated PVN cultures from neonatal rats were treated with KCl(50 mM), cocaine or dopamine (10µm) for one hour. Cultures were fixed one hour after treatment and stained immunocytochemically for cfos using a nickel enhanced chromogen to produce a purple reaction product. Cultures were then stained for oxytocin (OT) or tyrosine hydroxylase (TH) using DAB to produce a brown chromogen. In control cultures, 26% of OT and 33% of TH cells were also cfos-ir. These values were considered baseline levels of activation. Depolarization with KCl increased activation of both OT and TH cells. Cocaine and dopamine treatment increased activation of OT neurons, but had no effect on TH cells. Increases of 46.2% and 97.4% were noted with cocaine and dopamine, respectively. These studies demonstrate that dopamine and cocaine specifically activate OT neurons and may provide a neuronal mechanism for the neuroendocrine/cardiovascular effects of cocaine. *(Supported by PMA)*

Stimulant	KCl	Cocaine	Dopamine
OT	65.4% ± 13.4	46.2% ± 12.1	97.4% ± 12.2
TH	57.6% ± 6.0	-12.9% ± 6.5	22.8% ± 4.4

% change in cells immunopositive for cfos and OT or TH

346.4

MORPHINE WITHDRAWAL EXCITATION INDUCES c-fos GENE EXPRESSION IN RAT MAGNOCELLULAR OXYTOCIN NEURONES.

J.A. Russell*, M. Hamamura,† L. Johnstone, G. Munro (SPON: Brain Research Association), Department of Physiology, University Medical School, Edinburgh, U.K.
†Department of Physiology, Jichi Medical School, Japan.

Acute morphine inhibits oxytocin (OXT) neurones, but dependence develops during i.c.v. morphine infusion for 5 days (Bicknell et al J.Physiol. 396 319-347; 1988). In anaesthetized dependent rats naloxone (5mg/kg) increases OXT neurone firing-rate 3.5-fold, but has no effect in controls; consequently naloxone increases OXT secretion in dependent rats 10-fold more than in controls. Vasopressin neurones are unaffected. To determine whether withdrawal induces c-fos expression in dependent oxytocin neurones, cryostat sections through hypothalamus from anaesthetized dependent rats were hybridized with a ^{35}S-labelled oligonucleotide c-fos mRNA probe and exposed on film for quantitative autoradiography (Hamamura et al, J.Physiol. 444, 51-63; 1991). c-fos mRNA signal in the SON and PVN was detected only after naloxone (+40 min). In conscious dependent rats, 90 min after naloxone, posterior pituitary oxytocin (but not vasopressin) was depleted by 17% and plasma oxytocin doubled; Fos protein, detected by immunocytochemistry on cryostat sections, was strongly expressed in the SON and PVN. The data suggest rapid c-fos gene activation in magnocellular oxytocin neurones hyperexcited during naloxone-provoked morphine withdrawal.

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346.5

CENTRAL SEROTONIN INDUCES VASOPRESSIN AND OXYTOCIN SECRETION AND A SPECIFIC PATTERN OF C-FOS EXPRESSION IN RAT BRAIN. J.A. Saydoff*[1,2], F. Leanza[1], M. Carnes[3], and M.S. Brownfield[1,2]. Dept. Comparative Bioscience[1], Sch. Vet. Med., Neuroscience Training Program[2], Univ. Wisconsin, Madison, WI 53706, and Dept. of Geriatrics[3], Veterans Administration Hospital, Madison, WI 53705.

The aim of this study was to map potential brain pathways whereby central serotonin (5-HT) stimulates the secretion of vasopressin (VP) and oxytocin (OT). A chronic indwelling i.c.v. cannula followed by chronic arterial and venous femoral lines were surgically placed in rats. Freely moving, conscious rats had blood samples taken at -15, 5, and 15 min. and were perfuse fixed with 4% parafomaldehyde in PBS at 90 min. At time -5 min., rats were given i.c.v. vehicle or the 5-HT1C/2 antagonist LY53857 (10 ug). At time 0 min., rats were given i.c.v. vehicle, 10 ug 5-HT, or 200 ng angiotensin II (AII). 5-HT and AII induced large increases in VP and OT plasma levels. The increases in VP and OT induced by 5-HT were attenuated by LY53857.

Immunocytochemistry for c-Fos was performed on forebrain areas of the rat brains. 5-HT increased c-Fos expression relative to the vehicle control group in OVLT, nucleus medianus, lateral septum, periventricular area, supraoptic nucleus, and paraventricular nucleus (PVN). LY53857 attenuated 5-HT induced c-Fos expression primarily in the lateral septum, nucleus medianus, OVLT, and PVN. AII induced an overlapping, but distinct pattern of c-Fos expression.

These findings are consistent with central 5-HT1C/2 receptors contributing to serotonergic stimulation of VP and OT secretion and the pathway for this effect may involve the lateral septum, nucleus medianus, OVLT, and/or PVN.

Research supported by NIH MH45812, Univ. Wisc. Grad. Sch. JAS is a Fellow with the American Heart Assoc. Wisc. Affiliate.

346.6

THE LAMINA TERMINALIS IS A SOURCE OF THE GABAergic INNERVATION OF THE MAGNOCELLULAR NEUROSECRETORY SYSTEM. B.L. Roland* and P.E. Sawchenko, The Salk Institute, La Jolla, CA 92037.

GABA is an important inhibitory neurotransmitter in the neuroendocrine system. We recently used retrograde tracing and immunohistochemical methods to identify a number of local, intrahypothalamic, sources of glutamic acid decarboxylase-immunoreactive (GAD-ir) projections to the paraventricular nucleus (PVH). Complementary anterograde transport studies indicated that these local sources project principally to the parvocellular division of the PVH, with only meager contributions to the magnocellular parts of the PVH or to the supraoptic nucleus (SO). Three new lines of evidence raise the possibility that cell groups lining the lamina terminalis comprise an important source of the GABAergic innervation of magnocellular neurons. First, hybridization histochemical localization with cRNA probes revealed extensive expression of genes encoding both isoforms of GAD (i.e., GAD65 and GAD67) in the subfornical organ (SFO), the median preoptic nucleus (MePO) and the vascular organ of the lamina terminalis (OVLT). Second, combined localization of GAD mRNAs with retrograde tracer following injections into the PVH revealed retrogradely labeled cells with above-background GAD mRNA signal in the SFO, MePO and the OVLT. Third, discrete unilateral knife cuts in the coronal plane, just caudal the OVLT and extending 2 mm laterally from the midline and 2 mm dorsally from the base of the brain, reduced GAD-ir in the magnocellular division of the PVH and in the SO on the ipsilateral side. These results are consistent with those of electrophysiological studies demonstrating inhibitory components (some bicuculine-sensitive) of the response of identified magnocellular neurons to electrical stimulation of cell groups associated with the lamina terminalis.

346.7

PHARMACOLOGICAL DIFFERENTIATION OF SEROTONERGIC (5HT) STIMULATION OF OXYTOCIN AND VASOPRESSIN SECRETION IN THE CONSCIOUS RAT. M.S. Brownfield*[1,2], J. Armstrong[1], P. Rittenhouse[3], Q. Li[3], and L.D. Van de Kar[3]. Dept. Comp. Biosci.[1], Neurosci. Training Prog.[2], Univ. of Wisc., Madison, WI 53706 and Dept. Pharmacol., Loyola Univ., Maywood IL 60153.

Serotonin (5-HT) and an assortment of drugs that activate the brain serotinergic system including releasers, uptake inhibitors and agonists stimulate oxytocin (OT) and vasopressin (VP) secretion. Our results show that serotonergic control of oxytocin (Saydoff et al., J. Pharm. Exp. Ther. 257:95, 1991) and vasopressin (Brownfield et al., Neuroendocrinology 257:277, 1988) is mediated mostly by 5-HT1C/5-HT2 receptor mechanisms. This general conclusion is agreed on by work from other laboratories but some details are still controversial, particularly with regard to receptor mechanisms. In this abstract we present evidence which confirms this general finding but reveals that serotonergic regulation of these two hormones can be differentiated pharmacologically.

Stimulated OT and VP secretory responses are similar to the releaser/uptake inhibitor fenfluramine. However, OT secretion is more sensitive to 5HT agonists MK212 and mCPP than is VP. The 5HT releaser PCA has a weak stimulatory effect on OT and VP secretion but following blockade of alpha-1 receptors by prazocin PCA significantly stimulates the secretion of both hormones. RU24969, considered a 5HT1a,b agonist stimulates OT but not VP when given alone, but pretreatment with the 5HT2/1C antagonists: LY53857 inhibits the OT response but not VP; ritanserin stimulates both OT and VP; spiperone inhibits OT and elevates VP. The 5HT2/1C agonist DOI stimulates OT but not VP when given alone and pretreatment with ritanserin inhibits the OT response. Pretreatment with 5HT1A agonist ipsapirone before DOI significantly elevates OT and VP secretion over DOI treatment alone. These studies suggest that different pathways, each containing serotonergic elements, act on OT and VP cells. (Supported by NIH 45812)

346.8

ANGIOTENSIN-INDUCED SALT APPETITE IN RATS: AN INHIBITORY ROLE FOR CENTRAL OXYTOCIN. R.E. Blackburn*, A.D. Demko, G.E. Hoffman, E.M. Stricker, and J.G. Verbalis. University of Pittsburgh, Pittsburgh, PA 15261.

In several models of salt appetite in rats, NaCl intake can be inhibited by treatments associated with pituitary oxytocin (OT) secretion. We investigated the potential involvement of OT in the NaCl intake stimulated by angiotensin II (AII), a treatment similarly associated with OT secretion. Male rats with indwelling intracerebroventricular (icv) cannulae were maintained on pelleted chow, water, and 2% NaCl solution. On experimental days all food and fluids were removed 1 h prior to treatment. Rats were given icv injections of either 150 mM NaCl (NS, 5 µl) or an OT receptor antagonist (OVT, 10 µg/rat in 5 µl); 30 min later rats were given either AII (5 ng/rat in 5 µl) or NS icv. Intakes of water and saline were monitored every 15 min for the next hour. Cumulative fluid intakes (ml) are below (* $p < 0.01$ compared to NS + AII):

	15 min		1 h	
Group	H_2O	2% NaCl	H_2O	2% NaCl
NS + AII (n=17)	12.8 ± 1.9	2.3 ± 0.5	17.8 ± 1.9	5.4 ± 0.7
OVT + AII (n=17)	8.0 ± 2.0	7.3 ± 1.0*	16.9 ± 2.4	12.4 ± 1.4*
OVT + NS (n=11)	1.2 ± 0.5*	0.5 ± 0.2*	2.3 ± 0.6*	1.2 ± 0.5*
NS + NS (n=11)	0.6 ± 0.4*	0.5 ± 0.2*	1.7 ± 0.8*	0.9 ± 0.4*

Central OT-receptor antagonism potentiated the volume of 2% saline ingested by 2-3 fold following icv AII but did not interfere with the dipsogenic actions of AII; OVT alone did not increase saline intake. Immunocytochemical studies demonstrated that icv AII at this dose caused pronounced c-Fos expression in both magnocellular and parvocellular hypothalamic OT neurons. Our results confirm that central AII activates OT neurons, and suggest that some of these central OT projections may mediate an inhibitory component of the salt appetite induced by central AII, as well as other treatments, in the rat.

346.9

MORPHOLOGICAL CHARACTERIZATION OF SYNAPTIC INPUTS TO THE SUPRAOPTIC NUCLEUS (SON) FROM MEDIAN PREOPTIC NUCLEUS AND ADJACENT REGIONS IN THE RAT. W.E. Armstrong*, H. Wong & M. Tian. Dept. of Anat. Neurobiol., Univ. Tenn., Memphis, Memphis, TN 38163.

Light microscopic studies suggest the presence of direct projections to the SON from the median preoptic nucleus (MPN) and from nearby periventricular regions, areas known to be important for osmotic regulation. In the present investigation we examined anterogradely labeled varicosities in the SON with the electron microscope to determine the presence and nature of synaptic contacts after placement of the anterograde tracers phaseolus vulgaris leucoagglutinin or biotinylated-dextran in or near the MPN.

The densest labeling in the SON arose from injections involving the MPN, but substantial labeling was present from injections near the lamina terminals and from dorsal, anterior periventricular regions. However, only limited inputs were found with injections in the more caudal periventricular strata despite revealing a massive input to the bed nucleus of the stria terminalis and to much of the caudal hypothalamus (excepting ventromedial nucleus). Regardless of the density of labeling or injection placement, synaptic contacts in the SON were largely axo-dendritic and symmetrical, with terminal diameters on the order of 1 x 0.5 µm. Occasionally axo-somatic, and more rarely, axo-axonic synapses were observed, as were asymmetric contacts.

Symmetrical contacts are thought to mediate inhibitory postsynaptic actions, although exceptions are encountered. These results provide evidence for monosynaptic, putatively inhibitory connections from the MPN and rostral periventricular regions to the SON in the rat. The homogenous appearance of these contacts and the preference for dendrites as postsynaptic targets from both regions suggest a similarity in function regarding the control of SON neurons. Supported by NIH #NS23941(WEA).

346.10

PATCH-CLAMP ANALYSIS OF SPONTANEOUS SYNAPTIC CURRENTS IN RAT SUPRAOPTIC NEURONS J.-P Wuarin* and F.E. Dudek Mental Retardation Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024 and Dept. of Anatomy and Neurobiology, Colorado State Univ., Fort Collins, CO 80523.

Excitatory and inhibitory amino acids have been shown to mediate electrically evoked synaptic responses in neurons recorded in hypothalamic supraoptic and paraventricular nuclei. Using whole-cell patch-clamp recordings, we tested the hypothesis that amino acid transmitters mediate all spontaneous excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) in supraoptic magnocellular neuroendocrine cells. A total of 27 cells were recorded. Resting membrane potential was -63±2.5 mV (mean± SE, n=21) and input resistance was 726±50 MΩ (n=25). Numerous spontaneous EPSCs and IPSCs were observed in all cells. With tetrodotoxin (0.5 µg/ml, n=3) in the perfusion solution, EPSCs and IPSCs were still clearly distinguished from background noise, indicating that miniature spontaneous synaptic currents were detected. In bicuculline (10 µM), no IPSCs were seen (n=10). No IPSCs or EPSCs were detected in bicuculline (10 µM) and 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX) (10 µM, n=6). The decay phase of averaged spontaneous currents could be well fitted by single exponentials with time constants ranging from 5.5 to 6.6 ms for IPSCs (3 cells) and from 1.3 to 3.0 ms for EPSCs (4 cells). These results suggest that in supraoptic magnocellular neurons, glutamate or related excitatory amino acids mediate all the spontaneous EPSCs by acting primarily on non-NMDA receptors at resting membrane potential, and that activation of $GABA_A$ receptors mediates all spontaneous IPSCs.

346.11

PERSISTENT SUPRAOPTIC (SON) & SUPRACHIASMATIC (SCN) DYNORPHIN-A ABNORMALITIES IN ADULT ACTIVITY-BASED ANOREXIC (ABA) RATS EXPOSED TO NEONATAL MORPHINE. P.F. Aravich*, R.A. Howell, S.N. Downing, T.S. Rieg, L.E. Doerries. Dept. Anat./Neurobiol., Eastern Virginia Med. Sch., Norfolk, VA 23501; Christopher Newport Univ., Newport News, VA 23606; V.A. Med. Ctr., Hampton, VA 23667.

Opioid abnormalities have been linked to anorexia nervosa (AN) and may or may not be produced by neonatal morphine exposure. Because of interest in the relationship between exercise and AN, we have been exploring ABA in the rat and have reported dynorphin A abnormalities. This experiment determined if neonatal morphine exposure causes persistent changes in hypothalamic dynorphin A content in ABA rats and if these alterations affect susceptibility to the syndrome. Female Sprague-Dawley pups were injected on postnatal days 1-8 with morphine sulfate (0, 0.5 or 5.0 mg/kg, sc). They were weaned on day 23 and maintained on Purina chow until day 53, at which time they were subjected to ABA (1.5 h/day food; 22.5 h/day running wheel access). All rats were sacrificed following a weight loss of 25%. The SON and SCN were analyzed for dynorphin A (per mg protein) via RIA. It was found that neonatal morphine exposure had no reliable effect on 24-hr open-field activity just prior to ABA, body weight prior to ABA, susceptibility to ABA (i.e., number of days to weight-loss criterion) or food intake during ABA. It did, however, reliably decrease average wheel turns per day and had a differential effect on regional hypothalamic dynorphin A content, elevating it in the SON and decreasing it in the SCN. This investigation demonstrates that neonatal morphine exposure reduces ABA-induced running and has long-term, differential consequences on hypothalamic dynorphin systems. The effects of neonatal morphine in normal weight rats and on circadian and neural lobe function in ABA rats remain to be determined.

346.12

NEW EVIDENCE FOR CIRCUITS LINKING THE RETINA TO THE SUPRAOPTIC NUCLEUS IN RATS. J. D. Levine* and R. R. Miselis. Dept of Animal Biology, University of Pennsylvania, Philadelphia, PA 19104.

Previous studies have shown that the lateral component of the retinohypothalamic tract terminates dorsal to the caudal SON, as well as, sparsely throughout the SON. In this study, we ask whether pseudorabies virus (PRV), a transynaptic neuroanatomical tracer, will label cells in these areas. The Wild type (Wt; $4X10^8$ or $6X10^8$ pfu/ml) and Bartha (Ba; $5X10^8$ or $9X10^8$ pfu/ml) strains were employed to identify retinorecipient neurons located within the SON and peri-SON terminal field in the female albino rat. Following bilateral sympathectomy to eliminate the confounding sympathetic innervation of the eye, each rat received a uniocular injection of 20 µl CT-HRP and 2-3 µl Wt or Ba. A double labeling protocol was adopted to display both the CT-HRP reaction product and infected neurons in the same section. The Wt infected a small group of cells within the SON and within the CT-HRP labeled peri-SON terminal field. In contrast, Ba infected only an occasional cell in this region. These findings demonstrate that retinal ganglion cells may innervate the SON directly. This provides an anatomic basis for photic influence on magnocellular function. Currently, we are examining the possibility that cells in the peri-SON terminal field might be interneurons that provide retinal ganglion cells indirect access to magnocellular neurons.

Supported by GM27739

346.13

CHARACTERIZATION OF PROJECTIONS TO THE NEUROHYPOPHYSIS BY TRANSNEURONAL PASSAGE OF PSEUDORABIES VIRUS. R.C. McLear, B.J. Oldfield, L.W. Enquist, D.E. Pleasure* and R.R. Miselis. Dept. of Animal Biology, School of Veterinary Medicine, University. of Pennsylvania, Phila., Pa 19104

There are conflicting opinions as to the primary source of afferents to the magnocellular (Mg) neurons of the neurohypohyseal system for osmotic control of vasopressin secretion. One view has nearby neurons as the source of osmotic input. Another view proposes more distant neurons in the structures of the lamina terminalis as the primary source of osmotic information for the modulation of vasopressin secretion. We have used the transneuronal passage of Bartha (Ba-an attenuated strain of pseudorabies virus) to locate second order neurons innervating the Mg neurons of the neurohypophysis. At short survival times (≤ 16 hours) Ba labels only the Mg neurons of the paraventricular, supraoptic and accessory nuclei. At longer survival times (20-28 hours) Ba passed transneuronally into second order neurons which were confined to the subfornical organ, median preoptic nucleus, organum vasculosum of the lamina terminalis, the caudal ventrolateral medulla and vagal complex. In these time periods there was very little labeling of neurons nearby the magnocellular nuclei suggesting a primacy of input from the above more distant nuclei in the modulation of Mg function. Osmoreceptors may lie among these neurons. Supported by NIH GM 27739.

346.14

EFFECT OF ANGIOTENSIN II ON SUPRAOPTIC NEURONS OF THE ADULT RATS WITH SLICE PATCH CLAMP METHOD. T. Nagatomo, K. Inenaga, Y. Ueta, F. Izumi* and H. Yamashita. Depts. of Physiology and Pharmacology*, Univ. of Occup. and Environ. Health, School of Medicine, Kitakyushu 807, Japan.

We have already reported *in vitro* experiment that angiotensinII (AII) increases the firing rate of rat supraoptic neurosecretory cells. (Okuya et al 1987). To elucidate the mechanism of the action, we used patch-clamp techniques in the thin slice preparations and recorded from the microscopically identified supraoptic neurons of the adult Wistar male rats. Effects of AII on isolated potassium currents of the supraoptic neurons were investigated. To separate I_A and I_K, Na^+ free and Co^{2+} (2mM) containing perfusion medium were used to block Na^+ and Ca^{2+} related currents. I_A was isolated by substracting currents either at two holding potentials (-80mV and -50mV), or before and after application of 4-AP (5mM). In some experiments, TEA (5mM) was also added to the perfusion medium to suppress I_K components. Bolus injection of AII (10^{-5}M, 100µl) and bath application of AII (10^{-7}M) suppressed I_A amplitude in responsive neurons with decrease of 32% and 29%, respectively. On the other hand, I_K was suppressed only slightly. These results indicate that AII excites neurosecretory cells partly through suppression of I_A.

346.15

THE EFFECTS OF HISTAMINERGIC INPUTS ON SUPRAOPTIC NUCLEUS NEURONS IN RAT HYPOTHALAMIC SLICES. Q.Z. Yang* and G.I. Hatton, University of California Riverside, Riverside, CA 92521.

Earlier extracellular studies by others and intracellular studies in our laboratory showed that histamine (HA) has excitatory effects on phasically firing neurons in the supraoptic nucleus (SON). Our dual intracellular recordings revealed that HAergic cells of the tuberomammillary nucleus (TM) are directly presynaptic to phasically firing vasopressin (VP) cells and their activation elicited EPSPs and action potentials.

In the present study, we investigated the effects of electrical stimulation of the TM in horizontally cut hypothalamic slices, with particular interest in responses of continuously firing, putative oxytocin (OX) neurons. Extracellular electrical stimulation of the TM evoked short latency (3-4 ms) fast IPSPs in most putative and all immunocytochemically identified OX neurons. These responses could be blocked by H_2-, but not H_1- antagonists. IPSPs could also be blocked by picrotoxin, but not by 10 µM bicuculline. Taken together with the earlier studies, these data suggest that HAergic input serves to differentially modulate OX and VP release via monosynaptic contacts with SON neurons. Support by NS16942.

346.16

UP-AND DOWN-REGULATION OF κ-OPIOID MODULATION OF OXYTOCIN SECRETION IN THE NEUROHYPOPHYSIS THROUGH PREGNANCY. R.J. Bicknell*[1], A.J. Douglas[2], S. Dye[1] and G. Leng[1]. [1]AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT and [2] Dept. of Physiology, University Medical School, Edinburgh EH8 9AG, UK

During pregnancy in the rat an endogenous opioid inhibition over oxytocin (OXT) neurones is activated which may allow accumulation of neurohypophysial stores, prevent premature OXT secretion and, during parturition, regulate the spacing of deliveries. One site of opioid action is autoregulatory, involving κ-receptors in the neurohypophysis and mediated via opioids co-secreted with OXT. We have gauged the magnitude of autoregulatory restraint during pregnancy. We compared the rise in plasma OXT concentration produced by i.v. cholecystokinin (CCK; 20µg/kg) before (CCK1) and after (CCK2) injection of the κ-antagonist norbinaltorphinine (norBNI; 20µg/kg) in conscious rats. In non-pregnant rats the ratio CCK2/CCK1 was 2.00±0.13 indicating a doubling of CCK-induced OXT output in the presence of norBNI. In mid-pregnancy (days 8-14) the ratio CCK2/CCK1 was significantly higher at 2.56±0.20 ($P<0.05$) and in late pregnancy (days 15-21) was significantly lower at 1.67±0.08 ($P<0.05$ vs non-pregnant). *In vitro*, the opioid antagonist naloxone (10^{-5}M) potentiated electrically evoked OXT secretion by 265%. This potentiation was significantly less in neurohypophyses from 16 and 21 day pregnant rats (30% and 25% reduction respectively). Pro-dynorphin mRNA levels in the supraoptic nuclei were unchanged in late pregnancy as was neurohypophysial content of dynorphin A 1-8: content of met^5-enkephalin was however significantly reduced on day 21 ($P<0.01$). Our data suggest that an upregulation of autoinhibitory opioid mechanisms restraining OXT secretion occurs in early or mid-pregnancy, followed by downregulation of this mechanism. Altered synthesis/secretion of proenkephalin rather than prodynorphin peptides may be involved. Since overall opioid tone over OXT neurones is enhanced in late pregnancy, activation of central opioid systems is indicated at this time.

346.17

FUNCTIONAL ROLE OF APAMIN SENSITIVE K^+ CURRENT IN STEADY STATE FIRING OF RAT SUPRAOPTIC NEUROENDOCRINE CELLS. K. Kirkpatrick* and C.W. Bourque.
Montreal General Hospital and McGill University, Montreal, Canada.

Magnocellular neuroendocrine cells (MNCs) express an apamin sensitive gK(Ca) that is responsible for the afterhyperpolarization (AHP) that follows evoked trains of action potentials. Voltage and current clamp experiments on 29 supraoptic MNCs impaled in superfused hypothalamic explants examined the properties and function of the current underlying the AHP (I_{AHP}). Hybrid clamp analysis revealed I_{AHP} to have a linear I-V relation with an E_{rev} near -98 mV (n=4) which shifted by 56 mV/log$[K^+]_o$. While an apamin sensitive AHP did not follow single impulses (100 nM;n=14) I_{AHP} could be activated with trains of as few as 3 action potentials suggesting that in tonically firing MNCs, AHP channels may be active in the steady state. Application of a voltage clamp following a period of spontaneous firing revealed a decaying outward current reversing near -98 mV, that was absent in the presence of apamin (n=11). Bath application of 100 nM apamin to tonically firing cells caused a dramatic increase in firing rate (n=5) but had no effect on silent cells (n=3). This study suggests that in addition to it's recognized roles in spike frequency adaptation and post burst inhibition, I_{AHP} is tonically activated to limit the rate of MNC spiking activity. Modulation of I_{AHP} therefore presents a means to regulate neurohypophysial hormone release.

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REGULATION OF AUTONOMIC FUNCTION: OTHER

347.1

SYMPATHETIC-EVOKED MYDRIASIS IS ANTAGONIZED BY HISTAMINE H_3 RECEPTOR ACTIVATION IN ANESTHETIZED CATS. M.C. Koss*[1], G. Figueiras[2] and J.A. Hey[3]. University of Oklahoma College of Medicine[1], Oklahoma City, OK, University Autonoma de Guadalajara[2], Guadalajara, Mexico and Schering-Plough Research Institute[3], Bloomfield, NJ.

Frequency-dependent pupillary dilations were evoked by electrical stimulation of the preganglionic cervical sympathetic nerve (sympatho-excitation) or the hypothalamus (parasympatho-inhibition) in sympathectomized cats. Systemic administration of the selective H_3 receptor agonist R-α-methylhistamine (RαMeHA) produced a dose-dependent depression of mydriasis due to sympathetic activation but had no effect on responses elicited by parasympathetic withdrawal. RαMeHA was much more effective in depressing sympathetic responses obtained at lower frequencies when compared to higher frequencies of stimulation. This inhibitory effect of RαMeHA was antagonized by the H_3 blocker thioperamide (3 mg/kg i.v.) but not by combined pretreatment with H_1 and H_2 blockers, chlorpheniramine (300 μg/kg i.v.) and cimetidine (5 mg/kg i.v.). RαMeHA was without effect on pupillary responses elicited by i.v. (-)-epinephrine (1-30 μg/kg). These results demonstrate that histamine H_3 receptors modulate sympathetic activation of the iris at a site proximal to the iris dilator muscle. A likely mechanism would be prejunctional inhibition of NE release although a ganglionic action cannot be excluded. Supported by EY09344.

347.2

DIABETIC BB/W RATS HAVE IMPAIRED ADRENOMEDULLARY CATECHOLAMINE SECRETION. R.A. Wilke, P.I. Lelkes† and C.J. Hillard*. Dept. of Pharmacology, Medical College of Wisconsin, Milwaukee, WI 53226 and †Dept. of Medicine, University of Wisconsin, Milwaukee, WI 53201.

After years of having their disease, many insulin-dependent diabetics lose the capacity to secrete catecholamines (CA) from their adrenal medullae. The mechanism for this pathological change is unknown. In order to gain some insight into the nature of this abnormality, we have isolated rat adrenal glands, perfused them in a retrograde fashion, and fluorometrically measured the CA content of the perfusate as an index of adrenal medullary secretion. With this preparation, we have demonstrated that secretion of CA in response to transmural electrical stimulation of splanchnic nerve terminals (600 cycles at 80 V) is severely attenuated in glands from female BB/Wistar rats with diabetes of 4 month duration (65% reduction when compared to their age-matched, nondiabetic controls). Furthermore, we have found that (in the presence of 500 μM mecamylamine) CA secretion in response to direct stimulation of the medulla with 20 mM potassium is reduced 40% in adrenal glands from the diabetic rats, evidence that the adrenal medulla itself is hypofunctional. Finally, using HPLC, we compared the content of both epinephrine and norepinephrine in adrenal medullae from these rats. There were no significant differences between diabetics and controls, indicating that the above secretory defect is not due to a difference in the amount of stored catecholamines available for release. Mechanistic studies such as these continue to enhance our understanding of the etiology of long-term diabetic complications hypoglycemia unawareness and autonomic neuropathy.

347.3

EFFECT OF ANTIBODIES AGAINST ACETYLCHOLINESTERASE ON EXPRESSION OF PEPTIDES AND CATECHOLAMINE SYNTHESIZING ENZYMES IN THE RAT ADRENAL GLAND.
Å. Dagerlind[1]*, T. Hökfelt[1] and S. Brimijoin[2]. [1]*Dept. of Histology and Neurobiology, Karolinska Institute, P.O. Box 60400, S-104 01 Stockholm, Sweden and* [2]*Dept. of Pharmacology, Mayo Clinic, Rochester MN 55905*

In the rat, systemic administration of monoclonal antibodies (1.5mg, i.v.) against acetylcholinesterase (AChE) caused autonomic dysfunctions such as piloerection (30 min after the injection) and ptosis (1h). Four days after the antibody administration a total disappearance of AChE immunoreactive fibers was seen in the adrenal medulla. However, a small number of AChE immunoreactive chromaffin cells and intramedullary ganglion cells, also seen in the control adrenal medulla, remained positive. In addition, the antibody injection affected levels of several peptides present both in fibers and chromaffin cells in the adrenal gland. The number of cells expressing calcitonin gene-related peptide (CGRP), galanin (GAL) and enkephalin (ENK) was dramatically increased compared to the very few cells observed expressing these three peptides in the normal gland. The sparse networks of CGRP and GAL positive fibers were however unchanged. In contrast, the dense network of ENK fibers present in the normal adrenals totally disappeared after the antibody injection. Substance P (SP) and somatostatin (SOM) positive cells, not present in the normal gland, appeared after AChE antibody administration, although to a much lower degree as compared to CGRP, GAL and ENK cells. No certain differences between normal and treated animals could be observed with regard to neuropeptide Y (NPY) or either of the catecholamine (CA) synthesizing enzymes tyrosine hydroxylase (TH), aromatic l-amino acid decarboxylase (AADC), dopamine β-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) immunoreactive chromaffin cells. The data presented here suggest a decentralization of the adrenal gland due to a detruction of preganglionic fibers and terminals by the anti-AChE antibody.

347.4

ENDOGENOUS ADENOSINE RESTRAINS CATECHOLAMINE SECRETION FROM ADRENAL MEDULLA IN RATS.
C.J. Tseng*, W.Y. Ho, C.J. Kuan and C.S. Tung. Depts. of Pharmacology and Physiology, National Defense Medical Center, Taipei, Taiwan, R.O.C.

The purpose of this study was to test the hypothesis that endogenous adenosine restrains norepinephrine and epinephrine release from adrenal medulla. We examined the effects of an adenosine receptor antagonist, 1,3-dipropyl-8-p-sulfophenylxanthine (DPSPX), on the epinephrine and norepinephrine release. Plasma catecholamine was measured by HPLC with E.C. detection. In conscious, unrestrained rats, DPSPX significantly increased plasma catecholamine in control rats and rats treated with hydralazine. The effect of DPSPX on plasma catecholamine was significantly greater in rats treated with hydralazine compared with control rats. This effect was attenuated in rats pretreated with converting enzyme inhibitor, captopril. Epinephrine was significantly reduced in rats received acute adrenectomy. These results demonstrated that under basal physiological conditions, endogenous adenosine tonically inhibits catecholamine secretion from adrenal medulla, which is achieved partially by inhibitory effect of adenosine on renin-angiotensin system, and this effect is augmented whenever the sympathetic system is stimulated.

347.5

MAP-2 AND GAP-43 IMMUNOREACTIVITY IN THE INTACT AND DECENTRALIZED PELVIC PLEXUS OF RATS N.M. Minorsky* and W.G. Dail Anatomy Dept., Univ. New Mexico, Sch of Med., Albuquerque, NM 87131

Neurons in the pelvic plexus reacquire innervation after chronic interruption of preganglionic input. One explanation for the emergent innervation is that intrinsic synapses form between ganglion neurons. The present study uses immunocytochemical labelling for cytoskeletal proteins to examine whether dendrites or axons contribute to the newly formed synapses. Specifically this study examines the incidence with which Map-2-IR (dendritic) and Gap-43-IR (axonal) fibers appose penile neurons in the intact and chronically decentralized pelvic plexus of rats. Results indicate that in both the intact and decentralized ganglia, Map-2-IR fibers are scarce and appose fewer than 15% of the penile neurons. By comparison, Gap-43-IR fibers enclose 57% of the penile neurons in the intact ganglia. This value, which drops to 27% after acute nerve lesion, increases to 48% in the chronically decentralized animal. The paucity of Map-2-IR fibers, and the apparent restitution of Gap-43-IR plexuses following chronic nerve lesion, suggest that decentralized neurons are reinnervated by axons.

347.6

THE ROLE OF GLIA AND MACROPHAGES IN TRANSNEURONAL PASSAGE OF PSEUDORABIES VIRUS. J.P. Card[1], L. Rinaman[2], & L.W. Enquist[1]. [1]DuPont Merck Pharmaceutical Co., Wilmington, DE and [2]Medical College of Pennsylvania, Philadelphia, PA.

Pseudorabies virus (PRV) is a neurotropic swine alpha herpesvirus that has been used extensively to map multisynaptic circuits in the CNS. The most convincing evidence for specific transport of virus through synaptically linked populations of neurons has been achieved with attenuated strains of virus. However, all strains produce pathological changes in neurons after prolonged replication that could compromise the circuit-specific transport of virus. We previously demonstrated that infection of neurons with virulent and attenuated strains of PRV produces a predictable temporal response of glia and macrophages that appears to isolate and restrict spread of virus from chronically infected neurons. In the present study, we have extended that analysis to the ultrastructural level. Inoculation of the stomach wall with virulent and attenuated strains of PRV led to infection of neurons in the dorsal motor nucleus of the vagus (DMV). However, the basic structure of the DMV, and the neuropil in particular, were remarkably free of pathology, even in specimens from animals chronically infected with the most virulent strain of PRV. Infected neurons were invested with and effectively isolated by processes of reactive astroglia and polymorphonuclear macrophages. Some of these cells ultimately became infected by virus, but exhibited a defect in envelopment that prevented release of infectious virus. These findings are consistent with the conclusion that the non-neuronal response to viral infection of neurons contributes to specific transport of virus through multisynaptic circuits of neurons.

347.7

REPLICATION, ASSEMBLY & INTERCELLULAR TRANSPORT OF PSEUDORABIES VIRUS IN RAT CNS. B.H. Lee[1], R.B. Lynn[2], L.W. Enquist[3], J.B. Grinspan[4], R.R. Miselis[4], & J.P. Card[3]. [1]Geongsang Natl. Univ., Korea, [2]Thomas Jefferson University, Philadelphia, PA, [3]DuPont Merck Pharmaceutical Co., Wilmington, DE, and [4]University of Pennsylvania, Philadelphia, PA.

Pseudorabies virus has been used extensively to map multisynaptic circuits of neurons in the central nervous system. The utility of this method is based upon the assumption that transneuronal passage of virus occurs preferentially at synapses and that neurons are not infected by release of virus into the extracellular space. Although numerous projection specific patterns of neuronal transport have been offered in support of this conclusion, clear documentation of the pathways of viral replication, assembly, intracellular transport and egress from infected neurons have not been established. In the present study, we have used electron microscopy to characterize these processes in neurons of the dorsal motor vagal nucleus which were infected by injecting virulent or attenuated strains of PRV into the ventral wall of the stomach. Systematic examination of infected neurons in serial micrographs demonstrated a highly organized pathway of viral replication and transport which utilized existing cellular machinery and biased transport of virions to sites of afferent synaptic input. Among the most important aspects of this process was the application of a Golgi-derived envelope to newly replicated viral capsids. This bilaminar membrane appeared to be instrumental in intracellular transport and permitted fusion events that allowed virions to pass between neurons at sites of synaptic contact. Virions were distributed throughout the full extent of the dendritic arbor, but release only occurred at sites of afferent synaptic contact.

RESPIRATORY REGULATION: CHEMORECEPTION

348.1

ACTIVITY OF RESPIRATORY NEURONS DURING HYPOXIA IN CHEMODENERVATED CATS. J.E. Melton, S.J. England, M. Douse, J. Duffin and J.A. Neubauer*. UMDNJ-R. W. Johnson Med. Sch.,New Brunswick,NJ 08903 and Univ. of Toronto,Toronto,ONT M5S1A8.

In anesthetized, chemodenervated cats, phrenic nerve activity decreases during hypoxia culminating in apnea at an arterial O_2 saturation of 50%. To determine if decreased respiratory activity is due to inhibition of premotor inspiratory neurons by Bötzinger bulbospinal expiratory neurons, extracellular activity was recorded from expiratory neurons in the Bötzinger complex and bulbospinal inspiratory neurons in the dorsal (DRG) and ventral (VRG) respiratory groups during acute hypoxia. All neurons progressively decreased firing rates during hypoxia. Expiratory neurons became silent prior to, and inspiratory neurons with or immediately following, loss of phrenic nerve activity. During reoxygenation, phasic firing was evident in 35% of inspiratory and expiratory neurons before, with onset of the remainder coincident with, return of phrenic nerve activity. Firing rates increased during reoxygenation, returning to prehypoxic levels within 2 min. These data suggest: 1) Hypoxia inhibits/disfacilitates Bötzinger expiratory and DRG/VRG inspiratory neurons in the chemodenervated animal; 2) Bötzinger expiratory neurons are not the source of hypoxic inhibition of these inspiratory neurons; 3) Phasic activity of subpopulations of respiratory neurons precedes the onset of integrated motor output. Supported by HL16022 and MRC Canada.

348.2

SYNERGY BETWEEN THE AMOUNT OF TYROSINE HYDROXYLASE IN RAT NUCLEUS TRACTUS SOLITARIUS AND VENTILATORY ACCLIMATIZATION TO CHRONIC HYPOXIA. [1]M. Denavit-Saubié*, [3]P. Schmitt, [3]J.F. Pujol and [2]J.M. Péquignot. [1]Institut A. Fessard - CNRS, 91198 Gif-sur-Yvette (France) [2]URA 1195 - CNRS, Fac. de Médecine Grange-Blanche, 69008 Lyon (France) and [3]UMR105 -CNRS, Fac. de Médecine A. Carrel, 69008 Lyon (France).

Long term hypoxia causes a gradual rise in ventilation which is transmitted by peripheral chemosensory pathways to the caudal part of the nucleus tractus solitarius (NTS). Since this area is rich in catecholamines, the aim of this work was to study their evolution during ventilatory acclimatization by measuring Norepinephrine (NE) turnover and the quantity of tyrosine hydroxylase (TH) in the caudal and rostral parts of the NTS. Two groups of rats Sprague-Dawley were kept either in normoxia (21%O2) or in hypoxia (10%O2/90%N2) during 3, 7, 14 and 22 days. TH protein, NE turnover, tidal volume and respiratory frequency were measured. Ventilation increased progressively during the first week of hypoxia and then stabilized. In the caudal part of the NTS, there was a concurrent increase in both NE turnover and TH content which reached maximal levels after 14 days, while the rostral part of the NTS was unaltered. There is a strong relationship between the biochemical and the physiological responses to long term hypoxia and the distinction between caudal and rostral part of the NTS reflects the target of chemosensory inputs.

348.3

DEOXYGLUCOSE UPTAKE AND OXYGEN PARTIAL PRESSURE IN THE ISOLATED PERFUSED BRAINSTEM OF ADULT GUINEA PIG. T. Schäfer+, M.P. Morin-Surun, M. Denavit-Saubié and R. Naquet*. Institut Alfred Fessard, C.N.R.S., F-91198 Gif-sur-Yvette Cedex, France. +Dept. of Applied Physiology, Ruhr-University, W-4630 Bochum, FRG.

Recently we have demonstrated that in the adult brainstem preparation, perfused through the basilar artery, a functional respiratory network is preserved (Morin-Surun et al., 1992, Exp. Brain Res., in press). Electrophysiological studies showed that the ventral respiratory group (VRG) is functioning in this preparation. To detect the metabolic conditions and oxygen supply within this region we measured deoxyglucose uptake and oxygen partial pressures in the tissue. Deoxyglucose, given as a single bolus into the basilar artery, follows the same pathway as glucose, but remains trapped as deoxyglucose 6-phosphate and is not metabolized any further. The autoradiographic mapping of 2-[^{14}C] deoxyglucose in the adult guinea pig brainstems indicates glucose utilization in the whole cross sections of the entire brainstem. Tissue oxygen partial pressures were measured in steps of 100 μm using an O_2-sensitive microelectrode (Diamond, tip 25 μm), calibrated in two Krebs solutions saturated with air and nitrogen, respectively, at the same temperature as the perfusion solution (Krebs, 95% O_2, 5% CO_2, 25°C, no oxygen carrier). Mean pO_2 (n=5) was 476±105 mmHg on the ventral surface superficial to the VRG, 350±43 mmHg at 200 μm below the surface, 141±29 mmHg at 1200 μm, 33±16 mmHg at 2200 μm and 114±26 mmHg at 3200 μm. We conclude that both, glucose utilization and oxygen supply are preserved in the isolated perfused brainstem.

348.4

WHOLE CELL PATCH RECORDINGS OF VENTROLATERAL MEDULLARY NEURONS THAT ARE STIMULATED BY HYPOXIA. P.C. Nolan* and T.G. Waldrop. Dept. of Physiology & Biophysics, Neuroscience Program and College of Medicine, Univ. of Illinois, Urbana, IL 61801.

Recent studies suggest that neurons in the ventrolateral medulla (VLM) are involved in the mediation of cardiorespiratory responses to hypoxia. Prior results from this laboratory obtained with extracellular recordings have shown that a high proportion of VLM neurons increase their discharge frequency during hypoxia *in vivo* and *in vitro*. The present study employed whole cell current clamp recordings in a brain slice preparation to determine intracellular responses of VLM hypoxia-sensitive neurons. Neuronal morphology was examined by including lucifer yellow in some of the recording pipettes. Brain slices (300-500 µm) from male Sprague-Dawley rats were placed in an interface chamber and perfused with nutrient media equilibrated with 95% O_2/5% CO_2. VLM neurons had a resting V_M of -54.6±2.2 mV and a peak input resistance of 275.7±21.0 MΩ. A severe hypoxic stimulus (5% CO_2/95% N_2) elicited a depolarization (6.7 mV±1.7) that persisted for the duration of the stimulus (30-90 sec). A similar response (4.6±0.8 mV) was produced by a more moderate level of hypoxia (10% O_2/5% CO_2/85% N_2). Neurons studied were distributed from the caudal aspect of the facial nucleus to the rostral portion of the lateral reticular nucleus and ventral to the nucleus ambiguus. The hypoxia-sensitive neurons were multipolar with several projections from the cell body. These findings indicate that the excitation of VLM neurons by hypoxia is associated with a depolarization. Thus, activation of neurons in this medullary area may contribute to cardiorespiratory responses to hypoxia (Supported by NIH 38726 and AHA-IL Affiliate).

348.5

MODULATION OF THE RESPIRATORY RESPONSES TO HYPOXIA AND HYPERCAPNIA BY SYNAPTIC INPUT ONTO CAUDAL HYPOTHALAMIC NEURONS. E.M. Hom and T.G. Waldrop*. Dept. of Physiology & Biophysics, Neuroscience Program and College of Medicine, Univ. of Illinois, Urbana, IL 61820.

Prior results from this laboratory have demonstrated that the respiratory response to hypercapnia is accentuated by microinjection of GABA antagonists or GABA synthesis inhibitors into the caudal hypothalamus of both cats and rats. However, no evidence was found for involvement of hypothalamic GABA in the respiratory response to hypoxia. The purpose of the present study was to determine if mechanisms of synaptic input other than GABAergic onto caudal hypothalamic neurons affects the respiratory responses to hypoxia. The respiratory (diaphragmatic EMG) responses to hypoxia (10% O_2) and hypercapnia (5% CO_2) were recorded in anesthetized rats before and after bilateral microinjection of $CoCl_2$ (100 mM) into the caudal hypothalamus in order to block synaptic transmission. Both hypoxia and hypercapnia elicited increases in tidal diaphragmatic activity and respiratory frequency prior to the microinjections. The respiratory response to hypercapnia was increased (+27.3%) after $CoCl_2$ microinjections; this finding is consistent with prior results obtained with blockade of GABAergic input. In contrast, the respiratory response to hypoxia was diminished (-38.3%) after the $CoCl_2$ microinjections. The results of this study support our prior findings that neurons in the caudal hypothalamus modulate the respiratory response to hypercapnia. In addition, our findings suggest that an excitatory input onto hypothalamic neurons modulates the respiratory responses to hypoxia. (Supported by NIH 38726 and AHA).

348.6

OPTICAL ACTIVITY OF THE VENTRAL MEDULLARY SURFACE IN THE KITTEN DURING HYPOXIC STIMULATION. D. Gozal, X-W. Dong, D.M. Rector, J. Zhang* and R.M. Harper. Div. Neonatol. & Ped. Pulmonology, Children's Hospital, USC Sch. of Med.; Brain Res. Inst. and Dept. of Anatomy & Cell Biology, UCLA Sch. of Med., Los Angeles, CA.

The functional topography of cells within the ventral medullary surface (VM) was assessed in developing kittens during mild hypoxic exposure by optical imaging procedures. The VM was exposed through a ventral surgical approach in five kittens, 10-20 days of age, under sodium pentobarbital anaesthesia. An imaging device, consisting of a 3.2 mm diameter optical probe with 25 µ fiber resolution and charge coupled device camera, was placed on the VM surface, and tissue was illuminated by 700 nm light. Images were digitized at 2-3 sec intervals before, during, and for 30 min after hypoxic challenges with 12% O_2 in N_2. Images within each epoch were averaged and subtracted from baseline. The statistical significance for each pixel change was determined by analysis of variance (α=.05). Most kittens became apneic after an initial increase in ventilatory activity. Overall neuronal activity increased immediately after hypoxic exposure, and persisted unless apnea developed. Cessation of breathing resulted in a marked decline of activity. During mild hypoxia, neuronal activity in the VM surface of the kitten parallels ventilatory patterns. (Supported by HD22506 & NIDR DE 07212)

348.7

TRANSIENT AND SUSTAINED PATTERNS OF OPTICAL ACTIVITY IN THE CAT VENTRAL MEDULLARY SURFACE TO CO_2 STIMULATION. X-W. Dong*, D. Gozal, D.M. Rector, & R.M. Harper. Brain Res. Inst. and Dept. of Anatomy & Cell Biology, UCLA Sch. of Med.; Div. Neonatol. & Ped. Pulmonology, Children's Hospital, USC Sch. of Med., Los Angeles, CA.

Neuronal activity patterns in chemoresponsive fields of the ventral medullary surface (VM) were examined by measuring reflected light changes during hypercapnic ventilatory challenges. The VM was exposed through a ventral surgical approach in ten adult cats under sodium pentobarbital anaesthesia. A 3.2 mm diameter optical probe and charge coupled device camera were placed on the VM surface, and tissue was illuminated by 700 nm light. Images were digitized at 2-3 sec intervals before, during, and for 30 min after hypercapnic challenges with 3, 5, and 10 % CO_2 in O_2. Each image was subtracted from baseline, and reflectance changes in both overall and regional areas were assessed. Derivatives of these measures were then plotted sequentially. After an initial delay of approximately 30 sec from onset of CO_2 administration, marked transient changes in reflectance occurred, with subsequent baseline shifts during the stimulus. Upon return to room air, transient optical responses in both overall and regional activity were again observed. The direction and extent of the response depended on both the area of the VM surface and the CO_2 concentration of the gas, respectively. Chemoresponsive neurons in the VM apparently respond with both transient and sustained changes in activity; the transient responses are reminiscent of "on" and "off" discharges observed in other sensory systems. (Supported by HL22418 & NIDR DE07212)

348.8

ELECTROPHYSIOLOGIC EFFECTS OF CHANGES IN PCO_2 ON RAT MEDULLARY NEURONS *IN VITRO*. G.B. Richerson*. Department of Neurology, Yale University and VAMC, West Haven, CT. 06516

The central chemoreceptors for respiration have been localized to the medulla, but have not yet been identified. To characterize possible cellular mechanisms underlying respiratory chemoreception, modulation of neuronal excitability by changes in PCO_2 was compared in respiratory related and other regions of the rat medulla using whole cell patch clamp recordings in brain slices. Rats (10-21 days) were anesthetized, and slices (100 um) were prepared with a vibratome. Slices were maintained in a perfusion chamber on a fixed stage upright microscope. Low power visualization permitted neuroanatomical localization using histologic landmarks, and visualization at 400X with Nomarski optics allowed recordings from anatomically identified neurons.

To prevent dialysis of pH buffers, calcium, and other intracellular constituents which could lead to a change in the normal response to CO_2, the amphotericin perforated patch technique was used. Electrodes were filled with a solution containing KMethanesulfonate 135 mM, KCl 30 mM, HEPES 5 mM, EGTA 1 mM, and amphotericin B 1 mg/ml. After stabilization of access resistance, whole cell recordings of membrane potential were possible for 1-2 hours. PCO_2 was systematically changed from 5% to 7% and 9%, and the resulting changes in resting potential, firing rate, synaptic activity, and response to depolarizing current were measured. There was a diversity of responses to changes in PCO_2, with transient and prolonged, inhibitory and excitatory effects which varied by cell type and location, presumably reflecting differences in function. These differences are likely to contribute to control of respiratory output *in vivo*. Supported by Dept. of Veterans Affairs, and NIH.

348.9

CO_2 RECEPTION IN THE SNAIL, H. ASPERSA. J.S. Erlichman, J.C. Leiter, and F.V. McCann*, Dartmouth Medical School, Department of Physiology, Lebanon, NH 03756.

Gas exchange in the terrestrial gastropod, H. aspersa, is accomplished through a vascularized diffusion lung referred to as the mantle. The opening of the mantle cavity to the external environment is controlled by an occlusible breathing pore called the pneumostome. In these experiments we show that exposure of small regions of the visceral ganglia to low concentrations of CO_2 (4-6% CO_2) result in large increases in the diameter of the pneumostome, similar to that observed in the intact animal exposed to hypercapnia. Thomas (J. Physiol. 354:3-22, 1984) has shown that the recovery of intracellular pH (pHi) to acidification is dramatically reduced when Cl^- conductance is altered. To determine if inhibiting the rate of pHi recovery alters the rate or magnitude of the pneumostome opening, we perfused the snail brain with 4.5-6% CO_2 equilibrated saline solutions (pH 7.2) with and without the Cl^- channel blocker DIDS (10 uM). We found that the pneumostomes opened more quickly (11/13) and to a greater extent (10/13) when the brains were perfused with high CO_2/DIDS solutions compared to high CO_2 solutions alone. Similar results were obtained in Cl^- substituted solutions. We conclude that H. aspersa may be a useful model for examining chemoreception as it pertains to the control of breathing, and that the ventilatory effects of hypercapnia may be mediated by a) pHi, b) the rate of pHi recovery. (Supported by HL 19827).

348.10

MECHANISMS OF CO_2-INDUCED DEPOLARIZATION IN SNAIL *Lymnaea Stagnalis* GIANT NEURONS. F. Hayashi, C. Jiang, R. Takeda* & G. G. Haddad. Section of Respiratory Medicine, Dept. Pediatrics, Yale School of Medicine, New Haven, CT 06510.

To test the hypothesis that CO_2 has a direct effect on neuronal membrane properties, intracellular recordings were performed in snail (Lymnaea) neurons and the ionic mechanisms for the CO_2-induced changes were studied. Visceral and right parietal ganglia were dissected and maintained in-vitro in room air and room temperature. Membrane potential (Vm), input resistance (Rm) and intra-/extracellular pH (pH_i, pH_o) were measured in giant neurons (VD_{1-3}, RPD_{1-2}) in these ganglia. These cells had a baseline Vm of -61±6 mV (n=43, mean±SD), and an Rm of 32±8 MΩ (n=7). Superfusion of these cells with a gas mixture containing 15% CO_2 produced a depolarization of 14±6 mV (n=17) and an increase in Rm of 79%. Both pH_i and pH_o were reduced during CO_2 exposure by 0.6 (n=17) and 0.8 (n=5) pH units respectively, and perfusing cells with acidified solution (the same pH_o as during CO_2 exposure) mimicked the effect of CO_2 on Vm and Rm. K^+ channel blocker, Cs^+ (10 mM), markedly (60%) reduced the CO_2-D from 17.0±5.0 mV to 7.0±1.0 mV (n=4, P<0.01), but 4-AP and apamin did not produce any change in CO_2-D. Blockade of Ca^{++} channels with a Ca^{++}-free medium plus Co^{++} (5 mM) or Mg^{++} (6 mM) also significantly decreased (40%) the CO_2-D (n=4, P<0.05). Na^+ and Cl^- channels were probably not involved, since TTX (10 μM), Na^+-free or Cl^--free media did not have any effect. We conclude that 1) CO_2 induces a dose-dependent depolarization in snail giant neurons; 2) the CO_2-D seems to be mediated by intra- or extracellular acidification; and 3) inactivation of K^+ channels and/or activation of Ca^{++}-dependent mechanisms seem to underlie the CO_2-D.

348.11

CARDIORESPIRATORY EFFECTS OF KAINIC ACID ON BRAINSTEM CHEMOSENSITIVE ZONES R.M. Douglas, D.G. Bernard, Y. Pan, S.G. Burgest, R. Millis, J.A. Holloway, C.O. Trouth*

Dept. of Physiol. & Biophysics, Howard University Coll. of Med. Washington, D.C. 20059

Physiological investigations have indicated that neurons located on the ventral medullary surface (VMS) subserve central respiratory chemosensitivity and vasomotor control. This study purports an attempt to identify the respiratory component involved. In spontaneously breathing rats, medullary neuronal activity (MNA) in the caudal chemosensitive zone on the VMS was studied in response to inspired CO_2 and topically applied acetylcholine (ACh) before and after topical application of kainic acid (KA), an excitotoxic amino acid analogue of glutamate, to the VMS. Inspired CO_2 and ACh increased MNA and diaphragmatic activity (DA). KA, which purportedly destroys neuronal cell bodies but not fibers of passage, caused a marked increase in MNA and DA. This was followed by a gradual (over 30 min) decline in MNA and DA with eventual loss of activity and respiratory arrest. Within 10-15 min of artificial respiration, MNA and DA recovered, however, with reduced sensitivity to inspired CO_2. It was not possible to maintain MNA and DA for more than 5-6 minutes after weaning the animal from the respirator. These results suggest that (1) KA compromises the ability of the superficial neurons to maintain the central drive to respiration, (2) KA appears to deplete the neuronal metabolic stores of superficial CO2 sensitive neuronal elements, and (3) the cardiovascular component of the VMS seems unimpaired by the topical application of KA.

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348.12

AGE DIFFERENCES IN CHOLINERGIC AND CATECHOLAMINERGIC NEURONS IN RAT BRAINSTEM RESPIRATORY CHEMOSENSITIVE ZONES. Y. Pan*, R. M. Douglas and C. O. Trouth. Dept. of Physiol. & Biophysics, Coll. of Med., Howard Univ., Washington, D.C. 20059

Central respiratory chemosensitivity has been ascribed to cholinergic neurons on the ventral medullary surface (VMS) which are sensitive to changes in the H^+ ion concentration of the cerebrospinal fluid and to increased inspired carbon dioxide. We examined the chemosensitive areas of newborn and adult rats by immunocytochemical techniques for the presence and distribution of cholinergic and catecholaminergic neurons. Alternating cryostat sections from the rat medulla were studied using primary antibodies against choline acetyltransferase (ChAT) and tyrosine hydroxylase (TH). In the adult rat, multipolar ChAT positive neurons were found in the caudal chemosensitive area and in the nucleus reticularis lateralis. Bipolar TH positive neurons with fibers running parallel to the VMS were identified. The newborn rat, by contrast, was almost devoid of ChAT neurons in the caudal VMS. Lightly positive ChAT neurons began to appear about the 4th day. In the newborn, TH positive neurons have the same distribution as in the adult. Thus in the rat, the newborn period (1-4 days) is characterized by (1) paucity of cholinergic neurons and (2) reduced sensitivity to inspired carbon dioxide. These results suggest that age related differences in the responsiveness to physiological stimuli might be referable to the integrity or development of cholinergic neurons. *Support: NIH-NIGMS Grant # S06GM08016*

348.13

CHOLINERGIC MECHANISMS INVOLVED IN BRAINSTEM RESPIRATORY CO_2-CHEMOSENSORY CONTROL. D.G. Bernard*, L.M. Sexcius, R.M. Millis, C.O. Trouth. Dept. of Physiol. & Biophysics, Coll. of Med., Howard Univ., Washington, D.C. 20059.

The effect of cholinergic mechanisms on extracellular CO_2-sensitive neuronal activity recorded at the caudal brainstem respiratory chemosensitive area on the ventral medullary surface (cVMS) was investigated in chloralose-urethane anesthetized adult and newborn cats. Endogenous cholinergic activity was identified by measuring the cholinolytic effects produced by the topical application of atropine sulfate (ATR) to the cVMS during peak neuronal responses to inspired CO_2 (hypercapnic breathing). The responsiveness of newborns (1-6 days) to inspired CO_2 was markedly reduced compared to adult cats. cVMS CO_2-sensitive neurons were excited by topically applied acetylcholine and physostigmine. ATR reversed the CO_2 induced excitation of neuronal activity to near control values whereas hexamethonium was less effective. Application of prazosin and propranolol in mock cerebrospinal fluid pH 7.4 produced no significant change in either neuronal or respiratory activity. These results suggest 1) age differences in cVMS neuronal response to inspired CO_2, 2) ATR blocks the CO_2 induced excitation of these neurons suggesting muscarinic receptor involvement and 3) the cVMS contains CO_2-sensitive cells which are excited by ACh and Phy.

SUPPORT: NIH-NIGMS Grant #: S06GM08016

348.14

LOCUS COERULEUS (LC) NEURONS EXPRESS C-FOS IMMUNOREACTIVITY UPON STIMULATION OF CENTRAL CHEMOSENSORY SYSTEM. M.A. Haxhiu*, B. Erokwu, N.R. Prabhakar, N.S. Cherniack, and K.P. Strohl. CWRU, Cleveland, Ohio.

Neurophysiologic and pharmacologic studies showed that the central CO_2 sensitive structures are located in a relatively discrete region of the ventral medulla. Recently, we found in cats and confirmed in rats that chemosensory neurons can be identified by using c-fos expression in response to hypercapnia. Hence, it is now possible to study the location of activated cells by hypercapnia in other brainstem regions involved in autonomic and respiratory control. In these studies we utilized c-fos protein expression to investigate the effect of CO_2 on LC neurons, and to determine pathways which might mediate hypercapnic response. Experiments were performed on awake, and on anesthetized, paralyzed and artificially ventilated rats and cats. Awake animals were exposed for 1 hour to room air (6 rats and 1 cat), or to 15% CO_2 in room air (8 rats and 2 cats). Frozen sections of brainstem (50 μm) were immunostained for c-fos protein using avidin-biotinylated peroxidase complex (ABC). In both species, hypercapnia induced c-fos expression in LC neurons, a response which was not altered by deafferentation of peripheral chemoreceptors (3 rats). A similar effect was found in α-chloralose anesthetized cats artificially ventilated with hypercapnic gas mixture. Local application of 2% lidocaine on ventral surface of medulla (VMS) attenuated but did not abolish LC c-fos expression (n=2). Activation of cell bodies by topical application of NMDA (10 nmol) on VMS, induced strong c-fos expression in LC neurons (n=4). We conclude that LC show early gene responses to hypercapnia, and structures located on ventral surface of medulla may play a significant role in this response. Supp: HL-35830 and Isabelle and Bernard Zuckerman Fund for Respiratory Research.

348.15

WIDESPREAD SITES OF BRAINSTEM VENTILATORY CHEMORECEPTORS. E.L. Coates, A. Li, and E.E. Nattie*
Dartmouth Medical School, Department of Physiology, Lebanon, NH 03756

We have used a novel method to study the location of central chemoreceptors (J. Physiol. 441:433-451,1991). Microinjections (1 nl) of acetazolamide (AZ, 10^{-3}M), an inhibitor of carbonic anhydrase, were made in a number of locations throughout the brainstem of chloralose-urethan-anaesthetized, vagotomized, carotid denervated, paralysed, servo-ventilated rats and cats. We found that phrenic nerve activity was stimulated with AZ injections located beneath the ventrolateral medulla, in the region of the nucleus tractus solitarii, and in the region of the locus coeruleus. The rationale of our approach was to produce a local region of acidosis around the site of AZ injection. This is attributed to delayed CO_2 hydration and/or delayed formation of carbonic acid from metabolically produced H+. To determine the size of the region of the AZ induced tissue acidosis we measured the pH of the brain tissue with H+ selective electrodes (tip dia = 20um) placed at distances of 0 - 700um from the AZ injection pipette. We found that pH changes could not be detected at distances greater than 400um. These results indicate that the tissue acidosis produced by AZ injections is limited. In addition, the number and location of sites where AZ injections stimulated phrenic nerve activity indicates a widespread distribution of respiratory chemoreceptors. (Supported by HL 28066 and HL 07449).

348.16

DISTRIBUTED BRAIN STEM RESPIRATORY NEURAL NETWORKS AND PHRENIC NERVE DISCHARGE: CORRELATIONS DURING CAROTID CHEMORECEPTOR STIMULATION. K. F. Morris*, A. Arata, R. Shannon and B. G. Lindsey.
Physiol. & Biophysics, Univ. South Florida Med. Ctr., Tampa, FL 33612.

Stimulation of carotid body chemoreceptors (CStim) results in three changes in phrenic nerve activity: a large increase, a decaying afterdischarge (Eldridge et al. Resp. Phys. 40:49) and long term enhancement (Millhorn et al. Resp. Phys. 41:87). We studied the involvement of brainstem respiratory related neural networks in these responses. Spike trains of neurons were recorded simultaneously (max.=20, mean=12.7) in the ipsilateral n. tractus solitarius (NTS), rostral (region of retrofacial n.) and caudal ventral respiratory group (VRG), the contralateral VRG, and medullary raphe n. (RN) of 10, anesthetized, vagotomized, artificially ventilated cats. Phrenic nerve efferent activity was recorded. CStim was by injection of 200 ul of CO_2 saturated saline solution via the external carotid artery. We analyzed 275 spike trains with respiratory cycle-triggered and peristimulus-time histograms (PSTHs), and spike triggered average histograms of unrectified and full wave rectified phrenic multiunit activity (PSTAs). Cross-correlograms (CCs) of 1,829 pairs were analyzed. We detected, in PSTHs of spike trains from all five medullary regions, long-time scale (*sec.*) firing rate changes correlated with phrenic responses to CStim. Short-time scale (*ms.*) correlations included: positive lag peaks in CCs with chemoresponsive, respiratory modulated VRG trigger neurons and RN targets; central peaks and troughs in PSTAs with chemoresponsive triggers in RN; and positive lag peaks in PSTAs with chemoresponsive, respiratory modulated triggers in NTS and VRG. The data provide evidence for a subset of routes by which CStim evoked changes in central inspiratory drive are communicated to midline neurons implicated in cardiorespiratory integration (Lindsey et al. these proceedings) and to phrenic motor neurons. Supported by NS19814 & BRSG S07 RR05749.

SOMATIC AND VISCERAL AFFERENTS: TOUCH

349.1

MECHANICS OF THE PACINIAN CORPUSCLE: STATIC AND DYNAMIC MEASUREMENTS. B.Pietras* and S.J. Bolanowski.
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The input-output (IO) function relating the magnitude of the Pacinian corpuscle (PC) receptor-potential and stimulus amplitude is highly nonlinear showing saturation, hysteresis, and an asymmetric, full-wave rectification. These functions were obtained by monitoring the displacement of the stimulator and not the PC itself. Thus it is possible that the nonlinearities are introduced by inadequate stimulus definition and/or the mechanics of the accessory capsule interposed between the stimulus and the neural element. A series of experiments were conducted using video microscopy on cat mesenteric PCs to determine: 1) reactive forces as measured under static displacement conditions with stimulus probe and PC size as parameters; 2) whether and how the perimeter and internal portions (lamellae) of the PC's accessory capsule follow dynamic displacement stimuli in the steady-state (sinusoidal, 1-600 Hz, stimulus probe size, 125-500 μm dia.) and 3) the resultant reactive forces produced by such dynamic stimulation. It was found that for the static conditions the reactive forces are nonlinear, showing a quadratic relationship between force and displacement. Increases in probe size increase the reactive force, but they are not correlated with PC size. For the dynamic case, the capsule and internal lamellae closely follow the stimulus albeit with attenuated amplitude from the perimeter of the capsule to the neurite. Additionally, the dynamic reactive forces are nonlinearly related to stimulus displacements displaying prominent hysteresis. Thus the hysteresis in the IO function may be explained if the pertinent stimulus dimension is force-related, although none of the observed mechanical properties can explain saturation or the asymmetric, full-wave rectification. These latter two are likely a result of the neural transduction mechanisms.

349.2

QUANTITATIVE ANALYSES OF THE CYTOPLASMIC EXTENSIONS AND MITOCHONDRIAL LOCATION WITHIN PACINIAN CORPUSCLES (PC). J.E. Landcastle, D. Sulitka, N.B. Slepecky, S.C. Chamberlain* and S.J. Bolanowski.
Institute for Sensory Research and Dept. of Bioengineering, Syracuse Univ., Syr., NY 13244.

It is speculated that the sites of mechanotransduction within PCs are the cytoplasmic extensions (E) emanating along the major axis of the neurite which is elliptical in cross section. For example, we have proposed the existence of two groups of E responding in phase opposition. Furthermore, because transduction is an active process the presence of mitochondria (M) at these sites is required. To explore the possibility that there are two groups of E and to assess the organization of M, analyses were performed on 22 PCs. Regarding the E (n=137), four parameters were measured: height (H) of projection from the neurite (mean, 1.68 μm, s.d., 1.30 μm); length (L) parallel to the neurite (mean, 0.52 μm; s.d., 0.29 μm); width at their base (BW; mean, 1.49 μm; s.d., 1.20 μm) and widest width (WW; mean, 0.97 μm; s.d. 0.61 μm). The distributions relating the number of E to either H, BW or WW are unimodal but skewed to lower values and for L is bimodal (0.32 μm and 0.64 μm) suggesting a basis for the two E idea. The relationships among the various E dimensions are: $BW = -0.93 + 4.69L$; $WW = 0.06 + 1.53L$; $H = 0.18 \times 10^{0.934WW}$; $H = 0.217 \times 10^{1.4L}$, ; $H = -0.46 + 3.06BW - 1.7BW^2 + 0.37BW^3$. Analysis of the M shows that they are grouped close to the neural membrane. The relation between number of M and polar-coordinate degree values is bimodal with the M number greatest along the major elliptical axis consistent with the location of the E, although they are also present, but to a much smaller degree, around the entire perimeter of the neurite. Supported by NIH grant DC00380.

349.3

INTENSITY AND FREQUENCY CHARACTERISTICS OF FIBERS INNERVATING THE CAT HINDPAW. K. Grobaski, C.M. Checkosky* and S.J. Bolanowski.
Institute for Sensory Research and Dept. of Bioengineering, Syracuse Univ., Syracuse, NY 13244.

There have been conflicting reports in the literature regarding whether the neural innervation of the tactile pad of the cat's hindpaw is a suitable model for human glabrous skin. For example, it has been argued that the cat possesses only three physiologically defined fiber types, Pacinian corpuscle (PC), rapidly adapting (RA), and slowly adapting (SA) fibers, the SA category not divisible into SAI and SAII subgroups as is found for humans. To help clarify the issue, frequency (FC) and intensity characteristics (IC) of single units (n > 116) in the medial plantar nerve were obtained in response to bursts of sinusoidal vibrations. Additionally, adaptational properties and receptive-field (RF) organization were ascertained using punctate (von Frey) stimuli. The results that were obtained could be grouped readily into four categories, the categories reminescent of those found for fibers innervating human glabrous skin: a) PC fibers (22.2%) having very large RFs, fast adaptation, high sensitivity, U-shaped FCs in the 40-400 Hz range, phase-locking, and ICs showing plateaus at multiples of the stimulus frequency; b) RA fibers (22.2%) having small RFs, fast adaptation, moderate sensitivity, U-shaped FCs in the 10-150 Hz range, phase locking, and ICs showing plateaus at multiples of the stimulus frequency; c) SA I fibers (20.0%) having small RF, slow adaptation, moderate sensitivity, positive sloping FCs in the 10-150 Hz range, phase locking, and ICs also showing plateaus and d) SA II fibers (35.6%) having large RFs, slow adaptation, low sensitivity, negative sloping FCs in the 20-300 Hz range, little phase locking, ICs without plateaus and characteristic, low-rate, spontaneous activity. Supported by NIH grants DC00380 and DC00098.

349.4

MODALITY-BASED CLASSIFICATION OF THE RAT CUTANEOUS RECEPTORS BY MULTIVARIATE ANALYSIS. J.W. Leem*, W.D. Willis and J.M. Chung. Marine Biomed. Inst., Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX, 77555.

A series of natural stimuli that elicit characteristic responses for well-known types of cutaneous receptors in the rat has been defined in our laboratory. The aim of this study is to classify cutaneous receptors into functionally meaningful groups on the basis of response similarity using multivariate statistical procedures.

A total of 188 cutaneous afferent units was identified as belonging to one of 9 receptor types in the foot skin of 62 anesthetized rats. Responses of units to 11 predefined stimuli were examined using several multivariate statistical analyses.

More than 90% of total sample subjected to discriminant (93.1%) or cluster analysis (90.4%) of their responses to 11 stimuli were correctly categorized into their own receptor types. The test for the similarities among 11 stimuli using factor and cluster analyses revealed that 6 of them were distinct stimuli. Cluster analysis of responses of the same sample to those 6 stimuli yielded 6 groups, each of which contained members believed to contribute to a particular sensation.

The results indicate that cutaneous receptors can be divided into modality-specific groups according to the similarities of their responses to 6 stimuli by several multivariate analyses. (Supported by NIH grants NS21266, NS11255 and NS09743 and a grant from Bristol-Myers Squibb Co.)

349.5

DEVELOPMENT OF A SILICON MICROPROBE FOR CONTROLLED MECHANICAL STIMULATION OF SENSORY NERVE TERMINALS.

B. J. Kane, G. Kovacs, C. Storment, and D. L. Tanelian*. Departments of Electrical Engineering and Anesthesia, AEL 132, Stanford University, Stanford, CA 94305.

One of the factors limiting our understanding of mechanical transduction is the lack of means with which to apply precisely controlled mechanical stimuli to individual nerve terminals. The development of a device capable of this has been impeded by the small scale required in both the delivered force and the application area Such a probe must be small enough to activate single mechanosensitive nerve endings under visual guidance and measure the delivered force. In order to solve this problem silicon integrated circuit technology was used to produce miniature mechanical probes with passive force sensing circuitry on board. These probes are comprised of a cantilever silicon beam with a strain sensing piezoresistive element at the base to measure the strain as the beam deflects. Resistive bridge circuitry allows for temperature compensation of the probes. The probe fabrication process begins with a selective diffusion of boron into a silicon wafer to define the gross shape of the probe. This doped region is etched selectively in the final processing step to remove the probes from the bulk silicon wafer. Following the doping, an insulating silicon dioxide layer is grown over the boron doped regions and the thin-film piezoresistive material is then deposited and etched selectively to define the shapes of the strain sensing elements. Thin-film gold interconnects are then fabricated and the entire wafer is coated with a layer of insulating silicon nitride through which electrical contacts are etched. When etched free of the substrate the probes exhibit a proportional relationship between force applied at the tip and voltage output. These probes will eventually be mounted on an electromagnetic actuator to provide closed-loop feedback control of force and force application rate. Potential exists for these probes to be used in the study of mechanoelectric transduction in sensory nerves and other mechanosensory cells. Supported by the Stanford OTL Research Incentive Fund and NIH grant R01 NS28646-01A.

349.6

FINITE ELEMENT MODELLING OF MECHANORECEPTIVE TERMINAL DEFORMATION IN GLABROUS SKIN.

Z.Gao and J.R.Phillips* University Laboratory of Physiology, Parks Road, Oxford. OX1 3PT. U.K.

We have used the Finite Element (FE) method to investigate which stress/strain components in the skin are transduced by the different classes of mechanoreceptive terminals when the glabrous skin is stimulated. The FE skin model takes into account the surface ridges, the interdigitation of the dermal-epidermal border and the different mechanical properties of the epidermis, dermis and subcutaneous tissues. The behaviour of the model is examined under a variety of conditions and material properties (e.g. laterally moving and stationary stimuli; homogeneous elastic and viscoelastic media and layers of dissimilar materials). When the epidermis and dermis are modelled as one homogeneous material the predictions of the FE model corresponded to those of a simple continuum model. However, when the epidermal material is made much stiffer than that of the dermis (as is the case for real skin), the horizontal strain at locations where Meissner's corpuscles are found is much greater than that predicted by the homogeneous model under the same loading conditions. This observation provides quantitative support to the idea that the dermal/epidermal interdigitations act to amplify the distortion of mechanoreceptive terminals when the skin is stimulated. (Supported by Wellcome Trust grant 017733/1.27)

349.7

TACTILE DISCRIMINATION AND IDENTIFICATION OF THE SHAPES AND ORIENTATIONS OF ELLIPSOIDAL OBJECTS R.H. LaMotte, M.A. Srinivasan* and A. Klusch-Petersen, Dept. of Anesthesiology, Yale University School of Medicine, New Haven, CT 06510 and Research Laboratory of Electronics, MIT, Cambridge, MA 02139.

The shape of an object is physically defined by the principal curvatures and their orientations at each point on its surface. The magnitude and/or rate of change in the curvature of the skin brought in contact with the object is then represented in the discharge rates of certain cutaneous mechanoreceptors (Srinivasan and LaMotte, Wenner-Gren Intl. Symp. Series, Vol.57, Ch.5, 1991). As part of ongoing studies of the neural coding of tactually perceived shape, we measured the capacities of humans (1) to detect deviations from sphericity of ellipsoidal objects applied to the fingerpad and (2) to discriminate or identify differences in the orientation of the major axes of ellipsoids or a cylindrical bar, relative to the axis of the finger and/or the direction of stroking the object over the skin. Each object had a radius of 5 mm along one axis, and for the ellipsoids, radius along the orthogonal axis ranged from 4.75 to 1 mm. A servo-controlled stimulator with 4 degrees of freedom rotated the object to a desired orientation in the horizontal plane and then vertically indented the stationary fingerpad to a maintained force of 40 gwt. Either the contact center was fixed, or the object was stroked along a linear or circular trajectory over the skin.

The subjects detected deviations in sphericity on the order of 1mm in radius and, for the cylinder, discriminated differences in orientation of 5-10° and identified about 6 categories of orientation in 30° steps. Discrimination and identification of orientations deteriorated as the deviations from sphericity reduced. Results were interpreted in relation to the known sensitivities of rapidly and slowly adapting mechanoreceptive nerve fibers to changes in curvature of the skin brought about by contact with objects of different shapes. Supported by ONR Contract N00014-91-J-1566 and PHS grant NS 15888.

349.8

REPRESENTATION OF TEXTURE PATTERNS ON THE SKIN BY POPULATIONS OF CUTANEOUS AFFERENTS. *E.P. Gardner and D. Gardner** Depts. of Physiology and Biophysics, NYU Medical Center, and Cornell University Medical College, New York, NY

Spatial event graphs (SEGs) have been reconstructed from peripheral nerve responses to regular two-dimensional dot patterns scanned across the hand using an OPTACON stimulator. Textures of three spatial periods (4, 6 and 8 mm) and seven phase angles (0-135°) were evaluated. While each texture evokes a stereotyped SEG, the SEGs of individual afferents show varying degrees of overlap depending upon the relative positions of their receptive field centers. The firing patterns of a pair of cutaneous afferents are perfectly correlated when the spatial period and phase of the dot pattern precisely match the distance vector. Correlation of firing is reduced at other dot spacings. Thus Neuron A may be perfectly correlated with Neuron B for one pattern, and correlated only 60% of the time for another pattern, while correlations with Neuron C may measure 25% and 75% respectively. A correlation matrix relating the firing of a reference afferent, located near the center of the tactile array, with all other pairs of stimulated afferents was computed for each of the patterns, allowing us to relate synchrony of firing to stimulation parameters. We propose that the neural mechanisms underlying texture perception may involve unique combinations of simultaneously activated afferents. Textures could be represented by the correlation of firing of specific groups of cutaneous afferents as reflected in the total spike output of central neurons, rather than by isomorphic representation of the timing of stimulation of unique skin locations. *(Supported by NIH Research Grant NS11862 from NINDS).*

349.9

DISCRIMINATION OF PERIODIC TEXTURE PATTERNS ON THE HUMAN HAND. *C.E. Kops and E.P. Gardner** Dept. of Physiology and Biophysics, NYU Medical Center, New York, NY 10016

In order to evaluate a new theory of texture perception based upon the correlation of firing among individual afferents in the median nerve, we measured the ability of 15 human subjects to discriminate periodic dot patterns scanned across the tactile array of an OPTACON stimulator. Patterns differed in spatial period (4, 6 and 8 mm) and phase (0-135°), and were alternated twice in a two-alternative forced-choice protocol. Subjects were asked to indicate whether a given pair of patterns felt the same or different, using a computer keyboard to signal responses. Accuracy of performance in the discrimination task appears directly related to differences in the correlation matrix computed from afferent firing patterns recorded in monkeys. Subjects were unable to discriminate patterns of 4 mm spatial period which differed in phase, except for vertical patterns in which alternate columns on the tactile array were silent. Discrimination of phase at wider spatial periods improved as the dot spacing increased, with the most accurate performance occuring at 8 mm spacing. Patterns whose relative phase angle differed by < 45° were more poorly differentiated than those of greater angular disparity. Stimuli of the same phase but different spatial frequencies were less accurately discriminated than patterns differing in both phase and spatial frequency, except at the narrowest spacing where none of the patterns could be differentiated. These data support our hypothesis that the neural mechanisms underlying texture perception may involve unique combinations of simultaneously activated afferents. *(Supported by NIH Research Grant NS11862 from NINDS).*

349.10

SEROTONIN BIPHASICALLY ACTIVATES AND THEN INHIBITS TYPE I AFFERENT UNITS SERVING TOUCH DOMES *IN VITRO*. L. He, P.R. Burgess, R.P. Tuckett* and K.B. English. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, Utah, 84108

A skin/nerve preparation was utilized for in vitro study of the effects of serotonin (5HT) and its antagonists on single type I unit afferent discharge. Type I afferents terminate upon Merkel cells in the basal epidermis, and in rats both Merkel cells and type I nerves are immunopositive for serotonin.

Back skin (4 cm^2) from adult rats (n=15) was excised along with its accompanying dorsal cutaneous nerve and artery, and pinned epidermal side up to a silgar-coated perfusion chamber. The afferent nerve and artery were threaded through a hole into an adjacent oil-filled chamber for nerve recording and drug administration. Branches of rat dorsal cutaneous nerves are small, enabling discharge of single type I units to be monitored with whole nerve recording. Type I responses (n=17) were studied during controlled mechanical stimulation (indentation and retraction velocity = 8.3 μm/sec; plateau amplitude = 150 μm, 42 sec duration) of touch domes before, during and after 5HT (5, 50, 500 μM dissolved in perfusion fluid plus 0.1% hydralazine). Unit responses (n=17) to 5HT were also compared to controls (n=14) receiving drip infusion of perfusion fluid plus 0.1% hydralazine.

Mechanically evoked activity of seventy percent of the units tested was enhanced and subsequently inhibited upon repeated stimulation following 5HT. However, a majority (84%) of control units showed decreased responsiveness with repeated stimulation. In addition, ketanserin, a $5HT_2$ antagonist, inhibited mechanically initiated activity in 67% of the tested units (n=10) which subsequently recovered to pre-treatment responsiveness. These results suggest 5HT may be a neuromodulator or transmitter in the Merkel cell/neurite complex. Supported by NIH grant 07938.

349.11

SENSORY EFFECTS OF ELECTRICAL STIMULATION DURING PARTIAL NERVE BLOCK BY LOCAL ANESTHETICS IN HUMANS. F.A. Popitz-Bergez*, S.A.Raymond, J.G. Thalhammer. Anesthesia Research Laboratory, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115

When single cutaneous afferent axons in the rat are exposed to local anesthetics (LA) at concentrations which slow but do not block impulse conduction, electrical stimulation (ES) can produce conduction block. We are now studying the possible sensory correlate of this effect in human volunteers by applying ES (50-100 Hz just above sensory threshold for perception) during recovery from LA block of the superficial radial nerve via skin electrodes distal to the block.

Four volunteers, 20-48 yrs, were injected with 1.5 cc of 1% lidocaine at the wrist and a 2x2 cm skin area in the distribution of the blocked nerve was delineated on the injected and control hands for sensory testing. The subjects were asked to report the sensations evoked by repeated stimuli: touch (end of a smooth cotton swab), cold (steel rod at 0°C for 10 s), pinprick (25G spinal stylet), noxious heat (steel rod at 50°C for 10 s). Directional sensitivity and the capability to perceive the spatial extent of an area bounded by strokes on the skin were tested by outlining the square on the skin and "drawing" 2 cm long lines within the square using the swab.

Several observations were reported by all subjects in 6 trials: During block resolution: 1. When touch could be detected reliably on both hands, subjects could not identify the direction of stroking on the blocked side. This effect was accentuated by ES. 2. Cold sensation waxed (to even painful levels) and waned during some of the 10 s tests. It was abolished within a 1-2 s by ES, but recovered slowly over ~1 min after ES was switched off. 3. Cold was usually the last modality to become detectable during recovery, and its block always outlasted the block of pain. Without LA: ES alone reduced aftersensations evoked by cold and pinprick, and diminished the "brightness" of sensations, but did not prevent correct recognition of modality. We hypothesize 1) that the disruption of impulse discharge patterns by LA does not confuse modality recognition by subjects but does reduce sensory discrimination and 2) that ES potentiates LA blockade of sensation.

349.12

TACTILE AFFERENT FUNCTION AFTER HUMAN CERVICAL SPINAL CORD INJURY. C. K. Thomas* and G. Westling. The Miami Project to Cure Paralysis, Univ. of Miami Sch. of Med., Miami, FL 33136.

The function of tactile afferent units innervating the glabrous skin of the hand was examined in 11 individuals who had impaired or absent touch perception due to chronic (>1 year post injury) cervical (C3-C6) spinal cord injury. Such information is fundamental if the intent is to use signals from these natural sensors to control the electrical stimulation of paralyzed muscles[1]. Activity of single tactile fibers in the median nerve was recorded using microneurography[2]. Measurements were made of: 1) how the afferent response adapted (fast or slow; FA or SA) to maintained indentation; 2) the force required to activate the receptor mechanically (mechanical threshold); 3) the size of the receptive field using the von Frey hair which provided 4 or 5 times the threshold force [type I (FAI, SAI) and type II (FAII, SAII) units have small and large fields respectively]; 4) the conduction delay when the receptor was stimulated mechanically, then electrically. The proportion of FAI, FAII, SAI and SAII units sampled (33%, 24%, 32% 11%; n=66) and single afferent electrical conduction velocity were similar to control data[3,4]. There was also a significant delay in the afferent response when the receptive field was stimulated mechanically, as expected from control data[5]. However, mechanical threshold was higher for the FAI units and receptive field size was greater for the FAII units compared to control data[6,7]. Thus, tactile afferent signals may be useful to regulate force in paralyzed muscles during manipulation provided the information from different receptor types can be extracted and processed selectively.

[1]Hoffer and Haugland, In: Neural Protheses:Replacing motor function after disease or disability, Oxford University Press p99, 1992; [2]Vallbo et al., Physiol. Rev. 59:919, 1979; [3]Johansson and Vallbo, J. Physiol. 286:283, 1979; [4]Johansson and Vallbo, TINS 6:27, 1983; [5]Knibestöl, J. Physiol. 232:427, 1973; [6]Johansson et al., Br. Res. 184:343, 1980; [7]Johansson and Vallbo, Br. Res. 184:353, 1980.

349.13

A GATE THAT SWINGS IN THE OPPOSITE DIRECTION: INHIBITION OF TOUCH BY PAIN. A.V. Apkarian*, R.A. Stea, S. Bolanowski. Dept. Neurosurgery, SUNY Health Science Center, Syracuse, NY 13210 and Institute for Sensory Research, Syracuse University, Syracuse, NY 13244.

Regional blood flow studies show decreased neuronal activity in the somatosensory cortex during nociception in humans (Stea et al. Neurosci Abst 17:293 1991). This implies an alteration of touch perception during pain. The hypothesis, that touch thresholds are elevated during coincident pain, was tested in 10 volunteers in psychophysical studies which employed a tonic thermal stimulus and a vibrotactile stimulus to the right thenar eminence. Thresholds and absolute magnitude estimates of suprathreshold vibrotactile stimuli were assessed with the skin temperature at 31 °C (control), at the pain threshold and above pain threshold (42-47 °C). Vibrotactile thresholds were determined using ascending, descending and two alternative forced choice techniques. Vibrotactile magnitude estimates were done with an open scale. Three frequencies were tested: 1, 10, 100 Hz, these frequencies chosen since changes in skin surface temperature alone do not produce changes in threshold. Changes in auditory threshold (1 kHz) were also measured. Mild to moderate pain resulted in increased vibrotactile thresholds in all but one subject. The mean change was 9.9db at 100Hz, 8.2db at 10Hz and 8.3db at 1Hz. The changes occurred regardless of the method of threshold determination. Magnitude estimate curves during pain increased in slope, and showed recruitment. The threshold change was abrupt and first detected at temperatures just below the subjects' pain threshold. Auditory thresholds were unchanged during painful stimulation of the hand. The touch threshold changes are not attributed to attentional changes since they occurred just below pain threshold (where nociceptors become active) and were not observed in audition. The 2.5 fold increase in touch threshold during pain, indicates the existence of a gate which inhibits touch, perhaps to sharpen the perception of pain. Supported. by NIH Grant DC0098 to SJB.

PAIN PATHWAYS: SUPRASPINAL

350.1

ORGANISATION OF ASCENDING AXONS ORIGINATING FROM THE RAT SPINAL CORD STUDIED WITH THE PHA-L METHOD. R. Dallelt, P. Raboissont, J. F. Bernard, D. Le Bars and L. Villanueva. (SPON: European Neuroscience Association*).
INSERM, U-161, 2, rue d' Alésia, Paris, and †Faculté de Chirurgie Dentaire, 63000, Clermont-Ferrand, (France).

The location of ascending axons originating from different laminae of the cervical spinal cord was examined by using the anterogradely transported lectin Phaseolus Vulgaris Leucoagglutinin (PHA-L) coupled with immunohistochemistry.

Male Sprague-Dawley rats were anesthetised with chloral hydrate and PHA-L (5%) was electrophoretically injected into different laminae of the cervical spinal enlargement. Two weeks later, the rats were perfused transcardially, the spinal cord was removed and cut in serial sections which were reacted with PHA-L antibody and ABC complex. Ascending axons were observed in transverse sections from C1 level and were compared with those observed following large PHA-L injections in the lumbar cord enlargement.

The majority of ascending axons originating from all laminae of the cervical spinal enlargement were located ipsilaterally whereas those axons originating from the lumbar cord presented a widespread distribution, with a relevant contralateral component.
This study confirms previous observations showing marked differences in the organisation of cervical as compared to lumbar spinal ascending somatosensory pathways. Supported by DRET.

350.2

SPINAL AFFERENT PROJECTIONS TO THE SUBNUCLEUS RETICULARIS DORSALIS (SRD) OF THE RAT MEDULLA STUDIED WITH THE PHA-L METHOD. P. Raboissont, A. Mallart*, R. Dallelt, J. F. Bernard, D. Le Bars and L. Villanueva INSERM, U-161, 2, rue d' Alésia, Paris, and †Faculté de Chirurgie Dentaire, 63000, Clermont-Ferrand, (France).

SRD is a caudal area within the brainstem that processes specifically cutaneous and visceral nociceptive information from the whole body. Their neurones are exclusively activated by Aδ- and C-fibre inputs and encode cutaneous and visceral stimuli within the noxious range. We examined the spinal projections to the SRD following injections of Phaseolus Vulgaris Leucoagglutinin (PHA-L).

Male Sprague-Dawley rats were anesthetised with chloral hydrate and PHA-L (5%) was electrophoretically injected into different laminae of the cervical spinal cord enlargement. Two weeks later, the rats were perfused transcardially, the brain and spinal cord were removed and cut in serial sections which were reacted with PHA-L antibody and ABC complex.

Dense labelled terminals were found in the ipsilateral SRD and covered its rostrocaudal extent. The highest density of labelling was observed following injections in the deeper layers of the spinal cord. These data confirm that part of the spinal efferents to the SRD originate from the ipsilateral cervical cord. Together with our previous electrophysiological and anatomical data, this study suggests that the SRD provides a link in spino-reticulo-spinal loops implicated in the processing of pain. Supported by DRET.

350.3

ORGANIZATION OF THE PARABRACHIAL EFFERENT PROJECTIONS TO THE AMYGDALA: A PHA-L STUDY IN THE RAT. I.F. Bernard*, M. Aldén and J.M. Besson. INSERM U 161, 2 rue d'Alésia, F-75014 PARIS, FRANCE.

Microinjections of *Phaseolus Vulgaris* leucoagglutinin (PHA-L) restricted (n=43) or adjacent (n=20) to subnuclei of the pontine parabrachial area (pPB) and revealed by immunohistochemistry permitted to demonstrate that the pPB projections onto the amygdaloid central nucleus (Ce) were topically organized :

1) The pPB region including the medial (pPBm), the waist area (pPBwa) and a part of the ventral lateral (pPBvl) subnuclei projects primarily to the medial subdivision of the Ce (CeM). Projections were also found in the basolateral posterior (BLP), basomedial posterior (BMP) and anterior cortical (ACo) nuclei of the amygdala.

2) The pPB region including the remaining portion of the pPBvl, the rostral portion of the central lateral subnucleus (pPBcl) and the outer-rostral portion of the external lateral subnucleus (pPBel) projects primarily to the lateral subdivision of the Ce (CeL).

3) The pPB region including the dorsolateral subnucleus (pPBdl), the caudal and the inner-rostral portion of the pPBel and the external medial (pPBem) subnuclei projects primarily to the lateral capsular subdivision of the Ce (CeLC).

It was suggested that the pPB - CeM pathway is implicated in gustative processes; the pPB - CeL pathway is implicated in visceral and chemosensitive processes; the pPB - CeLC pathway is implicated in respiratory, cardiovascular and nociceptives processes.

350.4

EXPRESSION OF FOS-LIKE IMMUNOREACTIVITY AND PREPROENKEPHALIN mRNA IN THE RAT PARABRACHIAL NUCLEUS AFTER NOCICEPTIVE MECHANICAL STIMULATION OF THE SKIN. O. Hermanson*, H. Ericson, D. Larhammar and A. Blomqvist. Dept of Cell Biology, Univ. of Linköping, Dept of Neuropharmacol., Astra Research Centre, Södertälje, and Dept of Medical Genetics, Univ. of Uppsala, Sweden.

Previous anatomical studies have demonstrated that the parabrachial nucleus (PBN) is a major target for ascending fibers from the superficial lamina (lamina I) of the spinal and medullary dorsal horns. PBN neurons are the source of several peptidergic projections to the rostral brain stem and forebrain. In this study the expression of c-FOS-immunoreactivity and preproenkephalin A-mRNA (ppENK) was examined in PBN neurons after cutaneous nociceptive stimulation. Male Sprague-Dawley rats were pinched in the nape of the neck or the base of the tail. Another group of rats served as controls. Frozen sections were incubated with an antibody against FOS-protein or hybridized with a ^{35}S-labeled cRNA probe to ppENK. Neurons expressing moderate to heavy ppENK-labeling were most abundant in the internal lateral, the central lateral and part of the external lateral parabrachial subnuclei. Cutaneous nociceptive stimulation resulted in FOS-like immunoreactivity in neurons within the terminal field of the spino-parabrachial projection. Quantitative data from the *in situ* hybridization, obtained by analysis of the optical density on X-ray films, suggested that the expression of ppENK in PBN neurons is influenced by nociceptive stimulation.

350.5

"DEEP" AND "SUPERFICIAL" NOCICEPTIVE STIMULATION EVOKE DIFFERENT BEHAVIORS AND INCREASE FOS-LIKE IMMUNOREACTIVITY IN DISTINCT REGIONS OF SPINAL CORD AND MIDBRAIN PERIAQUEDUCTAL GRAY K.A. Keay A. Depaulis† and R. Bandler*. Dept. of Anatomy, University of Sydney, NSW, Australia, 2006. & †LNBC, Centre de Neurochimie, 67084 Strasbourg, France.

We have reported that, the lateral region of the caudal third of the midbrain periaqueductal grey (PAG) which mediates flight and hypertension receives inputs from lamina I and the lateral cervical nucleus [LCN] of the upper cervical spinal cord [UCC]; whereas the ventrolateral PAG region which mediates hypotension and immobility is targeted by cells in laminae VII & VIII. In the UCC, cells of lamina VII &VIII receive a significant afferent input from the deep neck muscles, whereas cells of lamina I and the LCN receive a large input from cutaneous nociceptors. Thus we investigated the hypothesis that nociceptive activation of the deep neck muscles might activate the spino-ventrolateral PAG projection, whereas cutaneous nociceptive stimulation might activate the spino-lateral PAG projection. We used the technique of immunohistochemical detection of Fos protein as a marker of neuronal activation following, in the lightly anesthetised rat, either: i) bilateral injection of 5% formalin into the deep neck muscles; ii) bilateral subcutaneous injection of 5% formalin into the neck; or iii) radiant heat of the neck.The data showed that deep neck muscle injections significantly increased (up to 3x's) the numbers of Fos-like immunoreactive cells in: i) the ventrolateral PAG and ii) laminae VII & VIII of the UCC. In contrast, the "superficial" noxious stimuli increased the numbers of Fos labelled cells in: i) the lateral & to a lesser extent the ventrolateral PAG and ii) in laminae I & the LCN of the UCC. Further experiments with freely moving rats demonstrated that "deep" noxious stimulation of the neck evoked a profound immobility seemingly identical to that evoked by excitatory amino acid [EAA] stimulation of the ventrolateral PAG, whereas "superficial" noxious stimulation of the neck evoked defensive behaviours similar to those evoked by EAA stimulation of the lateral PAG. On balance, these data suggest that deep and superficial noxious stimuli target respectively the functionally distinct ventrolateral and lateral regions of the caudal PAG, which in turn mediate quite distinct patterns of physiological response.

350.6

NOXIOUS HEAT-EVOKED FOS-LIKE IMMUNOREACTIVITY IN THE RAT MEDULLA. S.L. Jones* and R.W. Blair. Departments of Pharmacology and Physiology, University of Oklahoma, Oklahoma City, Oklahoma 73190.

The medulla receives nociceptive information from the spinal cord via multiple ascending pathways. In the present study, the expression of fos-like immunoreactivity was used to determine which populations of neurons in the medulla are activated by noxious heating of the hindpaw. Rats were anesthetized with pentobarbital, and the left hindpaw was immersed in 55-60°C water for 10sec for 30 consecutive trials at 2min intervals. Following a 2hr survival time, the rats were perfused, and the medulla was processed for fos-like immunoreactivity. Compared to control animals (n=5), animals exposed to noxious heat (n=5), demonstrated significantly greater numbers of fos-positive neurons in the lateral reticular nucleus and in the surrounding reticular formation on the side contralateral to the noxious stimulus, but not on the ipsilateral side. No differences in the numbers of fos-positive neurons in animals exposed to noxious heat compared to controls were noted in the: solitary nuclei, dorsal motor nucleus of the vagus, caudal raphe nuclei, spinal trigeminal nuclei and inferior olivary nuclei. Thus, noxious heat-evoked changes in fos-like immunoreactivity can be identified in medullary regions associated with pain, cardiovascular and motor function. (S.L.J. is supported by the Presbyterian Health Fdn. and OCAST and R.W.B. is supported by HL29618).

350.7

ENRICHMENT OF GLUTAMATE-LIKE IMMUNOREACTIVITY IN SPINOTHALAMIC TRACT TERMINALS IN THE NUCLEUS SUBMEDIUS OF THE CAT. A.-C. Ericson*, A. Blomqvist, A.D. Craig, O.P. Ottersen and J. Broman. Dept of Cell Biology, Univ. of Linköping, Sweden, Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ, U.S.A., and Dept of Anatomy, Univ. of Oslo, Norway.

Previous light and electron microscopic studies have demonstrated that the nucleus submedius (Sm) is a specific lamina I spinothalamic tract (STT) termination site in the medial thalamus. The identity of the neurotransmitter(s) in the STT terminals in Sm is unknown. In this study the presence of glutamate-like immunoreactivity in the STT terminals was investigated with an electronmicroscopic immunogold-labeling technique. The STT terminals were identified by anterograde transport of WGA-HRP and subsequent tetramethyl benzidine histochemistry. The density of gold particles over STT terminals was compared with that over peroxidase-negative terminals of the same type as STT terminals (RL terminals), terminals of presumed cortical origin (RS terminals), presynaptic dendrites (PSDs), large neuronal cell bodies, and randomly selected areas in Sm. The density of gold particles over STT terminals, RL terminals and RS terminals was significantly higher than that over PSDs, cell bodies and tissue average. These observations indicate that glutamate may serve as a neurotransmitter for nociceptive and thermoreceptive information between STT terminals and Sm neurons.

350.8

SPINOTHALAMIC TRACT TERMINALS IN THE CAT'S NUCLEUS SUBMEDIUS ARE PRESYNAPTIC TO GABA-IMMUNOREACTIVE LOCAL CIRCUIT NEURONS. A. Blomqvist*, A.-C. Ericson, J. Broman, and A.D. Craig. Dept of Cell Biology, Univ. of Linköping, Sweden, and Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ, U.S.A.

This study examines the synaptic relationships in the nucleus submedius (Sm) between spinothalamic tract terminals and GABA-immunoreactive profiles. Spinothalamic tract terminals were labeled with anterograde transport of WGA-HRP and subsequent tetramethylbenzidine histochemistry. GABA-immunoreactive profiles were identified in the electron microscope with an immunogold-labeling technique. GABA-like immunoreactivity was present in small neurons, presynaptic dendrites (PSDs), and F-type terminals. The spinothalamic tract terminals in nucleus submedius were presynaptic to GABA-negative dendrites that presumably originate from thalamocortical relay cells and to GABA-immunoreactive PSDs. The PSDs in turn were presynaptic to relay cell dendrites. In addition, the GABA-immunoreactive PSDs were in synaptic contact with other GABA-immuno-reactive PSDs and were postsynaptic to small GABA-negative terminals of presumed cortical origin. When reconstructed in serial sections triadic arrangements typical for thalamic sensory relay nuclei were found between spinothalamic tract terminals, PSDs and relay cell dendrites. These results indicate that processing of spinothalamic tract input to Sm involves inhibitory circuits through GABAergic interneurons.

350.9

ORGANIZATION OF SPINOHYPOTHALAMIC TRACT AXONS IN THE DIENCEPHALON OF THE RAT. Rami Burstein*, A. Strassman and D. Borsook. Pain Physiology Lab., Dept. of Neurol., Mass. General Hosp. and Dept. of Neurobiol. Harvard Med. Sch., Boston, MA 02114.

Recently, Burstein, Giesler, and colleagues described direct nociceptive projections from the spinal cord to the hypothalamus. These studies indicated that spinohypothalamic tract (SHT) axons could reach the hypothalamus by a lateral route (through the lateral diencephalon) or by a medial route (by crossing the midline within the hypothalamus through the supraoptic decussation). In order to define more clearly the anatomical organization of the SHT, we quantitatively examined the effects of selective lesions of either the lateral or medial SHT projection on the distribution of retrogradely labelled neurons in the spinal cord.

Restricted electrolytic lesions were made in the area of either the medial projection, the lateral projection, or both; control animals received a lesion dorsal to the diencephalon. The retrograde tracer Fluoro-Gold (FG) was then injected unilaterally into the hypothalamus. Our findings indicate that of the 2722 SHT neurons counted in the spinal cord about 57% project to the hypothalamus through the lateral projection only, 27% project through the medial projection only, and 10% project through both pathways. Approximately 6% were not eliminated by lesions of both pathways, and thus may reach the hypothalamus through other routes. Neurons that project through the lateral route were present mainly contralateral (73%) to the injection site. Neurons that project through the medial route were found mostly ipsilateral (62%) to the injection site. The presence of ipsilaterally located cells following the medial lesion, and contralaterally located neurons following the lateral lesion suggest that about 25% of SHT neurons ascend in the ipsilateral spinal cord.

These findings indicate the existence of 2 major diencephalic pathways in which spinal cord neurons project to the hypothalamus. This information may be useful for future ablation studies attempting to examine the role of the SHT in pain.

350.10

CHARACTERIZATION OF RESPONSES OF NEURONS IN THE VPL TO NOXIOUS COLORECTAL DISTENSION (CRD) IN THE RAT. R.M. Danzebrink* and G.F. Gebhart. The Department of Pharmacology, The University of Iowa, Iowa City, Iowa, 52242, USA.

One hundred twenty four neurons in the ventral posterolateral nucleus (VPL) of the thalamus were characterized for their responses to noxious CRD (80 mmHg, 20s) and to non-noxious and noxious cutaneous stimulation in pentobarbital anesthetized rats using standard extracellular recording techniques. Thirty three/124 neurons responded exclusively to non-noxious (brush, tap) and/or noxious (pinch) cutaneous stimulation. Ninety two of the total 124 neurons studied responded to noxious CRD and 82 of these neurons received convergent cutaneous input. Neurons were either excited (n=70) or inhibited (n=22) by noxious CRD and were distributed throughout the VPL. Thirty eight/82 neurons responded reproducibly and reliably to noxious CRD for 20-64 minutes. Eight/10 of these neurons tested responded in an intensity-dependent manner, suggesting that VPL neurons can encode the intensity of CRD. Thirty five of 55 neurons which responded to CRD also responded to distension of the esophagus, a presumed noxious stimulus (Meller and Gebhart, 1991), and these neurons were distributed throughout the VPL. These data suggest that neurons in the VPL receive convergent visceral and convergent cutaneous inputs and that they may be involved in sensory discriminative aspects of visceral nociception.

350.11

PAIN EVOKED BY MICROSTIMULATION IN THE THALAMUS OF CHRONIC PAIN PATIENTS. J.O. Dostrovsky*, K.D. Davis, F.E.B. Wells and R.R. Tasker, Dept of Physiology and Division of Neurosurgery, University of Toronto, Toronto, Ontario, Canada M5S 1A8

The role of the human thalamic ventrobasal complex (VB, or ventrocaudal nucleus) in nociception is puzzling since reports of nociceptive neuronal responses and stimulation-evoked pain are rare in the non-pain patient. In this study we investigated the incidence of painful sensations evoked by thalamic microstimulation in patients with chronic pain due to deafferentation or central lesions.

Data were obtained during stereotactic procedures for implantation of chronic deep brain stimulating electrodes. Tungsten microelectrodes were used to record neuronal responses and to deliver stimuli. Localization of VB was determined according to stereotactic coordinates and neuronal responses to innocuous somatic stimuli. At selected sites within VB, microstimulation (1s trains, 300Hz, 0.1-0.2ms pulses, $<100\mu A$) was performed and the patient was requested to describe the quality of the sensation and its peripheral location (projected field).

Microstimulation commonly evoked paraesthesia-type sensations. However, the majority of pain patients also reported painful sensations at stimulation sites in (and below) VB in more than half of the trajectories. These painful sensations were often described as 'sharp','shocking','burning' or 'unpleasant'. All patients were capable of differentiating paraesthesia from pain. There was often a mismatch between the location of the projected painful sensation and the receptive fields of the tactile neurons recorded at the same site. The incidence of the projected/receptive field mismatch and painful evoked sensations was unusually high when compared to patients with 'normal' pain sensibility. These results provide further support for previous hypotheses suggesting that alterations in the processing of nociceptive information in thalamocortical pathways occur in chronic pain conditions (supported by MRC of Canada)

350.12

AFFERENTS CONNECTIONS OF THE AMYGDALOID COMPLEX (AC) OF THE RAT. COMPARISON OF TWO RETROGRADE TRACERS, FLUOROGOLD (FG) AND PSEUDORABIES VIRUS (PRV). L. Jasmin*, H. Liang, K. Tarczy-Hornoch, J.P. Card# and A.I. Basbaum. Depts of Anatomy, Physiology and Neurosurgery, UCSF, CA and #Dupont-Merck, Wilmington, DE.

We are studying the cells of origin of the nociceptive inputs to the AC of the rat with the transneuronal tracer, PRV. Distinguishing directly- from indirectly-labelled PRV-positive cell groups, however, requires a careful comparison with labelling produced by a sensitive tracer that is *not* transneuronally transported. To this end, we made stereotaxic injections of FG or PRV into the AC. Retrogradely-labelled FG and PRV cells were identified immunocytochemically with the ABC method.

We confirmed the previously reported afferents to the AC with FG, including those few from the spinal cord. We also found retrogradely-labelled cells in locations that, to our knowledge, have not been described, including the *thalamus:* centrolateral, intermediodorsal, paracentral, posterior intralaminar, precommissural; *hypothalamus*: dorsomedial, tuberomammillary, dorsal hypothalamic area, supraoptic tract area, tuber cinereum; *pons*: raphe pontis; *medulla*: reticularis gigantocellularis, subnucleus reticularis dorsalis, lateral reticular and the A1 region.

Presumed transneuronally-labeled cells (PRV only) were not found in the *thalamus*. In the *hypothalamus*, far more PRV- than FG-labelled cells were recorded in the regions listed above. The same was true for the parabrachial complex of the pons. These results suggest that transneuronal labelling to local circuit neurons had occurred. PRV-only labelled cells were also recorded in *hypothalamus*: magnocellular, suprachiasmatic, subincertal, paraventricular; pons: subcoeruleus and some areas of the reticular formation; *medulla*: A5 region, area postrema, dorsal paragigantocellularis, gigantocellularis pars alpha, paratrigeminal, trigeminal pars caudalis, supragenual; *spinal cord*: laminae I and II. These data indicate that PRV reveals a remarkably diverse source of connections with the AC. Because of the great sensitivity of PRV, due to its replication in neurons, the possibility that some of the presumed transneuronally-labelled cells were directly-labelled must be considered.

Supported by the MRC (Canada) and NS14627 and NS21445.

350.13

COMPLICATIONS FOLLOWING CO-INJECTION OF WHEAT GERM AGGLUTININ-HRP (WGA-HRP) AND CHOLERAGENOID-HRP (B-HRP) INTO THE SCIATIC NERVE OF THE RAT. H. Liu, I.J. Llewellyn-Smith# and A.I. Basbaum*, Depts. of Anatomy, Physiology and Keck Center for Integrative Neuroscience, UCSF, San Francisco, CA and #Center for Neuroscience, Flinders University, Bedford Park, South Australia.

Peripheral nerve injection of WGA-HRP or B-HRP respectively results in transganglionic labelling of small and large diameter primary afferent terminations in the spinal cord (Robertson and Grant, '85; LaMotte et al, '91). In this study, we report that after combined injections of the tracers into the sciatic nerve of the rat, there is an almost complete loss of transganglionic labelling in the spinal cord.

In some rats one of the tracers (either 1.5µl 5% WGA-HRP or 1.5µl 1% B-HRP in 0.1 phosphate buffer and 5% DMSO) was injected into the sciatic on one side and a combination of the tracers into the opposite sciatic. After 48-72 hours the rats were perfused with 0.5% para and 2.5% glut and frozen sections of lumbar dorsal root ganglia (DRG) and cord were reacted with a TMB-tungstate procedure. Injections of WGA-HRP or B-HRP alone produced the expected pattern of labelling in DRG and spinal cord. After co-injection of the tracers we only detected a few small diameter DRG cells and some terminal labelling in laminae I and II; this was much less than with WGA-HRP alone. Only limited retrograde labelling of motoneuron somata was detected. In other rats either WGA-HRP or B-HRP was injected and 24 hours later the other tracer was injected, about 5mm proximal to the first. The rats were perfused at least 48 hours after injection of the second tracer. The labelling pattern in the DRG and cord was characteristic of the *first* tracer injected, both in location and density.

Since the mutual inhibitory interactions were observed at the level of the DRG as well as the cord, we cannot determine whether the problem lies in transport or in uptake of the tracers. The fact that reduced labelling was observed even when the injections were separated temporally and spatially suggests that the underlying mechanism is more complicated than mere creation of a WGA-HRP/B-HRP complex that cannot be taken up and transported. Supported by: PHS grants DA/NIDA, NS21445, NS14627.

350.14

PET ANALYSIS OF BRAIN STRUCTURES DIFFERENTIALLY ACTIVATED BY NOXIOUS THERMAL STIMULI. K.L.Casey[1,2,4]*,S.Minoshima[3],K.L.Berger[3],R.A.Koeppe[3],T.J.Morrow[1,2,4] and K.A.Frey[1,3],Depts.ofNeurology[1],Physiology[2],and Division of Nuclear Medicine[3],University of Michigan and Neurology Research Laboratories,VAMC,Ann Arbor,Mi.48105

We identified regional increases in cerebral blood flow(CBF) that are differentially produced by noxious thermal stimulation of the skin.Nine healthy subjects (20 to 39 yrs) gave magnitude estimations of 5 sec thermal pulses applied with a contact thermode to the left forearm(resting temperature 31.8 +/-1.0^{o}C.). Stimuli of 40^{o}C were rated as warm (2.1+/-1.0 s.d.) and stimuli of 50^{o}C as painful (8.9+/-0.9 s.d.)on a 0-10 scale with 7.0 as pain threshold. These stimuli were applied repetitively to 6 sites while positron emission tomographic(PET) scans were performed after the intravenous bolus injection of $H_2{}^{15}O$ (66mCi).Three scans of each subject were acquired during 40^{o}C and 3 during 50^{o}C stimulation. Subtraction images were made and oriented with a stereotaxic atlas to reveal the brain structures differentially activated by 50^{o}C stimuli. Guided by a priori hypotheses, the following structures were sampled at points of maximum difference over 6 ml volumes of interest and showed significant percentage increases in CBF (p=0.01(**),or p=0.05(*) by one-tailed t-test): **Contralateral thalamus,3.0%**; contralateral (but not ipsilateral) S2-insular cortex,2.9%**; ipsilateral thalamus,2.3%*; anterior cingulate gyrus,2.1%**; pontomesencephalic junction,1.9%*; and contralateral (but not ipsilateral) S1 cortex,1.6%*.** The cerebellar vermis showed a 3.1% increase, but was not included in the a priori hypotheses. These results emphasize the role of subcortical and limbic structures in pain.(Supported by Dept.of V.A. and a Bristol-Myers Squibb award).

350.15

THERMORECEPTIVE NEURONS AND BEHAVIORAL OUTCOME OF INJURY IN AREA 7B CORTEX OF MONKEYS. W.K.Dong*, V.J.Roberts, T.Hayashi, B.M.Fusco, and E.H.Chudler. Departments of Anesthesiology and Psychology and Multidisciplinary Pain Center, University of Washington School of Medicine, Seattle, WA 98195.

Two subgroups of thermoreceptive neurons have been identified in area 7b cortex (inferior parietal lobule) of monkeys during performance of an appetitive tolerance-escape task. Monkeys were allowed to initiate and terminate thermal stimulation of the face and were reinforced for completing trials that sometimes required tolerance of noxious temperatures (>44°-45° C). Reward was delivered after responding to an audiovisual cue. Subgroup 1: Low threshold, "warm" neurons had positively accelerating, non-linear stimulus-response functions when skin temperatures were increased from 32°C up to 43°-44°C. The S-R curves were steepest for final plateau temperatures between 38° and 44°C. Subgroup 2: High threshold, nociceptive-specific neurons crudely graded temperatures from 44°-48°C and encoded the entire duration of plateau temperatures without discharge adaptation. Pain tolerance thresholds were within the temperature range (46°-48°C) that elicited maximal discharge frequencies. Chronic focal compression of area 7 eliminated behavioral escape to all noxious temperatures including 51°-52°C. Ability to detect variable offset of thermal shifts in lieu of audiovisual cues for reinforcement indicates that thermosensitivity remained intact. Pain asymbolia has also been reported in man after posterior parietal cortex injury. Supported by NIH grants NS29459 and NS07217.

350.16

RESPONSES OF VENTROLATERAL ORBITAL CORTEX NEURONS TO NOXIOUS VISCERAL STIMULATION IN THE RAT. K.A. Follett* and B. Dirks. Division of Neurosurgery, University of Iowa Hospitals, Iowa City, IA 52242.

Cortical mechanisms of visceral nociception have not been well-studied. This study was undertaken to determine if neurons in ventrolateral orbital cortex (VLO) respond to a physiologic noxious visceral stimulus (colorectal distension, CRD).

In pentobarbital-anesthetized rats, spontaneously active single neurons were isolated in VLO using standard microelectrode recording techniques. Their responses to graded balloon distension of the colon (40-80 mm Hg) were recorded. Cutaneous receptive fields were studied in these same neurons.

Twenty-nine neurons were isolated. Twenty-one responded to CRD. Nineteen of the 21 (90 %) were inhibited by CRD and 2 (10 %) were excited. Thresholds were as low as 40 mm Hg. Large, bilateral cutaneous receptive fields were identified in 14 of the 21 neurons (67%).

We have demonstrated that VLO neurons respond to a physiologic noxious visceral stimulus in a rat model. The nearly uniform (inhibitory) responses and wide cutaneous receptive fields are distinct from the variable CRD responses and discrete, contralateral cutaneous receptive fields of neurons we have studied previously in primary somatosensory cortex.

PAIN MODULATION: SUPRASPINAL

351.1

SUPRASPINAL MORPHINE ANALGESIA: SYNERGY BETWEEN MESENCEPHALIC, PONTINE AND MEDULLARY SITES IN RATS. G.C. Rossi*, G.W.Pasternak, J.M.Kiefel and R.J.Bodnar. Dept. of Psychology, Queens Col., CUNY, Flushing, NY 11367 and Dept. of Neurology, Memorial Sloan-Kettering Cancer Center, NY, NY 10021.

Spinal and supraspinal opiate systems display multiplicative analgesic interactions following ventricular and intrathecal morphine (JPET 215: 633-642, 1980). Since morphine analgesia can be elicited from the periaqueductal gray (PAG), locus coeruleus (LC) region, nucleus raphe magnus (NRM) and nucleus reticularis gigantocellularis (NRGC), the present study evaluated whether morphine analgesia elicited from pairs of these sites displays synergy on the tail-flick test in rats. A morphine dose-response function was determined over 2 h for each site alone (ED_{50}: PAG: 1.9ug, LC region: 4.3 ug, NRM/NRGC: 2.5 ug). Morphine was simultaneously administered into either the PAG and NRM/NRGC or the LC region and NRM/NRGC such that the sites alternately received either a fixed (1 ug) or variable (0.2-1) dose. Isobolographic analyses revealed that simultaneous morphine administration shifted the dose-response curve to the left (2-5 fold) for PAG-NRM/NRGC placements, and produced lesser, but significant leftward shifts for LC region-NRM/NRGC placements. In keeping with mu_1 mediation of morphine analgesia from these placements (Brain Res. 447: 25-43, 1988), naloxonazine eliminated analgesic synergy between PAG and NRM/NRGC placements.

351.2

SEROTONERGIC AND OPIOID ANTAGONISTS IN THE VENTRAL MEDULLA INHIBIT MESENCEPHALIC MORPHINE ANALGESIA IN RATS. J.M. Kiefel*, M.L. Cooper and R.J. Bodnar. Dept. of Psychology, Queens Col., CUNY, Flushing, NY 11367.

Morphine analgesia elicited from the periaqueductal gray (PAG) is significantly inhibited by methysergide administered into the nucleus raphe magnus (NRM) and nucleus reticularis gigantocellularis (NRGC), indicating serotonergic mediation (Physiol. Behav. 51: 201-205, 1992). Given the existence of multiple serotonin receptor subtypes and an enkephalinergic PAG-NRM/NRGC pathway, the present study evaluated whether the $5HT_3$ antagonist, ICS 205930 (0.25-5 ug), the $5HT_2$ antagonist, ritanserin (0.25 -2.5 ug) and the opioid antagonist, naltrexone (10 ug) in the NRM/NRGC altered PAG morphine (2.5 ug) analgesia. PAG morphine analgesia was significantly inhibited by NRM/NRGC injections of: ICS205930 on the tail-flick (91%) and jump (70%) tests, ritanserin on the tail-flick (76%) and jump (67%), and naltrexone on the tail-flick (94%) and jump (100%) tests. Thus $5HT_2$ and $5HT_3$ receptor subtypes participate in the serotonergic mediation of mesencephalic morphine analgesia in the ventral medulla, and an enkephalinergic pathway is also involved in this supraspinal analgesic system. (Supported by PSC/CUNY 662225).

351.3

SEROTONIN RECEPTOR SUBTYPE ANTAGONISTS AND 2-DEOXY-D-GLUCOSE ANALGESIA: TEST-SPECIFIC EFFECTS IN RATS. M.C. Fisher and R.J. Bodnar*. Dept. of Psychology, Queens Col., CUNY, Flushing, NY 11367.

Multiple serotonin receptor subtypes modulate opiate and opioid-mediated analgesia (e.g., Neurosci. Lett. 95: 313-317, 1988; Brain Res. 500: 231-240, 1989; JPET 256: 983-992, 1991). Since 2-deoxy-D-glucose (2DG) analgesia is mediated by endogenous opioids, the present study examined whether 5HT (methysergide: 5-10 mg/kg, ip), $5HT_2$ (ritanserin: 2.5 mg/kg, sc) or $5HT_3$ (ICS 205930: 0.25-5 mg/kg, sc) antagonists altered 2DG analgesia on the tail-flick and jump tests over a 2 h time course in rats. Serotonergic antagonists exerted test-specific effects without altering nociceptive thresholds. On the tail-flick test, 2DG (450 mg/kg) analgesia was significantly reduced by ICS 205930 (40-47%), but not by methysergide (15-28%) or ritanserin (6%). In contrast, 2DG analgesia on the jump test was significantly potentiated across the time course by ritanserin (67-92%), methysergide (21-64%) and ICS 205930 (26-45%). Thus, whereas the $5HT_3$ antagonist ICS 205930 selectively reduces 2DG analgesia on a spinally-mediated (tail-flick) nociceptive measure, the above serotonergic antagonists, but particularly ritanserin, potentiate 2DG analgesia on a supraspinal (jump) nociceptive test. (Supported by PSC/CUNY 662225).

351.4

AGING,GENDER AND GONADECTOMY EFFECTS UPON MORPHINE ANALGESIA IN RATS. A.K. Islam* and R.J. Bodnar. Dept. of Psychology, Queens Col., CUNY, Flushing, NY 11367.

Morphine analgesia is sensitive to gender and aging differences in that analgesic magnitude is greater in male than in female rats, and is smaller in older (14-24 mo.) than in younger (4-9 mo.) female rats. The present study examined the roles of aging, gender and gonadectomy in morphine analgesia on the tail-flick test. Rats were anesthetized and received sham surgery or gonadectomy at 3 months of age, and tested in separate groups (n=8-10) at either 6,12,18, or 24 mo. of age. Analgesia was assessed at weekly intervals across a dose range (1-10 mg/kg, ip) and a 2 h time course. Whereas intact males and females displayed similar morphine dose-response curves at 6 mo. of age, morphine analgesia was significantly greater in older intact males. Aging increased analgesic sensitivity to morphine in intact males, and decreased sensitivity in intact females. Moreover, castration decreased and ovariectomy increased analgesic sensitivity to morphine relative to same-sex controls across all age groups. These data indicate that aging and gender variables interact with each other to alter the efficacy of morphine analgesia in rats, and that adult gonadectomy appears to modulate each gender's analgesic response across the aging process.

351.5

OPIATE REGULATION OF AMINO ACID RELEASE FROM THE NUCLEUS RAPHE MAGNUS (NRM) OF THE RAT. Liang Liu, Paul Green* and Allan I. Basbaum. Depts. of Anatomy, Physiology and Keck Center for Integrative Neuroscience, University California San Francisco, CA 94143

A glutamatergic synapse in the NRM has been implicated in descending antinociceptive mechanisms. In this study, we used *in vivo* microdialysis to study basal and evoked release of glutamate, aspartate, taurine and glycine in response to microinjection of morphine into the NRM. Male Sprague-Dawley rats were stereotaxically implanted with a concentric microdialysis probe (150μm i.d.) with an attached side catheter (Frothingham and Basbaum, J. Neurosci. Meth., 1992). Recovery of amino acids began at least one day after surgical implantation. The probe was perfused with a Ringer's solution at 2.0 μl/min. Samples were collected at 15 minute intervals. Four samples were collected to determine basal concentrations. Then morphine (5.0μg/0.5μl saline) was microinjected through the side catheter over a 2.0 min period. A tail pinch test was used to assess analgesia. Samples were analyzed with pre-column OPA derivatization and fluorescence detection, within 24 hours.

Basal concentrations of the amino acids were: glutamate--22.7pm/30μl; aspartate--0.5pm/30μl; taurine--16.3pm/30μl; glycine--3.4pm/30μl. Microinjection of morphine significantly increased the extracellular concentration of glutamate (40-300%). The increase was only detected in the two samples following the morphine injection. There was no change in the levels of the other amino acids. Injection of morphine vehicle produced no detectable change. In two animals in which the morphine microinjection did not produce analgesia, we did not detect an increase in glutamate levels.

It has been proposed that the analgesia produced by microinjection of opiates involves lifting of a tonic inhibitory (GABAergic) control of the spinally-projecting NRM neurons. The present results suggest that local injection of opiates may also "activate" these projection neurons, via the release of glutamate from the terminals of PAG neurons that arborize within the NRM. Our continuing studies are evaluating the receptors through which the morphine effect is generated and the Ca^{2+} dependency of this release. Supported by PHS grants DE/DA08973, NS21445 and NS14627.

351.6

ARE THE ANTINOCICEPTIVE EFFECTS OF THE KAPPA SELECTIVE AGONIST CI-977 SUPRASPINALLY MEDIATED? K.R. Gogas*, J.D. Levine and A.I. Basbaum. Depts. of Anatomy, Physiology and Medicine and Keck Center for Integrative Neuroscience, University of California San Francisco, CA, USA.

We previously reported that supraspinal administration of the mu opioid receptor agonist, DAMGO, produces a dose-related, naloxone-reversible inhibition of noxious stimulus-evoked pain behaviors and spinal cord fos-like immunoreactivity (FLI) in the rat, by activating descending inhibitory pathways. To examine the contribution of descending inhibitory pathways to kappa-receptor mediated analgesia, we have studied the effect of icv administration of the selective kappa agonist, CI-977, on pain behaviors and on spinal cord FLI evoked by unilateral formalin injection into the hindpaw of rats. We also tested the effects of lesions of the dorsolateral funiculi (DLF) of the spinal cord on CI-977-mediated antinociception.

Cannulae were implanted into the third ventricle of male Sprague-Dawley rats (200-225g; Bantin-Kingman) one week prior to testing. In some cases, animals received either sham or bilateral lesions of the DLF at the T3 level, immediately prior to cannula implantation. On the day of testing, CI-977 was administered into the third ventricle (icv) 10 min prior to injection of 5% formalin into the dorsal surface of the hindpaw. CI-977 produced a dose-related (0.06-2.0 μg) antinociception; the effect of the highest dose was reversed by either icv naloxone (10 μg) or icv nor-binaltorphimine (10 nmol). A lower dose of naloxone (1.0 μg), which reverses icv DAMGO-mediated antinociception, was ineffective against CI-977. In contrast to our findings with morphine and DAMGO, the antinociception produced by icv CI-977 was not associated with a concomitant attenuation of spinal cord fos-immunoreactivity. Furthermore, lesions of the DLF had *no effect* on the antinociception produced by icv CI-977. This lack of a dose-related inhibition of spinal cord FLI by behaviorally analgesic doses of icv CI-977, taken together with the DLF lesion data, suggests that supraspinal kappa opioid receptors can produce analgesia *without* activating descending inhibitory controls. Supported by the Pharmaceutical Manufacturer's Assoc. and NIH grants NS14627, NS21445 and DE/NIDA 08973.

351.7

CHOLINERGIC NEURONS IN THE PEDUNCULOPONTINE TEGMENTUM MODULATE NOCICEPTION BY ACTING AT NICOTINIC AND MUSCARINIC RECEPTORS IN THE VENTROMEDIAL MEDULLA L.F. Fitzgerald* and H.K. Proudfit. Dept. of Pharmacology, Univ. of Illinois at Chicago. Chicago, IL 60680.

Electrical or chemical stimulation of the cholinergic pedunculopontine tegmental nucleus (PPTg) produces antinociception in rats. Experiments were designed to establish that this antinociception is mediated by cholinergic receptors in the ventromedial medulla (VMM). Injection of atropine, pirenzepine or mecamylamine into the VMM blocked the antinociception produced by PPTg stimulation, but methoctramine had no effect. In a separate series of experiments, injection of nicotine, methacholine or physostigmine into the VMM produced antinociception. Atropine antagonized the antinociception produced by nicotine, and mecamylamine antagonized the antinociception produced by methacholine. Finally, antinociception produced by physostigmine was antagonized by mecamylamine and atropine. These experiments indicate that PPTg stimulation produces antinociception by releasing acetylcholine which acts at nicotinic and muscarinic receptors in the VMM. Supported by National Institute on Drug Abuse USPHS Grant DA03980.

351.8

AF64A ATTENUATES NICOTINE-INDUCED ANTINOCICEPTION IN THE PPTG AND NRM. M.K. Nihei* and E.T. Iwamoto. Graduate Center for Toxicology and Department of Pharmacology, University of Kentucky Coll.of Med., Lexington, KY 40536.

The effects of ethylcholine aziridinium ion (AF64A) pretreatment of the pedunculopontine tegmental nucleus (PPTg) or the nucleus raphe magnus (NRM) on nicotinic antinociception were examined in adult, male Sprague-Dawley rats. Nicotinic antinociception was induced by either nicotine administered s.c. or N-methyl carbamylcholine (NMC) microinjected into the PPTg or NRM. Seven days after the implantation of guide cannulas aimed at the PPTg or NRM, AF64A (0.06, 0.125, 0.25, 0.5 nmol), or phosphate buffer, was injected in a 0.06μl volume. One week later, 40 nmol of NMC in 0.5μl of buffer was injected into the same site. This dose of NMC induced maximal antinociception 5 to 15 minutes after its injection into the PPTg or NRM as assessed by the hot-plate and tail-flick tests. NMC-induced antinociception was inhibited by the 0.125, 0.25 and 0.5 nmol doses of AF64A adminstered in the NRM, and by the 0.5 and 1 nmol doses of AF64A administered in the PPTg. In separate experiments, 0.375 mg/kg s.c. of nicotine produced maximal antinociception 5 to 10 minutes after injection, in both the hot-plate and tail-flick tests. These responses were absent in animals pretreated 1 week earlier with 0.5, 0.25 and 0.125, but not 0.06 nmol of AF64A in the NRM. These data suggest that the antinociceptive effects of centrally administered NMC depend on the integrity of cholinergic neurons in the regions of the NRM or the PPTg. Furthermore, SC nicotine-induced antinociception depends on intact NRM cholinergic neurons. (Supported in part by NS28847 and the KTRB.)

351.9

ACTIVATION OF OFF-CELLS IS A COMMON ELEMENT IN THE ANTINOCICEPTION PRODUCED BY DIRECT LOCAL APPLICATION OF BOTH OPIOIDS AND $GABA_A$ RECEPTOR ANTAGONISTS IN THE RAT ROSTRAL VENTROMEDIAL MEDULLA (RVM). M.M. Heinricher* and V. Tortorici. Dept. Neurol., Univ. Calif., San Francisco, CA 94143 and Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela.

Blockade of GABA transmission in the RVM of lightly anesthetized rats produces inhibition of the tail flick reflex (TF), an effect consistent with the suggestion that opioids activate pain inhibiting neurons by inhibition of a GABA interneuron. The aim of the present study was to identify the neurons mediating the antinociceptive action of GABA antagonists by recording the activity of TF-related RVM neurons before and after local infusion of bicuculline methiodide (BIC) in doses sufficient to inhibit the TF (12.5-80 ng in 100 nl).

Cells of one class, "off-cells" are identified by a TF-related pause in firing, and are thought to have a net antinociceptive effect. Following BIC, these neurons showed a prolonged period of continuous activity, with a smooth increase to a peak followed by a more gradual decline. The time course of this active period paralleled that of TF inhibition. "On-cells" are identified by a TF-related burst of activity. These cells did not become continuously active following BIC infusion. At low doses, on-cells displayed a decrease in firing in those cases in which the TF was inhibited.

These results demonstrate that the activity of off-cells is highly constrained by GABA, suggesting that disinhibition of these neurons results in antinociception. Coupled with observations indicating that opioids acting in RVM activate off-cells indirectly, these data demonstrate that disinhibition of off-cells is central to the antinociceptive actions of both opioids and GABA antagonists in RVM.

Supported by PHS grant DA05608.

351.10

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

In this experiment, we examined whether spinal $GABA_A$ receptors mediate the antinociception produced by chemical stimulation of neurons in the nucleus raphe magnus (NRM). Rats were prepared under general anesthesia with an intrathecal (IT) catheter and a chronic guide cannula aimed at the NRM. One week postoperatively, baseline nociceptive sensitivity was determined using the tail flick test. Rats were then injected IT with either 0.3 μg bicuculline methiodide (a selective $GABA_A$ antagonist) or with vehicle (0.9% saline). Three minutes later, 30 nmol L-glutamate was microinjected into the NRM and tail flick latency (TFL) was redetermined at fixed intervals thereafter. Microinjection of glutamate significantly increased TFL in vehicle-pretreated rats compared to baseline TFL 1, 2, 5, and 15 minutes following glutamate administration ($p < 0.01$). However, TFL in bicuculline-pretreated rats was not significantly greater than their baseline TFL ($p > 0.05$) at any time after glutamate administration. These results suggest that GABA in the spinal cord may mediate descending inhibition from the raphe via an action at $GABA_A$ receptors. Supported by PHS Grant DA 07004.

351.11

MODULATION BY ANGIOTENSIN III OF ARTERIAL PRESSURE-RELATED AND PAIN-RELATED NEURONAL RESPONSIVENESS IN THE NUCLEUS RETICULARIS GIGANTOCELLULARIS OF RATS. S.H.H. Chan* and H.-F. Tsai. Inst. of Pharmacology, Natl. Yang-Ming Med. Coll., Taipei, Taiwan, R.O.C.

We combined extracellular single-neuron recording and microiontophoresis to evaluate the modulation by angiotensin III (AIII) of arterial pressure (AP)- and pain (P)-related neuronal responsiveness in the nucleus reticularis gigantocellularis (NRGC). Spontaneously active NRGC neurons in adult, male Sprague-Dawley rats anesthetized with pentobarbital were evaluated for their responsiveness to pain (tail clamp) and transient hypertension elicited by phenylephrine (5 µg/kg, i.v.). The responses of NRGC neurons to hypertension and tail clamp, when delivered individually, were suppressed by microiontophoretically-applied AIII. These actions were antagonized by AIII receptor antagonist Ile^7-AIII. In NRGC neurons that manifested both AP- and P-relatedness, the responsiveness to tail clamp was reduced upon a simultaneous elevation in systemic arterial pressure. Interestly, this preferential response pattern was reversed to one that favored nociception in the presence of AIII. These results reveal that neuropeptides such as AIII may differentially modulate neuronal responsiveness according to the prevailing physiologic status of the animal.

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351.12

Examining a possible GABAergic link between ON and OFF cells in the rostral ventromedial medulla. **S. McGaraughty*, S. Reinis, J. Tsoukatos, & M. Lemon. Department of Biopsychology, University of Waterloo, Waterloo, Ontario CANADA N2L 3G1.**

Two classes of cells in the rostral ventromedial medulla (RVM), ON and OFF, are important in the descending modulation of noxious input. It has been previously hypothesized that these two types of cells communicate locally by GABAergic interneurons (Heinricher, Morgan, & Fields, 1991; McGaraughty, Reinis, & Tsoukatos 1991). Systemic morphine (10 mg/kg) followed by systemic naloxone (3 mg/kg), 30 min later, was given to rats anaesthetized with either pentobarbital (a GABA agonist) or with ketamine (a non-competitive NMDA receptor antagonist) and xylazine. In the ketamine paradigm picrotoxin (1 mg/kg), a GABA antagonist, was given 30 min after the injection of naloxone. Under both anaesthetic paradigms the animals were kept in a deep state of anaesthesia. The spiking activity of the ON and OFF cells was recorded. The ON cells were typically inhibited by morphine under both anaesthetics. However, the OFF cells demonstrated an excitatory response only under ketamine anaesthesia suggesting a possible GABAergic inhibition of OFF cells in the pentobarbital paradigm. The excitation of ON cells may increase the local release of GABA inhibiting OFF cell transmission.

351.13

INVOLVEMENT OF AN ANTIANALGESIC EFFECT OF NEUROTENSIN IN THE NRM IN THE RESPONSE TO MORPHINE ADMINISTRATION IN THE PAG. M.O. Urban* and D.J. Smith. Depts. Anesth. & Pharmacol., WVU-HSC, Morgantown, WV 26506

The neuropeptide neurotensin has been implicated in pain modulation in the CNS and has been localized in the descending projections from the periaqueductal gray (PAG) to the nucleus raphe magnus (NRM) (Beitz. J. Neurosci. 2: 892, 82). These studies were designed to determine if these neurotensin projections participate in pain modulation from opioids administered into the PAG. The experiments were conducted by utilizing intracerebral microinjections in awake male Sprague-Dawley rats and measuring the noxious heat induced tail-flick response (TFL). Microinjection of neurotensin into the NRM produces a biphasic effect as both antinociceptive (increased TFL) and hyperreflexive (decreased TFL) responses are observed. The hyperreflexive (antianalgesic) response is most prominent at lower doses of neurotensin (0.3 and 1 nmol) being exhibited by 86% and 50% of the animals, respectively. It was most evident at 10 min following injection and disappeared within 30 min. The antinociceptive effect of neurotensin is observed only at the higher doses (3-30 nmol). Morphine (6 nmol) injected into the PAG produces an antinociceptive effect that is enhanced by administration of the neurotensin antagonist [D-Trp11]neurotensin (3 pmol) or anti-neurotensin antiserum in the NRM. In contrast, β-endorphin (10 nmol) injected into the PAG produces an antinociceptive response that is unaffected by these substances in the NRM. The data demonstrate that PAG-NRM neurotensin neurons are responsible for an antianalgesic component of morphine's action. In contrast, this pathway is apparently not involved in the antinociceptive effect of β-endorphin. This is consistent with the notion that separate descending pain inhibitory neuronal pathways are responsible for the action of these opioids from the PAG (Tseng and Tang, J. Pharmacol. Exp. Ther. 252: 546, 90).

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351.14

INTRA-VTA INJECTIONS OF THE SUBSTANCE P ANALOGUE, DI ME C-7, CAUSES ANALGESIA IN THE FORMALIN TEST FOR CHRONIC PAIN. N. Altier* and J. Stewart, Center for Studies in Behavioral Neurobiology, Dept. Psychology, Concordia University, Montréal, Canada, H3G 1M8.

Experiments were designed to investigate the analgesic effects of substance P (SP) injected into the ventral tegmental area (VTA). Rats were tested in the formalin test for tonic pain following an injection of 0.05 ml of 2.5% formalin into one hind paw immediately after bilateral intra-VTA infusions of 3.0 µg/0.5 µl/side of the synthetic SP analogue, DiMeC-7, or saline using a within subjects design. Pain responses were recorded for 72 min. Two weeks later, the same rats were assessed in spinal reflex tail-flick test for pain. Tail-flick latencies were recorded following immersion of the tail in 55° C hot water at 10 min intervals for one hour immediately following intra-VTA infusions of DiMeC-7 or the vehicle. In the formalin test, DiMeC-7 reduced pain responses during the first 30 min. In the tail-flick test, DiMeC-7-treated rats were, if anything, hyperalgesic compared to saline-treated rats. In a second experiment, the same formalin test procedure was used except that rats received intra-VTA infusions 25 min following a formalin injection. DiMeC-7 attenuated pain responses for at least 30 min. These results parallel those of Morgan and Franklin (1990) who showed that intra-VTA injections of morphine produced analgesia in the formalin, but not the tail-flick test, and reinforce the idea that different neural systems mediate tonic and phasic pain. The fact that SP release in the VTA has been implicated in the activation of dopamine neurons in stress (Bannon et al, 1983) suggests that SP might mediate a form of stress-induced analgesia.

351.15

Fentanyl causes morphine - like increases in PAG histamine release when coupled with tail pinch.

K.E. Barke* and L.B. Hough Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208.

Recent studies in our laboratory employing the techniques of antinociceptive measures, intracerebral injections and intracerebral microdialysis support the hypothesis that a portion of morphine's supraspinal antinociceptive effect is due to histamine (HA) release in the periaqueductal grey (PAG). Presently, the actions of fentanyl, a highly selective μ agonist, were simultaneously studied on PAG HA release (with *in vivo* microdialysis) and on antinociceptive responses in the same animal. In animals repeatedly tested for antinociception (tail pinch method), fentanyl (0.3 mg/kg s.c.), significantly increased both extracellular HA levels (0.5 - 3.0 hr) and tail pinch latencies (0.5 - 2.5 hr). In contrast, the same dose of fentanyl had no effect on HA release in animals that were not tested for antinociception. Similarly, saline injections with or without tail pinch measurements had no significant effect on HA release, suggesting that tail pinch alone was not sufficient to cause HA release. Although previous results show that antinociceptive doses of opiates like morphine induce PAG HA release, the present results suggest that PAG HA release may not be obligatory for μ opiate antinociception. Even though simultaneous measurements of transmitter release and nociceptive responses would appear to be a powerful approach to study analgesic mechanisms, the present results also show that such behavioral testing can influence in vivo neurochemical events, perhaps by induction of stress responses (Supported by DA-03816, and DA-05460).

351.16

Histamine antinociception in the periaqueductal grey. K.K. Thoburn, L.B. Hough, J.W. Nalwalk, S.A. Mischler and M. Aschner*, Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208.

Since histamine (HA) has been previously implicated as a mediator of antinociception (ANC), its effects on hot plate (52°) antinociceptive responses were presently studied in detail after intracerebral injection. When administered into the rat ventrolateral periaqueductal grey (PAG), HA (0.03 - 0.3 µg/0.5 µl) induced significant ANC. The largest response (21% of maximum possible effect after 0.3 µg) occurred 5 and 10 min after HA injection. Larger doses of HA (10 - 100 µg) had biphasic effects on ANC. However, preliminary histological results suggest that these doses of HA induce tissue damage. Mapping studies showed that HA (1 µg, 5 min) induced significant ANC when given into the PAG, but not when administered into the median raphe, mesencephalic reticular formation or the dorsal PAG. HA ANC (0.3 µg, PAG) was significantly antagonized by co-administration of the opiate antagonist naloxone (1 ng), the H_2 antagonist tiotidine (1 ng) and the H_1 antagonist temelastine (20 pg). These results suggest that: 1) HA induces ANC in the PAG in non-toxic doses, 2) the response is anatomically specific, and 3) both HA receptors and opiate receptors may play a role in HA ANC. Since intracerebral tiotidine (1 ng/PAG) attenuates systemic morphine ANC (Hough et al., Brain Res. 1992, in press), inhibition of HA ANC by this treatment (shown presently) further supports the hypothesis that endogenous HA is a mediator of morphine analgesia (Supported by DA-03816).

351.17

INFLUENCES OF MORPHINE AND NALOXONE ON THE FUNCTION OF NOCICEPTIVE NETWORKS IN THE VENTRAL (POSTERIOLATERAL AND MEDIAL) THALAMUS OF THE RAT. J. Tsoukatos*, S. Reinis, and S. McGaraughty. Dep. of Psychology, University of Waterloo, Waterloo, Ont., Canada, N2L 3G1.

We have previously identified three types of neuronal responses to noxious tail heating in the VPL and VM thalamus of ketamine-xylazine anaesthetised rats, i.e activation, inhibition, and no response. We have analyzed our results using a novel method developed in our laboratory (Reinis, Weiss, Mcgaraughty, and Tsoukatos, 1991).We have reported that morphine appeared to mimic the effects of heat, and that the effect was reversed by naloxone.

We have tried to replicate the above results studying the effects of noxious stimulation, when the network was under the influence of morphine or naloxone. Morphine (10 mg/kg) decreased the firing rate of the heat inhibited cells (the most common response) and increased the firing rate of the heat activated cells. These effects were reversed by naloxone (2 mg/kg).

The effects of morphine appeared shortly after the injection (2-4 minutes). The reversing action of naloxone was delayed by 10-20 minutes following the injection.

351.18

INTRAVENOUS MORPHINE INCREASES CSF NOREPINEPHRINE IN SHEEP. C. Tong,* J.C. Eisenach, Department of Anesthesia, Wake Forest University Medical Center, Winston-Salem, NC 27157-1009

Opioids alleviate pain by multiple mechanisms, including activation of bulbospinal inhibitory pathways, some of which are noradrenergic. To test this hypothesis in conscious animals, 6 adult sheep were prepared, after ACUC approval, with intravenous, arterial, and thoracic spinal catheters. On separate days, beginning at least 3 days after surgery, animals received iv morphine (0.5 or 1 mg/kg) followed by sampling of CSF at intervals for 1 hr for norepinephrine analysis by HPLC. Morphine caused an increase in CSF norepinephrine from 150±33 pg/ml at baseline to 300±190 pg/ml after 0.5 mg/kg and 530±200 pg/ml after 1.0 mg/kg ($P<0.001$). These results in a conscious, non-rodent species support previous investigations, primarily in anesthetized rats, demonstrating systemic or brainstem opioid induced spinal inhibition which is noradrenergic in nature and inhibited by spinally administered α-adrenergic antagonists. Previous studies in sheep suggest opioid induced norepinephrine release is likely acting on α_2-adrenoceptors, based on anti-nociceptive trials and radioligand binding studies in this species. Supported in part by grant GM35523 from the National Institutes of Health.

RETINA AND PHOTORECEPTORS: PHOTORECEPTORS, HORIZONTAL AND BIPOLAR CELLS

352.1

ROD SYNAPSE GEOMETRY AFFECTS TRANSMITTER CONCENTRATION AT THE BIPOLAR CELL. R. Rao*[1,2], G. Buchsbaum[2] and P. Sterling[1]. Depts. of Neuroscience[1] and Bioengineering[2] Univ. of Pennsylvania, Philadelphia, PA 19104.

The cat rod synapse contains a single active zone: a linear array of about 100 vesicle release sites arching over the invaginating tips of a pair of bipolar dendrites. A pair of invaginating horizontal cell spines creates an extracellular volume of 0.1 μm^3. We calculated some functional consequences of this unique geometry.

Assuming an intravesicular glutamate concentration of 100 mM, a 32 nm synaptic vesicle contains ~1000 glutamate molecules. If these are evenly distributed within the extracellular volume, the glutamate concentration is about 20 µM. At this concentration 90% of the receptors are bound (2 non-cooperative binding sites/receptor; K_d = 0.7 µM). Thus, in darkness, tonic release of one vesicle at a time could maintain a saturating glutamate concentration within the invagination. This implies a low probability of release at each of the 100 sites.

A simple diffusion model predicts that following the release of one vesicle, glutamate concentration would peak at the bipolar dendritic tip (presumed site of APB receptors) in less than 0.5 ms. The height of the peak depends strongly on the locus of the release site along the 1.8 µm arch. Sites at the base and peak of the arch lie respectively 220 and 500 nm from the bipolar tips and this leads to a 5-fold difference in peak concentrations. Were the active zone not arched, but straight, the ratio of distances between closest and farthest sites to the bipolar tip would be 4-fold and this would lead to a 16-fold difference in peak concentrations. Thus, the arched form of the active zone minimizes the difference in peak concentrations due to different loci of release. EY00828

352.2

MODULATION OF CA CHANNEL PROPERTIES BY EXTRACELLULAR pH IN ROD PHOTORECEPTORS. Farid Mahmud & Steven Barnes*, Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

L-type Ca channels of many species undergo changes in gating and permeation with changes of extracellular pH. Here we extend these observations to the Ca channels of rod photoreceptors where there may be important physiological ramifications of endogenous interstitial pH changes shown to accompany the normal response to light.

Barium currents were recorded with whole-cell patch-clamp techniques from mechanically isolated tiger salamander rods under conditions designed to eliminate current contributions from other ion channels (bath (in mM): 20 $BaCl_2$, 10 TEABr, 5 CsCl, 65 NaCl, 2.5 KCl, 8 glucose and 10 HEPES; intracellular solution: 100 CsCl, 3.5 $MgCl_2$, 1 EGTA, 10 HEPES, pH 7.2). Current-voltage relations (I-V's) measured at test pH were always bracketed by I-V's in pH 7.4 to control time-dependent dialysis-related effects. Peak inward currents nearly doubled over the pH range of 6.9 to 8.1. Part of this increase is accounted for by pH-induced shifts of the Ca channel activation curve midpoint, which amounted to a 10 mV negative shift between pH 6.9 to 8.2. Proton block underlies a significant reduction of the fully-activated Ca channel conductance.

We show that synaptic transmission from rods depends on the third power of the Ca influx function and that proton-induced Ca channel changes account for observations of pH-regulated synaptic transmission. Proton modulation of Ca channels may lead to other effects on cell function involving the activity of Ca-gated channels and Ca-regulated enzymes.

Supported by the Alberta Heritage Foundation and the MRC.

352.3

MODULATION OF SYNAPTIC TRANSFER BY EXTRACELLULAR pH AT THE PHOTORECEPTOR OUTPUT SYNAPSE. Vaishali Merchant* and Steven Barnes, Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

We show that interstitial pH strongly modulates the transfer properties of the glutamatergic photoreceptor output synapse via presynaptic actions.

Dark-adapted larval tiger salamander retinal slices were used where control of extracellular pH is possible and where retinal neurons can be identified by their position in the slice and by their morphology (using 1% Lucifer yellow). Light-elicited current changes in horizontal cells and bipolar cells were measured with the whole-cell patch-clamp technique. Postsynaptic current changes were eliminated when the retinal slice was bathed in media of pH 7 and enhanced five-fold when bath pH was increased from 7.6 to 8. Overall the current changes showed an exponential dependence on extracellular pH, exhibiting an e-fold increase per 0.23 pH unit between pH 7 and 8. Horizontal cell responses to glutamate (2 to 50 uM) were unchanged between pH 7.6 and 8.0, and the light response of cones was fairly constant between pH 7.6 and 8.0, so neither explains the strong enhancment of postsynaptic response.

We account for transmission modulation via the pH-sensitivity of presynaptic Ca channels. The Ca channel activation curve slides along the voltage axis as pH changes. Postsynaptic response shifts are predicted by laterally shifting Attwell *et al.*'s synaptic transfer function for the photoreceptor-horizontal synapse by amounts determined by the shift of Ca channel activation.

Light-induced alkylinization of the photoreceptor environment would shift the Ca channel activation curve negative, increase Ca influx and increase transmitter release. This could reset the photoreceptor synaptic output range during prolonged light or dark exposure.

Supported by the Alberta Heritage Foundation and the MRC.

352.4

RHODOPSIN-LIKE GENE EXPRESSED IN "GREEN" CONES IN GOLDFISH RETINA. P. A. Raymond*, L. K. Barthel, J. K. Knight, S. A. Sullivan, Anat. & Cell Biol. and Neurosci. , Univ. Michigan, Ann Arbor, MI 48109

We used a bovine rhodopsin probe to isolate and sequence a cDNA clone (3.1.4) coding for goldfish rhodopsin, and we obtained additional goldfish visual pigment clones from K. Nakanishi. All clones were from the same retinal cDNA library (D. Goldman). Two of Nakanishi's clones (7.4.2 and 7.3) are highly homologous to human rhodopsin (>70% amino acid identity) and to the chicken rhodopsin-like gene (Wang, *et al.*, Biochem.31:3310, 1992) thought to represent the chicken "green" pigment. In order to determine whether the goldfish rhodopsin-like gene is expressed in rods or cones, we made sense and antisense digoxygenin-labeled riboprobes for *in situ* hybridization with 3 µm cryosections of light- and dark-adapted whole eyes and isolated retinas.

The principal member of goldfish double cones (DC) contains a long wavelength-sensitive pigment (red) and the accessory member a middle wavelength-sensitive (green) pigment; long single cones (LSC) are either red or green. Riboprobes generated from clone 3.1.4 hybridized to rod photoreceptors in two locations: the perinuclear region and the myoid process adjacent to the ellipsoid, which is displaced up to 30 µm from the nucleus. Riboprobes to clones 7.4.2 and 7.3 hybridized to the perinuclear region of the accessory member of DC pairs and some but not all LSC. In dark-adapted retinas, where cone myoids are elongated, signal was not associated with the displaced ellipsoid as it was in rods. Sense probes in all cases gave no signal. These results show that green cones in goldfish retina contain a rhodopsin-like pigment. EY04318.

352.5

BLUE CONES CONTACT OFF-MIDGET BIPOLAR CELLS

K. Klug, N. Tiv, Y. Tsukamoto[1], P. Sterling[2] and S.J. Schein*. Dept of Psychology, UCLA, Los Angeles, CA, [1]Hyogo Coll Med, Hyogo, Japan and [2]Dept of Anatomy, Univ of Pennsylvania, Philadelphia, PA

"Blue cone" bipolars resemble On-midget bipolars in the location of their terminals in the inner plexiform layer, but many are contacted by more than one (widely spaced) cone. To determine whether S cones contact Off-midget bipolars as well as "blue cone" On bipolars, we examined 80 contiguous cone pedicles and their bipolars using electron micrographs of serial sections from macaque foveal retina.

Three of the 80 pedicles did not contact an On-midget bipolar. These three were smaller and had more synaptic ribbons (22) than did neighboring pedicles (17 - 20). The three unusual pedicles were widely spaced and did contact the dendrites of "blue cone" bipolars, suggesting that we have identified three S cones and their associated "blue cone" bipolars.

Each S cone pedicle also contacted its own Off-midget bipolar. This finding is consistent with the observation that S cones make both invaginating and triad-associated flat contacts with bipolars (Kouyama & Marshak 1992). Our finding, that S cones contact Off-midget bipolars as well as "blue cone" On bipolars, raises the question of why so few blue-Off, yellow-On responses have been found in ganglion and dLGN cells (Malpeli & Schiller 1978, de Monasterio 1979).

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352.6

THE CONE PHOTORECEPTOR MOSAIC OF THE GREEN SUNFISH. D. A. Cameron* and S. S. Easter, Jr. Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109-1048.

Recent evidence has implicated the geometrical birefringence of the double cones of the green sunfish (*Lepomis cyanellus*) as the biophysical basis of this vertebrate's polarization sensitivity. Because of the intimate link between the organization of the cone photoreceptor mosaic and the psychophysical details of polarization sensitivity, we have examined the structural features of the *L. cyanellus* cone mosaic: (1) the arrangement of the cones is constant across the retina (except for two regions), with double cones being nearly orthogonal to each other and aligned roughly ± 45° to the nearest retinal margin tangent; (2) the ratio of double to single cones is everywhere the same; (3) cone density is highest in the temporal retina; (4) retinal growth is circularly symmetric; (5) the retina maintains a roughly constant density of cones during growth by intercalating peripheral rows of cones amongst more central rows. These results will be presented as they relate to polarization vision and visual resolution.

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352.7

PHOTORECEPTOR TOPOGRAPHY IN THE RETINAE OF ANUBIS BABOONS. Q. Fischer[1], M. Kirby[2], V. Jennings[2] and J. Mielke[3]. [1]Dept. of Psych., Univ. of Calif., Riverside, CA 92521; [2]Dept. of Peds., Loma Linda Univ., Loma Linda, CA 92350; [3]Sch. of Opt., Univ. of Calif., Berkeley 94720.

The number and distribution of rods and cones in the retinae of anubis baboons (*Papio anubis*) were compared with the distribution of ganglion cells in this species. These values were then compared with retinal topography described in other primate species.

We have estimated the number and distribution of rods and cones in wholemounted retinae using Nomarski differential interference phase contrast (NDIC) optics in conjunction with video-enhanced microscopy. The total number of rods (mean=74 million) and cones (mean=4 million) was intermediate between that described for the smaller retinae of macaques (Packer et al. 1989 J.C.N. 288:165) and the larger retinae of humans (Curcio et al 1990 J.C.N. 292:497). As observed for the retinae of macaques the distribution of cones in *P. anubis* was flattened peripherally, but dominated by a sharp central increase which peaked at the fovea (mean=217,000/mm^2) and rod distribution increases slowly to a peak (mean=204,000/mm^2) forming an annular ring which surrounds the central retina at the approximate eccentricity of the optic disk (4 mm). Within this ring there is a region of higher density in superior retina similar to the "dorsal rod peak" described by Packer et al. 1989 (J.C.N. 288:165). However, unlike other primate species (Wikler & Rakic 1990 J. Neurosci. 10:3390) the isodensity contours for both rods and cones in *P. anubis* are horizontally elongated peripheral to the optic disk in the same retinal position as the visual streak in ganglion cell distribution previously described for this species (Fischer & Kirby, 1991, Brain Behav. Evol. 37:189). Further, this correlates with the prominent streak in the ganglion cell distribution in the retinae of *P. anubis* compared to the retinal ganglion cell distribution of other primate species (Fischer & Kirby 1991).

352.8

PHOTORECEPTOR SHEETS ISOLATED FROM THE NEONATAL RAT RETINA LACK SYNAPSES AND OTHER RETINAL CELLS. T.L. Valentino[1], and M.S. Silverman*. Sensory Neuroscience Lab, Central Institute for the Deaf, and [1]Dept. of Biology, St. Louis University, St. Louis, MO. 63110.

We have presented evidence suggesting that photoreceptors transplanted in an intact outer nuclear layer (ONL) sheet form synapses with host retina in which endogenous photoreceptors and their synaptic connections were almost entirely eliminated (Silverman *et al.* Exp. Neurol. 115:87, 1992). However, the possibility remained that these synapses formed between transplanted photoreceptors and non-photoreceptor retinal cells that were inadvertently included in the transplant.

To evaluate this possibility we analyzed ONL photoreceptor sheets isolated by vibratome sectioning (Silverman and Hughes, IOVS 30:1684, 1989) for the presence of ribbon-type synapses and non-photoreceptor retinal cells. The isolated ONL appeared as a uniform, almost transparent sheet (nonuniform areas were excluded from use). Electron micrographs were constructed from the photoreceptor sheet and a comparable area (superior retina) in the 8-day-old retina. Ribbon-type synapses were identified as an electron-dense presynaptic ribbon structure surrounded by a cluster of vesicles apposed to postsynaptic processes. Determination of cell type was based on morphological characteristics such as cell size and nuclear chromatin pattern.

The normal 8-day-old Sprague-Dawley rat retina contained an average of 27.5 synapses/100μm of outer plexiform layer (OPL). All were found within the OPL. In contrast, we have seen no synapses nor any non-photoreceptor cells in the isolated photoreceptor sheets. Because no photoreceptor synapses nor contaminating non-photoreceptor cells were seen in the isolated ONL, the significant increase in ribbon-type synapses we reported following photoreceptor transplantation apparently were formed between the transplanted photoreceptors and host retinal neurons.

352.9

IMMUNOCYTOCHEMICAL IDENTIFICATION OF PHOTORECEPTOR POPULATIONS IN THE RETINAS OF NORMAL AND RED-LIGHT-REARED TREE SHREWS. H.M. Petry*, J.T. Erichsen and Á. Szél. Departments of Psychology and Ophthalmology & Visual Science, Univ. of Louisville, KY 40292; Department of Neurobiology & Behavior, SUNY at Stony Brook, NY 11794; Lab I of Electron Microscopy, Semmelweis Univ. of Medicine, Budapest Hungary

Microspectrophotometry shows the tree shrew retina to contain short-wavelength-sensitive (SWS) cones, long-wavelength-sensitive (LWS) cones, and rods (Petry & Harosi, 1990). We present immunocytochemical data on the presence and retinal distributions of these photoreceptor types based on their affinity for monoclonal antibodies OS-2, COS-1 and anti-rhodopsin, which have been shown to be specific, respectively, for SWS cones, mid/LWS cones, and rods in other mammals (Szél et al, 1988). Retinas were obtained from normal adult tree shrews (Tupaia belangeri) and from adult tree shrews reared from birth to 8 wks of age in deep red light (>600nm). Rearing tree shrews in red light, which deprives SWS cones of photic stimulation, produces long-term deficits in chromatic/achromatic discriminations (Petry & Kelly, 1991). One goal was to explore the possibility that the discrimination deficits resulted from a reduced population of SWS cones. Results in normal shrews showed clear immunocyto-chemical differentiation of the three photoreceptor types, each with retinal variation in density. Counts from flatmounted retinas showed a total cone density of 12,000/mm^2 - 30,000/mm^2. SWS cones comprised 4% - 11% of cones and displayed the hexagonal packing typical of this cone type. Percentages of SWS cones in the retinas of red-light-reared shrews were within the range determined for normals and no difference in SWS packing was apparent. These results argue that SWS cone integrity is not dependent on photic stimulation during early post-natal life. Supported by NIH R29-EY07113 (HMP) and R01-EY04587 (JTE).

352.10

AN ULTRAVIOLET-SENSITIVE CONE IN THE GERBIL RETINA. **G. H. Jacobs* and J. F. Deegan II. Dept. of Psychology, University of California, Santa Barbara, CA 93106**

Gerbils are unusual among rodents in that their retinas contain relatively large numbers of cones. Earlier, electrophysiological experiments established that Mongolian gerbils (*Meriones unguiculatus*) have a cone pigment with a peak of c. 493 nm. Under photopic test conditions the gerbil retina behaved univariantly over the range of 440 to 620 nm leading us to conclude that the gerbil has only a single class of cone (Jacobs & Neitz, *Experientia*, 1989, *45*, 317). Govardovskii et al. (*Vision Research*, 1992, *32*, 19) employed the monoclonal antibody OS-2 to label a second class of cone in the gerbil retina. By analogy to results on other mammals, they suggest these receptors to be "blue cones" with a sensitivity peak of c. 430 nm. Electroretinogram flicker photometry and behavioral discrimination tests have been used to show that the conclusions from both of these studies were in error: the gerbil indeed has a second cone type, but it is not a "blue" cone. In the presence of intense, long-wavelength adaptation the second cone of the gerbil is revealed to have peak sensitivity in the ultraviolet (UV), at c. 360 nm. Adaptation experiments show the sensitivities of the two cone types can be independently manipulated, and the behavioral results demonstrate that gerbils can utilize signals from the UV cone to make visual discriminations. (Supported by EY-02052)

352.11

AGE-RELATED CHANGES IN LIPID PEROXIDATION IN ISOLATED ROD OUTER SEGMENTS OF RAT RETINA. Ohia, S.E.*, Bagchi, M. and Stohs, S.J. Dept. of Pharmaceutical Sciences, Creighton University, School of Pharmacy & Allied Health Professions, Omaha, NE 68178.

Lipid peroxidation has been implicated in retinal degenerations such as those induced by constant illumination and exposure to oxygen. The aim of the present study was to investigate the effect of age on membrane lipid peroxidation in photoreceptor cells of the rat retina. Rod outer segments were isolated from Long-Evans rats aged between 23-90 days old as described by (1). Lipid peroxidation was determined colorimetrically based on the formation of thiobarbituric acid reactive substances (TBARS) using malondialdehyde as the standard. Absorbance values were measured at 535 nM and an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} was used. The following values were obtained for TBARS content in isolated rod outer segments from animals at different ages:

Age	TBARS Content (nmoles/mg protein)
23 days	4.28 ± 0.17
45 days	5.96 ± 0.21
90 days	8.66 ± 0.31

Values give are means ± S.E.M. of 4-6 experiments.

TBARS content in rod outer segments from 90 days old rats was about two-fold higher than in those obtained from 23 days old animals. Our results demonstrate that lipid peroxidation in isolated rod outer segment increases with age in retina from Long Evans rats.

(1) Chaitin, M.H. & Hall, M.O.: Invest. Ophthalmol. Vis. Sci. 24, 812-820, 1983

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352.12

THE SPATIAL PROPERTIES OF LIGHT ADAPTATION IN CAT HORIZONTAL CELLS. M.J.M. Lankheet, A.W. Przybyszewski and W.A. van de Grind (SPON: European Neuroscience Association). Utrecht Biophysics Research Institute, Department of Comp. Physiology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Light adaptation in cone driven cat retinal ganglion cells has been shown to be a localized process. The size of the adaptation pool for the center mechanism was found to be at least as small as the size of an X cell center (Cleland and Freeman, 1988). In order to investigate whether photopic light adaptation takes place in the cones or, alternatively in the bipolars, we have studied the spatial spread of light adaptation in cat horizontal (H-) cell responses. H-cell activity was recorded in the optically intact, *in situ* eye. The mean luminance levels were in the high mesopic and photopic range and H-cell responses were mainly cone driven.

It was found that, in the photopic range, light adaptation is not a strictly local process. Adaptation signals effectively spread into non-illuminated parts of the H-cell receptive field. The size of the adaptation pool was quantified by measuring the increment threshold as a function of the diameter of an adapting background spot. The integration area for adaptation signals was found to be comparable to the size of the receptive field for the response proper.

These findings suggest that the spread of adaptation occurs in H-cells rather than in the photoreceptors. The results will be discussed in the light of ganglion cell receptive field organization and light adaptation mechanisms for ganglion cell centre and surround mechanisms.

352.13

DIFFERENT FORMS OF GLUTAMIC ACID DECARBOXYLASE (GAD) IN CAT AND MONKEY HORIZONTAL CELLS. N. Vardi*, A. Tabaeezadeh, D. Kaufman[1] and P. Sterling. Dept. Anat., Univ. of Pennsylvania, Philadelphia, PA 19104-6058. [1]Dept Psychiatry and Biobehavioral Sciences, UCLA, Los Angeles, CA 90024-1759.

Evidence that mammalian horizontal cells are GABAergic has been equivocal. Both cat and monkey horizontal cells are immunoreactive for endogenous GABA but do not accumulate exogenous GABA. Cat horizontal cells label with a mRNA probe for GAD, but they do not react with a widely used antiserum to GAD (from Oertel et al.). Monkey horizontal cells show the opposite pattern: they are negative for the mRNA probe but some do react with the Oertel antiserum. Since GAD was recently shown to be of 2 forms, 65 kDa and 67 kDa, we considered whether the apparently conflicting results might arise from a mismatch between these probes and the forms of GAD present in horizontal cells of the two species. Accordingly, we stained sections of aldehyde-fixed cat and monkey retina with antibodies specific for GAD65 (Chang and Gottlieb, '88; J. Neurosci 8: 2123) and GAD67 (Kaufman et al., '91; J. Neurochem 56: 720). Cat horizontal cells (types A & B) were immunonegative for GAD65 but positive for GAD67. Somas stained, as did their processes in the outer plexiform layer, including the type B axon terminal that invaginates the rod spherule. Monkey horizontal cells (types H1 & H2) showed the opposite pattern: somas and processes were immunopositive for GAD65 but negative for GAD67. The immunoreactivity for GAD67 in cat and its absence in monkey fit the hybridization results which showed presence of GAD mRNA in cat and absence in monkey. Indeed, this mRNA prob was directed at GAD67. This reconciles previous inconsistencies in the chain of evidence identifying GAD in mammalian horizontal cells and strengthens the conclusion that these cells are GABAergic.

352.14

GLUTAMATE MODULATES A CALCIUM CURRENT IN CATFISH HORIZONTAL CELLS THROUGH CHANGES IN INTRACELLULAR pH (pH_i). D.B. Dixon*, K-I Takahashi and D.R. Copenhagen. Dept. Ophthalmology, UCSF San Francisco, CA 94143

Glutamate is thought to be released tonically from photoreceptor and bipolar cells. Light evoked excitation of all classes of neuron postsynaptic to the rods and cones is via activation of APB, AMPA, and NMDA type receptors. We report here that glutamate can have a modulatory role on voltage-dependent currents in horizontal cells.

We used the whole-cell patch-clamp technique to record from isolated retinal horizontal cells. We studied a high threshold, non-inactivating calcium current (ICa^{++}) which is blocked by nifedipine and cadmium. This current activates at potentials above -30 mV and contributes to the resting potential in darkness.

Earlier studies demonstrated that ICa^{++} is altered by pH_i (Takahashi et al, 1992). Intracellular acidification induced by bath application of acetate, or increased CO_2 or washout of NH_4Cl reduced ICa^{++}. Here we report that bath applied glutamate (1-5 μM) reversibly reduces the peak ICa^{++} by 27.8 ±6.5% (n=6). Horizontal cells loaded with the pH indicator dye BCECF-AM were acidified by glutamate. We tested the hypothesis that the glutamate-induced reduction in ICa^{++} was mediated via pH. This was done by loading the cells with patch pipet solutions containing high concentrations of pH buffer (25 mM HEPES). Under these conditions, glutamate suppression of ICa^{++} was reduced. Additionally, simultaneous measurements of pH_i and ICa^{++} in single cells indicate that glutamate-induced acidification and reduction of ICa^{++} can be recorded concurrently.

We propose that glutamate may have dual roles in the retina. It is a conventional excitatory neurotransmitter and may also be a neuromodulator which can influence cell excitability and transmitter release through modulation of ICa^{++}.

352.15

DOPAMINE REDUCES CHANNEL OPEN TIME IN ZEBRAFISH HORIZONTAL CELL ELECTRICAL SYNAPSES. D. G. McMahon,* Dept. of Physiology and Biophysics, University of Kentucky, Lexington, KY 40536.

My laboratory is interested in defining the molecular mechanisms of synaptic modulation in the teleost retina. The zebrafish (Brachydanio rerio) is an attractive preparation for combining biophysical and molecular approaches to the study of retinal function. I now report in vitro studies of zebrafish horizontal cell electrical coupling and its modulation by dopamine.

Horizontal cells were dissociated and maintained in cell culture by standard techniques. Electrical coupling between contacting cell pairs was assayed by dual whole-cell patch clamp recording. Junctional conductance averaged 1.7 nS (23 pairs). Application of 50 uM dopamine reduced coupling in 5 of 7 pairs to an average of 45% of control, while 100 uM dopamine reduced coupling in 5 of 6 pairs to 41% of control. Dopamine's action peaked after approximately 1 minute and recovery took 2-5 minutes. Dopamine did not alter extrajunctional voltage-gated conductances. In poorly coupled pairs, junctional channel activity of 25-35 pS, 65-75 pS and 130-140 pS was observed. In 3 such pairs, application of dopamine reduced coupling by reducing the frequency of junctional channel openings. The duration of channel openings, as analyzed by exponential fits of dwell-time histograms, was reduced by dopamine. At the 65-75 pS conductance level the mean time constant was reduced from 22.1 msec to 16.6 msec (1038 and 302 events). The mean unitary conductance was unchanged (71 pS pre dopamine vs 73 pS post). Similar results were observed at the 25-35 pS level.

These results provide a primary description of horizontal cell electrical coupling in the zebrafish and indicate that dopamine modulates coupling in a manner similar to its previously described action in other teleosts (perch, bass, catfish). Supported by NIH EY09256 and the UKMC Research Fund.

352.16

MEMBRANE CURRENTS OF XENOPUS RETINAL HORIZONTAL CELLS A. Akopian and P. Witkovsky* Dept. Ophthalmology, New York University Medical Center, New York, N.Y. 10016

We studied membrane currents of horizontal cells using the whole cell version of the patch clamp. Horizontal cells were isolated and maintained in short term culture by standard methods. The cells resembled those isolated from salamander retina (Gilbertson et al. J. Neurophysiol. 66:2002, 1991) and were distinguished from other cell types we tested by the presence of a prominent, fast-activating inward rectifier. In normal Ringer solution, the current-voltage function was triphasic, with a region of negative slope conductance between ca. -30 and -10 mV. The following currents were distinguished by pharmacological treatment: an inward rectifier active below -70 mV, a fast inward Na current, a prominent A-current blocked by 4-AP and a delayed outward rectifier, blocked by TEA. In the presence of a cocktail of TTX, 4-AP and TEA, two calcium currents were identified. A sustained, L-type current, blocked by Cd, was activated near -20 mV and peaked at 0 mV. A Cd-resistant, transient Ca current activated near -50 mV and peaked at -20 mV. Supported by EY 03570 to P.W.

352.17

THRESHOLD EFFECTS OF DOPAMINE IN THE XENOPUS RETINA. D. Krizaj* and P. Witkovsky, Depts. Ophthal. and Physiol. & Biophys. NYU Med Ctr., New York, N.Y. 10016

The extracellular concentration of dopamine (DA) in the Xenopus retina ranges from 150-600 nM as a function of lighting conditions and time of day. DA levels were high in light near subjective dawn and low in darkness. We reported previously (1) that a threshold dose for the DA-induced alteration and rod/cone input to horizontal cells (HC) was 2 um. We now find that if ascorbate is added to the solution, DA effects are obtained reliably at 0.5 μm. We recorded simultaneously from rods and HCs in superfused eyecups. Rod responses were elicited with scotopically balanced red and green flashes to test for possible cone input to the rod, as reported for salamander rods (2), but none was found. Under scotopic conditions, red and green LED's were modulated sinusoidally in counterphase and adjusted in intensity to produce a null response for both rod and HC. Both cells followed flicker to 4-6 Hz. The gain of the synaptic transfer was $\leqslant 10$. Addition of 0.5 - 1.0 μM DA was without effect on the rod null but it evoked a large imbalance in the HC. Red stimuli now elicited a greatly increased HC response that followed to 15 Hz. The DA effect was blocked by cis-flupenthixol. These data indicate that DA levels in the physiological range influence transmission of rod and cone signals through the retinal network. 1) Witkovsky et al., J. Neurophysiol. 62:864,1989 2)Hare & Owen, J. Physiol. 445:741, 1992. Support EY 03570

352.18

DOPAMINE AND cAMP ACTIVATORS RELEASE INTRACELLULAR CALCIUM AND MODULATE THE CALCIUM CURRENT IN ISOLATED CATFISH HORIZONTAL CELLS.

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Dopamine has been implicated in the regulation of the voltage sensitive calcium channel in retinal ganglion cells (Liu & Lasater, ARVO, 1992). Its effect is thought to be due to cAMP activation of protein kinase A. We have measured the effects of dopamine, forskolin, 8-bromo-cAMP and other cAMP/PKA modulators on both the calcium current and calcium release from intracellular stores in isolated cone horizontal cells. Dopamine either increases, decreases or has no effect on the calcium current measured in voltage clamped cells. Forskolin, a cAMP activator, always decreases the calcium current and this decrease can be reversed in some cases by dopamine

Catfish horizontal cells have caffeine sensitive intracellular calcium stores. Dopamine, forskolin and 8-bromo-cAMP increase intracellular calcium measured in fura-2 loaded cells. The release is immediate, reaches a maximum within several seconds and declines slowly. The amount of the calcium increase is concentration dependent. These results support the hypothesis that the rise in intracellular calcium can modulate directly the calcium channel activity by a calcium dependent process.

Supported by Grant NIH EY-01897.

352.19

SEROTONIN-LIKE IMMUNOREACTIVITY AS A MARKER TO IDENTIFY ISOLATED BIPOLAR CELLS FROM THE SKATE RETINA.

R.L.Chappell*, E.Schlemermeyer, C.V.Logan, F.Mouly and M.Artinian. Hunter College and Graduate Center of the City University of New York, New York, NY 10021.

The all-rod nature of the retina of the skate (*Raja erinacea*) makes it of special interest when considering the role bipolar cells play in rod pathways from the outer to the inner plexiform layer. Our earlier studies using immunocytochemical techniques demonstrated PKC-like immunoreactivity in one class of skate bipolar cell having a morphology typical of "rod" ON-bipolar cells in duplex retinas. By preloading the retina with serotonin in the presence of pargyline, we were also able to confirm the presence of bipolar cells showing serotonin-like immunoreactivity (SLI). The SLI bipolar cell morphology, however, was found to be characteristic of OFF-bipolar cells with terminals branching in layers 1 and 4 of the inner plexiform layer. Double labelling confirmed that these two classes of skate bipolar cell were indeed distinct.

We have now applied techniques for SLI developed on the intact skate retina to the identification of its isolated bipolar cells in culture, thus providing a basis for identification of SLI bipolar cells with their electrophysiological and pharmacological properties when studied using whole-cell patch recording techniques following retinal dissociation. Successful labelling of a population of isolated skate bipolar cells for SLI was accomplished using both FITC immunofluorescence and the ABC method with diaminobenzidine as the chromophore. In each case, the isolated bipolar cells were pre-loaded for one hour in skate Ringer containing 20μM serotonin and 1mM pargyline. (Supported by NIH Grant EY-00777.)

352.20

QUANTAL EXCITATORY TRANSMISSION TO OFF-CENTER BIPOLAR CELLS IN THE SALAMANDER RETINA.

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Spontaneous excitatory miniature synaptic currents were studied in voltage clamped bipolar cells under conditions in which presynaptic calcium influx was suppressed (2 mM Cobalt, 0 mM Calcium superfusate). Bandpass filtered excitatory events were fit to a gaussian-filtered difference of exponentials, and from this two time constants, an amplitude, and an integrated charge were obtained for each hypothetical unfiltered event. Plotted as a density in an amplitude vs. charge graph, the events tended to cluster at regular intervals along several different slopes. This suggested the existence of several kinetically distinct types of quanta, each of which corresponded to the synchronous discharge of some number of presynaptic vesicles. Events were also scattered in a manner consistent with a process in which release was not perfectly synchronous, but tended to occur in clusters.

A useful measure of event kinetics was the charge/amplitude ratio, which was related to event "duration". For each kinetically distinct class of event, the amplitude distribution was heavily skewed towards the fundamental subunit. The following fundamental subunits (in terms of "duration" and peak amplitude) were observed at -50 mV: 2.3 msec, 0.9 pA and 2.4 pA; 4.3 msec, 2.3 pA; 7.4 msec, 1.2 pA and 5.5 pA. All three classes of kinetics were observed in most cells (even when axotomized), but in greatly varying proportions. Cells with telodendria ramifying in the distal 1/4 of the inner plexiform layer were strongly dominated by the 2.3 msec events, while "Off" cells with telodendria ramifying more centrally in the IPL were dominated by the 7.4 msec events. 20 uM CNQX greatly reduced the mean amplitudes for all charge/amplitude ratios. Most likely, these quantal events are inputs from photoreceptor and dendrodendritic bipolar cell synapses.

Since the concentration of glutamate in synaptic vesicles is probably extremely large compared to the $K_{1/2}$ for bipolar cell glutamate receptors, the apparently independent action of vesicles released synchronously at the same active zone suggests that uptake is extremely rapid, and that the kinetic diferences observed reflect different glutamate receptor types.

AUDITORY SYSTEM: CENTRAL PHYSIOLOGY III

353.1

FREQUENCY-RELATED DIFFERENCES IN THE SPEED OF HUMAN AUDITORY PROCESSING.

David L. Woods*, Claude Alain, Oren Zaidel, and Diego Covarrubias, Dept of Neurology, U.C. Davis, VAMC, Martinez, CA, 94553.

Two experiments were performed comparing auditory evoked potentials (AEPs) elicited by loudness-matched 250 Hz and 4000 Hz tone pips. Randomized sequences of stimuli were presented at short interstimulus intervals (mean 40-200 ms). The EEG was digitized continuously from 31 electrodes at a high rate (833 Hz/channel) to permit the simultaneous analysis of wave V (mean peak latency 6.8 ms), Na (20.1 ms), Pa (29.8 ms), Nb (38.4 ms), Pl (51.1 ms) and N1 (101.1 ms) components. In comparison with peak latencies following the 4000 Hz tones, the peak latencies of all components were delayed by 12.0-23.1% following 250 Hz tones. Frequency-related differences increased with component latency, ranging from 0.9 ms for wave V, to 23.0 ms for the N1. Interpeak latency differences were also prolonged. The results indicate central delays in the processing of low frequency auditory signals. The behavioral significance of these delays was evaluated in a a third experiment comparing simple reaction times (RTs) to the same stimuli. RTs were delayed by 15 ms following the 250 Hz tones. The rapid processing of high signal frequencies may play a role in the localization of sounds in space. Supported by a grant from the NIDCD to DLW and by a FCAR postdoctoral fellowship to CA.

353.2

RESPONSES OF CELLS IN AN AUDITORY THALAMIC NUCLEUS OF THE BARN OWL TO SOUND LOCALIZATION CUES.

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In the Barn Owl, nucleus ovoidalis is a thalamic nucleus which receives ascending input from a midbrain auditory nucleus and projects to the forebrain auditory area, Field L. Field L contains neurons which are highly sensitive to sound location, as does the external nucleus of the inferior colliculus (ICx) (Knudsen & Konishi, Science, 202:778-780, 1978). ICx space-specific units respond only to sounds with a particular combination of interaural time difference (ITD) and interaural level difference (ILD). To date, ICx has been found to project exclusively to the optic tectum. This study was undertaken to determine the role of nucleus ovoidalis in processing ITD and ILD information and relaying this information to the forebrain.

We have recorded extracellular single unit responses from cells in nucleus ovoidalis which were stimulated with digitally synthesized sound (tone or noise) presented dichotically. Frequency is mapped along the dorsal-ventral axis of the nucleus, with higher frequencies represented dorsally. Isolated units had best frequencies which ranged from 10 KHz down to 500 Hz. The majority of units had rather narrow frequency tuning (approx 1 KHz wide at half peak) although a few units were isolated with much broader frequency tuning (approx. 5 KHz wide at half peak). Units typically were inhibited below spontaneous activity levels at frequency side bands flanking the best frequency peak. The majority of units isolated thus far were tuned to both ITD and ILD. Of the units which were tuned to ITD, none exhibited side peak suppression to noise stimuli, indicating tha phase ambiguity is preserved in these cells. The majority of ILD tuned neurons which have been isolated were either excited by stimuli from the contralateral ear and inhibited by stimuli from the ipsilateral ear (EI), or inhibited by the contralateral ear and excited by the ipsilateral ear (IE), with neither population apparently overrepresented. A few units were isolated which were selective for ILD (bell shaped tuning curves). Additionally, a few cells have been isolated which responded selectively either to ITD or ILD but not both.

353.3

CODING ASSOCIATIVE AUDITORY INFORMATION BY SPECTRAL GRADIENT ENHANCEMENT. F. Ohl, C. Simonis, H. Scheich*. Dept. of Zoology, Tech. Univ., 6100 Darmstadt, Germany.

Learning-induced physiological plasticity in the adult auditory cortex has previously been demonstrated by a variety of techniques. A basic finding in imaging studies is the enlargement of representational areas which might correspond to the enhanced firing probability of single neurons as revealed by unit recordings. In addition, using the 2-deoxyglucose method we demonstrated asymmetric shifts of activated areas in the primary auditory cortical field A1 of Mongolian Gerbils towards lower frequencies during the aquisition phase of an associative learning task ("tone bursts predict foot shocks") (Scheich, H., Simonis, C., Soc. Neurosci. Abstr., 17, 1991, p. 450). This implies that beside activity changes at the frequency-specific location within a tonotopic map there must exist learning mechanisms that produce oriented shifts of the frequency representation.

To gain further insight into the relevant dynamic mechanisms we recorded electrophysiologically from 74 neurons in A1 of awake gerbils. Presenting mild electrodermal stimuli (EDS) to the animals' tails alone or pairing them with tone bursts of different frequencies increased or decreased the spike rate of 63% of the neurons up to 90%. The effects were non-frequency-specific in that the changes were proportional to the unit's tuning before conditioning. If, however, the EDS were paired with only one of the frequencies presented (CS+) the change of spike rate in response to frequencies neighbouring the CS+ was more positive than to the CS+ itself. This causes the CS+-response to lie on a point on the tuning curve (and supposedly in the map) where the change of activity along the spatial frequency gradient became enhanced. Hence, the increase of this spectral gradient (contrast) could be the most consistent and relevant feature of the dynamic rearrangement. This interpretation is also in accordance with the occurence of the maximum increase of the CS+-response istself reported by other authors (Weinberger, NM. et al., Concepts in Neurosci., 1, 1990, p. 91), because there the CS+ would come to lie on a spot of maximum spectral gradient, too. *Supported by DFG, SFB 45.*

353.4

PHYSIOLOGICAL MECHANISMS FOR CATEGORICAL RESPONSES REFLECTING THE VOICE ONSET TIME BOUNDARY IN AUDITORY CORTEX. M. Steinschneider*, C.E. Schroeder, J.C. Arezzo and H.G. Vaughan, Jr., Albert Einstein College of Medicine, Bronx, NY, 10461.

Temporal information processing is a key component of speech perception. Its importance is exemplified by the selective impairment for discriminating rapidly changing sounds in dysphasic children. We studied the temporal pattern of neural activity which encodes the voice onset time (VOT) speech parameter by examining laminar profiles of current source density and multiunit activity to the syllables /da/ and /ta/ in auditory cortex of awake monkeys. VOT, the time interval between stimulus onset and stimulus periodicity, was varied from 0 to 80 msec in 20 msec increments.

VOT is reflected by responses time-locked to stimulus onset and to the onset of stimulus periodicity. Phasic responses to the latter speech segment are present with VOTs of 40 msec or longer and diminished for shorter VOTs. These patterns correlate with the /da/-/ta/ perceptual boundary. Two different mechanisms are responsible for this response differentiation: (1) hyperpolarizing events suggestive of active inhibitory processes follow an initial excitatory response to stimulus onset and diminish the second excitatory response to /da/, and (2) prolonged depolarizing events following stimulus onset attenuate the second excitatory response to /da/.

We conclude that both excitatory and inhibitory auditory cortical mechanisms determine the categorical VOT perceptual boundary, and suggest that dysfunction of these temporal processing mechanisms may contribute to the speech perception deficits of dysphasic children.
(supported by DC00657, MH06723 and the J.S. McDonnell Foundation).

353.5

SENSITIVITY OF NEURONS IN THE INFERIOR COLLICULUS OF THE UNANESTHETIZED RABBIT TO INTERAURAL TEMPORAL DISPARITIES OF THE ENVELOPES OF HIGH-FREQUENCY TONES. R. Batra*, S. Kuwada, and T.S. Stanford, Dept. of Anatomy, Univ. of Conn. Health Center, Farmington, CT 06030.

Interaural temporal disparities (ITDs) have traditionally been considered a cue for the azimuthal localization of low-frequency tones, that is, tones which have a half-wavelength greater than the width of the head. It is now apparent that ITDs may also be a cue in locating a high-frequency sound, if the sound has a low-frequency envelope. Neurons in the auditory pathways are indeed sensitive to this cue. Here we report the properties of neurons in the inferior colliculus (IC) of the unanesthetized rabbit to ITDs of the envelopes of sinusoidally amplitude-modulated, high-frequency (≥ 2 kHz) tones.

Neurons that were sensitive to ITDs fell into two groups: those that were maximally excited at the same ITD at modulation frequencies > 250 Hz (MSO-like neurons) and those that were maximally suppressed at the same ITD (LSO-like neurons). We restricted our analysis to modulation frequencies > 250 Hz because at lower frequencies many neurons were sensitive to large ITDs which were unlikely to be associated with sound localization.

Most neurons of both types had characteristic delays in the range which the rabbit is likely to encounter in the free field (± 300 μs). Most delay curves of MSO-like neurons had peaks within ± 300 μs, while most delay curves of LSO-like neurons had troughs within this range.

The peaks of the delay curves of MSO-like neurons were narrower than the troughs of the delay curves of LSO-like neurons, and nearly as narrow as the peaks of the delay curves of neurons sensitive to ITDs of low-frequency pure tones.

Thus, the coding for ITDs of envelopes is nearly as precise as that for ITDs of low-frequency pure tones. The MSO-like neurons may encode the location of a complex high-frequency sound, while the LSO-like neurons may help to sharpen the receptive fields of MSO-like neurons.

This study was supported by NIH grant NS 18027 to S.K.

353.6

EFFECTS OF INFERIOR COLLICULUS LESIONS ON THE AUDITORY BRAINSTEM RESPONSE IN RATS. D.G. Campbell and J.R. Coleman*, Univ. South Carolina, Columbia, SC 29208.

The auditory brainstem response (ABR) is used experimentally and clinically to monitor the integrity of peripheral and central auditory pathways. The role of the inferior colliculus (IC) as a contributor to averaged surface potentials was examined in the rat. Bilateral IC lesions were made in male Long-Evans rats (90 days of age) and responses compared to age-matched controls. Electrocoagulative lesions were made stereotaxically under Ketamine/Acepromazine (100:1 mg/kg) anaesthesia. The ABR generating stimuli were 85 dB peSPL noise bursts (1.5 ms duration, 11.1/sec) presented monaurally to both left and right ears. Surface potentials were recorded (20 ms window, 512 presentations) from subdermal electrodes under Ketamine/Acepromazine (60:1 mg/kg) anaesthesia. Control animals displayed five major wave components (Cooper et al., Hear. Res. 43:171-180, 1990) followed by a negative deflection around 6.5 ms. Significant differences in the amplitudes of the IV-V wave complex ($p<.0001$) and the following negative deflection ($p<.01$) were detected. Histology confirmed that experimental subjects had bilateral lesions of the caudal IC. It was concluded that the IC contributes to both the IV-V wave complex and the negative deflection at 6.5 ms in the rat.

Supported by NSF DBS-9200624 and the Deafness Research Foundation.

353.7

RESPONSE OF NEURONS IN LATERAL SUPERIOR OLIVE AND MEDIAL NUCLEUS OF THE TRAPEZOID BODY TO REPETITIVE STIMULATION: A BRAIN SLICE STUDY OF TEMPORAL INTEGRATION IN THE SUPERIOR OLIVARY COMPLEX. S.H. Wu* and J.B. Kelly. Laboratory of Sensory Neuroscience, Psychology Department, Carleton University, Ottawa, Canada K1S 5B6.

Temporal integration in the superior olivary complex was investigated by recording the response of neurons in a mouse brain slice to repeated electrical stimulation of the trapezoid body. A 400 μm brain slice was taken through the brain stem and maintained in an oxygenated saline solution following the procedure of Wu and Kelly (1991). Stimulating electrodes were placed on the trapezoid body and both extracellular and intracellular responses were recorded from the lateral superior olive (LSO) and the medial nucleus of the trapezoid body (MNTB). In both structures, a single current pulse of sufficient strength elicited a single action potential. Excitatory postsynaptic potentials were apparent in intracellular recordings. In the MNTB, the neurons were capable of following very high rates of stimulation without reduction of response probability. Almost every MNTB neuron was capable of following repetition rates of 500 Hz and most were capable of following 1000 Hz. In contrast, most LSO neurons were incapable of following high rates of stimulation. Only a few neurons were capable of following rates of 500 Hz. The difference between LSO and MNTB in following repeated current pulse stimulation supports the idea that MNTB neurons are specialized for preservation of temporal information, whereas LSO neurons are specialized for binaural integration.

Research supported by NSERC and Human Frontier Science Program.

353.8

AUDITORY CORTICAL EVOKED POTENTIALS IN THE CAT: SENSITIVITY TO SMALL BINAURAL TIME DIFFERENCES. J.B. Kelly[1]* and D.P. Phillips[2]. [1]Laboratory of Sensory Neuroscience, Dept. of Psychology, Carleton University, Ottawa, Ontario K1S 5B6 and [2]Departments of Psychology and Otolaryngology, Dalhousie University, Halifax, Nova Scotia B3H 4J1.

The effect of binaural stimulation on the amplitude of click-evoked cortical potentials was investigated in barbiturate-anesthetized cats. Recordings were made with silver ball electrodes placed on the cortical surface and time-locked responses were averaged on line to obtain measures of latency and peak-to-peak amplitude. Evoked response amplitude was found to vary as a function of the interaural time difference (ITD) between paired clicks delivered to the two ears. Generally, the response amplitude was greatest when the leading click was delivered to the ear contralateral to the recording site. However, there was considerable diversity among recordings in the shape of the binaural response curve and the magnitude of the change in response amplitude. For some recordings, the response was reduced as much as 30% as the ITD was shifted in favor of the ipsilateral ear. For others, the reduction was as little as 5%. The effect of ITD was reflected in the ratio of response amplitudes recorded from the two hemispheres. The dynamic range of ITDs over which response amplitude was found to vary was around 1000 μs in the cat. Comparable ITD data from the albino rat revealed a larger depth of response modulation, typically more than 50% amplitude reduction, and a smaller dynamic range, approximately 500 μs. These differences are presented in terms of neural mechanisms for binaural processing in the two species.

Research was supported by NSERC grants to J.B.K. and D.P.P.

353.9

INTERICTAL CHANGES AND CENTRAL AUDITORY PROCESSING.
D M Daly*. Box 210855, Dallas, TX 75211.

Focal seizures disrupt function of the cortical areas in which they occur. When balance of inhibitions declines/fails, patients with seizures involving auditory cortex have reported certain sets of synthesized stop-like sounds as nasals, 'bleats', and then undifferentiable buzzes[1]. We here report changes in central auditory processing during supervised withdrawal of medication.

A 22 year old man developed partial seizures characterized by translational vertigo, blurred vision, and difficulty comprehending (but not hearing) speech; head and eyes may deviate left and left upper extremity may elevate; he then briefly has difficulty finding words and may appear mute. Spells occurred when he was inactive or not alert. EEGs with sphenoidals revealed a sleep sensitive left mesial temporal spike focus and spike discharges over right frontal regions.

Auditory testing with concurrent EEG began 24 h after last medication (CBZ, VPA). Performance differed significantly ($p<0.0001$) from standards (16 healthy; 16 patients with CPS medically controlled at least 6 mo), and from patient's own controlled performance. Sounds the patient had previously classified as [be]-[de]-[ge] he reported now as 'bleats' or buzzes; on [ge]-[ye] the mid point of the boundary decreased 20 ms. These changes persisted over 100 min. From the EEG, spikes occurred infrequently after responses; on occasion bursts of spikes occurred in the interval between sets and stopped when testing resumed.

Results indicate that diminished inhibition, and even disinhibition can persist without overt electrical concomitants in EEG.

[1] J Neurophysiol (44:1, 200, 1980); *Kindling II* (219, 1981). Testing contributed by inventor who retains all proprietary rights and interests.

353.10

RATE REPRESENTATION OF SECOND FORMANT FREQUENCIES OF /ɛ/-LIKE STEADY-STATE VOWELS IN CAT AUDITORY NERVE.
R. A. Conley* and S. E. Keilson Johns Hopkins Center for Hearing Sciences Baltimore, MD 21205

The encoding of steady-state vowels in the firing rate of auditory nerve fibers has been well studied (Sachs & Young, 1979; Sinex & Geisler, 1983; Miller & Sachs, 1983; Delgutte & Kiang, 1984; Delgutte, 1984; Deng & Geisler, 1987). These studies have shown that rate profiles from high spontaneous rate fibers encode formants of steady-state vowel stimuli at low to moderate sound pressure levels and that low and medium spontaneous rate fibers provide a good representation at high sound levels.

In this study, coding of the second formant of the vowel /ɛ/ was studied in the auditory nerve using synthetic vowel stimuli. Alternate forms of /ɛ/ with second formant peaks located at 1400, 1700 and 2000 Hz were generated synthetically and used as stimuli. Responses of a population of auditory nerve fibers to these three stimuli were recorded at two sound levels, 50 and 70 dB SPL.

Plots of mean spike rate or normalized rate vs. fiber characteristic frequency (CF) show second formant peaks, even in the high spontaneous population at 70 dB. Such peaks decrease as sound level increases and as formant frequency is lowered. The formant peaks are clearly represented in plots of rate differences between two vowels. Such plots closely resemble the ratio of the magnitudes of the two vowel spectra. The results suggest that information concerning the position of vowel formant peaks is present in the spike rate of the auditory nerve. These data will be used to predict cats' performance in discriminating vowel second formant frequency. Supported by grants from NIDCD.

353.11

UNMASKING OF AUDITORY TARGETS AND ENHANCEMENT OF SELECTIVITY TO A SOUND LOCALIZATION CUE BY SIMULATED MOTION IN THE OWL'S INFERIOR COLLICULUS.
TT Takahashi and CH Keller*Inst. of Neuroscience, Univ. of Oregon, Eugene OR 97405.

We tested, at the neuronal level, whether motion facilitates the detection of acoustic targets, as it does visual targets. Auditory neurons in the central nucleus of the barn owl's inferior colliculus (ICc), due to their selectivity for interaural phase difference ($\Delta\Phi$), are tuned to the azimuth of sound-sources and form a topographic map of $\Delta\Phi$. While recording from single ICc neurons, we presented tones that simulated either moving or stationary sound sources with or without background noise. The neuron's $\Delta\Phi$-specific response, quantified by the vector strength (analagous to the standard deviation), served as a measure of the neuron's ability to detect the tone.

Results suggest that neurons of the ICc are capable of exploiting the motion of sound sources. Tones that mimicked a stationary source could be obscured by noise, as judged by a neuron's vector strength, but if the $\Delta\Phi$ changed continuously thus mimicking motion, the neuron's $\Delta\Phi$-specific response re-emerged. The latter was not observed when the stimulus mimicked a stationary, amplitude-modulated tone-source, suggesting that a change of position was required rather than a simple time-varying stimulus. The cells also showed a higher selectivity for $\Delta\Phi$ in the absence of noise if the target-tone simulated motion. These results suggest that the place-code for space in the ICc is of finer grain for moving sources.

These phenomena were demonstrated with tonal stimuli, thus they can be subserved by neuronal interactions within a single isofrequency layer of the ICc. Since modulations in $\Delta\Phi$, but not in amplitude, could produce these phenomena, it is likely that the mechanism itself involves interactions between cells that represent adjacent $\Delta\Phi$ values.

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353.12

TOPOGRAPHIC REPRSENTATION OF MULTIPLE ACOUSTIC TARGETS BY A NEURAL NETWORK.
MK Fleming, WJ Goodwin, and TT Takahashi*. Dept of Psych., Stanford Univ., Stanford, CA & Inst. of Neurosci., Univ. of Oregon, Eugene, OR.

The barn owl's inferior colliculus contains a visual-system-like map of space built from auditory units with spatial receptive-fields. Visual maps can easily represent more than one stimulus. In the auditory system, however, sound-waves from multiple sources will sum at the eardrums, and, if two or more sound sources broadcast common spectral components, the binaural cues for the shared components, such as interaural phase and intensity differences, assume values that are the vector averages of those frequencies from the individual sources. For an auditory space-map to represent multiple targets accurately, other cues are therefore required. We investigated whether the owl's auditory space map could represent two acoustic targets, given that the two targets had some unshared spectral components. Using standard back-propagation algorithms we trained a two-layered network to parse simulated sound-sources into hotspots of neural activity along a one-dimensional space map. The input layer consisted of units, which, like the cells of the central inferior collicular nucleus (ICc), were selective for a frequency and an interaural phase difference ($\Delta\Phi$). Simulated sources at given spatial loci can be represented as a spectrum of $\Delta\Phi$s by this input layer. The output layer, like the external inferior collicular nucleus (ICx), consisted of neurons that were selective for a particular $\Delta\Phi$ spectrum. Following training, the network was capable of accurately representing the targets' number and locations on the one-dimensional space map, provided that the simulated sources broadcast some unshared frequencies and that shared frequencies were represented with differing amplitudes. Most interestingly, analysis of the connectional weights revealed a projection pattern between input and output layers that was strikingly similar to the known connectivity between the ICc and ICx in the owl.

353.13

Quantitative Analysis of the Neurophonic Frequency-Following Potential in the Central Auditory Pathway of the Long-Evans Hooded Rat.
D.S. Weiss* and S. Reinis. *Dept of Psychology, Univ of Waterloo, Waterloo, ONT, Canada, N2L 3G1.*

The neurophonic potential (NP) was observed in the auditory pathway of the Long-Evans rat in the lateral lemniscus (LL), lateral superior olive (LSO), trapezoid body (TB), and inferior colliculus (IC). The NP was not observed in the medial geniculate body nor was it organized tonotopically in the IC. However, a rudimentary organization of NP frequency was observed along the rostrocaudal dimension. The rostral IC was characterized by low frequency NPs between 400 to 1900 Hz. The response at the central nucleus reached the upper limits of the sound system at 4500 Hz. More caudal aspects of the IC were characterized by NP frequencies between 600 to 2700 Hz. Bands of neurophonic activity in the IC were interspersed with regions of NP absence. The NP persisted following a small electrolytic lesion suggesting that volume conduction played a role in the local transmission of the NP. Lesioning the LSO or TB ipsilateral to the stimulus abolished the neurophonic from the contralateral IC. The mass activity of cells was evaluated using high resolution histograms and correlograms of interspike intervals. Raw waveforms were passed through a narrow band digital filter to remove the neurophonic sinusoid. Histograms constructed from both filtered and unfiltered data were characterized by peaks at regular multiples of the stimulus period. Two consistent findings in the mass activity correlograms for the filtered data were (i) a significant dip near the origin of the graph, and (ii) the dip was followed by one or more significant peaks at multiples of the stimulus period. It is suggested that the NP was generated by a graduated fluctuation in the baseline activity of an ensemble of fibers or cells phase-locked to the stimulus tone. These results are discussed in relation to the volley principle of hearing.

353.14

FREE FIELD RESPONSES OF CELLS TO STATIC AND MOVING SOUND STIMULI IN THE ANTERIOR ECTOSYLVIAN CORTEX (AEC) OF CATS.
H. Jiang*, P. Poirier, F. Lepore, M. Ptito, and J.-P. Guillemot. Groupe de Rech. en Neuropsy. Exp., Université de Montréal and Université du Québec, Montréal, Qué., Can. H3C 3J7.

The posterior part of the anterior ectosylvian cortex (AEC), surrounded by a number of auditory areas (AI, AII and AAF), has been shown to contain neurons which respond to different characteristics of auditory stimuli. In the present study, single unit activity was recorded along the banks of the posterior part of the anterior ectosylvian sulcus in anesthetized-curarized cats.The stimuli consisted of static bursts of broad band noise or simulated moving sounds emitted from a series of 16 loud-speakers each separated by approximately 10°. These speakers were located at the azimuth on a perimeter surrounding the animal's head in an anechoic room. Most of the units encountered responded to a static sound presented at each of the 16 positions (78° ipsilateral to 78° contralateral). They showed, however, mainly contralateral dominance. Various forms of responses were obtained, including on, off and post-stimulus rebound responses. Some neurons showed long sustained discharges to a static or/and a moving sound. Those units showing directional selectivity responded more strongly to the onset of static sound stimuli presented in the appropriate hemifield. A feature analogous to velocity tuning was also observed in some of the motion sensitive units. The movement sensitivity and the long sustained post-stimulus cell discharges suggest that the AEC may be involved in the processes mediating stimulus induced action in animals.

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353.15

NEURONAL RESPONSES TO FREQUENCY MODULATED SOUNDS IN THE ANTERIOR AUDITORY FIELD (AAF) OF THE CAT'S CORTEX. B. Tian* and J.P. Rauschecker. Neuroethology Unit, NIMH, Poolesville, MD 20837, U.S.A. and Max-Planck-Institut für biologische Kybernetik, W-7400 Tübingen, Germany.

Frequency modulated (FM) sounds are important components of auditory communication signals in many different species, including birds, bats, and primates. In addition, FM sweeps are the auditory equivalent to moving bars or spots of light in the visual domain, which have proved so effective for the stimulation of visual cortical neurons. We have studied responses of neurons to frequency modulated sounds in the cat's anterior auditory field (AAF), which has been suggested on the basis of neurophysiological and anatomical data to be the first area in the ascending auditory cortical pathway besides AI (Rouiller et al., 1991).

Neurons in AAF responded well to FM sweeps with rates ("speeds") between 4 and 600 Hz/ms. Almost half of the units (96/210=46%) responded best to the higher FM rates (high-pass neurons), only 4 % were low-pass units. Fourty-eight percent showed band-pass characteristics with a preference for a certain range of FM rates. The instantaneous frequency at the response peak generally matched the best frequency in the pure-tone tuning curve. However, one third of the neurons responded with multiple peaks when tested with FM sweeps, while most of them were broad-band tuned with a single peak when tested with tone bursts. Two thirds of the neurons in AAF had a preference for one direction of the FM sweep over the other, when a 50%-criterion was applied to the difference in both responses.

The anterior auditory field seems to specialize in the processing of fast-changing, transient sounds, since neurons in other cortical areas more often prefer slower rates of frequency and amplitude modulation (Mendelson & Cynader, 1985; Schreiner & Urbas, 1988). An analogy with the visual cortex of the cat and its parallel organization into areas with different velocity preferences (areas 17 and 18) is still speculative, but could be tested further with anatomical and physiological techniques. The involvement of AAF in the processing of transient sounds contained in species-specific or prey-specific vocalizations and in auditory spatial analysis has to be considered.

353.16

BINAURAL UNMASKING IN FERRETS. J.E. Hine, R.L. Martin and D.R. Moore*. University Laboratory Physiology, Parks Rd., Oxford OX1 3PT, U.K.

In humans it is well-known that the detectability of noise-masked low frequency tones in one ear can be enhanced by presenting the same masking noise to the other ear. We have developed a technique to show binaural unmasking in ferrets using three different free-field speaker configurations. Each task requires discrimination between a 500Hz tone-in-noise stimulus and a noise-alone stimulus. The tone-in-noise is always presented from a speaker positioned 90° right of the ferret's head. The difference between the tasks lies in the presence and position of a second source of identical noise. If present, the second speaker is either placed 90° left or right of the animal's head.

Results have been obtained for four normal adult ferrets using positive conditioning procedures and a descending method of limits. The mean masked threshold for two speakers 90° right (64.5±1.8dB SPL) was significantly higher than both one speaker 90° right (57±0.7dB SPL, $p<0.05$) and two speakers placed 180° apart (54±2.2-dB SPL, $p<0.01$). Thus, there is an improvement in detectability of the tone due to the angular separation of the second source of noise. Data from two of the same ferrets who underwent unilateral cochlear removal suggests that this improvement is due to binaural processing. We intend to use this paradigm to study the functional, binaural consequences of hearing loss.

353.17

AUDITORY BRAINSTEM PROJECTIONS TO THE SUPERIOR COLLICULUS OF THE FERRET A.J. King*, Z.D. Jiang and D.R. Moore Lab. of Physiology, Parks Road, Oxford OX1 3PT, U.K.

The superior colliculus (SC) is not tonotopically organized, but, instead, contains a map of auditory space. We have identified the source of ascending projections to the SC in the ferret, a species in which the auditory representation has been extensively studied. The distribution of retrogradely labelled neurons in auditory brainstem nuclei was examined, following injections of either horseradish peroxidase or rhodamine- and green-latex microspheres into the deeper layers of the SC. The heaviest projections arose from the nucleus of the brachium and the external nucleus of the inferior colliculus (IC), although back-labelled neurons were also found in rostral regions of both the dorsal and ventral nuclei of the lateral lemniscus. While the input from the IC to the SC is predominantly ipsilateral, labelled neurones were found in all these auditory nuclei on both sides of the brainstem. Discrete injections of the tracers revealed a much larger projection to the caudal than the rostral region of the SC, although the distribution of labelled neurons was broadly similar in each case. However, few neurons were double-labelled following injections of both types of fluorescent microsphere into distinct regions of the SC.

These findings suggest that the generation of a map of auditory space in the SC may be based on a convergent input from several auditory nuclei in the brainstem.

353.18

RESPONSE LATENCY OF UNITS OF AUDITORY (A_1) CORTEX AND THEIR ABILITY TO SUPPORT SHORT LATENCY AUDITORY CONDITIONED BEHAVIOR. E. Zotova,* C.D. Woody, and E. Gruen. UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024.

Patterns of unit activity were recorded from A_1 during conditioned blinking produced by 70 db click CS (forward paired with glabella tap and hypothalamic electrical stimulation; 570-10ms ISI; see Hirano et al., Br. Res. 1987) but not by a backward paired hiss DS of comparable intensity.

Averages of activity from 136 units in 3 cats showed onset latencies of 8-12 ms of response to CS, but the magnitude of response in the first 16 ms after CS presentation did not change significantly after conditioning. Thus elicitation of short (20ms) latency blink CRs by this CS is not enhanced by transmissions through A_1. Enhancement of the response to the CS in units of the motor cortex at latencies 8-12 ms after stimulus presentation must be supported by other pathways or changes.

Interestingly, the onset latency of spike discharge activity elicited by the same click CS at the level of the inferior colliculus was 12-16 ms, suggesting that some transmissions to A_1 by classical auditory pathways may be slower than previously thought. (Supported in part by The Deafness Research Foundation and The Univ. Calif. - St. Petersburg State University Exchange Program.)

353.19

CORTICAL RESPONSES TO CORRELATES OF AUDITORY MOTION: EFFECTS OF INTENSITY AND RATE OF INTENSITY CHANGE. J.M. Toronchuk and M.S. Cynader*. Depts. of Ophthalmology & Psychology, University of British Columbia, Vancouver, British Columbia, Canada, V5Z 3N9.

We have previously demonstrated that neuronal response properties, such as phasic vs. tonic, onset vs. offset, monotonicity vs. non-monotonicity and E/E vs. E/I can act synergistically, suggesting underlying mechanisms for selectivity to binaural correlates of auditory motion. Using AM ramps presented to the two ears, we have further investigated subtle interactions of these response properties which enable certain cells to be selectively responsive to particular combinations of intensity changes in the two ears. By holding the rate and absolute level of intensity change in the contralateral ear constant and varying either the rate of intensity change or the absolute intensity of the AM ramp in the ipsilateral ear, we have found that response selectivity is influenced by the rate/intensity function of a cell's response to ipsilateral sound. Cells with non-monotonic interaction between the two ears may respond preferentially to specific rates of relative intensity change. Cells with both on and off responses may have different binaural interactions for the two components resulting in a further refinement of selectivity. Specific examples will be shown illustrating these effects. Thus, the response of a specific cortical cell to auditory motion is dependent on both absolute intensity difference and also relative rate of intensity change at the two ears. Supported by MRC (Canada) MA-98556 to M.S.C.

353.20

A BINAURAL AFTEREFFECT THAT TRANSFERS FROM ONE EAR TO THE OTHER. *Z. Shu*, N. V. Swindale, C. A. Laszlo†, and M. S. Cynader, Departments of Ophthalmology and †Electrical Engineering, University of British Columbia, Vancouver, B.C., Canada V5Z 3N9.*

Distortions of visual perception following prolonged exposure to an unvarying stimulus have been frequently observed. The most familiar of these is the motion aftereffect in which, after a few minutes of viewing objects moving in a single direction, a stationary object appears to move in the opposite direction. By using an auditory stimulus that has either a notch- or a peak-shaped spectrum with the position of the notch or peak varying with time over a certain frequency range, we demonstrated [Shu *et al.* (1991), *Society for Neuroscience Abstracts* 17:304] an auditory aftereffect analogous to the visual motion aftereffect. Particularly, we showed that after adapting to upward motion of a moving spectral peak, a stationary peak appeared to move downwards, and vice versa. By adapting to one ear and testing with the other ear we are now able to show that this aftereffect is binaural: After prolonged listening with one ear to a moving peak stimulus that repeatedly moves in one direction, a stationary peak heard by the other ear appears to move in the other direction.

In a two-alternative forced choice experiment the task of the listener was to judge the direction of motion of the peak in a test stimulus heard with one ear, after listening to an adapting stimulus with the other ear. Probit analysis was used to estimate the 50% response rate on the resulting psychometric function, giving a measure of the stimulus velocity which sounded stationary to the listener. Our findings suggest that the auditory system may have a previously unsuspected binaural sensitivity to the motion of peaks and troughs in the spectra of complex sound sources. Furthermore, the binaural nature of the observed aftereffect implicates that this sensitivity is due to neural processing in the *central* auditory system, after the site of binaural combination.

354.1

VOLTAGE-DEPENDENT SODIUM CURRENTS RECORDED FROM DISSOCIATED RAT TASTE CELLS. M.S. Herness.* Lab. of Neurobiology & Behavior, Rockefeller University, New York, NY 10021.

A detailed description of voltage-dependent sodium currents, obtained with whole-cell patch-clamp techniques on cells dissociated from foliate and circumvallate papillae, is presented to better understand transductive mechanisms of taste receptor cells. Best estimates suggest that 50% of taste receptor cells possess these sodium currents which were similar in current-voltage, time to peak current, and h_∞ relationships as well as tetrodotoxin-sensitivity. Currents activate around -50 mV, reach a maximal inward current of 250 - 700 pA at -10 mV, and have reversal potentials at approximately 60 to 80 mV (126 mM $[Na^+]_{out}$). Maximal sodium conductance (3 to 12 nS) occurs at +10 mV. Instantaneous current-voltage relationships appear linear. Voltage-dependent inactivation becomes evident as low as -90 mV and is complete by -30 mV. About 20% inactivation would exist at a resting potential of -70 mV. Temporal dependence of inactivation appears long, often lasting seconds. These data help to delimit the expanse and puissance of membrane excitability available to taste receptor cells for gustatory stimuli response.

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354.2

EFFECTS OF ACID STIMULI ON ISOLATED HAMSTER TASTE CELLS. T.A. Gilbertson*, S.D. Roper and S.C. Kinnamon. Dept. of Anatomy and Neurobiology, Colo. State Univ., Ft. Collins CO 80523 and Rocky Mtn. Taste and Smell Center, UCHSC, Denver, CO 80262.

The transduction of acid (sour) stimuli has been suggested to involve a variety of mechanisms including block of apical K^+ channels, gating of Ca^{2+} channels and changes in intracellular pH. Using a non-invasive recording technique, we recently showed indirect evidence suggesting that protons can permeate amiloride-sensitive (AS) Na^+ channels causing taste cell excitation (Gilbertson et al. *Soc. Neurosci. Abs.* **17**: 1216). To provide direct evidence that protons can permeate the AS Na^+ channels, we have recorded whole-cell currents in taste cells isolated from hamster fungiform taste buds. In an extracellular solution containing 140 mM NaCl, approximately half of all cells exhibit amiloride-sensitive currents. To test for the presence of a direct proton current, cells were placed in an extracellular solution containing 0 mM Na^+ and 20 mM TEA to eliminate Na^+ and K^+ currents, respectively. In over two-thirds of cells tested, pressure application of citric acid (pH 4.5) directed toward the cells' apical membrane elicited an inward current apparently carried by protons (mean amplitude ± S.E. = 13.8 ± 3.6 pA). At all physiological potentials (-100 to +20 mV), this current was inward, consistent with it being a proton current ($E_{rev} \approx +150$ mV @ pH 4.5). In most cells, amiloride (30 μM) completely blocked the proton current suggesting that protons were permeating the AS Na^+ channels. In taste cells showing no amiloride sensitivity, acid stimuli decreased a standing current. This was accompanied by a decrease in the cell membrane conductance. Amiloride had no effect upon this reduction in current. Thus, protons may affect a variety of apically localized ion channels in taste cells, all of which may contribute to the detection of acid stimuli.

354.3

THE PERMEABILITY OF THE TASTE BUD AND LINGUAL EPITHELIUM TO HORSERADISH PEROXIDASE AND LANTHANUM IONS IN GERBIL CIRCUMVALLATE PAPILLAE. K.S. Lu* and M.H. Chen, Department of Anatomy, College of Medicine, National Taiwan University. Taipei, TAIWAN, ROC.

The permeability barrier of the lingual epithelium is still controversial and that of the taste bud remains an enigma. This paper reports a series of experiments in which horseradish peroxidase (HRP) and lanthanum ions (La^{+3}) were used as tracers to investigate the barrier properties of the lingual epithelium and taste buds. HRP was introduced (1) either topically on the dorsal surface or subepithelially into the connective tissue of circumvallate papillae and (2) intravascularly into the anterior jugular vein. La^{+3} was applied through intracardial perfusion. The subsequent fate of both tracers was followed with the electron microscopy.

In gerbil circumvallate papillae, the lingual epithelium on the dorsal surface of the papilla is partially cornified and that on the trench wall is uncornified or slightly cornified. When introduced subepithelially, HRP was visible in the connective tissue and extended through basal lamina into (1) the intercellular space, but not beyond the apical intercellular junction of taste buds, (2) superficial layer of the trench wall epithelium, and (3) the granular layer of dorsal lingual epithelium. Intravenous injection of HRP revealed that HRP penetrated the fenestrated endothelium of connective tissue and extended into the taste bud and the lingual epithelium as in subepithelial studies. Topically applied HRP failed to cross the lingual epithelium and the apical intercellular junction of the taste pore. La^{+3} deposited over the intercellular space of the taste bud and the lingual epithelium. The extent of La^{+3} deposition was essentially similar to that of HRP administered subepithelially or intravenously.

These preliminary data suggest that a permeability barrier to HRP or La^{+3} exists in the lingual epithelium of dorsal lingual surface as well as in the epithelium and taste buds on the trench wall of the circumvallate papillae. (Supported in part by grants Nos. NSC-80-0412-B002-159 and NSC-81-0412-B002-558 from National Science Council, ROC)

354.4

DIFFERENCES IN MATERNAL NACL INTAKE ALTER THE TERMINAL FIELD ORGANIZATION OF TASTE AFFERENTS IN THE NUCLEUS OF THE SOLITARY TRACT OF ADULT RATS. Robert J. Contreras*, I. L. Vandevelde, and E. K. Basco. Florida State University, Department of Psychology, Tallahassee, FL 32306-1051.

Using horseradish peroxidase (HRP) anterograde transport, King & Hill (*J. Comp. Neurol.* 303: 159-169, 1991) have recently shown that the distribution of chorda tympani afferents within the nucleus of the solitary tract (NST) was altered in adult rats exposed to a sodium-deficient diet from the third day of gestation to postnatal day 35. It is well established that the chorda tympani nerve, which innervates salt-sensitive taste receptors within the anterior tongue, is critical for the control of salt intake in the rat. Our prior research indicates that the amount of NaCl consumed by adult rats is linearly related to the level of dietary NaCl their mothers consumed during pregnancy and lactation. Differences in NaCl intake are more striking when the adult offspring are examined after sodium depletion. We hypothesize that there is a sensitive period in development when maternal NaCl intake can influence the terminal field organization of salt taste neurons within the brain, critical in determining long-term levels of NaCl ingestion under conditions of sodium repletion and depletion. Adult female rats were fed diets containing either basal 0.15% or high 3% levels of NaCl from conception to postnatal day 30. The offspring were then fed mid 1% NaCl for at least 2 months before analysis. The central terminal field of taste afferents was assessed after anterograde transport of wheat germ HRP applied to the central end of the chorda tympani of 10 rats. Our preliminary analysis of 6 cases (3 basal & 3 high NaCl) indicates that there is a re-distribution of afferent terminal labelling among the dorsal, intermediate, and ventral zones of the NST. (Supported by NIH grant HL38630).

354.5

DIFFERENTIAL RESPONSE CHARACTERISTICS OF CHORDA TYMPANI SINGLE FIBERS TO SALT STIMULI IN 129/J AND C57BL/6J MICE. K. S. Gannon* and R. J. Contreras. Program in Neuroscience, The Florida State University, Tallahassee, FL 32306-1051.

129/J and C57BL/6J inbred mouse strains exhibit differential consumption of and preference for NaCl solutions. It has been well documented that 129/J mice have a significantly greater preference for 0.08M NaCl than C57BL/6J mice. In the rat, the chorda tympani nerve, which innervates salt-sensitive taste receptors on the anterior tongue, is critical for the control of salt intake. Previously, we found that chorda tympani electrophysiological responses to NaCl were similar in the two inbred mouse strains. However, following treatment of the tongue with the sodium channel transport blocker, amiloride hydrochloride, strain differences appear which may underlie the behavioral difference in sodium consumption seen in 129/J and C57BL/6J mice. Amiloride inhibition of the chorda tympani response to NaCl was significantly greater in C57BL/6J mice than in the salt-preferrring 129/J mice. Until now, only whole nerve recordings have been obtained from the chorda tympani in these two strains of mice. To obtain a comprehensive understanding of peripheral gustatory neural coding of salt stimuli, salt-sensitive fibers within the chorda tympani need to be identified and their individual responses measured. At present, our research is devoted to assessing the response characteristics of single chorda tympani units to NaCl, KCl, and NH_4Cl in 129/J and C57BL/6J mice before and after amiloride treatment of the tongue. (Supported by NIH grant HL38630).

354.6

AN IN VITRO ASSAY FOR IRRITANTS. ELECTROPHYSIOLOGICAL PROPERTIES AND CHEMICAL REACTIVITY OF TRIGEMINAL (GASSERIAN) NEURONS IN TISSUE CULTURE. J.Eugenin*, S. Roberts, S. Carlyle, K. Rapp, and R.P. Tuckett. Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, UT 84108, U.S.A.

Enzymatically dissociated trigeminal ganglion (TG) neurons from neonatal Sprague Dawley rats were plated on collagen coated dishes and cultured in DMEM/KGM (20/80) supplemented with 2% HS, 2% FBS, 2.3 mM sodium bicarbonate, 4.4 mM Hepes and 0.1 (μg/ml) 7-S NGF at 37 °C, 100% humidity and 5% CO2 in air.

After 3-12 days, membrane properties were measured in 142 units (membrane potential, 44.5 ± 0.6 mV; input resistance 41.9 ± 2.3 MΩ; input capacitance 77.0 ± 3.5 pF; time constant 3.1 ± 0.5 ms, mean ± SEM). Depolarizing electrical pulses (5 ms) evoked action potentials (APs) 74.8 ± 0.9 mV in amplitude and 6.8 ± 0.2 ms in duration, followed by afterhyperpolarization of 7.9 ± 0.3 mV and 99.19 ± 9.5 ms in duration. Forty four percent of cells showed time-dependent rectification to inward current and 63% generated multiple (2-4) APs to suprathreshold depolarizing pulses (100 ms). Chemicals pressure injected into the surrounding environment produced depolarization (up to 40 mV), depolarization plus APs, or APs only. Over 50% of tested neurons responded to administration (30 s) of either 1 μM capsaicin or 90 pM bradykinin. About 15% and 50% responded to administration (250 ms) of 10 mM serotonin and 300 μM histamine, respectively. TG neurons also responded to irritant substances such as hydrazine (60 pM) and nonadecafluorodecanoic acid (1 μM).

These findings support the notion that TG neurons in culture form an heterogenous, chemosensitive population and suggest that cultured sensory neurons can be used as a model for studying cellular mechanisms of irritancy and sensitization.

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354.7

A CALCIUM-DEPENDENT ANION CONDUCTANCE IN *NECTURUS* TASTE RECEPTOR CELLS. Randall S. Taylor & Stephen D. Roper* Dept. of Anat. & Neurobiol., Colorado State University, Ft. Collins CO 80523 and The Rocky Mtn Taste & Smell Center, Denver CO 80262.

It was first shown in 1983 that taste cells are excitable and thus possess voltage-dependent ionic conductances. Kinnamon and Roper (J. Gen. Physiol., **91**:351. 1988) demonstrated the presence of a Ca^{2+}-dependent K^+ conductance in *Necturus* taste receptor cells. McBride and Roper (J. Memb. Biol. **124**:85. 1991) demonstrated that taste cells also possess TEA-insensitive, Ca^{2+}-dependent currents that were affected by altering the chloride equilibrium potential. Although the data were indirect, they concluded that taste cells possess a Ca^{2+}-dependent Cl^- conductance in addition to the other ionic conductances previously described. Here we report the results of whole-cell patch-clamp studies on this presumptive Ca^{2+}-dependent Cl^- current. Upon depolarization of taste cells in thin lingual slices or of isolated taste cells, we have recorded a Ca^{2+}-dependent Cl^- current in *Necturus* taste cells. That is, after replacing all permeant ions but Na^+, Ca^{2+} and Cl^- and adding TTX to the bath (**bath**: 96 mM NaCl, 20 mM $CaCl_2$, 10 mM HEPES; **patch pipette**: 140 mM NMDG-tartrate, 10 mM NaCl, 1 mM $MgCl_2$, 0.1 mM EGTA (=10^{-8} M Ca^{2+}), 5 mM ATP, 0.5 mM GTP, 0.5 mM cAMP, 5 mM HEPES), we found a slowly activating outward currentng outward current that disappeared when Cl^- was replaced with isethionate or other impermeant anions. The outward current was also eliminated when Ba^{2+} was substituted for Ca^{2+}, even in the presence of Cl^-. The pharmacology of these chloride channels is the next step in understanding the role these channels may play in taste transduction. We speculate that Ca^{2+}-dependent Cl^- currents may be involved in distinguishing between the taste of salts that have different anion compositions. Supported by NIH grants DC00244 and DC00374.

354.8

FINE STRUCTURE OF TASTE BUDS ON THE LAMB EPIGLOTTIS. R.D. Sweazey*, C.A. Edwards and B.M. Kapp. School of Dentistry, University of Michigan, Ann Arbor, MI 48109.

Previous investigations have described in detail the fine structure of taste buds located in the oral cavity and aryepiglottal folds. However, similar detailed information is currently lacking for taste buds located on the laryngeal surface of the epiglottis. Therefore, we examined the fine structure of taste buds on the lamb epiglottis using electron microscopy. Five lambs aged 30-45 days were perfused using a modified Karnovsky's fixative, the epiglottis removed and processed to epoxy using standard procedures. Thin sections of epiglottal taste buds were examined by T.E.M. Taste buds were located in small groups and ranged in size from 25-35 um. In addition to undifferentiated basal cells, three general groupings of cells similar to those previously observed in taste buds of the oral cavity could be distinguished. Type I cells were most numerous and contained typical apically located membrane-bounded dense vesicles, numerous free ribosomes and rough endoplasmic reticulum, spindle shaped nuclei with numerous small invaginations, and numerous slender microvilli projecting into the taste pore. Type II cells contained no apical membrane-bound vesicles, had less rough endoplasmic reticulum, spherical nuclei, and small numbers of blunt microvilli projecting into the pore. Type III cells had round to oblong nuclei that were frequently deeply invaginated, large numbers of vesicles ventral to the nucleus, and a peg like apical projection into the taste pore. In addition to these different cell groups, numerous small nerve processes were observed adjacent to all three cell types. In many of these nerve-cell boundaries synaptic-like thickenings of the membrane and clear and dense-cored vesicles could be identified. Our results suggest that the fine structures of taste buds located on the epiglottis is similar to those previously observed in taste buds of the oral cavity and aryepiglottal folds. Supported by N.I.H. Grant DC00735.

354.9

SELECTIVITY OF LINGUAL FIBERS TO CHEMICAL STIMULI ON RAT TONGUE. Y. Wang and S. A. Simon*. Department of Neurobiology, Duke University, Durham, NC 27710.

The mechanisms by which chemical stimuli enter lingual epithelia and elicit responses from lingual fibers are largely unknown. To obtain such information, single unit extracellular recordings from 88 rat trigeminal ganglion cell bodies were measured in response to chemical stimuli flowed over rat tongue. Fiber types were identified by measurements of conduction velocity and responses to mechanical and thermal stimuli. Chemical stimuli included NaCl, KCl, NH4Cl, CaCl2, LaCl3, nicotine, hexanol, capsaicin and menthol. About 75% of C fibers, 25% of Aδ fibers and 0% of Aβ fibers responded to hyperosmotic (to 2.5 M) concentrations NaCl, KCl and NH4Cl. In contrast, no responses were elicited by CaCl2 (to 1.0 M) and LaCl3 (1.0M). Thus, the irritating and/or painful sensations elicited by these salts arise only from C and Aδ fibers.

The responses to these monovalent salts were reversibly inhibited by 3.5 mM LaCl3. These data suggest that La+++ blocks tight junctions in lingual epithelia, thus preventing salts from diffusing into the epithelia where they can interact with C and Aδ fibers. Also consistent with this interpretation is that La+++ does not inhibit the responses to hydrophobic stimuli such as nicotine, capsaicin, menthol and hexanol which permeate the epithelia by partitioning into plasma and axonal membranes. Work was supported by NIH DC0165 and from the Smokeless Tobacco Research Council.

354.10

VOLTAGE CLAMP STUDIES OF THE AMILORIDE-INSENSITIVE NEURAL RESPONSE. Q.YE, G.L.Heck, D.L.Hill* and J.A.DeSimone. Dept.of Physiology, VCU, Richmond, VA 23298. *Dept.of Psychology, UVA, Charlottesville, VA 22903.

The chorda tympani (CT) response of many mammals to Na^+ salts is anion dependent. This anion effect is mediated in part by anion-dependent field potentials set up across apical shunt pathways [1]. In these studies, we found another anion effect which was related to the amiloride-insensitive component (AIC) of the Na^+ salt CT response [2]. The AIC is negligible for non-chloride salts and for NaCl at concentrations under about 50mM. Accordingly the neural responses to 25mM NaCl and sodium gluconate (NaGlu) were identical at any clamping voltage, with enhancement at negative voltages and suppression at positive voltages. At 90mV the suppressed response approached the AIC. At 0.2M, NaGlu and NaCl responses converged at -60mV, however, at more positive voltages NaCl responses exceeded those of NaGlu. At 90mV the NaCl response approached the AIC for NaCl. Furthermore, the AIC was independent of voltage at any NaCl concentration. The voltage-independence of AIC can be explained by an electroneutral penetration of NaCl across the shunt barrier giving NaCl access to basolateral channels or possibly to cells without an apical projection. The existence of Na^+ channels on the basolateral side of the taste bud can be demonstrated immunohistochemically and supports shunt penetration as a second stimulation mechanism available to NaCl but not other Na^+ salts. Studies of the voltage sensitivity of the CT in rats deprived of Na^+ throughout development, which exhibit no amiloride sensitivity, may help us to understand the Na^+ salt taste mechanism in depth. (Supported by NIH grant DC 00122)

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354.11

IMP AS STIMULUS IN *PARAMECIUM* CHEMORECEPTION. J. L. Van Houten* and W. Q. Yang University of Vermont, Dept. of Zoology, Burlington, VT 05405-0086.

Paramecia are attracted or repelled by chemical stimuli. Generally attractants are fermentation or other bacterial products that function as food cues. Repellents seem to signify non-optimal pH or ionic strength. IMP is a stimulus for paramecia and, because it is an indication of bacterial action on food (used in the fish industry) it could serve as a food cue for paramecia. Instead, it seems to repel the cells. The behavioral responses to IMP in T-mazes are inhibited by GMP or AMP but not by cAMP or CMP. Interestingly, K-glutamate also inhibits, drawing a parallel with putative MSG taste.

In order to indirectly assay the signal transduction and physiological events underlying IMP chemoresponse, we analyzed swimming speed and frequency of turning by computer motion analysis. These analyses show slight decreases in swimming speed and increases in frequency of turning in IMP, behaviors which are consistent with small, sustained depolarizations of the cells in K_2-IMP relative to KCl. Small depolarizations would slow ciliary beating and slightly increase frequency of calcium action potentials that cause changes in swimming direction. Direct electrophysiological recording will verify this.

Pawn mutants are not repelled from IMP, probably because, for lack of action potentials, they fail to make abrupt turns in their swimming paths. Mutant K ShyA/B (courtesy of T. Evans) is repelled by IMP and LiCl has little effect on IMP chemoresponse, implicating a sensory transduction pathway that differs from that for most other stimuli. This work supported by NIH and the Vermont Cancer Center.

354.12

CLONING THE PLASMA MEMBRANE CALCIUM PUMP GENE IN *PARAMECIUM*. N.L. Elwess#, G. Mawe*, and J.Van Houten#. #Dept. of Zoology, *Dept. of Anatomy and Neurobiology, University of Vermont, Burlington, VT, USA 05405

Paramecium can detect metabolites of bacteria, its food source. These attractants are thought to be detected through one of at least two chemosensory transduction pathways found in *Paramecium*, the one which seems to involve a plasma membrane calcium pump. Attractants hyperpolarize the plasma membrane and, for most attractants, this hyperpolarization appears to be dependent upon a pump current, such as from a calcium plasma membrane pump. Through the use of the polymerase chain reaction, we have identified sequences which seem to represent a family of ion translocation ATPase genes. This family of ion pump genes is not just restricted to the plasma membrane calcium pump that we believe is involved in chemoreception, but should include other calcium pumps, such as those found on the ER, mitochondria, and alveoli (a specialized calcium sequestering organelle of ciliates). Therefore, to ensure that the gene of interest is the plasma membrane calcium pump primers for PCR were designed to include the calmodulin binding domain which is unique to the plasma membrane calcium pump among other calcium pumps. PCR products of the appropriate size as judged from published mammalian plasma membrane pump sequences are being sequenced. Cloning the plasma membrane calcium pump gene should provide the necessary and sequence information to produce nucleotide probes and antibodies against peptides to more directly localize the pump and determine its role and function in the chemosensory transduction pathway.

This work is supported by NIH and the Vermont Cancer Center.

355.1

DIRECT INPUT FROM PRIMARY SOMATOSENSORY CORTEX TO PYRAMIDAL TRACT CELLS IN THE RAT MOTOR CORTEX. T. Xiong* and L.L. Porter, Dept. Anat. & Cell Biol., USUHS, Bethesda, MD 20814.

The connection between axons arising from primary somatosensory cortex (SI) and pyramidal tract (PT) cells of the motor cortex was investigated in adult rats. The anterograde tracer, Dextran Tetramethylrhodamine (DT) was injected into the electrophysiologically identified forelimb representation of SI. Fast Blue (FB) was stereotaxically injected into the medullary corticospinal tract to identify PT cells. The dendritic arbors of the PT cells were visualized by intracellular injection of Lucifer Yellow into FB labeled PT cells of the motor cortex in lightly fixed tissue slices (150μm, sagittal plane) under fluorescence microscopy. The DT injection resulted in patch-like labeling in motor cortex. Most labeling was found in layers II-III with some in layers V-VI. Bouton-like swellings along DT labeled axons were often found apposed to both the apical and basal dendrites of PT cells, indicating synaptic connectivity. Our results suggest that PT cells receive direct input from SI. Thus the activity of the motor cortex output neurons may be influenced by this projection.

355.2

CONNECTIONS OF VIBRISSAL REGIONS IN RAT SENSORY-MOTOR CORTEX. L.L. Porter*, R. Izraeli, Dept. of Anat. & Cell Biol., USUHS, Bethesda, MD 20814.

Projections to the vibrissal region of motor cortex were studied in the rat as a step toward understanding information processing in the sensory-motor cortex. Intracortical microstimulation (ICMS) was delivered to the motor cortex of lightly anesthetized rats. Tungsten-in-glass microelectrodes were used to identify sites at which movement of a single vibrissa was elicited by low threshold current (5-20 μA). The number of vibrissae which moved depended on the level of anesthesia. A WGA-HRP (2.5%) filled pipette (15 μm) was placed into the site of stimulation and a small amount of the tracer was delivered by iontophoresis. Series of alternate sections were cut in the coronal or tangential plane and processed for HRP or cytochrome oxidase. Retrogradely labeled cells were observed in the ipsilateral barrel field of SI. They were organized in a columnar array with cells in both supragranular and infragranular layers. Analysis of adjacent cytochrome oxidase stained sections revealed that labeled cells were aligned with barrel structures representing the vibrissae. They were not restricted to the barrel corresponding to the whisker which responded to ICMS in motor cortex, but spread to adjacent barrels. Labeled neurons were also found lateral to the barrel field, in the second somatosensory cortex. Here, label was diffuse but extensive and bilaminar. In addition, labeled cells were observed in the ipsilateral thalamus and in the contralateral vibrissal motor cortex. Future studies will be performed to determine the functional role of sensory cortex input on motor cortex activity. Supported by NIH Grant No. NS 27038.

355.3

INPUT-OUTPUT ORGANIZATION OF THE RAT VIBRISSAE MOTOR CORTEX. E. Miyashita[1], H. Asanuma[1]* and A. Keller[2]. [1]The Rockefeller University, New York, NY 10021, [2]Dept. Anatomy, USUHS, Bethesda, MD 20814-4799.

In order to study cortical control mechanisms of the rat vibrissae motor area, retrograde and anterograde tract tracing techniques were used to define the input-output organization of the rat vibrissae motor area. Wheat germ agglutinin conjugated horseradish peroxidase (Sigma type VI, 3% solution) or *Phaseolus vulgaris* leucoagglutinin (Vector Lab., 2.5% solution) were injected iontophoretically into physiologically defined rat vibrissae motor area. The rat vibrissae motor cortex was defined using intracortical microstimulation method (10 trains of 300 Hz, 250 μsec pulses; stimulus strength < 50 μA).

The rat vibrissae motor area had reciprocal connections with the lateral agranular cortex (AGl) in the contralateral side and the ipsilateral somatosensory cortex (SCx). Labeling in the vibrissae region of the SCx consisted of several distinct patches. In the thalamus, it had dense reciprocal connections with the dorsolateral part of the ventral lateral nucleus and the ventrolateral part of the intralaminar nucleus and less dense reciprocal connections with the ventromedial nucleus. Labeled terminals were found mainly in the dorsolateral part of the striatum, the intermediate and deep layers of the superior colliculus, the lateral part of the central gray, the mesencephalic reticular formation, the parvocellular portion of the red nucleus, the pontine nucleus, and the pontine reticular formation. Especially in the pontine nucleus, the labeled terminals formed discrete dense patches. Supported by NIH grants NS 31078 and NS 10705.

355.4

MEASUREMENT OF LESION PARAMETERS FOR STUDIES OF MOTOR RECOVERY AFTER SENSORIMOTOR CORTEX INJURY IN THE RAT. L.B. Goldstein* and R. Kamis. V.A & Duke Medical Centers, Durham, NC 27705

The ability of rats to traverse a narrow elevated beam has been used to quantitate recovery of hindlimb motor function after unilateral sensorimotor cortex injury. Lesion extent is an important variable to consider in studies of the effects of drugs on beam-walking recovery. However, histologic evaluation precludes neurochemical measurements in brain tissue. The present study was carried out to determine how well the dimensions of cortex lesions measured at the surface of the brain correlate with beam-walking recovery in comparison with standard histology. After behavioral training, rats (n=20) underwent suction-ablation of the right sensorimotor cortex. Beam-walking was measured over the next 12 days. The maximum medial extent of the lesion (closest approximation of the lesion to the inter-hemispheric fissure) and lesion surface area were measured from digitized images of the dorsal surface of each brain. Maximum % cingulate cortex damage and lesion volume were measured from serial coronal histologic sections. Both lesion maximum medial extent and maximum cingulate cortex damage (r=0.83 and 0.76, p=0.0001, respectively) but not lesion surface area or lesion volume (r=0.27, p=0.27 and r=0.43, p=0.06, respectively) were correlated with beam-walking recovery. Measurement of lesion surface maximum medial extent also was reliable (intra- and inter-observer reliability were r=0.82 and 0.82, p=0.0001, respectively). Lesion surface maximum medial extent provides a valid and reliable measure for studies of beam-walking recovery after sensorimotor cortex suction-ablation injury.

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355.5

PHYSIOLOGICAL HETEROGENEITY OF IDENTIFIED NON-PYRAMIDAL CELLS IN RAT FRONTAL CORTEX Y. Kawaguchi* and Y. Kubota, Lab. for Neural Systems, Frontier Research Program, RIKEN, Wako 351-01, Japan

Physiological and morphological properties of layer V non-pyramidal cells were studied in slices of rat frontal cortex by whole cell, current-clamp recording and intracellular staining by biocytin. One class (fast-spiking (FS) cells) had input resistances lower than 400 MΩ and spike-widths at half amplitude shorter than 0.8 ms; the other (low-threshold spike (LTS) cells) had higher input resistances than 400 MΩ and spike-widths longer than 0.8 ms. Afterhyperpolarizations (AHPs) following action potentials consisted of a single component in FS cells, but two components with early and late peaks were observed in LTS cells. AHPs of FS cells had faster time-to-peak and larger amplitude than the early component of the AHPs of LTS cells. Low threshold spikes were observed in hyperpolarized potentials in LTS cells, not in FS cells. Spike trains elicited by depolarizing pulses in FS cells showed almost no spike-frequency adaptation, while those in LTS cells showed adaptation. A combination of stimulation-induced EPSPs with depolarization caused repetitive firing in FS cells which was abolished by NMDA receptor blockers. Repetitive firing was not observed in LTS cells under these conditions. Dendrites of FS cells were mostly smooth, but those of LTS cells possessed a modest but consistent population of spines. Axons of FS cells tended to run horizontally along layers and on this basis some resembled basket cells. Axons of LTS cells were more vertically oriented and extended in columnar fashion through layers I to VI. These suggest layer- and column-specific differentiation of neocortical intrinsic neurons with different firing modes.

355.6

FURTHER EVIDENCE THAT THE RETICULAR FORMATION IS THE MAJOR SOURCE OF MEP IN THE RAT. J.-H. Sohn*, B.R. Park[1], J.J. Kim[2], K.S. Cho, J.H. Kim The Miami Project To Cure Paralysis, Dept. of Neurol. Surg., Univ. of Miami, Miami, FL 33136, [1] Dept. of Physiol., Won Kwang Univ., Iri, Korea [2] Dept. of Anat., Chosun Univ., Kwangjoo, Korea

Motor evoked potentials (MEPs) produced by electrical stimulation of rat sensorimotor cortex (SMC) have been shown to originate from the reticular formation (RF). However, the possibility that other brain structures might also be activated during SMC stimulation has not been completely ruled out. The object of this study was to investigate whether any component of the MEPs originates from activation of red nucleus (RN) and/or vestibular nucleus (VN). Evoked potentials (EPs) were monitored epidurally from 20 Sprague-Dawley female rats (250-300 g) at T10 and L3 spinal cord levels, using two pairs of teflon-coated wire electrodes with a 1 mm exposed tip. The right SMC was stimulated using a screw (0.5 mm in diameter) while RN, VN and RF were stimulated through microelectrodes placed stereotaxically in the target nuclei. A striking similarity was found in waveform and conduction velocity between MEPs and RF-EPs. Selective spinal cord lesions placed at T8 to disrupt either rubrospinal or vestibulospinal tracts did not affect the amplitudes and latencies of MEPs. However, lesion of the ventral cord dramatically reduced the amplitudes and conduction velocities of both MEPs and RF-EPs. Intracerebral injection of local anesthetics, which blocked RN-EP or VN-EP, did not alter either the amplitudes or latencies of the MEPs, indicating that RN and VN do not contribute to MEPs generated by cortical stimulation. The results indicate that the MEPs originate from the reticular formation and conduct along the ventral funiculus. (Supported by NIH Grant NS28059, The Miami Project Research Fund, and GE International Scholar Training Fund)

355.7

PRESYNAPTIC ACTION OF 5-HYDROXYTRYPTAMINE IN RAT CORTICAL NEURONS. E. Tanaka, H. Hasuo* and R. A. North
Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

Intracellular recordings were made from layer V neurons of the rat anterior cingulate cortex in vitro. Electrical stimulation of the subcortical white matter or layer V evoked synaptic potentials with four components; fast epsps blocked by CNQX, fast epsps blocked by APV, fast ipsps blocked by bicuculline (500 nM - 10 μM), a slow ipsps blcoked by saclofen (100 μM). 5-HT(1 -300 μM) reduced the amplitude of the epsps; this was concentration-dependent with a 50% reduction at about 23 μM. Both CNQX-sensitive and APV-sensitive components were equally inhibited by 5-HT; the $GABA_A$ and $GABA_B$ components were not affected. Depolarizations evoked by brief superfusion of glutamate were not affected by 5-HT. The presynaptic inhibition of epsps was blocked by cyanopindolol (100 nM), and was mimicked by TFMPP (100 - 300 nM). These results show that glutamate terminals express 5-$HT1_B$ receptors, activation of which presynaptically inhibits transmitter release.

355.8

NEUROTROPHIN INDUCTION IN THE ADULT RAT BRAIN FOLLOWING ADMINISTRATION OF THYROID HORMONE. J. B. Pan*, D. Casuto, S. Watanabe, S. P. Arneric, and T. Giordano. Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064.

Neurotrophins, in particular NGF, have been proposed as a potential therapy in the treatment of Alzheimer's disease; however, the size of these compounds preclude their passage across the blood brain barrier. Induction of the neurotrophins by small molecules may circumvent this problem. In this study rtPCR was used to measure the abundance of NGF, BDNF, and NT-3 RNA relative to that of cyclophilin RNA following peripheral administration of thyroid hormone (TH) to aged (20-22 month old) male Sprague-Dawley rats. NGF and NT-3 exhibited a statistically significant increase (+50%, $p<0.05$) in the hippocampus relative to cyclophilin following TH administration. These increases did not correlate with an increase in ChAT activity, an enzyme which had been shown to be enhanced by NGF and BDNF, in the hippocampus. In the cerebral cortex, TH administration increased levels of all neurotrophins (49%-NT-3; 30%-NGF; and 30%-BDNF), but these effects were not statistically significant, whereas ChAT activity did demonstrate a statistically significant increase (28%, $p<0.01$). The fact that BDNF did not increase significantly in either region was supported by the absence of a thyroid responsive element in the upstream region of the BDNF gene. These results indicate that TH administration was capable of co-ordinately increasing the accumulation of NGF and NT-3 transcripts thus offering the potential of modulating multiple neurotrophin systems with a single small molecule.

355.9

NEURAL FIRING PATTERNS IN CULTURES OF RAT CEREBRAL CORTEX. T.S. Donta* and J.A. London. Division of Neurological Sciences and Dept. of Biostructure and Function, University of Connecticut Health Center, Farmington, CT 06030.

We have been characterizing the suitability of slices of rat cerebral cortex maintained *in vitro* for multiple-site optical recordings using voltage-sensitive dyes and a photodiode array. The goal is to observe how the activities of neurons from different cortical layers are synchronized, and to then infer a role for this activity in cortical processing. Anatomical analysis has shown that normal cortical cell types exist in the cultures, and that cortical layering is maintained *in vitro* for two to three weeks. Reported here are some typical neural firing types found using extracellular glass electrodes. Recordings from cultures of somatosensory (SI) and agranular insular cortex (AI) were made. Spontaneous activity, and activity after bath application of L-glutamate has been recorded. Regularly spiking neurons with firing rates of 30-100 Hz were found, as well as bursting neurons. Neurons firing doublet spikes were also seen. Both regularly spiking neurons and bursting neurons were found in SI and AI, and regularly spiking neurons were recorded from more often after addition of L-glutamate. These types of activities are consistent with activities found *in vivo*. Combined with multi-site optical electrophysiology, the *in vitro* cortical model has considerable potential for studying cortical function. [Supported by a Klingenstein Fellowship and USPH Grant 2P01-NS16993-09.]

355.10

IMMUNOHISTOCHEMICAL ANALYSIS OF THE ACETYLCHOLINE (ACh) INNERVATION OF NEOCORTICAL AREAS IN ADULT CAT. C. Avendaño*, D. Umbriaco, L. Descarries and R.W. Dykes. Centre de recherche en sciences neurologiques (Départements de physiologie et de pathologie), Université de Montréal, Montréal, Québec, Canada H3C 3J7.

The ACh innervation of the primary somatosensory, visual and auditory areas of adult cat cerebral cortex was studied using monoclonal antibodies against purified rat brain choline acetyltransferase (ChAT; Cozzari et al., *Soc. Neurosci. Abstr.* 16: 200, 1990). ChAT positive fibers were found in all layers of every area examined. Both laminar and areal differences were noticed. Common features included a dense innervation of layer I, mostly from horizontally running fibers, a relatively sparse innervation of layers II, III, V and VI, and a denser innervation of layer IV. The latter, however, was heavily innervated in the somatosensory and auditory areas, but only slightly more than layer III in the visual area. Most ACh fibers appeared very thin and bore many varicosities of various sizes, both as *en passant* and terminal boutons. The small varicosities were the most numerous, but many fibers showed a combination of large and small varicosities. There were also a few thick and strongly immunoreactive fibers with a course parallel to the pial surface in layers I and II, or ascending through the deeper layers. Moreover, sparsely distributed strands or clusters of unusually large varicosities were observed, mainly in infragranular layers and sometimes forming pericellular arrangements. The fibers bearing such varicosities were variable in thickness. Lastly, a few ChAT-immunoreactive nerve cell bodies were found in the somatosensory cortex. Most of these occupied layers I-III and had a small, oval or polygonal soma with 1-3 primary dendrites oriented in various directions. (Supported by a DGCyT fellowship, the FCAR and MRC grants MT-3544 and MA-8700).

355.11

INVOLVEMENT OF D1 DOPAMINE RECEPTORS IN PREMOTOR AND MOTOR CORTICAL FUNCTIONS IN MONKEYS. T. Sawaguchi*, I. Yamane, and K. Kubota. Dept. of Neurophysiol., Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan.

To examine the roles of dopamine receptors in cortical motor functions, selective dopamine antagonists (SCH23390 for D1 and sulpiride for D2 receptors) were locally injected into premotor and motor cortices of two rhesus monkeys who performed a reaching task with their arms. The monkeys initiated the task by pressing a central lever. After receiving a visual go signal, they then released the lever and reached out to one of three target locations (left, upper, or right) that had been indicated 2-5 s before. Local injections of SCH23390 (10-50 μg/3-5 μl) into postarcuate areas of the premotor cortex (over 10 sites) increased reaction time (from the go signal to movement onset), decreased the frequency of task trials and created no clear change in reaching time (from movement onset to offset). SCH23390 injections into hand-arm areas of the motor cortex (over 7 sites) increased the reaching time without clear change in the reaction time or the frequency of task trials. By contrast, injections of sulpiride (50 μg) or SCH23388 (50 μg, an inactive analogue of SCH23390) into the effective sites by SCH23390 in either the premotor or motor cortex had no clear effects on task performance. These results suggest that the activation of D1 dopamine receptors is normally involved in the functions of the premotor and motor cortices; i.e., initiation of directional reaching movements for the premotor cortex and motor execution for the motor cortex.

355.12

SPATIAL EXTENT AND BEHAVIORAL DEPENDENCE OF COHERENCE OF 25-35 HZ OSCILLATIONS IN PRIMATE SENSORIMOTOR CORTEX. **V. N. Murthy, D.F. Chen and E. E. Fetz*, Dept. of Physiol. & Biophys. and Regional Primate Research Ctr., Univ. of Washington, Seattle, WA 98195.**

Local field potentials (LFPs) in sensorimotor cortex of awake monkeys intermittently exhibit coherent oscillations at 25-35 Hz; the strength and spatial extent of coherence were examined during different behaviors. Sliding-window cross-correlation functions were computed for pairs of LFPs recorded at separate sites. The amplitude of the correlation peak closest to the origin (correlation strength) increased during challenging sensorimotor tasks -- e.g., picking raisins from a Klüver board (0.89±0.05), or retrieving food from unseen locations (0.92±0.04). With the monkey inactive, oscillatory episodes at the same sites occurred infrequently and showed lower correlation strengths (0.60±0.16). With greater separation of the recording sites, the oscillations were coherent less often and their correlation strength decreased. Natural stimulation of the contralateral arm or leg evoked oscillations in the lateral or medial part of motor cortex, respectively. Simultaneous stimulation of hand and leg increased LFP oscillations at both sites, but did not increase their coherence. To determine if coherence increased during behavior that involved coordinated activation of sites, LFPs were recorded simultaneously from left and right motor cortices when the monkey manipulated objects with one hand alone and with both hands. Oscillatory episodes increased in both hemispheres when the monkey used either hand alone or both hands together. The coherence of oscillations across hemispheres occurred just as often and was just as strong during single-handed movements (0.82±0.11) as during bimanual manipulations (0.76±0.10). Findings to date suggest that if coherent cortical oscillations are involved in sensorimotor associations, they would occur at a finer level of spatial or behavioral resolution than revealed by our recordings. [NS12542 & RR00166]

355.13

BRAIN STATE MATRIX COMPUTATIONAL APPROACHES TO NEOCORTICAL LIMBIC RHYTHMS, Creighton University, Omaha, NE 68178, C. C. Turbes. ✗

The brain information processing between stimulus inputs and motor out puts is expressed behaviorally as correlates of such cognitive processes as attention, perception and memory. It is necessary to partition these processes into first order functions so that different brain systems which subserve these separable functions can be delineated and their relationships studied.

Most important in such studies is the development of measurements operations for those component brain functions, a brain states matrix.

The EEG and sensory and cognitive "40HZ" a frequency band of the gamma range (35 - 85 Hz) which centers on specific coherent frequencies with focused arousal. These measurement operations have been used to show the interaction of the neural substrate, neural mechanisms, behavioral correlates and chemical alterations of focused arousal and behavioral immobility states and (13 to 17 Hz) SMR rhythms.

The coherent and resonant properties of these cell assemblies of the neocortex and the limbic system are demonstrated in these studies. Coherent oscillations are determined by using coherence, partial coherence, phase spectral and tau coherence spectral analyses of templates of synaptic connections.

355.14

MOTOR CORTEX OSCILLATORY NEURAL ACTIVITY DURING VOLUNTARY MOVEMENT IN *MACACA FASICULARIS*. G. Gaál*, J. N. Sanes, and J. P. Donoghue. Dept. Neuroscience, Brown University, Providence, RI 02912

This experiment evaluated oscillatory local field potentials (LFPs) in primary motor cortex (MI) and area 6 during instructed delay movements. Microwires (70 μm) were implanted in the forelimb region of MI and area 6 and EMG wires were implanted in arm muscles. Sites were identified and classified according to movements evoked by intracortical electrical stimulation. Simultaneous recordings from up to 12 electrodes for LFPs (10-80 Hz filtered) and unit discharge were evaluated for task related activity across several days during wrist movements in a visually guided instructed delay (1.5-3.5 sec) task.

LFPs oscillating between 20-40 Hz could be recorded from most MI and area 6 sites. Oscillations could occur for several sec or only for 100-300 msec. The oscillations were most prevalent during the instructed delay and the beginning of the reaction period, but were typically suppressed during movement. Sites at which digit movements were recorded tended to have the greatest suppression effect, whereas the one wrist site in MI continued to exhibit oscillatory activity throughout the movement. Cross correlograms often showed relatively high amounts of synchronous activity from the different sites during the instructed delay period. Synchronization appeared greatest for sites at which digit movements were evoked; activity was desynchronized abruptly at these sites around movement onset.

Single units showed phasic, task related activity at movement onset. To date, we have not found neurons with activity in phase with LFP oscillations. Discriminated neurons also did not show significant evidence of oscillation in discharge rate.

These data indicate that synchronous activity occurs widely in motor cortex during instructed delays. LFPs in nearby and distant sites can be highly correlated, though we have yet to find evidence of synchrony between individual neurons and LFPs. Changes in correlation of LFPs at nearby and distant sites may suggest signals related to preparation for movement, with coherence diminishing during actual movement. Support: NS25074, Culpeper and Whitehall Foundations

355.15

COHERENT OSCILLATIONS IN PRIMARY MOTOR AND SENSORY CORTICES DETECTED USING MEG AND MFT.

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It has recently become possible to compute the 3-D distribution of electrical brain activity from recordings of the scalp MEG (Ribary et al., 1991, PNAS, 88:11037-41). This method, Magnetic Field Tomography (MFT) was implemented in the analysis of a distinct oscillatory activity (15-30 Hz) observed during the execution of simple hand movements. The dynamics of the MEG field during this task could not be accounted for by a single stationary cortical source (Lado et al., Soc. Neurosci Abstr., 1991, 126.14). Rather, MFT analysis demonstrated two spatially separate superficial sources during oscillatory bursts. The anatomical origins of the signals were determined by superimposing the 3-D distribution of brain activity on 3-D MRI reconstructions. Oscillatory activity usually originated in the hand region of primary motor cortex. Frequently, however, activity was also observed in the hand region of primary somatosensory cortex. When present, activity in these two regions was synchronized, although a phase shift in the time course of activity in the two sources was also observed. Comparison of superficial with depth recordings indicated that activity in the primary motor cortex frequently alternated with deep activity at the same frequency. From these observations we conclude that synchronized oscillatory activity is present in anatomically distinct regions of the brain. We speculate that this activity serves to dynamically group an ensemble of neurons active in a behavior.

355.16

NON-HOMOGENOUS DISTRIBUTION OF CALCIUM ELECTROGENESIS ALONG THE DENDRITES OF NEOCORTICAL PYRAMIDAL NEURONS.

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Under conditions of 24 mM TEA and 1 μM TTX, intracellularly recorded pyramidal neurons generate prolonged Ca^{++} plateaus; these often (>50% of cells) show step-wise repolarization to one or more lower-amplitude plateau levels. Using NEURON (Hines, Int. J. Biomed. Computing, 24:55-68, 1989), the existence of several stable voltage levels was simulated by two different models:1) the presence of multiple, electrotonically separated sites of Ca^{++} spike electrogenesis in the dendritic tree, and 2) the presence in a single compartment of several different types of Ca^{++} channel, each with its own voltage dependence. In experiments, brief hyperpolarizing pulses during the large plateau terminated it without terminating the lower "step"; this could be simulated in both computer models. However, the observed effect of prolonged depolarizing or hyperpolarizing currents on the breakpoint from which each plateau level repolarized could only be simulated with the multicompartmental model. Thus, the more depolarized the breakpoint, and hence the more proximal the spike initiation zone, the less it was affected by the injected current. In experiments, the ratio of the breakpoint voltages for the different plateau levels was equal to the ratio of the highest repolarization velocities. This indicates that at the sites of Ca^{++} electrogenesis, breakpoint voltage and time course of repolarization was the same for the spatially disparate spikes. Because it could be shown that the breakpoint voltage was closely related to a specific region on the voltage activation curve of the Ca^{++} current underlying the spike, the findings strongly suggest that the same Ca^{++} channel is involved both proximally and distally in the dendrites.

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355.17

SOME TECHNIQUES FOR PROCESSING MULTICHANNEL NEURONAL RECORDINGS.

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We are engaged in a project in which simultaneous, multi-unit recordings will be obtained from 24 to 64 closely spaced sites in the precentral motor cortex of the awake, behaving monkey, and from 8 to 16 intramuscular EMG electrodes. Such a deluge of data requires new technologies for visually scanning patterns of neural and muscular discharge, for quantitative comparison of these patterns, for studying statistical variability in the patterns during apparently identical movements, and for detecting slow but consistent changes in them as new motor skills are acquired.

For visual, on-line monitoring, we have developed software for displaying multichannel data in an **n X m** matrix, where the color of each matrix cell represents the mean or integrated level of activity over a finite (20-50 ms) time interval at a single recording site. By storing selected matrices, statistical comparisons can be made of activity patterns across brain and muscular domains during different movements, at different times during a given movement, and at different stages of skill acquisition. We are also developing pattern recognition hardware and software, based upon cluster analysis, which allow changing neuronal patterns to be tracked quantitatively as new skills are acquired, or to detect (from brain signals) the emergence of intended limb movements. Such data can potentially be used for control of neural prosthetic devices or functional neuromuscular stimulation in paralyzed patients. Video tapes illustrating the operation of these software systems will be presented.

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355.18

ENSEMBLE NEURON ACTIVITY IN MEDIAL FRONTAL CORTEX OF AN AWAKE BEHAVING ANIMAL: ADVANCES IN METHODOLOGY.

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The purpose of this study was to develop a new technical approach for recording ensembles of single neuron activity in the medial frontal cortex in awake rats. The medial frontal cortex is a complex brain region proposed to play a role in the temporal organization of behavior as well as providing an anatomical substrate for the reinforcing properties of drugs of abuse. We have recently obtained initial results recording ensemble unit activity in the neostriatum of behaving rats. Two bundles of 8 microwires were stereotaxically implanted into the medial frontal cortex of male Long-Evans rats. Microwire localization was verified from X-rays and histology at the completion of the experiment. Neuronal activity, consisting of groups of isolated single units, was recorded during spontaneous activity and compared to that obtained while the animal was locomoting on a treadmill. In a preliminary study, nine clearly defined stable units in a single rat were monitored over a two-month period. Two units demonstrated alterations in activity during phasic treadmill walking. During the 30 sec treadmill ON periods, one unit decreased activity while the other increased firing. An interesting finding for this latter unit was a characteristic endogenous 3 sec cycling of activity during the 30 sec treadmill ON periods. The activity of the remaining units was not related to treadmill-enslaved locomotion. These alterations in neuronal activity were not elicited by presentation of either a conditioned tone paired with the treadmill ON period or an unconditioned tone. These findings indicate that routine study of the activity of neuron ensembles across long-term behavioral states is now feasible. *Supported by DA-05381 (JMP), DA-02338, MH-44337, AFOSR-90-0416 and Biological Humanics Foundation (DJW).*

355.19

CORTICORETICULAR ACTIVITY DURING VOLUNTARY GAIT MODIFICATIONS IN THE CAT. B. Kably* and T. Drew. Dept. Physiology, Université de Montréal, Canada, H3C 3J7

Single unit activity was recorded from the peri-cruciate cortex during a task in which cats modified their gait in order to step over obstacles attached to a moving treadmill. A cat was implanted for recording electromyographic activity from flexor and extensor muscles of each of the four limbs; a recording chamber was placed over the right pericruciate cortex, and microwire electrodes (50μm diameter) were implanted into the caudal medulla to identify pyramidal tract neurones (PTNs). In addition, arrays of microwire electrodes were implanted into both sides of the medullary reticular formation (MRF) to identify corticoreticular neurones (CRNs). In continuing experiments, 24 PTNs have been recorded during locomotion; 6 of these PTNs were identified as CRNs. All 6 neurones were identified from the left MRF (contralateral to the recording site), from regions in which stimulation (11 pulses, 0.2ms duration, 330Hz) evoked flexion of the left forelimb and extension of the right forelimb. Four (4/6) of the CRNS were identified from electrodes on at least two of the bundles (separated in the antero-posterior direction by 2mm). All 6 CRNs were phasically modulated during locomotion and 4/6 changed their pattern of discharge when the cat modified its gait in order to lift the left limb over the obstacles. It is possible that, during gait modifications, such neurones, via their projections to the MRF, may be involved not only in increasing the activity of flexor muscles in the left, contralateral, limb but also in augmenting the level of extensor muscle activity in the supporting, right limb. (Supported by the MRC, FRSQ and FCAR).

BASAL GANGLIA AND THALAMUS V

356.1

VOLTAGE-GATED CURRENTS CONTRIBUTE TO SPONTANEOUS MEMBRANE POTENTIAL SHIFTS IN NEOSTRIATAL PROJECTION NEURONS. C.J. Wilson*, Department of Anatomy and Neurobiology, University of Tennessee, Memphis, TN

In unanesthetized animals, and in animals anesthetized using urethane, neostriatal spiny projection neurons *in vivo* undergo spontaneous membrane potential shifts, alternating between a very polarized state (-80 to -90 mV) and a subthreshold depolarized state (-45 to -55 mV) from which may arise action potentials. The transition to the depolarized state has been show to require afferent synaptic input, but the existence of a preferred membrane potential and the shape of the membrane potential transition may depend upon active membrane properties. To test this hypothesis, cells were loaded intracellularly with QX-314 (10-50 mM in the electrode) to block Na^+ currents, or with Caesium (0.5 to 2 M in the electrode) to block K^+ currents during *in vivo* intracellular recording in rats anesthetized with urethane.

For neurons not firing spontaneous action potentials (most cells), blockade of Na^+ currents using QX-314 had no effect on the membrane potential or on the time spent at either membrane potential state. In contrast, blockade of K^+ currents with Cs shifted the membrane potential in the depolarized state in the depolarizing direction, causing all cells to become spontaneously active. Additionally blocking action potentials with QX-314 caused the depolarized state to elicit large plateau potentials (-20 to -10 mV). When these were prevented by passage of constant hyperpolarizing current, spontaneous membrane potential variations were continuous, showing no preferred membrane potentials. These results indicate that while the spontaneous membrane potential shifts are timed by synaptic input, their shapes and amplitudes are determined by intrinsic properties of the spiny projection cells, and especially by potassium currents engaged at subthreshold membrane potentials.

356.2

DEVELOPMENTALLY REGULATED TRANSIENT CURRENTS IN RAT NEOSTRIATAL GRAFTS. Z.C. Xu*, C.J. Wilson Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis. Memphis, TN 38163

Previous studies of embryonic and adult rat neostriatum have shown that some transient currents are expressed at different developmental stages. For example, the voltage-dependent, slowly-inactivating K^+ current appears only in striatal neurons of adult animals. In contrast, the low-threshold Ca^{++} current is present only in embryonic striatal neurons. Using these two currents as indicators of maturity, the present study investigated the developmental status of rat neostriatal grafts.

A cell suspension of striatal primordia collected from 16 day gestation embryos was implanted into the kainic acid-lesioned neostriata of adult rats. Intracellular recording *in vitro* was performed two to six months after transplantation. A voltage-dependent, slowly-inactivating transient current, which caused a sag in the response to depolarizing current steps, was evident in graft neurons. This component of the response to injected current steps was sensitive to low concentrations of 4-AP (30 μM). In addition, a low-threshold Cd-sensitive component was found in graft neurons. This component and a TTX sensitive component were responsible for the short duration depolarizing hump elicited by a depolarizing current pulse. These results indicate that two different developmentally regulated currents exist in graft neurons and suggest a heterogeneous maturation of ionic currents in neostriatal graft neurons. Supported by NS26473.

356.3

A SLOWLY-INACTIVATING K^+ CURRENT CONTRIBUTES TO RAMP POTENTIALS IN STRIATAL NEURONS. E.S. Nisenbaum*, Z.C. Xu, and C.J. Wilson; Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee-Memphis, Memphis, TN 38163

Recent voltage-clamp studies using acutely dissociated cells have demonstrated that rat striatal neurons possess a slowly-inactivating A-current (I_{As}) which can be distinguished from other K^+ currents on the basis of voltage-dependence, kinetics, and sensitivity to 4-aminopyridine (4-AP). Using current injection protocols inspired by voltage-clamp studies designed to isolate I_{As}, the present experiments have examined the voltage-dependence of activation and inactivation of I_{As}-like responses in striatal neurons recorded from an *in vitro* corticostriatal slice preparation.

In response to near-threshold depolarizing current pulses, striatal neurons displayed a slow, ramp-like potential. Moreover, when spike discharge occurred, the time to first spike was delayed toward the end of the depolarization. Application of a low concentration (30 μM) of 4-AP, which selectively blocks I_{As}, reduced the slope of the ramp depolarization and decreased the latency to first spike discharge. When cells were hyperpolarized to -90 mV with a pre-pulse, the slope of the ramp potential was increased. In contrast, a depolarizing pre-pulse to -60 mV reduced the slope of the ramp depolarization. The time-course of recovery from inactivation showed that long (3-4 s) hyperpolarizing pre-pulses were required in order to remove inactivation of I_{As}-induced ramp potential. Together, these data indicate that a slowly inactivating K^+ current is present in neurons within the slice, and that the voltage-dependence, kinetics, and pharmacology of this current are similar to the I_{As} current previously described in acutely dissociated cells. (Support: ONR: N00014-92-J-1113, NARSAD, Tourette's Syndrome Association).

356.4

CONTRALATERALLY PROJECTING CORTICOSTRIATAL AXONS INNERVATE BOTH COMPARTMENTS OF THE STRIATUM. A.E. Kincaid* and C.J. Wilson. Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, TN 38163.

Recently it has been shown that neurons located in different layers of the cortex project preferentially to different compartments of the striatum. Neurons located in the deeper cortical layers project to the calbindin-poor patches and neurons in the more superficial layers project to the calbindin-rich matrix. This suggests that corticostriatal neurons can be divided into at least two different classes based on which compartment they innervate. Since crossed corticostriatal neurons represent a single well-defined cell type, we tested the hypothesis that these neurons innervate one compartment preferentially.

We used a combination of biocytin tract tracing and calbindin immunohistochemistry to determine the pattern of axonal innervation of crossed corticostriatal neurons. Biocytin was visualized using nickel chloride-enhanced DAB, producing a black reaction product, and calbindin was visualized using DAB alone, producing a brown reaction product.

Axons were traced to the contralateral hemisphere where they entered the striatum from the dorsal or lateral subcortical white matter. Axons with apparent boutons en passant were located in both the patch and matrix compartments of the contralateral striatum, similar to the pattern in the ipsilateral striatum. These results suggest that a single cell type can project to both compartments of the striatum and demonstrate that both compartments of the striatum receive information from neurons located in the contralateral cortex. Supported by NS20743.

356.5

DOPAMINERGIC DEPLETION AFFECTS THE TIME CONSTANT OF MEMBRANE TRANSIENTS PRODUCED IN RAT STRIATAL NEURONS BY HYPERPOLARIZING CURRENT PULSES. M. J. Twery*, L. A. Thompson and J. R. Walters. NINDS, NIH, Bethesda, MD 20892.

Deficits in brain dopamine alter striatal function and the response of neurons in striatal output areas to dopaminergic stimulation. The present study investigated whether dopaminergic deficiency produced by either a unilateral lesion of dopamine cells or reserpine altered passive membrane characteristics exhibited by striatal neurons in tissue slices. Intracellular recordings from striatal neurons located ipsilateral (n=45) to a 6-hydroxydopamine (6-OHDA)-induced lesion of midbrain dopamine cells (6-18 wk) revealed a decrease ($p<0.05$) in the mean time constant (τ^*, the larger tau of a double exponential curve fit) of the membrane transient produced by small hyperpolarizing current pulses compared to contralateral control neurons (n=37). The reduction in τ^* was not accompanied by changes in either membrane potential or input resistance (R_{in}). When striatal neurons were differentiated according to current-voltage relations, the mean neuronal capacitive parameter, τ^*/R_{in}, was significantly lower among the ipsilateral neurons exhibiting relatively linear relations compared to contralateral controls ($p<0.05$). The mean τ^*/R_{in} of neurons exhibiting a strong pattern of inward rectification associated with depolarization did not appear to be affected by denervation. Ongoing studies in reserpinized rats (1 mg/kg/day, 6 days), suggest that reductions in τ^*, but not τ^*/R_{in} occur as a result of acute dopamine depletion (n=12). The results suggest that intrinsic membrane properties in striatal neurons with relatively linear current-voltage relations may be under the influence of factors closely associated with the function of nigrostriatal nerve terminals.

356.6

A ROLE FOR EXCITATORY AMINO ACID (EAA) RECEPTORS IN MEDIATING THE BASAL ACTIVITY OF RAT GLOBUS PALLIDUS (GP) NEURONS AND THEIR ACTIVATION BY THE SUBTHALAMIC NUCLEUS (STN). R.P. Soltis*, J.R. Walters and M.D. Kelland. ETB, NINDS, NIH, Bethesda, MD 20892, USA.

EAA receptors play an important role in modulating basal ganglia function. The STN, as a source of putative glutamatergic projections within the basal ganglia, has received particular attention in this regard. To examine the effects of local EAA receptor blockade on GP neuronal activity under basal conditions and following activation of the STN input, drugs were infused directly into the GP and/or the STN while recording extracellular single-unit activity of Type II (+/- waveform) GP neurons in anesthetized rats. Local infusion of NMDA (30-300 pmol/200 nl) or AMPA (0.01-1.0 pmol) elicited increases in the firing rate of GP neurons in a dose-dependent fashion. In contrast, the NMDA antagonist dizocilpine (MK 801; 0.01-0.1 nmol) produced no discernable effect on basal firing rate; local infusion of the AMPA receptor antagonist NBQX (0.1-1.0 nmol) reduced the firing rate up to 50% of baseline. These data agree with our observations that systemic administration of NBQX inhibits the firing rate of GP neurons whereas dizocilpine produces no significant effect on the firing rate of these cells (Thompson et al., Neurosci. Abs., 1992). Infusion of the GABA-A receptor antagonist bicuculline methiodide (10 pmol/100 nl) into the STN elicited increases in the firing rate of GP neurons that were reversed by infusion of the non-selective EAA antagonist kynurenic acid (30 nmol) into the GP. Thus, EAA receptors mediate, at least in part, the basal activity of GP neurons as well as the increase in neuronal activity resulting from activation of a presumed glutamatergic input from the STN.

356.7

TONIC EXCITATORY INFLUENCE ON CAUDATE (CD) AND GLOBUS PALLIDUS (GP) NEURONS IS MEDIATED BY AMPA BUT NOT NMDA RECEPTORS. L. A. Thompson*, R. P. Soltis, J. R. Walters, and M. D. Kelland. ETB, NINDS, NIH, Bethesda, MD, 20892, USA.

Manipulations of excitatory amino acid (EAA) transmission with selective receptor antagonists have been suggested as having therapeutic potential for disorders of the basal ganglia such as Parkinson's disease. In order to evaluate the tonic influence of EAA systems on spontaneous neuronal activity in the basal ganglia, we have examined the effects of systemic administration of the AMPA receptor antagonist NBQX and the NMDA antagonist dizocilpine (MK-801) on spontaneously active CD Type I, GP Type II (+/- waveform), substantia nigra pars reticulata (SNpr), and entopeduncular nucleus (ENT) neurons. Extracellular single unit recordings were carried out in locally-anesthetized, paralyzed rats. NBQX (cumulative dosing, 1.0 - 33.3 mg/kg i.v.) had a significant dose-related inhibitory effect on the firing of CD (n=6) and GP (n=5) neurons. At the highest dose, firing rate was decreased to 22 ± 7% and 9 ± 4% of the basal rate in the CD and GP, respectively. NBQX had no effect on the activity of SNpr neurons (n=5). Some ENT neurons (n=5) showed either excitatory or inhibitory responses to NBQX that were not related to dose in the range tested. In contrast, MK-801 (0.003 - 3.3 mg/kg i.v.) had no significant effect on spontaneously active neurons in the CD (n=6), GP (n=6), or SNpr (n=7), but inhibited ENT neurons (n=4) in a dose-dependent manner. These results suggest that AMPA receptor activation plays a role in the tonic activity of CD Type I and GP Type II neurons, while NMDA receptor activation may have a relatively greater influence on ENT neurons.

356.8

ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL EVIDENCE FOR TWO DISTINCT CELL TYPES IN THE RAT GLOBUS PALLIDUS WITH DIFFERENT RESPONSES TO SYSTEMIC APOMORPHINE. M.D. Kelland*, R.P. Soltis and J.R. Walters. ETB, NINDS, NIH, Bethesda, MD 20892, USA.

Extracellular single-unit recording techniques were used to examine the rat globus pallidus (GP). In both locally-anesthetized, paralyzed rats and ketamine-anesthetized rats we observed two distinct extracellular waveforms, which we have labelled Type I (-/+) and Type II (+/-). There were no significant differences in the mean firing rate, firing pattern or number of cells/track between these cell types, but there was a significant trend towards higher numbers of fast-firing Type II cells. Surprisingly, Type I GP neurons were inhibited by systemic apomorphine. This is in contrast to the well-established excitation of Type II GP cells by apomorphine. Pretreatment with a subthreshold tachyphylactic dose of apomorphine reduced the responsiveness of both cell types to a subsequent high dose of apomorphine; pretreatment with dizocilpine (MK 801) changed the pattern of response to apomorphine only with regard to Type II GP neurons. Relative to locally-anesthetized, paralyzed rats, ketamine-anesthesia reduced the firing rate of both cell types, but did not alter the firing patterns or the direction of response of each cell type to apomorphine. Thus, two distinct GP cell types have been identified based on their observed extracellular waveform, and they exhibit differential pharmacological profiles. These data may necessitate a re-evaluation of general theoretical models of basal ganglia function in order to account for different effects of DA receptor stimulation on globus pallidus output.

356.9

COMPARATIVE TIME-COURSE OF HALOPERIDOL-INDUCED CATALEPSY AND ALTERATION IN ENKEPHALIN (ENK) mRNA LEVELS IN RAT STRIATUM. J.M. Delfs*, C. R.Smith, B. S.Neal and M-F Chesselet. Inst. of Neurol. Sci., and Depts of Psychiatry and of Pharmacol. U. of Penn. Phila, PA 19104

ENK-containing striato-pallidal neurons are thought to be critical for the control of movement by the basal ganglia. The cataleptogenic drug haloperidol (HAL) increases levels of ENK mRNA in the striatum. The goal of this study was to determine whether a direct relationship exists between HAL-induced catalepsy and striatal ENK mRNA levels. Adult rats received daily sc injections of vehicle (Tween 80) or HAL (1 mg/kg). All rats were tested for catalepsy 2 and 4 hrs after the injection every 3-4 days. HAL-treated rats consistently exhibited marked catalepsy after 3-21, but not 28 days of treatment. Rats were sacrificed 24 hrs after the last drug injection and sections from the striatum processed for in situ hybridization histochemistry with a ^{35}S-RNA probe for ENK mRNA (S. Sabol, NIMH). ENK mRNA was increased in the striatum and nucleus accumbens of rats sacrificed after 7, 14, 21 and 28, but not 3, days of treatment with HAL. There was no correlation between the magnitude and time course of ENK mRNA changes and the catalepsy score of individual rats. The data indicate that, with this regimen, sustained changes in striatal ENK mRNA precede and outlast HAL-induced catalepsy. Supp. by PHS grants MH44894, GM34781 and MH17168.

356.10

SPATIALLY SPECIFIC SPREAD OF EXCITATION IN THE MOUSE NEOSTRIATUM IN VITRO

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The complex organisation of the striatum is believed to provide the basis for a topographic processing of information. In order to investigate the spread of activity in different regions of the striatum, we performed photometric multisite measurements using voltage-sensitive dyes and laser scanning microscopy.

Coronal slices (400 μm) of the mouse neostriatum were incubated for 1 h in artifical cerebrospinal fluid containing the fluorescent dye RH 414 (30 μM). Twenty positions for registration of optical signals were arranged within an area of 1x1 mm, covering most of the striatum. The stimulation electrode was placed into the center of the striatum.

Intrastriatally induced optical signals were detected over the entire striatum with a strong preference for the ventrolateral direction. This spread of activity was associated with an enhancement of amplitude. All optical responses were reversibly abolished by perfusion with Ca^{2+}-free solution, by adding TTX (0,6 μM) or by the AMPA-receptor antagonist CNQX (10 μM). The GABA-A antagonist bicuculline (10 μM) did not significantly influence the amplitude of the optical signals but prolonged their duration. This effect was partly and reversibly blocked by the NMDA-antagonist APV (30 μM).

The data demonstrate that intrastriatal stimulation evokes AMPA-receptor dependent and GABA-A modulated synaptic activity which spreads over the striatum in a characteristic pattern.

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356.11

DOPAMINE-DEPLETION OF THE NUCLEUS ACCUMBENS LEADS TO INCREASED ACTIVATION OF THE LOCAL CIRCUITS.

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The Nucleus Accumbens (Acb) or limbic striatum receives important excitatory (glutamatergic) input from the hippocampal formation via the fornix fibers and a dopaminergic input from the ventral tegmental area. A characteristic field potential in the Acb is evoked by electrical stimulation of the fornix fibers. It consists of two positive waves peaking at 10 and 25 ms, corresponding to firing activity of the Acb neurons. To investigate the interaction between the excitatory and the inhibitory input, we examined the effects of denervation of the latter.

22 male Wistar rats (± 160g) were unilaterally injected with 6-OHDA in the Acb. The contralateral Acb was sham lesioned. The rats were divided in short-term (ST) and long-term (LT) survival groups of 1-2 weeks and 24 weeks, respectively. Field potentials were measured in the Acb. In ST rats, a striking increase (up to 4 times) of the amplitude of the P10 component at the site of the lesion, compared to the sham-lesioned contralateral Acb and to untreated rats, was found. LT rats still displayed an increase of 1.5 to 2 times. In 7 ST, 6 LT and 12 control rats unit-activity was measured using iontophoretical application of L-Glutamate (L-Glu). For each single cell we estimated the threshold ejection current (TEC) for a L-Glu response and the current at which the maximal firing rate (MFR) was obtained. MFR minus TEC was called the effective range (EFR). The results (mean ± sem) are given in the table.

	TEC (-nA)	EFR (-nA)	MFR <40 Hz (Hz)	MFR >40 Hz (Hz)
Controls	24.1 ± 3.1	18.8 ± 1.8	18.4 ± 1.9 (28 cells)	56.2 ± 5.6 (6 cells)
St Rats	21.8 ± 1.3	28.3 ± 2.8	24.0 ± 5.6 (19 cells)	78.9 ± 3.4 (25 cells)
Lt Rats	23.6 ± 2.0	36.9 ± 5.5	26.2 ± 2.3 (13 cells)	57.7 ± 4.9 (11 cells)

We conclude that dopamine denervation leads to an altered activational state of the Acb, resulting in an increase of the effective range by which higher firingfrequencies can be reached. This is also reflected in the increase in amplitude of the field potential.

356.12

ASPECTS OF MORPHOLOGY AND SIGNAL TRANSMISSION OF DOPAMINERGIC, CHOLINERGIC AND GLUTAMATERGIC ELEMENTS IN THE STRIATUM OF THE RAT AND THE LIZARD GEKKO GECKO

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A characteristic feature of the neostriatum of mammals is that the release of acetylcholine (ACh) can be stimulated by glutamate through activation of a N-methyl-D-aspartate receptor and subsequently inhibited by dopamine (DA) through activation of a D2 receptor. Using a superfusion technique we found that the release of ACh is not significantly inhibited upon D2 receptor activation in the caudomedial part of the nucleus accumbens of the rat nor in the striatal complex of the reptile *Gekko gecko*. We furthermore investigated the effects of D1 receptor activation and forskolin on the second messenger adenylate cyclase in the presence and absence of D2 receptor activation in the striatum of both species. To study the morphological substrate of dopamine-acetylcholine interactions in the striatal complex of *Gekko* we conducted single- and dual immunocytochemistry using antibodies against tyrosine hydroxylase (TH) and choline acetyltransferase (ChAT). In addition, we studied the ultrastructural characteristics of dopaminergic fibers and cholinergic neurons using antibodies against dopamine itself and ChAT, respectively. Analysis of our findings suggests that the striatal complex of Gekko bears more similarity to the caudo-medial (shell) part of the nucleus accumbens of the rat than to the rat neostriatum.

356.13

COMPARATIVE EFFECTS OF D1 AND D2 DOPAMINE AGONISTS ON SOMATOSTATIN AND NEUROPEPTIDE Y RELEASE IN CULTURED STRIATAL NEURONS.

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The neuropeptides somatostatin (SS) and neuropeptide Y (NPY) are co-localized in medium-sized spiny neurons of the striatum. These neurons, which also contain nitric oxide synthase and are spared in Huntington's Disease, are known to have dopamine receptors. This study was designed to investigate the effects of D1 and D2 dopamine receptor agonists on SS and NPY release in primary cultures of striatal neurons. The D1 agonist, SKF 38393 had no effect on the basal release of SS and NPY. By contrast, the D2 agonist quinpirole potentiated SS release 3-fold without affecting NPY. The ability of 56mM KCL to provoke a 12-fold increase of SS and a 5-fold increase of NPY was not affected by the D1 agonist but was attenuated by quinpirole. Neither the D1 nor the D2 agonist influenced the kainic acid-induced secretion of SS and NPY, whereas both agents reduced NMDA-stimulated release of SS and NPY. These data indicate that D1 and D2 receptor agonists may produce different responses in the medium spiny neurons of the striatum. They further suggest that SS and NPY release from these neurons may be dissociated under some circumstances.

356.14

ELECTROPHYSIOLOGY OF AGING IN THE NEOSTRIATUM: I. INTRINSIC Ca^{2+}-MEDIATED POTENTIALS.

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Evidence supports both age-related increases and decreases in basal levels of intracellular Ca^{2+} of mammalian neurons. We measured two Ca^{2+}-mediated potentials in the rat neostriatum to determine whether these cells express an age-related change in Ca^{2+} physiology. The afterhyperpolarization (AHP) that follows a single action potential or a series of action potentials was examined in young (<6 mo.) and aged rats (>23 mo.). Measurements were made of the peak amplitude and duration of the AHP. Although durations were much shorter than those previously reported in the hippocampus (50% decay = 5.8 ± 0.3 ms; mean ± sem; n=30), no significant difference in either amplitude or duration was observed between young (n=22) and old (n=8) rats.

The second Ca^{2+}-mediated potential studied was the plateau potential elicited by a depolarizing current injection in the presence of 30 mM TEA. Peak amplitude and duration were measured. The duration in young rats (n=7) varied from 300 to 1300 msec (800 ± 150 msec). By contrast the duration was significantly reduced in the aged rats (200 ± 70 msec; n=5; $p<0.01$; Student's T-test). Our results, which show that age-related changes in the Ca^{2+} physiology lead to decreased Ca^{2+} influx, are consistent with the hypothesis that the Ca^{2+} regulatory mechanism in aged neurons is altered. Supported by NIA grants AG00093 and AG09793.

356.15

ELECTROPHYSIOLOGY OF AGING IN THE NEOSTRIATUM: II. SHORT-TERM PLASTICITY.

X. Qu*, S. Bottjer, T. DeFazio, R. Dunia and J.P. Walsh. Andrus Gerontology Center & Department of Biological Sciences, USC, Los Angeles, CA 90089-0191.

Age-related changes in Ca^{2+} physiology affects many neuronal properties, including the release of neurotransmitter by the presynaptic terminal. A paired-pulse activation paradigm for excitatory corticostriatal afferents was used to determine whether one form of Ca^{2+}-mediated plasticity (paired-pulse potentiation) was altered in the neostriatum of the aged rat. A potentiation index was made by measuring the amplitude of the second synaptic potential relative to the first. Each cell was tested at various interstimulus intervals (150 to 30 msec; 10 msec decrements). We examined 14 cells from 10 young rats (3-6 mo) and observed paired-pulse potentiation in 9 cells (at intervals < 100 msec), no change in 3 of the cells, and slight paired-pulse depression in 2 cells (all interstimulus intervals studied). By contrast, paired-pulse depression was observed in all 10 aged neurons (>24 mo) recorded from 8 animals.

We also studied synaptic depression using a 15 pulse paradigm. Afferents received 15 stimuli at interstimulus intervals ranging from 30 msec to 1 sec. In aged rats, the EPSP exhibited, on average, more depression at all inter-stimulus intervals tested. Furthermore, the onset of the synaptic depression was more rapid in the aged animals. Supported by NIA grants AG00093 and AG09793.

356.16

BENZODIAZEPINE, GLUTAMATE, MUSCARINIC AND DOPAMINE RECEPTORS IN PUTAMEN AND GLOBUS PALLIDUS OF ALZHEIMER'S AND PARKINSON'S DISEASES.

JB Penney*, LS Dure IV, MV Catania, M Carlson, RH Price, Z Hollingsworth and AB Young. Neurology Service, Massachusetts General Hospital, Boston, MA 02114

We used ligand binding autoradiography to measure binding sites for 9nM [^{3}H]mazindol, 10nM [^{3}H]flunitrazepam, 10nM [^{3}H]AMPA, 60nM [^{3}H]kainate, 65nM [^{3}H]glutamate (NMDA conditions), 100nM [^{3}H]glutamate (metabotropic conditions), 1 nM [^{3}H]QNB, 0.5nM [^{3}H]SCH 23390, and 0.25nM [^{3}H]spiperone in the lentiform nuclei and choline acetyltransferase activity in putamen of 9 control, 9 Alzheimer's (AD), 8 Parkinson's (PD) and 9 AD plus PD brains. Data were analyzed by 2 way ANOVA. There were significant reductions of PD putamen dopamine uptake sites by 83% (96±32 vs 551±41 fmol/mg protein; mean±sem), NMDA sites by 26% (81±7 vs 110±10) and muscarinic sites by 9% (2264±82 vs 2487±72). AD insular cortex NMDA sites were reduced by 39% (214±36 vs 350±40), type 2 metabotropic sites by 33% (222±26 vs 334±45) and muscarinic sites by 17% (1560±123 vs 1875±123). Benzodiazepine sites were increased by 20% (289±17 vs 240±13) in AD putamen and by 25% (183±10 vs 146±13) in AD external globus pallidus. The binding reductions are consistent with the known pathology and pharmacology of AD and PD. The increases in benzodiazepine binding imply striatopallidal hypoactivity in AD. Current concepts of basal ganglia function suggest that such hypoactivity ought to reduce Parkinsonian symptoms in patients with combined AD and PD.

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356.17

A MODEL OF STRIATAL FUNCTION IN HABIT FORMATION,
Peter Dominey(1), Jean-Paul Joseph(2)*, Michael Arbib(1), (1) University of Southern California,LosAngeles 90089, (2) INSERM Unité 94, 69500 Bron,France.

We hypothesize that sensory activity (from inferotemporal Cx - IT) becomes associated with spatially selective saccade-related cells in the anterior striatum (Str) as a result of learning, by trial and error, to associate central visual cues (colored shapes) with saccades to one of four uniform peripheral targets. Learning, modification of IT-Str synapses, is regulated by reward-related nigrostriatal dopamine activity. This hypothesis is formalized in a model that includes IT, posterior parietal Cx (PP), FEF, Str, substantia nigra, and thalamus (VA, MD).

Cue and target input is provided by a spatial array where each element codes color and shape. Output is in the form of activity in an oculomotor map representing FEF. Spatial/movement selective Str cells receive topographic FEF and PP projections, and non-topographic IT projections. The model learns to associate many cues to each spatial target. For a new cue, noise provides initial "guessing" between the four targets. With a correct response, reward related activity strengthens the involved IT-Str synapses. Mistakes yield synaptic weakening. Significant learning dominates guessing effects of noise, and performance approaches 100%.

In our model, a new cue produces non-selective Str activity. With learning, the cue-related activity increases in Str cells having a spatial preference for the correct saccade, and decreases in cells with the non-preferred direction, yielding a net decrease in Str activity. Initial correct saccades that occur before significant learning (guesses) precede these cue-related changes. Different cues associated with the same saccade produce similar activity increases and decreases in cells with the respective spatial preferences and non-preferences. Cue similarity (eg. same color, different shape) produces generalization favoring previous associations. Strengthening IT-Str synapses on correct responses without weakening them on errors severely impairs learning conflicting associations, and less severely impairs learning non-conflicting ones, similar to 6-OHDA nucleus accumbens lesions in rodent. We are now training a monkey in this habit formation task to test these predictions.

356.18

MODULAR NETWORK ARCHITECTURE FOR PLANNING AND CONTROLLING MOTOR BEHAVIOR FORMED BY TOPOGRAPHIC PROJECTIONS BETWEEN CEREBELLUM, BASAL GANGLIA AND CEREBRAL CORTEX. J. C. Houk* and S. P. Wise, Dept. of Physiology, Northwestern University Medical School, 303 E. Chicago Ave, Chicago, Illinois 60611, and Lab. of Neurophysiology, National Institute of Mental Health, P.O. Box 289, Poolesville, Maryland 20837.

We outline a theory of motor behavior that will synthesize recent discoveries concerning the anatomy and physiology of the cerebellum, basal ganglia and cerebral cortex (Houk, J.C., Wise, S.P. *Technical Report 5. Center for the study of Neuronal Populations and Behavior.* Institute for Neuroscience, Northwestern University, 1992). This theory attempts to draw upon diverse knowledge coming from many disciplines of neuroscience. A consideration of regional specializations in neuronal architecture combined with the cellular properties of the principal neurons of these three brain regions leads to the concept that there are a few basic types of information processing module, and that these modules are used iteratively, in a cooperative manner, to control behavior. At one level of iteration, groups of similar modules form modular arrays that solve specific classes of problem. At a higher level of iteration, different types of modular array are interconnected to function in parallel in the control of overt behavior. (Supported by NIMH P50 MH48185 and ONR N00014-90-J-1822.)

CEREBELLUM II

357.1

THE ABNORMAL PRENATAL DEVELOPMENT OF THE EGL AND THE CONSEQUENCES IN THE CEREBELLUM OF THE MEANDER TAIL MUTANT MOUSE. J.A. Napieralski* and L.M. Eisenman, College of Graduate Studies, Thomas Jefferson University, Philadelphia, PA 19107.

The cerebellum of the mutant mouse meander tail is characterized by the presence of an apparently normal posterior lobe and an abrupt transition to a disorganized anterior lobe (abnormal foliation, agranular, Purkinje cell (PC) ectopia, and a paucity of Bergmann glial fibers (Ross et al 1990)). An analysis of the postnatal development of the meander tail cerebellum revealed a reduction in the EGL of the anterior portion of the cerebellum as early as the day of birth. This study reports the results of an examination of prenatal cerebellar tissue (E15-E18) from mea/mea and +/mea littermates. The data suggest that differences between the cerebellum of the meander tail and normal mouse are apparent as early as embryonic day 17 (E17) in the anterior most portion of the developing cerebellum. At E17 it is clear that there is (1) a thinner EGL and an incomplete migration of this layer over the surface of the cerebellum, (2) an incomplete migration of the PC to form the multilamina normally found beneath the developing EGL, and (3) the continuing presence of fibers in the dorsal and rostral portion of the cerebellum which are last seen in the normal animal at E16. These results suggest that the reduction in GC is due to a problem in cell proliferation in the EGL with a consequent failure to populate the EGL in the anterior lobe of the meander tail cerebellum. Supported by NIH grant NS22093.

357.2

DEVELOPMENTAL EXPRESSION OF CGRP mRNA IN THE OLIVOCEREBELLAR SYSTEM OF THE RAT. S. Morara[1], C. Sternini[2], L. Provini[3], A. Rosina[1](SPON: ENA). [1] Ist. Fisiol. Centri Nervosi, CNR, 20131 Milano, Italy, [2] CURE, Dept. of Medicine, UCLA & VAMC, Los Angeles, CA 90073, [3] Ist. Fisiol. Gen. Chim. Biol., Univ. Milano, 20133 Milano, Italy.

Our previous data showed that CGRP is transiently detected by immunohistochemistry in the rat olivocerebellar system, at a postnatal stage coincident with the "multiinnervation period" of the Purkinje (Pk) cells by the climbing fibers (CFs) (see also Rosina et al., this Meeting). High affinity CGRP binding sites were found neonatally in the Pk cell layer and in the adulthood in the molecular layer. Thus, it was of interest to study the expression of the CGRP mRNA, to analyze a) its time course and distribution in the olivary complex during development, and b) its presence in the adult. "In situ hybridization" with probes for $\alpha-$ and β-CGRP mRNA was used.

The expression of $\alpha-$CGRP mRNA in the olivary complex was found to overlap in time the expression of the peptide in the olivocerebellar system, i.e. it was present from birth to the end of the second postnatal week. Moreover, α-CGRP mRNA was found to be expressed in the medial sector of the caudal part of the medial accessory olive, in the caudal part of the beta and dorsal cap nuclei, and in a small medial region of the caudal part of the dorsal accessory olive, i.e. in the same olivary subnuclei as already described by immunohistochemistry. No $\beta-$CGRP mRNA was found to be present in the olivary complex either during the development or in the adulthood. The results indicate that the expression of mRNA encoding for CGRP in the olivocerebellar system is developmentally regulated and covers the period of transient CF connections with Pk somata, preceding the stage of CF climbing on the mature Pk dendritic tree. Thus, a role for the peptide in the plastic processes taking place in the cerebellum during development is confirmed.

357.3

CLIMBING FIBER INTERACTIONS WITH PURKINJE CELLS AS REVEALED BY CGRP IMMUNOSTAINING. A. Rosina[1], S. Morara[1] and L. Provini[2](SPON: ENA). [1]Ist. Fisiol. Centri Nervosi CNR, 20131 Milano and [2] Ist. Fisiol. Gen. Chim. Biol., Univ. Milano, 20133 Milano, Italy.

The neuropeptide Calcitonin Gene-Related Peptide (CGRP) is neonatally expressed in the rat olivary afferents to the cerebellum for a short time window, which coincides with the multiinnervation period of Purkinje (Pk) cells by climbing fibers (CFs). CGRP mRNA analysis confirms the time course of this transient expression (see Morara et al., this Meeting). By using double-immunostaining and confocal laser-scanning imaging we analyzed the interactions which occur at this developmental stage between CGRP-stained CF terminals and Pk somata, immunostained with Calbindin-D (28 kd).

The results show that from postnatal day (PD) 0 to PD 3, CGRP-positive terminal boutons design a diffuse net through the multilayer of Pk cells. From PD 4 to PD 12, Pk neurons align in monolayer and protrude perisomatic processes all over their perikaryon, while developing the dendritic tree. As visualized by confocal imaging and 3D reconstruction, CGRP-positive CFs progressively intensify their contacts with the Pk perisomatic processes throughout this period. The boutons first contact the inferior part of Pk soma in the form of a nest, later the whole soma up to the trunk of the primary dendrite. Between PD14 and PD21 the Pk cells adopt their adult morphology and the CGRP-positive boutons are no longer detectable. Thus, CGRP is expressed in the CF terminal boutons at the stage of perisomatic transient synapses, while the selection of a single CF axon collateral, which will form mature dendritic synapses, takes place. The factors underlying this plastic reshaping remain unknown. However, in view of the fact that CGRP receptors are expressed at the Pk cell layer at the same developmental stage,CGRP is a very likely candidate as a transmembrane signalling factor.

357.4

CEREBELLAR PROJECTIONS TO THE FORELIMB RECIPIENT ZONE OF THE CAT DORSAL ACCESSORY OLIVE ARE GABAERGIC. H.H. Molinari*. Department of Anatomy, Albany Medical College, Albany, N.Y. 12208

Most of the inferior olive receives GABAergic inputs from either the deep cerebellar nuclei or the lateral vestibular nucleus (Nelson and Mugnaini, 1989). Within the dorsal accessory olive (DAO) of rat, however, the hindlimb recipient zone receives such input but the forelimb recipient zone does not. The present study sought to determine whether the forelimb recipient zone of cat DAO likewise fails to receive GABAergic input from the deep cerebellar nuclei.

Wheat germ agglutinin conjugated to horseradish peroxidase was injected into the deep cerebellar nuclei of adult cats. The anterogradely transported tracer was visualized with tetramethyl-benzidine. The tissue was trimmed to include only the forelimb recipient zone of DAO, embedded for EM, and processed for GABA immunocytochemistry.

The vast majority of cerebellar terminals identified to date were also GABA-immunogold labeled. Thus, in contrast to rat, GABA-ergic terminals in the forelimb recipient zone of the cat DAO arise, at least in part, from the cerebellum. The terminals contacted dendritic shafts and dendritic spines in a ratio of about 2:1, the same ratio described by de Zeeuw et al. (1989) for cerebellar terminals in other olivary regions These data indicate that input from the cerebellum serves a common function throughout much of the inferior olive (NSF-BNS-8809840 and NIH-1R55NS29771).

357.5

PALE CELLS OF THE FLOCCULO-NODULAR LOBE ARE CALRETININ POSITIVE. A. Floris, M.E. Dunn, A.S. Berrebi *, D.M. Jacobowitz and E. Mugnaini, Lab. of Neuromorphology, Univ. of Connecticut, Storrs, CT 06269-4154 and Lab. of Clinical Science, Bldg. 10- 3D48, NIMH, Bethesda, MD 20892.

Immunostaining with antiserum to the calcium binding protein calretinin (CR) revealed no immunoreactivity within cerebellar Purkinje cells and stellate/basket cells of the molecular layer, but showed moderate staining of the granule cells and, more conspicuously, intense staining of non-granular neurons in the granular layer that are enriched in the ventral folia (I,IX,X). By their features, these densely stained cells were correlated with Lugaro and Golgi cells (Arai et al., J. Comp. Neurol. 310, 21-44, 1991). In this immunocytochemical light and electron microscopic study we demonstrate for the first time, in both rat and mouse, that densely CR-like immunoreactive cells in the flocculo-nodular lobe and the dorsal cochlear nucleus have features previously attributed to the so-called pale cells. Pale cells are a particular type of neuron situated in the granular layer of the cerebellar cortex. They occur at the highest density in the caudal cerebellum. Altman and Bayer (Exp. Brain Res. 29, 265-274, 1977) discovered that they are generated rather late in ontogeny (E19-P2 in the rat). We show that CR-like immunoreactive cells in the caudal cerebellum have dendrites which are laden with non synaptic evaginations and form conspicuous synaptic contacts with mossy fibers. These dendrites have special intermediate filaments and a rich complement of mitochondria; they also contain a fair number of dense core vesicles and occasional clusters of clear synaptic vesicles, suggesting some sort of paracrine function. The CR-positive dendrites receive symmetrical synapses from boutons containing pleomorphic vesicles, similar to Golgi cell axonal boutons. Neurons resembling pale cells were also identified in dissociated cerebellar cultures. In the cerebellum of the reeler mutant mouse, where cell migration is impaired, pale cells collect near the roof of the fourth ventricle, supporting the notion that they originate from the ventricular epithelium and not from the external granular layer. *Supported in part by grant NS 09904.*

357.6

EVIDENCE FOR THE PRESENCE OF THE NUCLEO-OLIVARY PROJECTION AT E18 IN THE MOUSE. M.A. Urban* and L.M. Eisenman, College of Graduate Studies, Thomas Jefferson University, Philadelphia, PA 19107.

The nucleo-olivary system is the reciprocal projection of the olivocerebellar system and displays a similar topographic pattern. The postnatal development of this GABA-ergic projection has been investigated immunohistochemically in the rat using antibody to glutamic acid decarboxylase (Gotow and Sotelo, '87). Fibers immunopositive to GAD were localized almost exclusively to perinuclear and interlamellar regions at P0, progressively invading the nuclear groups between P0 and P5. This study suggested that the projection reaches the olive at birth, but is established postnatally. Recent neuroanatomical tracing studies performed in E18 mice using applications of horseradish peroxidase crystals into the dentate nucleus reveal the presence of labelled fibers ascending in the brachium conjunctivum, crossing in the decussation of the superior cerebellar peduncles, running caudally in the descending limb of the brachium and into and among the cells of the contralateral principal olive. HRP application into the fastigial nucleus resulted in labelled fibers traversing the same course but which terminated in the ipsilateral medial accessory olive. These studies demonstrate the presence of nucleo-olivary fibers in the inferior olive prenatally and suggest that the projection is established prior to birth in a pattern comparable to that of the adult.

357.7

IMMUNOCYTOCHEMICAL LOCALIZATION OF L-BACLOFEN-SENSITIVE $GABA_B$ BINDING SITES IN CEREBELLAR CORTEX. G.R. Holstein*G.P. Martinelli and B. Cohen, Departments of Neurology, Cell Biology/Anatomy, Surgery and Physiology/Biophysics, Mount Sinai School of Medicine, CUNY, New York, N.Y. 10029.

Radiohistochemical studies have indicated that $GABA_B$ receptors are predominant in the molecular layer of rat cerebellar cortex, and may be located on Purkinje cell dendrites, as well as parallel fiber and/or climbing fiber terminals. These observations are supported by pharmacologic and physiologic data suggesting the existence of both pre- and post-synaptic $GABA_B$ sites. We have used an immunocytochemical approach to study the synaptic interactions involving L-baclofen-sensitive $GABA_B$ binding sites in rat (Sprague-Dawley) and monkey (M. fascicularis) cerebellar cortex. Experimental animals received an i.m. injection of L-baclofen-HCl (5 mg/kg); controls received an equal volume of saline. Ninety min. post-injection, animals were sacrificed by perfusion. Fifty µm vibratome sections were exposed to a monoclonal antibody raised against L-baclofen, and then further processed with the unlabeled antibody PAP procedure.

Saline-injected control animals exposed to primary antibody showed no immunoreactivity, and there was no immunostain in sections from experimental and control animals that were incubated in a solution lacking primary antibody. Immunoreactivity was present only in the drug-injected animals. At the light microscopic level, the molecular and Purkinje cell layers were markedly immunostained. Some of this staining appeared to be associated with the Purkinje cell dendritic trees. In addition, the granular layer showed pale immunoreactivity which was particularly evident in the cerebellar islands. At the ultrastructural level, the reaction product was manifest as dense deposits adherent to the inner plasma membrane of immunostained profiles. The label was also present on the outer membranes of cisternae and mitochondria. Most of the stained profiles were less than 0.5 µm in diameter. In general, these immunoreactive profiles exhibited dendritic morphology, and occasionally appeared to occupy postsynaptic sites. These results suggest that $GABA_B$ receptors are present on Purkinje cell dendrites. Aided by NIH Grants # NS-24656, NS-00294. L-baclofen was generously provided by Ciba-Geigy, Ltd.

357.8

SAGITTAL ORGANIZATION OF THE LATERAL RETICULO-CEREBELLAR PROJECTION IN THE MOUSE: A COMPARISON WITH THE MOSSY FIBER COLLATERAL SYSTEM BY DOUBLE ANTEROGRADE TRACING. J.A.Heckroth*. Indiana University School of Medicine, Terre Haute Center for Medical Education, Terre Haute, IN, 47809.

Mossy fiber afferents which arise from the lateral reticular nucleus of the medulla (LRN) have been shown to terminate in the cerebellar cortex in a longitudinally organized pattern in several species. The present study compares this pattern with the organization of the mossy fiber collateral system previously demonstrated by Heckroth and Eisenman (J. Comp. Neurol. 270:385-394, 1988). Adult B_6CBA mice received pressure injections of 1% WGA-HRP in the LRN, and injections of 20% rhodamine conjugated dextran (Molecular Probes) in lobule VIII of the cerebellum. Serial frozen sections were processed by the DeOlmos, Harding and Heimer TMB method and examined by epifluorescent and bright-field illumination. Sagittal stripes of the granular layer containing rhodamine labeled mossy fiber rosettes (from lobule VIII) were observed in the anterior lobe, and were compared *in the same sections* with the organization of TMB labeled mossy fibers (from LRN). Comparison of labeled regions and boundaries indicates that **these two mossy fiber systems respect common sagittal boundaries** which extend throughout the longitudinal extent of the cerebellar anterior lobe. Even where TMB labeled mossy fibers occupied two contiguous zones, the boundary between the zones remained visible as a thin label-free raphe. These results provide direct evidence that the cerebellar granular layer is divided by fixed sagittal boundaries into longitudinal compartments, each of which receives a unique combination of afferents.

357.9

AUTORADIOGRAPHIC LOCALIZATION OF CHOLECYSTOKININ (CCK) BINDING SITES IN ADULT OPOSSUM CEREBELLUM. P.C. Madtes Jr., G.A. Bishop and J.S. King*. Neuroscience Program, The Ohio State Univ., Columbus, OH 43210

A previous study has shown that CCK immunoreactive mossy fibers are present in the granule cell layer throughout the opossum's cerebellar cortex (King and Bishop, 1990, JCN 298:373). The intent of the present study is to localize CCK binding sites in the cerebellum. Serial frozen sections were analyzed using the method of Akesson et al. (Neuroendocrinology 1987, 45:257). Specific binding was determined using [125]I-labeled sulfated CCK_8 by subtracting nonspecific binding (measured in the presence of unlabeled sulfated CCK_8) from total binding (measured in the absence of unlabeled sulfated CCK_8). Binding sites were localized by apposing Kodak X-OMAT AR film to the slides for specific time periods. The films were processed and analyzed using a computer-based densitometry system. The K_D value (0.1nM) agrees with values reported for CCK receptors in other mammals. CCK binding sites are present in all lobules of the cerebellar cortex. With respect to laminar distribution there is a gradient of binding sites ranging from the highest density in the molecular layer to the lowest in the granule cell layer. Thus, the CCK binding site localization matches the lobular distribution of the peptide; however, there is a receptor-peptide mismatch with respect to the molecular layer which suggests CCK could act through volume transmission. That is, CCK may reach receptors in the molecular layer by diffusing through the extracellular space, from its localization in mossy fiber terminals within the granule cell layer. Preliminary findings based on the iontophoresis of CCK and extracellular recordings indicate that this peptide primarily has a facilitatory effect on the glutamate- and aspartate-induced firing rate of neurons in the cerebellar cortex. These data suggest that CCK may act as a neuromodulator within the opossum cerebellum. (Supported by NS 08798.)

357.10

THE ORIGIN OF SEROTONINERGIC PROJECTIONS TO THE CEREBELLAR NUCLEI OF THE CAT. P.H. Kitzman* and G.A. Bishop, The Department of Cell Biology, Neurobiology, and Anatomy. The Ohio State University. Columbus, OH. 43210.

Serotoninergic (5-HT) axons are present throughout the granule cell layer and the cerebellar nuclei of the cat cerebellum, where they form a dense beaded plexus. In a previous study, 5-HT axons in the cortex were found to arise from neurons located in the paramedian reticular nucleus, lateral reticular nucleus, peri-olivary reticular formation, and the lateral tegmental field (Kerr and Bishop, 1991, JCN. 304:502). As the source of the 5-HT fibers in the cerebellar nuclei is unknown, the medial, interpositus, and lateral cerebellar nuclei were selectively injected with either rhodamine or fluorescein-labeled latex microspheres which were retrogradely transported to brainstem neurons. Transverse sections of the brainstem were processed for immunohistochemistry with a primary antibody to 5-HT and secondary antibody tagged with either rhodamine or fluorescein. The location of neurons containing both serotonin-like immunoreactivity and retrogradely transported microshperes was plotted. All three of the cerebellar nuclei receive 5-HT afferents from the nucleus locus coeruleus, the dorsal nucleus of the raphe (median division), the dorsal tegmental nucleus (pericentral division), and the central superior nucleus. In addition, the medial nucleus receives projections from the nucleus raphe pallidus and the interpositus nucleus receives afferents from the lateral tegmental field. These brainstem sources of 5-HT to the cerebellar nuclei are distinct from the more caudal brainstem origins of 5-HT afferents to the cerebellar cortex. In a previous study, 5-HT was shown to have a suppressive effect on cortical neurons (Kerr and Bishop, 1992, Brain Res. in press). At present, the effect(s) of 5-HT on neurons in the cerebellar nuclei are unknown. (Supported by NS18028).

357.11

MORPHOLOGICAL CHARACTERISTICS AND SYNAPTIC RELATIONSHIPS OF BASKET CELLS IN THE CAT'S CEREBELLUM. G.A. Bishop*, Y.F. Chen and J.S. King, Dept. of Cell Biology, Neurobiology and Anatomy and Neuroscience Program. The Ohio State University, Columbus, OH 43210

The intent of this study is to analyze the distribution and synaptic relationships of basket cell axons and to quantify the number of GAD immunoreactive terminals on their somata. Basket cells were intracellularly filled with horseradish peroxidase or neurobiotin. The axons of most basket cells distribute either toward the base or the apex of the folium; only rarely are cells found that have a bilateral distribution of the axon around the cell of origin. The parent axons extend 232-682 μm (mean = 348 μm) from the parent cell body and give rise to 5-11 (mean = 8) collaterals that descend to arborize around subjacent Purkinje cell bodies. Descending collaterals that arise in proximity to the parent cell body (within 73-100 μm) arborize extensively forming a complex terminal "pinceau". Descending collaterals that arise distally from the parent cell are much simpler in form. Fine beaded branches (mean = 4/cell) arise from the axons of most basket cells and ascend into the molecular layer. Ultrastructurally both labeled and unlabeled basket cell axons are evident around the somata of Purkinje cells. Few synaptic specializations have been identified. The number of GAD positive terminals surrounding the basket cell body was determined from ultrastructural experiments in which a monoclonal antibody was used. On average, 16% of the synaptic contacts on the basket cell body are GAD positive, whereas 84% have the morphological characteristics of parallel fibers. In summary, these data indicate that 1) collaterals of a basket cell axon arborize to a greater degree around Purkinje cells located close to the parent basket cell; 2) Purkinje cells receive input from more than one basket cell; and 3) the predominant input to basket cell bodies appear to be derived from excitatory afferents. (Supported by BNS 8919796.)

357.12

ELECTRON MICROSCOPIC IDENTIFICATION OF PONTOCEREBELLAR AXON TERMINALS IN THE CEREBELLAR NUCLEI OF THE RAT. G. A. Mihailoff*, Department of Anatomy, Univ. of Mississippi Medical Center, Jackson, MS 39216-4505.

Previous studies in this laboratory have used a light microscopic anterograde axonal transport method, *Phaseolus vulgaris* leucoagglutinin (PHAL) to demonstrate in rats that axons which arise from neurons in the basilar pontine nuclei (BPN) or nucleus reticularis tegmenti pontis (NRTP) distribute to the cerebellar cortex (CTX) and the cerebellar nuclei (CN). Those axons that reached the granular layer of the CTX exhibited the morphology typical of mossy fibers while those distributing to the CN formed thin preterminal fibers that gave rise to small round boutons which have no apparent resemblance to the large, lobulated terminals in the granular layer of the CTX. Further, Shinoda et al. (J. Neurophys. 67:547) using intraxonal recording and filling with HRP have shown that single BPN axons distribute to both the CTX and CN. The objective of the present study then was to use electron microscopy to characterize the boutons formed by BPN and NRTP axon collaterals in the CN. PHAL was iontophoretically injected into the BPN or NRTP of rats and 7 to 12 days later the animal sacrificed, the CN sectioned on a vibratome and the sections reacted immunocytochemically and processed for electron microscopy to visualize the orthogradely labeled CN and CTX boutons. PHAL labeled boutons were observed in the medial and lateral CN, as well as the anterior and posterior interposed nuclei. Such boutons were ovoid or elongate in shape, appeared to contain round clear vesicles, and formed asymmetric synaptic contacts primarily with small dendritic profiles. The labeled boutons in the CN were smaller than those observed in the CTX which clearly exhibited the typical features of mossy fiber terminals. These observations confirm the existence of synaptic terminations in the CN formed by axons of BPN and NRTP origin. Supported by NIH grant NS12644.

357.13

Reticular Projections of the Cat Lateral Cerebellar Nucleus P.L.E. van Kan,* B. van Duin, A.R. Gibson, K.M.Horn, and J. Voogd. Barrow Neurol. Inst., Phoenix, AZ, 85013

Organization of cerebellar output follows a general schema whereby outputs of medial regions are directed more caudally than outputs of lateral regions. Medial nucleus projects mainly to medullary and pontine reticular formations, interpositus projects mainly to magnocellular red nucleus and cerebral cortex via thalamus, and lateral nucleus projects mainly to cerebral cortex via thalamus. Major exceptions to this schema are the nucleus reticularis tegmenti pontis and inferior olive, which receive input from interpositus and lateral nuclei.

Here we report anatomical evidence for another, less recognized, exception to the schema: The cat lateral nucleus projects to extensive regions of the pontine and medullary reticular formation.

We injected WGA-HRP into brainstem regions or cerebellar nuclei and analyzed the pattern of retrogradely labeled nuclear cells or anterogradely labeled brainstem terminations. Injections into pontine or medullary reticular areas labeled cells in the medial nucleus and dorsal regions of lateral nucleus. The same injections labeled few cells in interpositus. Anterograde tracing confirmed the retrograde results: Injections into dorsal regions of lateral nucleus resulted in widespread terminal label throughout brainstem reticular regions; injections into ventral regions of lateral nucleus produced little reticular label.

The reticular projections suggest that dorsal regions of the lateral nucleus are functionally distinct from its more ventral regions as well as from adjoining regions of interpositus.

357.14

ORGANIZATION OF RAT CUNEOCEREBELLAR PROJECTIONS ANALYZED IN 2-D UNFOLDED RECONSTRUCTIONS OF THE ANTERIOR LOBE. D.L. Tolbert*, C. Skillington, P. Puckett, J.M. Alisky and B.R. Clark. Department of Anatomy and Neurobiology, St. Louis University School of Medicine and Program in Physical Therapy, Washington University School of Medicine, St. Louis, MO. 63104.

Cuneocerebellar topography was studied using an image analysis system (Bioquant PM, R&M Biometrics) which graphically unfolds sagittal sections of the anterior lobe while maintaining spatial relationships between labeled terminals and the 2-D reconstructed cortex. WGA-HRP injections into the rat internal and/or external cuneate nuclei labeled mossy fiber terminals in the cerebellar cortex. Cuneocerebellar projections were primarily directed to the ipsilateral hemicerebellum. Projections from the internal cuneate nucleus were mainly localized to a discontinuous, sagittally oriented stripe which extended from the vermis in lobules I and II laterally through lobules III and IV to the lateral paravermis in lobule V. In lobule V an additional stripe was present laterally near its junction with lobule VI. Medially, outside the stripe a relatively small number of labeled terminals were present in the vermis. Injections of both the internal and external cuneate nuclei labeled additional terminals within the sagittally oriented stripe that was only partially labeled by the internal cuneate nucleus injections. The sagittal organization of cuneocerebellar projections is significantly different from the transverse organization of lower thoracic-upper lumbar segmental spinocerebellar projections. Supported by NIH grant NS20227.

357.15

PROJECTIONS OF INDIVIDUAL PURKINJE CELLS OF IDENTIFIED ZONES IN THE RABBIT FLOCCULUS TO THE VESTIBULAR AND CEREBELLAR NUCLEI. C. I. De Zeeuw*, P. L. DiGiorgi, D. R. Wylie, D. Wang, E. Marsh, and J. I. Simpson. Dept. Physiology & Biophysics, NYU Med. Ctr., New York, N.Y. 10016.

The rabbit flocculus can be divided both anatomically and physiologically into three large zones (1, 2, and 3) and two small zones (4 and C2). The projections of Purkinje cells (Pcells) in the three large zones were studied using biocytin as an anterograde tracer. The zones were identified by determining whether the Pcell climbing fiber response to moving visual patterns was best for rotation about particular horizontal axes (zones 1 and 3) or the vertical axis (zone 2). Small extracellular injections of biocytin resulted in intracellular labeling of only a few Pcells, the axons of which could be traced from the cell body to the terminals in the vestibular and cerebellar nuclei. To confirm anatomically the identification of the injected zone, some sections were stained for acetylcholinesterase. The Pcells of zone 1 projected to the ventral dentate nucleus, group y, and the superior vestibular nucleus (SVN); the Pcells of zone 2 projected to the magnocellular and parvocellular parts of the medial vestibular nucleus; and the Pcells of zone 3 projected to group y and the SVN. Some axons of Pcells of zones 1 and 3 branched and innervated two nuclei. The branching axons from zone 1 either innervated both the ventral dentate nucleus and group y or both group y and the SVN. The branching axons from zone 3 innervated both group y and the SVN. The terminals of individual Pcells were apposed to cell bodies of different shapes (round, triangular, and fusiform). In summary, Pcells of each of the large floccular zones project to a specific set of nuclei and an individual Pcell can innervate more than one nucleus.

357.16

DIFFERENCE OF AFFERENT AND EFFERENT CONNECTIONS OF MONKEY FLOCCULUS AND VENTRAL PARAFLOCCULUS AND ITS RELEVANCE TO OCULOMOTOR FUNCTIONS.

S. Nagao, N. Nakamura, J. Yamada[*1)] and T. Kitamura[1)]. Dept. of Physiol., Jichi. Med. Sch. and Dept. of Anatomy, Nippon Med. Sch.[1)], Japan

Our recent unit recording study suggests that the primate flocculus (FL) and ventral paraflocculus (vPFL) control differentially reflex and smooth pursuit eye movements. This view is also supported by the present anatomical investigation. WGA-HRP solution was injected locally into the FL or vPFL in 4 anesthetized monkeys. After survival periods of 3-4 days, they were perfused by saline, followed by a standard fixative. Frozen sections of brains were prepared frontally at 60 μm, and reacted with tetramethylbenzidine or diaminobenzidine. The mossy fiber inputs to the FL came mainly from neurons of the vestibular ganglion, vestibular nuclei, and nucleus reticularis tegmenti pontis (NRTP), while those to the vPFL came mainly from neurons of the pontine nuclei and NRTP. The sources of climbing fiber inputs were similar. The efferents from the FL terminated mainly in the vestibular nucleus, while those from the vPFL terminated mainly in the dentate and interposed nuclei of the cerebellum.

358.1

THE PRIMATE FRONTAL EYE FIELD IS THE TARGET OF NEURAL SIGNALS FROM THE SUBSTANTIA NIGRA, SUPERIOR COLLICULUS, AND DENTATE NUCLEUS. J.C. Lynch*[1], J.E. Hoover[2], and P.L. Strick[2]. Depts. of Anatomy and Ophthalmology, University of Mississippi Medical Center, Jackson, MS 39216[1] and VAMC and Depts. of Neurosurgery and Physiology, SUNY-HSC, Syracuse, NY 13210[2].

We used transneuronal transport of herpes simplex virus, type 1 (HSV-1) to label second-order neurons that send information to the frontal eye field (FEF) in monkeys (*Cebus apella*). The FEF was localized using low-amplitude (< 50μA) intracortical stimulation and then injected with a strain of HSV-1 that is transported transneuronally in the retrograde direction. Thalamic nuclei and cortical regions that are known to project to the FEF contained many neurons labelled with virus-specific antigen. In addition, we found neurons labelled by retrograde transneuronal transport in three subcortical sites: laterally in pars reticulata of the substantia nigra; ventrally in posterior portions of the dentate nucleus of the cerebellum; and in the optic and intermediate gray layers of the superior colliculus. All three of these regions are known to project to the thalamic subdivisions which contained labelled neurons and are known to contain neurons related to eye movements. These observations provide evidence that the output of the FEF is the product of neural signals originating in the substantia nigra, superior colliculus, and dentate nucleus, combined with input signals originating in other cortical areas.

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358.2

RECIPROCAL CONNECTIONS BETWEEN THE ZONA INCERTA AND MIDBRAIN IN THE CAT. Wensi Sun[1*], Paul J. May[1,2], and William C. Hall[3]. Depts. of Anatomy[1], and Ophthalmology[2], Univ. of Mississippi Med. Ctr., Jackson, MS, and Dept. of Neurobiology[3], Duke Univ., Durham, NC.

The relationship between the zona incerta (ZI) and saccadic eye movements was examined by tracing pathways connecting the ZI with the dorsal midbrain of cats. Following ZI injections, both retrograde and anterograde label was consistently found in the ipsilateral pretectal area and superior colliculus. In the pretectum, the labelled cells and terminals were located mainly in the anterior nucleus, and to a lesser extent in the posterior, and medial pretectal nuclei. In the superior colliculus, labelled terminals and a few labelled cells were found in the intermediate and deep grey layers. These pathways were further examined by making injections into the superior colliculus and pretectum. The collicular injections produced retrogradely labelled cells located predominantly in the ventral incertal sublamina and scattered terminal labelling throughout ZI. Following pretectal injections, the labelled cells were again concentrated in the ventral sublamina. However, the density of labelled terminals was much greater following pretectal injections, particularly in the ventral lamina. Following collicular, pretectal and medial ZI injections, labelled cells and terminals were observed curving from ZI dorsolaterally toward the thalamic reticular nucleus. Caudally, terminal and cell labelling continued into the ventral lateral geniculate nucleus (VLGn). This pattern of labelling may indicate that the extent of the cat ZI and/or VLGn is greater than previously recognized. In conclusion, these results indicate the presence of reciprocal connections between ZI and both the pretectum and superior colliculus in the cat. Thus, ZI may contribute to midbrain control over head and eye movements.

Supported by NEI grant EY07166(PJM) and EY08233(WCH).

358.3

A LIGHT AND ELECTRON MICROSCOPIC EXAMINATION OF THE LIGHT EVOKED BLINK REFLEX PATHWAYS IN THE MACAQUE. Paul J. May* Depts. of Anatomy and Ophthalmology, Univ. of Miss. Med. Ctr., Jackson, MS.

In the cat, there is evidence of a pathway from the olivary pretectal nucleus to the facial nucleus that subserves the light evoked blink reflex. The circuitry mediating this reflex in the primate was investigated by simultaneously injecting the pretectum, levator palpebrae muscle and orbicularis oculi muscle with WGA/HRP. The pretectal injection retrogradely labeled numerous cells and scattered terminals in the contralateral pretectum, mainly in the nucleus of the optic tract. However, with respect to consensual responses to light, no labeled elements were present in the contralateral olivary pretectal nucleus. Labeled axons were present rostrally in the ventral periaqueductal gray, in and around the Edinger-Westphal nucleus. Caudally, however, these anterogradely labeled axons appeared to avoid the retrogradely labeled levator motoneurons. Similarly, examination of the facial nucleus did not reveal a labeled terminal field overlying the labeled orbicularis oculi motoneurons, although labeled axons were present ventromedial to the facial nucleus and the inferior olive contained dense terminal label. The ultrastructure of these labeled levator and orbicularis motoneurons was investigated in order to confirm the lack of a monosynaptic pretectal input. Initial examination had revealed that both motoneuron populations exhibited the same three basic terminal types in synaptic contact with labeled somata and dendrites: profiles with 1. spherical vesicles, 2. pleomorphic vesicles, and 3. both pleomorphic and dense-cored vesicles. However, axosomatic contacts were more numerous on orbicularis motoneurons. Following a pretectal injection, no labeled terminals were observed contacting either type of motoneuron. These results suggest that in primates, the light evoked blink reflex must be subserved by polysynaptic excitatory and inhibitory CNS pathways to the orbicularis oculi and levator palpebrae motoneurons, respectively.

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358.4

STRIATE CORTICOPRETECTAL PROJECTIONS IN THE GUINEA PIG. F. Lui, R.A. Giolli*, R.H. Blanks, Y. Torigoe and S.V. Pham Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717 U.S.A.

Guinea pig has a significant nasotemporal component of horizontal optokinetic nystagmus (hOKN) when tested in monocular vision (Hayes & Ireland, 1969; personal observation). This functional finding raises the question, whether the nucleus of the optic tract (NOT) and its associated dorsal terminal nucleus, the recognized braistem centers for hOKN, receive any relevant input from the visual cortex, as Hoffmann (1986) postulated necessary for a nasotemporal hOKN response.

In order to answer this question, 15 guinea pigs were injected into the visual cortex with the tracers biocytin or horseradish peroxidase; after 48-72 hr. survival the guinea pigs were intracardially perfused and coronal sections of the brains (first preincubated with HRP-Avidin D in biocytin injections) were reacted using TMB as a chromogen.

The present study shows the usual projections of the striate cortex of mammals to the thalamus, tectum and pons; the projection to the pretectal complex is restricted to a single zone of pericellular terminals situated in the deep sector of the caudal portion of the NOT and/or in the more lateral portion of the posterior pretectal nucleus; contrary to reports in other species, no terminations are observed in the anterior pretectal nucleus.

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358.5

TOWARD A FUNCTIONAL ANATOMY OF THE CAT EDINGER-WESTPHAL NUCLEUS. Jonathan T. Erichsen*[1], Craig Evinger[2] and Paul J. May[3]. Depts. of Neurobiology & Behavior[1,2] and Ophthalmology[2], SUNY Stony Brook, Stony Brook, NY and Depts. of Anatomy and Ophthalmology[3], U. of Mississippi Med. Ctr., Jackson, MS.

In the cat, the preganglionic parasympathetic motoneurons that subserve pupilloconstriction and lens accommodation have been difficult to investigate because they do not form a discrete nucleus. We have therefore attempted to better specify their organization. Ciliary ganglion injections retrogradely labeled cells: 1. within (20.1%) and ventrolateral to (29.5%) the anteromedian nucleus [AM]; 2. within the Edinger-Westphal nucleus [EW] (6.8%) and the surrounding supraoculomotor area (10.7%); and 3. beneath the oculomotor nucleus (32%). Morphological analysis disclosed two populations: fusiform cells (8x25 um) found primarily in AM, and multipolar neurons (15-20 um), which were the major cell type at other locations. Labeled dendrites were observed crossing the midline, providing a possible basis for conjugate lens and pupil responses. In a series of transsynaptic experiments, WGA was injected either into the anterior chamber or the ciliary muscle to label preferentially the pupil and lens preganglionic neurons, respectively. In anterior chamber cases, the transsynaptically labeled motoneurons had a rostral distribution, i.e., more cells were in and lateral to AM. In contrast, ciliary muscle injections produced much higher levels of transsynaptic labeling caudally, in and around EW and beneath the oculomotor nucleus. Thus, a functional subdivision of the preganglionic motoneurons appears to exist along the rostrocaudal axis, in which pupilloconstriction is rostral and lens accommodation is caudal.

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358.6

DIVERGENT AXON COLLATERALS FROM PONTINE PRECEREBELLAR REGIONS TO MID-VERMIS OF THE CAT. H. Sato* and R. Noda. School of Optometry, Indiana University, Bloomington, IN 47405.

In the macaque, neurons in the fastigial oculomotor region (FOR) project to saccade-related nuclei of the contralateral brainstem, while they receive bilateral mossy-fiber input from pontine precerebellar neurons and ipsilateral input from Purkinje cells of the oculomotor vermis, lobules VIc and VII (Noda and Yamada, 1989; Noda et al., 1990). However, it is not known if the same cells project to the cerebellar oculomotor vermis of both sides or if different cells project to each side, possibly intermingling in the pontine regions. After injecting fast blue into the right half and diamidino yellow into the left half of the lobule VII of cats, and vice versa, topographical differences in the distribution of double- or single-labeled neurons were examined in the pontine precerebellar structures, pontine nuclei and nucleus reticularis tegmenti pontis (NRTP) of both sides. Single-labeled cells were distributed in the NRTP and four longitudinal columns of pontine nuclei, the dorsolateral, peduncular, lateral and medial (median and paramedian) pontine nuclei. The majority of single-labeled cells were found in the dorsolateral and peduncular pontine nuclei confirming an early observation (Hoddevik et al., 1977). Each nucleus contained a mixture of cells which project to either the left or the right vermis. Several cells in the dorsolateral and peduncular pontine nuclei and a few neurons in the NRTP, lateral and medial pontine nuclei send axon collaterals to both sides of the mid-vermis. (Supported by NIH grant EY04063).

358.7

EXCITING THE BLINK THROUGH INHIBITION. BASAL GANGLIA CONTROL OF THE BLINK REFLEX AND THE SUPERIOR COLLICULUS. M.A. Basso*[1] and C. Evinger[2]. Depts of [1]Psychology, [2]Neurobiology & Behavior and Ophthalmology, SUNY Stony Brook, Stony Brook, NY 11794.

Lesions of the nigrostriatal dopamine system produce an increase in blink reflex magnitude and excitability. This effect on the blink reflex presumably results from an increase in the activity of the inhibitory projection neurons of the substantia nigra pars reticulata (SNr). We tested this hypothesis by examining the consequences of reducing SNr activity with muscimol injections.

In urethane anesthetized rats, we elicited reflex blinks before and after injection of muscimol, a GABA-A agonist, into the SNr. We quantified blink reflex magnitude and excitability by presenting paired electrical stimuli to the cornea and measuring the amplitude of the orbicularis oculi EMG (OOemg) response. At short interstimulus intervals (<800ms), the OOemg response to the second stimulus was always less than that to the first. In animals injected with 6-OHDA, which should increase SNr activity, the blink evoked by the second stimulus was often larger than normal rather than suppressed. In contrast, injection of muscimol (0.1μg) into the SNr which should decrease its activity, transiently increased blink reflex suppression and decreased blink reflex magnitude. We tested the possibility that the SNr modulated the blink reflex excitability and magnitude through the SNr's inhibitory, GABAergic input to the superior colliculus.

We monitored the magnitude and excitability of the blink reflex following injection of 0.1μg of muscimol into the superior colliculus. This inhibition of collicular activity transiently increased the magnitude of the blink reflex but inconsistently altered blink reflex excitability. This result suggests that there are multiple pathways through which the basal ganglia can regulate the magnitude and excitability of the blink reflex, one of which is the superior colliculus.

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358.8

INTRACOLLICULAR PROJECTIONS OF THE SUPERFICIAL GREY LAYER IN THE TREE SHREW. P. Lee, W. C. Hall*, D. Fitzpatrick, and I. T. Diamond. Department of Neurobiology, Duke University, Durham, N.C. 27710.

The correct explanation of the physiological differences between the layers of the superior colliculus depends on precise knowledge of their interconnections. The tree shrew, with its large and distinctly laminated superior colliculus, is a good choice for studies of these connections. We have examined the projections of the superficial grey layer, stratum griseum superficiale, in this species after small extracellular injections of biocytin. The main intracollicular projection of neurons in the superficial grey layer is to the optic layer, stratum opticum. By comparison, the projection from the superficial grey layer to the layers beneath the optic layer is extremely sparse. The projections from the superficial grey layer to the optic layer have a columnar organization; the vast majority of the terminals are restricted to the sector of the optic layer that is immediately ventral to the injection site. These results indicate that a direct pathway from the superficial grey layer is unlikely to be responsible for the visual responses of cells in the deep layers, and suggest either that the cells of the optic layer serve as a relay between the superficial and deep grey layers, or that visual information reaches the deep layers primarily by indirect pathways through the cortex and basal ganglia. Supported by NIH Grants EY08233 and EY06821.

358.9

THE NUCLEUS OF THE OPTIC TRACT (NOT) IN THE OPOSSUM (Didelphis marsupialis aurita): AFFERENTS,EFFERENTS, IMMUNOCYTOCHEMISTRY FOR GABA AND DOUBLE LABELLING ANALYSIS. Vargas, C.D., Volchan, E., Hokoç, J.N.*, Bernardes, R.F., Rocha-Miranda, C. E.; Instituto de Biofísica Carlos Chagas F°/UFRJ, 21944, Brazil.

The NOT afferents and efferents were investigated by means of WGA-HRP injections. The injection sites attained the NOT, DTN, other pretectal nuclei and the anterior portion of SC. Labelled cells and terminals were present in contralateral NOT-DTN, LTN, INFSp, vLGN and ipsilateral MTN, LTN, vLGN and INFSp. Labelled cells and terminals could be distinguished bilaterally in praepositus hypoglossus and in medial and lateral vestibular nuclei. Labelled terminals were present bilaterally in the inferior olive and ipsilaterally in NRTP. These and eletrophysiological evidences suggest for the opossum's NOT a role in the horizontal OKN comparable to that of other mammals. The presence of GABAergic cells and terminals in NOT-DTN, MTN, INFSp and LTN was verified immunocytochemically. To investigate the existence of commissural GABAergic cells, rhodamine-beads were injected in the NOT and brainstem slices were processed for GABA. Double labelled cells were not found in the opposite NOT. A density analysis of GABAergic and retrogradely labelled cells in NOT-DTN showed that they superimpose in the posterior half of the complex, where the density of GABAergic cells is highest. These results suggest that the inhibitory modulation between the NOT and its opposite counterpart verified in eletrophysiological experiments might be mediated by GABAergic interneurons. Supported by: FINEP, CNPq, CAPES, FAPERJ.

358.10

THIRD NERVE EFFERENTS TO THE ORBIT AND THE RETINA IN THE RAT. P.M. Young, F. Mullin & J. Robertson-Rintoul#. Dept. of Psychology, University of Central Lancashire and #Dept. of Biol. & Preclin. Med., University of St. Andrews, U.K. (Spon: Brain Research Association.)

The subdivisions of the oculomotor nuclei of the rat projecting to the orbit and the retina were demonstrated by simultaneous unilateral injections, under tribromethanol general anaesthesia, of 10 µl of 2% Fluoro-gold, and 10 µl of cholera toxin-HRP conjugate. One injection was made to the posterior chamber of the eye and one to the orbit, with or without optic nerve section. Animals were sacrificed twenty four to seventy two hours later and 80 µm frozen sections were processed according to the technique of Mesulam using TMB as the chromogen. Fluoro-gold was visualised on a Leitz Diaplan using a wide band U.V. excitation filter. Five or six axonal fascicles 50-70 µm in diameter and 70-80 µm apart project ventrolaterally from the oculomotor nucleus to form several radicles emerging from the base of the cerebral peduncles. Dendritic processes were largely orientated rostro caudally and horizontally. A significant population of branching processes passes through the supraoculomotor central gray, through the parvocellular oculomotor nucleus to terminate close to the cerebral aqueduct and throughout the central gray. A subpopulation of oculomotor neurons was labelled following intravitreal injection, and tracer labelled-fibres were found apparently leaving the ventral component of the medial terminal nucleus of the accessory optic tract.

358.11

CHANGES IN HORIZONTAL OPTOKINETIC NYSTAGMUS (HOKN) FOLLOWING HEMILABYRINTHECTOMY. R.H.Blanks, C. G. Fowler, C. A. Zizz, Y. Torigoe, F. Lui and S. R. Whitaker. Depts. Anat. and Otolaryngol., Univ. of Calif. Irvine, CA 92717 and Audiol., VA Med. Cent. Long Beach, CA 90822.

HOKN was examined in 6 patients with acoustic neuromas and 17 controls with normal health, hearing and vision. Eye position was recorded with conventional electrooculography. Full-field optokinetic stimuli (projected 15° vertical stripes) consisted of velocity steps (15-60°/s) lasting 20-60s. With controls, eye velocity matched stimulus velocity (i.e., gain = 1.0) in all conditions (monocular, binocular) through 45°/s, but decreased to a gain of 0.8-0.9 in the range 45-60°/s. The excursion of the eye during a slow phase increased with stimulus velocity, e.g. 5-15° at 15°/s; 5-40° at 60°/s. Maximum eye velocity was achieved quickly (<3-4 s). Optokinetic afternystagmus (OKAN) was of low gain (<.15-.2) or absent in total darkness and, therefore, provided an unreliable indicator of vestibular pathology. The gain in all six patients was asymmetrical. Before surgery values were normal with stimuli towards the side of the tumor, but reduced for high stimulus velocities towards the contralateral side. Following the initial phases of compensation (no spontaneous nystagmus, ca. 11 days postoperation), there was a reduction in the HOKN gain to both sides. All patients showed fewer number of slow phase eye movements of uniformly low excursions (5-15°). On postoperative day 42, there was improvement of slow velocity gain to ipsilateral stimulation, whereas contralateral stimulation remained low. At this late postoperative period, there was an increase in the number of slow phase eye movements and a recovery of the full range of slow phase excursions. These findings confirm the asymmetry in HOKN gain before and after surgery for acoustic neuroma. The asymmetry was present only under monocular conditions. Finally, the partial recovery of slow velocity gain by day 42 and the near complete recovery of eye movement excursions during slow phase HOKN suggest that there is a late vestibular compensation which long outlasts the cessation of nystagmus.

358.12

Head braking and release during gaze shifts in the monkey. L.O. Phillips*, L. Ling, C. Siebold, and A. F. Fuchs. Departments of Psychology and Physiology and Biophysics, University of Washington, Seattle, WA 98195

It is not known how head-free gaze shifts are controlled. Vestibular signals may play an important role, but it is not clear when and/or how these signals can influence the gaze movement. To address this issue, we perturbed head movements during natural gaze shifts by unexpectedly stopping and/or releasing the head before, during, and following primate gaze movements to a visual target.

If the head is stopped during a large (up to 80 deg.) gaze shift, we observe a brief, compensatory, re-acceleration of the eye 10-12 msec following the perturbation. Eye movement amplitudes do not increase significantly, so gaze falls well short of the target, indicating that the eye/gaze burst generator does not compensate for the decreased amplitude of the head movement. The animal does, however, produce corrective saccades that bring the eye on target without further head movement. This shows that orbital mechanics are not limiting the initial gaze shift.

If the head is stopped near the end of the eye saccade, the eye does not re-accelerate. This suggests that the vestibular interaction during the ongoing gaze movement may occur at the level of the eye/gaze burst generator, which is no longer driving the eye at this point. If the head is stopped after the gaze shift is over, the compensatory counter-rotation of the eye stops following a delay of 10-12 msec. This eye movement is vestibular in origin.

If, during fixation of a stationary target, the head is rotated passively away from the target and then released, a high velocity head movement results and gaze remains stable. If a target step accompanies the release, an accurate gaze shift begins with a decrease in the compensatory counter rotation of the eye in the orbit. A saccadic eye movement only occurs well into the ongoing gaze shift. Gaze movement and eye saccades can, therefore, be initiated and controlled independently.

These results can be explained by a limited gaze control mechanism in which the VOR is titrated by a signal proportional to gaze error.

This work was supported by NIH grants EY00745 and RR00166.

358.13

METABOLIC ACTIVATION OF THE VESTIBULO-OCULOMOTOR SYSTEM BY INTRAVENTRICULAR ENDOTHELIN-1: MEDIATION BY CALCIUM L-CHANNELS. S.W. Shaver*, R.J. Beninger, H.M. Lawler, D.S. Wainman, D.F. Weaver, F.J. Espinosa and P.M. Gross, Departments of Psychology, Surgery, Medicine, Chemistry & Physiology, Queen's University & Kingston General Hospital, Kingston, Canada K7L 3N6

Intracerebroventricular (icv) injection of endothelin-1 (ET) in rats produces oculoclonus, nystagmus and barrel-rolling convulsions, responses indicating that vestibulo-ocular systems are stimulated. Nuclei controlling proprioception and extraocular muscles have appreciable quantities of ET receptors (Kohzuki et al., *Neuroscience* 42:245, 1991). We examined whether rates of glucose metabolism in oculomotor and visual-vestibular nuclei would be elevated by icv ET, and whether such stimulation was regulated by dihydropyridine-sensitive Ca^{2+} L-channels as shown previously in other brain regions (*Neuropeptides* 21:211, 1992). Male albino rats bearing a lateral ventricular cannula for icv injection were studied by use of the autoradiographic [^{14}C]deoxyglucose method for measuring regional rates of brain glucose metabolism (GM). The effects of saline (3 µl) or ET (9 pmol) were studied alone or after icv treatment with a Ca^{2+} L-channel antagonist, nimodipine (72 nmol in 1 µl). In comparison to saline treatment, ET evoked: 1) significant locomotor alterations assessed by an automated activity monitor, and 2) metabolic stimulation of oculomotor neurons, including the n. of the IIIrd, IVth, and VIth cranial nerves (+38-80%), interstitial n. of Cajal (+45%), Edinger-Westphal and Darkschewitsch n. (+46-80%), and the medial terminal n. of the accessory optic tract (+104%) in which ET receptors are particularly dense. Visual-vestibular structures, including the superior colliculus, prepositus hypoglossal n., inferior olivary n., cerebellar flocculus, and medial and superior vestibular n., had elevated GM following ET. All these stimulatory effects were attenuated or abolished by nimodipine. The findings indicate Ca^{2+}-sensitive behavioral and metabolic stimulation by ET of vestibulo-oculomotor nuclei that appear to be part of an intrinsic ETergic neural system.

358.14

SENESCENCE OF HUMAN VISUAL-VESTIBULAR INTERACTIONS (VVI). G.D. Paige*, Dept. of Neurology and Ctr. for Visual Sci., Univ. of Rochester, Rochester, NY 14642.

Visual and vestibular inputs work together to maintain target fixation and spatial orientation throughout life, but these tasks are compromised as age increases. Horizontal eye movements were recorded (search-coils) from subjects 18-89 years of age in response to visual (smooth pursuit, SP, and optokinetic, OK), vestibular (VOR), and combined stimuli (0.025-4 Hz, 50°/s peak). Effects of aging on the VOR were small and largely limited to a decline in performance at $\leq$0.1 Hz. SP and OK responses were closely correlated, and both deteriorated progressively with aging across all frequencies. OK trials at 0.025 Hz produced circular vection. The effect became increasingly robust with aging, suggesting that visual influences on equilibrium are enhanced in the elderly, perhaps in compensation for vestibular deterioration. VVI with an earth-fixed scene more closely approached perfect compensation than the VOR, SP, or OK alone, but performance declined slightly with aging at low frequencies. VVI with a head-fixed scene closely resembled the VOR at 4 Hz, where visual suppression is ineffective, but vision increasingly overpowered the VOR as frequency decreased. Visual suppression became progressively less effective with aging, in concert with the general deterioration of visual following. Other VVI mechanisms (e.g. mental set, context) may also deteriorate with aging. Suppt. by NIH Grants RO1-AG06442, P30-EY01319.

358.15

EFFECTS OF FUNCTIONAL ABLATION OF IRREGULAR AFFERENTS ON SECONDARY ,VESTIBULAR NEURON ACTIVITY IN ALERT SQUIRREL MONKEYS C.J. Chen-Huang* , R.A. McCrea, L.B. Minor and J.M. Goldberg Comm.Neurobiology, Univ. of Chicago, Chicago, IL 60637

Anodal galvanic currents, applied to both ears, can be used to functionally ablate irregular vestibular-nerve fibers. The procedure shows that there is no *net* irregular input to the vestibulo-ocular reflex (VOR) (J. Neurosci., 11: 1636). To understand the cellular basis for this finding, the responses of horizontal canal-related neurons in the vestibular nuclei during passive sinusoidal vestibular stimulation (0.5 and 2.0 Hz, 20-40°/s peak velocity) were compared in the presence and absence of the ablating currents. Position-vestibular-pause (PVP) neurons were studied as they are major contributors to the VOR.

The currents decreased the spontaneous firing of PVP neurons from typical values of 100 ± 40 to 60 ± 50 spikes/s. Because the polarizing currents decrease background discharge, they increase inhibitory saturation and decrease the peak-to-peak modulation in firing during rotations. On average, the decrease in modulation amounted to 10-15%, which might be expected to decrease VOR gain. Since the reflex is unaffected, there must be some central compensation for inhibitory saturation. Head-velocity gains were estimated from records corrected for eye-position and saccade-related activity and for inhibitory saturation. While the mean gain for the population was unaffected by the currents, individual units could have their gains changed by up to 60%. Half the units showed gain decreases; the other half, gain increases. The gain changes show that, despite the fact that the VOR is unaffected when irregular afferents are ablated, these afferents contribute to the firing of presumed VOR neurons, most likely by way of inhibitory and/or excitatory multisynaptic pathways (Supported by NIH Grants DC 00070 and EY 08041 and by NASA Grant NAG 2-148).

358.16

SPATIAL ORIENTATION AND DYNAMIC CHARACTERISTICS OF POSTROTATORY AND OPTOKINETIC AFTER-NYSTAGMUS FOLLOWING PASSIVE HEAD TILTS: I. HORIZONTAL VOR AND OKN. B.J.M. Hess* and D.E. Angelaki. Neurology Dept, University Hospital, CH-8091 Zürich, Switzerland.

If optokinetic afternystagmus is elicited about the yaw axis while the animal is in a static tilt position, the central vestibular system tends to align the eye rotation axis with the earth-vertical (Dai et al, JNP 1991). We examined the spatial characteristics and dynamics of postrotatory (PRN) and optokinetic afternystagmus (OKAN) following short-lasting (< 1s) passive head tilts away from the earth-vertical which were delivered immediately after cessation of rotation about the yaw axis (±90 °/s for VOR and ±60 °/s for OKN). Rhesus monkeys were implanted with dual scleral coils for measuring horizontal, vertical, and torsional eye position. The head tilt evoked a transient VOR in the appropriate head plane (e.g., roll induced a torsional, and pitch a vertical response) and decreased the time constant of horizontal PRN and OKAN. In addition, an orthogonal eye movement component was elicited during PRN or OKAN whose magnitude was proportional to the tilt angle. The direction and relative amplitude of the orthogonal component were such that the resultant three-dimensional eye velocity vector moved toward alignment with the spatial vertical. As to the horizontal system, the spatial and dynamic properties of the velocity storage appear to be similar under static and dynamic conditions during both VOR and OKN.

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358.17

SPATIAL ORIENTATION AND DYNAMIC CHARACTERISTICS OF POSTROTATORY AND OPTOKINETIC AFTER-NYSTAGMUS FOLLOWING PASSIVE HEAD TILTS: II. VERTICAL AND TORSIONAL VOR AND OKN. D.E. Angelaki* and B.J.M. Hess. Neurology Dept, University Hospital, CH-8091 Zürich, Switzerland.

Vertical and torsional VOR and vertical OKN were elicited in rhesus monkeys implanted with dual search coils for 3d eye movement recordings. Rotation of the animal in ear down and supine or prone position about the earth-vertical axis (± 90 °/s) elicited vertical and torsional VOR, respectively; rotation of the visual surround (± 60 °/s) generated vertical OKN in ear down position. At termination of the velocity step, animals were tilted within < 1 s in the roll plane during vertical VOR /OKN, in the pitch plane during torsional VOR , and in the yaw plane during both vertical VOR/OKN and torsional VOR. The time constant of vertical afternystagmus was smallest in the upright and in prone or supine positions. Similarly, decay of torsional nystgmus was fastest in upright and ear down positions. In addition to a transient VOR, an orthogonal eye movement component was elicited during vertical or torsional afternystagmus whose magnitude and direction depended on the angle and direction of tilt and tended to align eye velocity with the spatial vertical. Such property maintains the direction of stored angular velocity aligned with earth-vertical, and has not been described for the vertical and torsional system during static tilt (Dai et al, JNP 1991). Thus, dynamic canal and/or otolith activity appear to be necessary to specify the plane of the head tilt and to stabilize the axis of the velocity storage in space. Supported by SNF # 31-32484.91.

358.18

SUPPRESSION OF THE VESTIBULO-OCULAR REFLEX DURING ACTIVE GAZE PURSUIT T.E.Belton*, R.D. Tomlinson and R.A. McCrea Comm. on Neurobiology, Univ. of Chicago, Chicago, Ill 60637 and Dept. Otolaryngol., Univ. of Toronto

We have examined the gain of the vestibulo-ocular reflex (VOR) in squirrel monkeys during combined eye-head tracking of a visual target. While tracking a predictably moving target that was moved at 40°/s the monkeys' head movement was perturbed by a high acceleration transient generated by a brushless torue motor linked to the monkey's head. The perturbations caused the head to be accelerated 1,100 – 24,000°/s^2 (peak velocity 22 - 350°/s) either in the same or in the opposite direction of pursuit.

The gain of the VOR evoked by the perturbations was close to 1.0 when eye velocity during gaze pursuit was near zero, or in the opposite direction of the pursuit movement. On the other hand, the VOR was usually suppressed at a short latency (20 - 35 msec) when the purturbation occured while monkeys were pursuing targets with a combination of smooth pursuit eye and head movements and the head velocity was less than gaze velocity. Peak VOR gain in these instances ranged from .04 to .8. These observations were similar, regardless of the direction of the perturbation. They suggest to us that the VOR operates at a normal gain during gaze pursuit if the target is moving predictably and can be tracked with a head movement alone. However, if the speed of head movement during gaze pursuit is inadequate to maintain target stability on the retina, the VOR is reduced by a short latency mechanism.

359.1

THE NEUROCHEMISTRY OF THE MOTONEURONS OF THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS. C. Priest*, P. Popper and P. E Micevych. Dept. Anatomy and Cell Biology, Laboratory of Neuroendocrinology, UCLA School of Medicine, Los Angeles, CA 90024.

Because of its sensitivity to testosterone, the spinal nucleus of the bulbocavernosus (SNB)-target muscle system is a convenient model in which to study the regulation of expression of molecules used by motoneurons in intercellular communication. It has been shown that the number of gap junctions and the levels of preprocholecystokinin mRNA are decreased in the SNB following castration and are restored by testosterone. The expression of αCGRP is increased in the SNB motoneurons following castration because of decrease in the activity of the target muscles. In an effort to further characterise the neurochemistry of the SNB, we investigated the regulation by testosterone of the expression of choline acetyltransferase (ChAT) and galanin, a neuroactive peptide which also is expressed in α-motoneurons. Adult, male Long-Evans rats were castrated or castrated and implanted with testosterone-containing Silastic™ capsules (40 mm). Four weeks later, the rats were perfused with 4% paraformaldehyde and 20 μm thick sections through the SNB were processed for in situ hybridization with radiolobeled RNA probes complementary to the mRNAs coding for ChAT and galanin. Castration decreased, and testosterone replacement restored, the levels of ChAT mRNA in SNB motoneurons, as well as in motoneurons of the RDLN. Since the activity of the skeletal muscles innervated by RDLN motoneurons does not change following castration, this indicates that the effect of testosterone on the expression of ChAT mRNA is at the level of the neurons. Supported by NS21220.

359.2

α-CGRP mRNA EXPRESSION IN MOTONEURONS IS RELATED TO MYOSIN HEAVY CHAIN COMPOSITION OF SPECIFIC RAT HINDLIMB MUSCLES. C. E. Blanco*, P. Popper, and P. E Micevych. Dept. of Anatomy & Cell Biology, Laboratory of Neuroendocrinology, UCLA School of Medicine, Los Angeles, CA 90024.

The purpose of this study was to investigate whether the percentage of motoneurons within defined motor pools expressing the mRNA for α-calcitonin gene-related peptide (CGRP) was associated with the myosin heavy chain (MHC) composition of the target muscles. Motoneurons innervating the soleus (SOL), extensor digitorum longus (EDL) and contralateral tensor fascia latae (TFL) muscles of male rats (body weight approximately 300 g) were identified by injecting each muscle with a fluorescently labelled retrograde tracer (Fluorogold™, fluororuby and Fluorogold™, respectively). In the rat, the SOL muscle expresses the MHC-S (80%) and the MHC-IIA (20%) isoforms, the EDL expresses the MHC-IIA (25%) and MHC IIB (75%) isoforms and the TFL only expresses the MHC-IIB isoform. The animals were killed by transcardial perfusion under pentobarbital anesthesia 10 days after the retrograde tracers were injected and processed for CGRP *in situ* hybridization using a ^{35}S-labelled ribonucleic acid probe. CGRP mRNA positive motoneurons constituted 23% of the SOL motor pool, 82% of the EDL motor pool and 85% of the TFL motor pools. These observations suggest that CGRP mRNA positive motoneurons innervate muscle units where the constitutive fibers express MHC-IIB isoform. Furthermore, we have previously reported that approximately 10% of phrenic motoneurons (13% MHC-IIB in the diaphragm) and 90% of the spinal nucleus of the bulbocavernosus innervating the bulbocavernosus/levator ani muscles (100% MHC-IIB). Supported by NS 21220.

359.3

PHARMACOLOGICAL PROPERTIES OF NUCLEUS GIGANTOCELLULARIS NEURONS: AN IN VITRO STUDY IN GUINEA PIG BRAINSTEM SLICES. M. Serafin* and M. Mühlethaler. Dept. de Physiologie, CMU, 1 rue Michel-Servet, 1211 Genève 4, Switzerland.

Nucleus Gigantocellularis neurons (NGCn), located in the medullary reticular formation, belong to networks subserving heterogeneous functions. In a first step, two main neuronal cell types, A and B NGCn, differing by their intrinsic membrane properties, were identified in the guinea pig using either an isolated whole brain preparation or brainstem slices (Serafin et al., Soc. Neurosci. Abstr. 1987, 239.6; Serafin et al. 1990, Neurosci. Letters 120, 5-8). Moreover, intracellular injections of Lucifer Yellow have shown that both A and B NGCn were gigantocellular neurons. Thusfar, no attempts have been made to study the neuromodulation of these cells in vitro. Therefore, we recently undertook to study their response to different neurotransmitters.

Preliminary results indicated that monoamines such as serotonine, noradrenaline and histamine reversibly depolarized and excited A and B NGCn. Excitatory amino acid agonists such as NMDA, AMPA or trans-ACPD, also appeared to play a crucial role in controlling the activity of A and B NGCn. Indeed, their bath-application resulted in a reversible membrane depolarization of these neurons with a concomitant increase in their firing rate. In contrast, both A and B NGCn were hyperpolarized and inhibited by $GABA_A$ and $GABA_B$ agonists (muscimol and baclofen respectively). (Supported by grants no 3.288-0.85, 3.560.0.86 and 31-26495.89 from Swiss NSF).

359.4

DIFFERENTIAL GLUTAMATERGIC AND CHOLINERGIC PROJECTIONS TO THE PONTINE INHIBITORY AREA. Y.Y. Lai*, J.R. Clements and J.M. Siegel. Dept Psychiatry, UCLA, VAMC, Sepulveda, CA 91343 and Dept Vet Anat, Texas A&M Univ, College Station, TX 77843.

We have found that both glutamatergic (glut) and cholinergic (Ach) mechanisms in the pontine inhibitory area (PIA) are involved in the suppression of muscle tone. Glut is co-localized with Ach in the neurons of dorsolateral tegmental (LDT) and pedunculopontine nucleus (PPN) in rat (Clements et al., 1991). We investigated whether the glut and Ach afferents of PIA originate from the same neurons. WGA-HRP was microinjected into the PIA of either the decerebrated or intact cat (n=8). Ach neurons were identified by NADPH-diaphorase and glutamate-like immunoreactive cells were identified with a glut antibody. Of the LDT neurons projecting to PIA, 15% were cholinergic while 29% were glutamatergic. Of the PPN neurons projecting to PIA, 8% were cholinergic and 44% were glutamatergic. A few WGA-HRP/glut-labeled neurons in caudal PPN also contained NADPH-d staining. These neurons may not be cholinergic since the NADPH-d staining is weak. We conclude that Ach and glut afferent inputs to PIA in the cat originate from separate neuronal populations. The coordinated activation of these populations may be responsible for REM sleep atonia.

359.5

AN *IN VIVO* MICRODIALYSIS STUDY OF THE RELEASE OF SEROTONIN IN THE SPINAL CORD VENTRAL HORN OF TREADMILL-RUNNING RATS. C. GERIN, L. MARLIER*, A. LEGRAND and A. PRIVAT. D.P.V.S.N., INSERM U-336, Case Courrier 106, U.S.T.L., Place E. Bataillon, 34095 Montpellier, Cedex 05, France.

The ventral horn of the spinal cord is profusely innervated by 5-HT terminals that presumably modulate locomotor activity through motoneurones (Roberts et al., 1988). We have monitored with microdialysis the release of 5-HT and 5-HIAA in the spinal cord of rats spontaneously running on a treadmill.

A loop-shaped dialysis probe was implanted stereotaxically in the anterior horn of adult rats. Eight days later, the rats underwent a microdialysis session of four hours, divided in three parts: rest (90 min.), exercise (60 min.), post-exercise rest (90 min.). Samples were collected at fifteen minutes' intervals and 5-HT and 5-HIAA were monitored by HPLC-EC. The animals were sacrificed 30 to 40 days after the implantation of the probe, and the spinal cord was serially sectioned for the location of the probe and immunocytochemical detection of GFAP and 5-HT.

In most of the animals (n=6) the probe was located in the ventral horn, the glial reaction appeared moderate, and 5-HT neuritis were present at the contact of the probe. These animals did not show a significant difference of 5-HT and 5-HIAA levels in-between rest and exercise. At variance, levels were significantly decreased of respectively 36% and 23% in-between rest and post-exercise. In two animals, where the probe was partly located in the white matter of ventral funiculi and surrounded by many 5-HT fibers, releases of 5-HT and 5-HIAA were respectively increased of 287% and 482% during exercise, and returned to almost basal values thereafter.

It is hypothesized that during exercise, the release of 5-HT or 5-HIAA is increased in the ventral horn, but that this increase is buffered in the grey matter, whereas such buffering does not occur in the white matter. This increase is then followed by a compensatory post-exercise decrease.

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359.6

RAT SPINAL CORD NEURONS CONTAIN NITRIC OXIDE SYNTHASE. S. Saito, G. J. Kidd, D. F. Hanley*, D. S. Bredt, T. M. Dawson, D. Wilson, R. J. Traystman, and S. H. Snyder. Dept. of Anesth. & CCM and Neurology, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205.

Nitric oxide (NO) produced from arginine by nitric oxide synthase (NOS) has been identified as a neuronal messenger in the brain, but its presence and possible functions in spinal cord is not clear. In this study we investigated the distribution of NOS containing neurons in the rat spinal cord, using immunocytochemistry to identify NOS containing neurons. Uptake of the retrograde tracer Fluoro-Gold into preganglionic sympathetic and parasympathetic neurons was used to characterize NOS positive neurons. NOS intensly reactive neurons were found in Laminae I-IV, and X throughout the spinal cord. Neurons in the intermediolateral cell column of the thoracic to sacral spinal cord were also intensely stained for NOS, and some neurons in Laminae V, VI, VII, and VIII were weakly stained. Colocalization of NOS and Fluoro-Gold in the intermediolateral cell column indicated that some sympathetic and parasympathetic neurons contained NOS. In addition, punctate NOS staining throughout Laminae I-III and surrounding some motor neurons suggested the presence of NOS at synapses.

Localization of NOS containing neurons in the spinal cord suggests that NO may paly role in spinal cord neurotransmission, including sympathetic, parasympathetic, sensory, motor, and possibly visceral pathways.

359.7

TRH AND 5-HT REGULATION OF ONUF'S NUCLEUS MOTONEURONS. G.M. Holmes*, R.C. Rogers, J.C. Bresnahan and M.S. Beattie. Departments of Cell Biology, Neurobiology and Anatomy, and Physiology, Ohio State University, Columbus, OH, 43210.

Recent anatomical and physiological evidence suggests supraspinal peptidergic and aminergic control of lumbosacral cord motoneurons (MNs). These neuroeffector substances include thyrotropin releasing hormone (TRH) and serotonin (5-HT) which are co-localized in terminals in the ventral horn. The individual and interactive effects of these systems upon the external anal sphincter (EAS) MNs of Onuf's nucleus were studied in adult female cats.

Composite multibarrelled micropipettes containing artificial CSF plus 0.01% bisulphate preservative vehicle (VEH), TRH (10mM), 5-HT (0.136 mg/ml) and the TRH analog RX77368 plus 5-HT (0.025 and 0.136 mg/ml, respectively) were lowered into Onuf's nucleus following laminectomy. Once EAS MNs were identified by electrical stimulation (25-100 uA, 3Hz, 5 sec trains) nanoliter volumes of VEH, TRH, 5-HT, or RX77368+5-HT were injected. Compared to VEH injections, TRH elevated EAS pressure (measured by rectal manometer) within 5 min of injection. 5-HT, alone, did not produce any appreciable effects. Preliminary results of RX77368+5-HT injections indicate a marked reduction in EAS baseline muscle tension. These results indicate that EAS MNs are modulated by TRH and 5-HT and that these substances may be involved in the CNS regulation of continence.

Supported by NIH grants NS10165 and NS07291.

359.8

GABA-IR AXONAL BOUTONS IN THE VENTROLATERAL MOTOR NUCLEUS OF THE CAT S1 SPINAL SEGMENT. Brun Ulfhake* and Vania Ramirez, Department of Anatomy, Karolinska Institutet, S-104 01 Stockholm, Sweden.

The motoneurons (MNs) in the upper sacral spinal segments of the cat are grouped in 3 nuclei, namely: the dorsolateral (DL), the ventrolateral (VL) and the ventromedial (VM). The dendrites of the MNs in the VL are arranged in rather dense rostro-caudally oriented bundles, a property that simplifies ultrastructural analysis of their synaptic input. A number of studies have provided data on peptidergic and aminergic innervation of the VL nucleus. In this study we describe the innervation pattern of GABA-immunoreactive (IR) axons and the fine structure of GABA-IR axonal boutons.

Tissue specimens from adult cat spinal cord were used. Transverse vibratome sections were incubated according to the peroxidase-antiperoxidase (PAP) technique (Sternberger et al., 1970) using a rabbit anti-GABA serum (Chemicon, Temecula, CA, USA) as primary serum.

The VL nucleus receive an extensive innervation of GABA-IR axons, surrounding both cell bodies and dendritic profiles. The fine structure analysis revealed that GABA-IR axonal boutons contained flat or pleomorphic small agranular vesicles and often also a number of large dense-core vesicles. The synaptic complex appeared to be asymmetric and the postsynaptic membrane specialization was often rather thin. A frequent finding was that one and the same GABA-IR bouton made synaptic contact with two adjacent dendrites. This type of synaptic arrangement in conjunction with the frequent dendro-dendritic contacts among these MNs may be of importance for synchronizing their activity. Taken together, the present results indicate that GABA may be used as a transmitter by a large fraction of the synaptic input to the sacral MNs.

359.9

DISTRIBUTION OF SYNAPTIC VESICLE PROTEINS IN THE MONKEY WITH SPECIAL REFERENCE TO 5-HT- AND GAD-LIKE IMMUNOREACTIVE NERVE TERMINALS IN THE SPINAL CORD VENTRAL HORN Ulf Arvidsson[1,2,*], Fredrik Piehl[1], Staffan Cullheim[1], Oleg Shupliakov[1,3], Lennart Brodin[3], Paul Greengard[4], Reinhard Jahn[5] and Tomas Hökfelt[2]
Departments of [1]Anatomy, [2]Histology and Neurobiology, and [3]Nobelinstitute for Neurophysiology, Karolinska Institutet, Stockholm, Sweden. [4]Laboratory of Cellular and Molecular Neuroscience, The Rockefeller University, New York, New York, USA. [5]Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030, USA

Spinal cord motoneurons receive information from a large number of different sources and rough calculations indicate that one single motoneuron is contacted by at least 50,000 nerve terminals. Immunocytochemical techniques reveal that these terminals in close contact to motoneurons harbor different transmitter substances/modulators, e.g. monoamines, excitatory and inhibitory amino acids as well as several peptides. Based on differences in vesicle shape and synaptic complex formation, several distinct types of terminals in close association to motoneurons have been described. In order to study possible molecular differences between different types of synapses on motoneurons we have chosen to analyze the distribution of three synaptic vesicle proteins in two, not overlapping, transmitter identified systems that both have a substantial input to motoneurons, namely the 5-hydroxytryptamine (5-HT) and the gamma-aminobutyric acid (GABA) systems. Thus, the distribution of synapsin-, synaptophysin- and rab3-like immunoreactivity (LI) was studied in the grey monkey *(Macaca fasicularis)* spinal cord ventral horn by use of indirect immunofluorescence, peroxidase-antiperoxidase and immuno-gold techniques. Double labeling immunofluorescence experiments were performed in order to study co-localization with 5-HT and glutamic acid decarboxylase (GAD), which is the synthesizing enzyme for GABA. A dense punctate labeling of synaptophysin-, synapsin- and rab3-LI was found around the motoneurons as well as in the neuropil in the ventral horn. The density of synaptophysin-immunoreactive (IR) profiles was higher than that of synapsin which was in turn higher than for rab3. Preliminary results from double labeling experiments indicate that 5-HT-IR varicosities in the ventral horn also contain rab3-LI to a low degree, while no synapsin- or synaptophysin-LI was detected. On the other hand, GAD-IR varicosities seemed to harbor synapsin- and synaptophysin-LI to a high degree but no rab3-LI. The results of this study suggest that, in the ventral horn, 5-HT nerve terminals differ from GABA terminals with respect to their content of synaptic vesicle proteins.

359.10

STUDIES OF SYNAPTIC EXCITATION IN THE RAT SPINAL CORD AT LOW-CALCIUM SOLUTIONS. M. Pinco & A. Lev-Tov*, Dept. of Anatomy, The Hebrew University Medical School, Jerusalem, Israel.

Monosynaptic transmission in the neonatal rat spinal cord is characterized by a prolonged depression which is evident at stimulation intervals as long as 30-50 seconds. This depression has been shown to be of presynaptic nature and can be partially alleviated by reduction in the basic level of transmitter release induced by decreasing the Ca^{2+}/Mg^{2+} ratio of the bathing medium or by addition of low concentration of baclofen (Lev-Tov & Pinco, J. Physiol, 447: 149-169, 1992). In the present study we characterized monosynaptic transmission between dorsal root afferents and α-motoneurons using sharp-electrode intracellular recordings from hemisected spinal cord preparations of the neonatal rat bathed in low-Ca^{2+} high-Mg^{2+} Krebs saline. Double-pulse stimulation under these conditions transformed the synaptic depression into short term (50-60 ms) facilitation with maximal values (R2/R1) of 1.3-1.4. The facilitated responses could be further augmented by short (5-pulse) tetanic trains applied at 20-60 ms interpulse intervals. At longer intervals potentiation was over dominated by tetanic depression. Tetanic stimulation (5-20Hz) at longer durations (200 impulses) revealed much lower tetanic depression than that observed in normal Krebs saline. This low-level depression was accompanied by substantial post-tetanic potentiation lasting up to 5-10 minutes. These findings show that facilitation, potentiation and post-tetanic potentiation of monosynaptic excitation are developed at early postnatal stages, but cannot be expressed normally due to a complete masking imposed by the prolonged synaptic depression. Supported by grant #363/90 from the Israel Academy of Sciences and Humanities, Jerusalem, Israel.

359.11

PATCH CLAMP RECORDINGS OF NEURONS AROUND THE CENTRAL CANAL IN *THICK* SLICES OF RAT SPINAL CORD: EFFECTS OF SEROTONIN AND N-METHYL-D ASPARTATE. S. Hochman*, M.W. Salter, C. A. Livingston[1], L.M. Jordan[1] and J.F. MacDonald. Dept. of Physiology, Univ. of Toronto, Toronto, ON., Canada M5S 1A8. [1]Dept. of Physiology, Univ. of Manitoba, Winnipeg MB., Canada R3E 0W3.

Locomotor activity can be elicited in the *in vitro* rat spinal cord (Kudo and Yamada, Neurosci. Lett. 75:43-48, 1987). Interneurons controlling locomotion must be capable of rhythmic acitivity and be under descending modulation. Recent evidence suggests that neurons near the central canal (lamina X and dorsomedial lamina VII) are activated during locomotion (Dai et al., Soc. Neurosci. Abstr. 16:368.9) and may generate rhythmic bursting (Jordan and Noga, Soc. Neurosci. Abstr., 17:484.6). The present study involves an analysis of the effects of serotonin (5-HT) on the genesis of bursting behaviour in similarly located interneurons.

Neurons near the central canal (laminae X, VII and VI) were recorded in the whole cell configuration in transverse sections (400 μm) of caudal lumbar and rostral sacral spinal segments of 12-21 day rats. Bath application of 5-HT (100 μM) and NMDA (20 μM) produced an initial membrane hyperpolarization followed by depolarization and rhythmic fluctuations in membrane potential. Membrane conductance was increased. Further membrane depolarization increased the frequency of rhythmicity ultimately leading to sustained firing. In 3/4 cases the afterhyperpolarization following the action potential was reduced. These observations occurred in the presence of APV (40 μM) or by application of 5-HT alone (50μM). Local pressure applications of NMDA (250μM with 1 μM glycine) in voltage-clamped neurons produced I-V relations with a negative slope conductance and peak inward current at -20mV. Bath application of 5-HT was found to decrease the inward current evoked by NMDA by 27% (V_h -40mV). Supported by Canadian Networks of Centres of Excellence.

359.12

ULTRASTRUCTURAL EVIDENCE FOR SEROTONERGIC INPUT TO CHOLINERGIC NEURONS IN THE LATERODORSAL TEGMENTAL NUCLEUS IN THE RAT. T. Honda* and K. Semba, Dept. of Anatomy, Dalhousie Univ., Halifax, N.S. B3H 4H7 Canada.

We have recently shown that application of serotonin (5HT) decreases excitability and markedly modulates the discharge pattern of cholinergic bursting neurons in the laterodorsal tegmental nucleus (LDT) [Luebke et al., PNAS, 89(1992):743]. In the present study, we used electron microscopy to investigate synaptic relations between serotonergic axon terminals and cholinergic LDT neurons, by combining PAP-DAB pre-embedding immunohistochemistry for choline acetyltransferase (ChAT), and post-embedding immunogold staining for 5HT. ChAT-immunoreactive somata were mostly medium-sized and appeared oval, triangular or multipolar. Their somatic surface was covered by glial processes and both myelinated and unmyelinated axons. ChAT-positive neurons received many synaptic contacts on somata, dendrites and dendritic spines. In post-embedding immunogold-stained material, deposition of gold particles was seen in some of these axon terminals in contact with ChAT-immunoreactive neurons. These serotonergic synaptic contacts appeared to occur more frequently with somata and proximal dendrites than with distal dendrites or dendritic spines. The presence of serotonergic synaptic contacts predominantly with the somata and proximal dendrites of cholinergic LDT neurons is consistent with the suggested role of serotonin in modulating rapid eye movement sleep phenomenology through its action on cholinergic neurons in the mesopontine tegmentum.

359.13

Neuronal NADPH-diaphorase are related to survival and regeneration after severe neuronal damage. Wutian Wu*, Microsurgical Research Center, Department of Plastic Surgery, Eastern Virginia Medical School, Norfolk, VA 23501

The function of the enzyme nicotinamide adenine dinucleotide phospate diaphorase (NADPH-d) in central nervous system is still unknown. Using a histochemical method, the present study has, for the first time, discovered that some groups of spinal neurons, which are normally NADPH-d negative, turn into NADPH-d positive during the time period of regeneration in a severe peripheral and/or central nervous lesion animal model (rat). With peripheral lesion, spinal motoneurons began to appear positive NADPH-d reaction seven days following ventral root avulsion and reached the maximum in 2-3 weeks after the lesion. Regrowing NADPH-d positive neurites were found far away from the cell bodies to the white matter and gray matter. These NADPH-d positive motoneurons existed for a few weeks and disappeared 3-4 months after the lesion. No NADPH-d positive motoneurons were found when root lesions (axotomy or crush) were made more than 0.5 cm distant from the cord. In central lesion, seven days following hemisection in low thoracic spinal cord, neurons with their regrowing neurites in ipsilateral lamina IV, V, and nucleus dorsalis, and interneurons in bilateral lamina VII and VIII, became NADPH-d positive. These NADPH-d positive neurons only existed for a few days and disappeared 2-3 weeks after the lesion. Heavy NADPH-d positive fibers were also observed within the lateral and ventral funiculus of the lesion side. Some of those fibers were apparent from the neurons of the nucleus dorsalis and the preganglionic sympathetic neurons in the intermediolateral cell column. The latter are normally NADPH-d positive neurons.

Results of this study indicate that neuronal NADPH-d are closely related to survival and regeneration after severe neuronal lesion. Mechanism of NADPH-d on neuronal regeneration needs to be further studied. Supported by the Department of Plastic Surgery, Eastern Virginia Medical School.

359.14

DETECTION OF mRNAs ENCODING THE TWO ISOFORMS OF GLUTAMIC ACID DECARBOXYLASE IN THE RAT SPINAL CORD. S. Feldblum*, A. Dumoulin, A. Joyeux, C. Maurin-Richaud, F. Sandillon and A. Privat. Inserm U336, Montpellier, France, 34000.

Recently we have demonstrated that GABA synthesis in the CNS depends on two forms of glutamic acid decarboxylase (GAD), differing in size, subcellular location and affinity for the cofactor pyridoxal-5'-phosphate. We have suggested that GABA may serve functional roles other than as a neurotransmitter. To further investigate the role of the two GAD isoforms, GAD_{65} and GAD_{67}, we have studied their distribution in the spinal cord where coexist different types of inhibition (recurrent, reciprocal, presynaptic, post-synaptic). Using in situ hybridization to mRNAs encoding GAD_{65} and GAD_{67}, we have identified, with both probes, labeled cells scattered through layers I to V of the dorsal horn, in the intermedio-medial column and in the ventral horn. GAD_{67} mRNA levels were higher than GAD_{65} mRNA levels, as reported in most CNS structures. GABA-immunoreactivity confirmed this cellular distribution. Labeled cells of the ventral horn, of larger size (20-30 µm) than cells of the dorsal horn (10 µm), were, however, visible after a colchicine treatment only, suggesting that they may be projection neurons. We are currently investigating wether this cell type might be motoneurons, as reported in others mammals, or intersegmental neurons. Supported by the Garches and IRME fundations to SF.

359.15

ENDOGENOUS LECTIN (RL-29) EXPRESSION OF THE SPINAL AUTONOMIC PREGANGLIONIC NEURONS IN THE RAT. M.J. Park, A.A.Cameron, W.D.Willis, S.H. Barondes, H. Leffler and K.Chung*. Sch. Allied Health Sci. & Marine Biomed. Inst., Univ. Texas Med. Branch, Galveston, TX 77550.

Recent studies have demonstrated that an endogenous lactose-binding lectin RL-29 was selectively expressed in subsets of embryonic and postnatal DRG neurons and their fibers in the superficial dorsal horn of the spinal cord. The present study tested the possibility of RL-29 expression in other spinal neurons. Four adult normal male albino rats were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. Various segments of the spinal cord were removed, vibratome sectioned, and immunostained for RL-29 by the ABC method. The antibody to RL-29 was used at a concentration of 1:3,000 for immunoreaction. We report that a subset of spinal motor neurons, parasympathetic and sympathetic preganglionic neurons, selectively demonstrate intense immunoreactivity to anti-RL-29. Although the functional implications of RL-29 in spinal autonomic pregnalionic neurons are not known, the data suggest that RL-29 is a useful marker for these neurons. (supported by NS11255)

359.16

SUBSTANCE P MODULATES GLUTAMATE-ACTIVATED SYNAPTIC POTENTIALS BUT NOT SEROTONIN-INDUCED EXCITATION IN NEONATAL MOTONEURONS. P. Hernandez*, B.S. Seebach, and L. Ziskind-Conhaim. Dept. Physiol. and Ctr. Neurosci., Univ. of Wisconsin, Madison, WI 53706.

Substance P (SP) and serotonin (5-HT) coexist in medullary neurons that descend to rat spinal cords. To determine whether SP modulates 5-HT- or glutamate-mediated neuronal excitation, its effects were recorded intracellularly in motoneurons of newborn rats. SP (1 μM) generated an average depolarization of 13 mV, which was associated with a 40% increase in membrane resistance and an increase in the frequency of synaptic potentials. The induced depolarization was reduced following inhibition of synaptic activity. SP reduced, however, the amplitude of dorsal root-evoked potentials, and this reduction persisted despite motoneuron desensitization. Preliminary results suggest that the effect of SP on dorsal root-evoked potentials is probably not due to its action on glutamate receptors.

SP-mediated neuronal responses are similar to those induced by 5-HT (Ziskind-Conhaim and Newcomer, Soc. Neurosci., 1990, 16:727). However, our data suggest that there is no interaction between the postsynaptic mechanisms of their actions. SP and its analogues had no effect on 5-HT-evoked potentials. Therefore, SP and 5-HT independently modulate synaptic activity in the spinal cord. Supported by RCDA (NS01314) and NS23808 to LZ-C.

CONTROL OF POSTURE AND MOVEMENT: CLINICALLY RELATED STUDIES

360.1

EFFECTS OF TREADMILL SPEED AND INCLINE ON THE WALKING PATTERN OF NORMAL AND SPASTIC PARETIC SUBJECTS. A. Pépin* and H. Barbeau. School of Physical and Occupational Therapy, McGill University. Montréal, Québec, Canada. H3G 1Y5.

The purpose of this study was to investigate the changes on the locomotor pattern of spastic paretic (SP) subjects as a result of changes in speed and incline during treadmill walking. Electromyographic (EMG) activity of lower limb muscles, angular displacements and temporal-distance (T-D) parameters were recorded for three SP subjects and two normal subjects evaluated at comparable ranges of speed (0.1 - 0.7 m/s) and upward incline (0-15%). It was noted that the long cycle duration and large stance to swing ratio observed in SP subjects' comfortable speed (0.2 - 0.3 m/s) were also present in normal subjects for matched speed. For normal subjects, knee flexion during early stance and ankle plantarflexion at push-off were greatly reduced at low speeds, as observed in SP subjects. Changes in T-D parameters with increased speed were similar for all subjects. In addition, EMG activity showed greater amplitude with generally preserved timing for normal subjects. In SP subjects, the increase in the amplitude of EMG activity was limited, and mostly, the recruitment pattern was changed, with longer burst duration and increased stretch activation in the triceps surae. For all subjects, the T-D data did not change with the incline. In normal subjects, the amplitude of the EMG activity was increased in triceps surae and other extensor muscles, whereas such increase was limited in SP subjects. Our results suggest that some characteristics of the gait of SP subjects cannot be solely attributed to their deficit and are associated in part with their low walking speed. Adaptation to changes in speed and incline is limited in SP subjects and we are in the process of further quantifying those limitations. Supported by MRC Canada.

360.2

COMPARISON OF THE EFFECTS OF CYPROHEPTADINE, CLONIDINE AND BACLOFEN ON LOCOMOTOR PATTERN IN INDIVIDUAL SUBJECTS WITH SPASTIC PARESIS. K.E. Norman* and H. Barbeau. School of Physical and Occupational Therapy, McGill University. Montréal, Qué. Canada. H3G 1Y5.

Studies from our laboratory have shown that cyproheptadine and clonidine can improve locomotion in spastic paretic subjects. In this study, we compared the effects of these two drugs, and of baclofen, a standard antispastic drug, on locomotor pattern in subjects at least one year post-spinal-cord-injury. Evaluations were conducted for each subject at entry to the study, after three weeks on each drug, after each two-week washout period, and after a period of a drug combination. Treadmill locomotor pattern was analyzed by means of electromyographic and kinematic data. Overground locomotion was also evaluated when possible. Most subjects used wheelchairs full-time and they were able to perform limited stepping on the treadmill only with a partial support harness, parallel bars, and minimal speed. Two subjects made gains in treadmill speed and extent of locomotor independence in response to cyproheptadine. The improvement was associated with a reduction in muscle stretch activation and coactivation of antagonists, as well as changes in hip, knee and ankle kinematic patterns. These two subjects also showed a reduction in stretch activation during gait in response to baclofen, although triceps surae clonus was still present, and functional changes in gait pattern were less evident. A third subject showed no changes in muscle activation patterns during drug periods although there was an improvement in gait speed in response to baclofen. These preliminary findings show different effects on locomotor pattern associated with each drug. We are in the process of quantifying these differences.

Supported by NCE Canada, MRC, and the Man in Motion Legacy Fund.

360.3

ADAPTATION OF THE LOCOMOTOR PATTERN TO NEURO-MUSCULAR LESIONS IN NORMAL AND CHRONIC SPINAL CATS.

L. Carrier, E. Brustein, J. Provencher and S. Rossignol*. Cntr for Res. in Neurol. Sci., Fac. of Med., U. de Montréal, Québec, Canada H3C 3J7.

This work presents preliminary data on the adaptation of the locomotor pattern of cats walking on a treadmill after inactivation of the ankle flexor muscles on one side, first in the intact condition and, in one cat, after spinalization. Four cats were implanted chronically with Electromyographic (EMG) electrodes inserted, under general anesthesia, in hindlimb muscles. EMGs synchronized to video images were recorded in all sessions. One cat had a distal tenotomy of TA (Tibialis Anterior) and EDL (Extensor Digitorum Longus) and 3 cats had a neurectomy of TA and EDL. One of these was, in addition, spinalized at T13. 51 days after the neurectomy. The tenotomized cat adapted rapidly. However, the autopsy revealed that the TA and EDL tendons had regrown. The three other cats showed a remarkable adaptation of locomotion soon after the neurectomy. The kinematics showed a small increase in hip and knee flexion as well as some degree of abduction during swing so that the cats brought their limb forwards using an increased flexion mainly of the knee and some circumduction. These kinematic changes were accompanied by a consistent increase in the hip flexors such as Iliopsoas and Sartorius anterior as well as the knee flexor Semitendinosus. Most of the contralateral extensor muscles were also increased in amplitude. The one spinal cat showed some remarkable differences compared to the usual spinal cats. On the neurectomized side, the cat made shorter steps, landed on the dorsum of the toes and hyperflexed its knee during swing but could not bring its foot forward. The increased in activity of contralateral extensors also disappeared. It is concluded that locomotor adaptive mechanisms to neuromuscular deficits involve mainly supraspinal structures although some aspects of the adaptation (i.e. knee hyperflexion) may be due to some long-lasting changes in the spinal cord. (Supported by the MRC, the NCE and the FCAR).

360.4

SENSORY-ORGANIZATION TRAINING FOR OLDER ADULTS WITH BALANCE IMPAIRMENTS.

S. Moore*, M-S Hu, MH Woollacott. Dept. Exercise and Movement Science, University of Oregon, Eugene, OR 97403.

The problem of falling in older adults is multifactorial. In order for intervention to be effective, contributing factors which are modifiable must be identified. The primary hypothesis of this study is that impaired CNS processing of information from the balance senses (vision, somatosensory, vestibular) is a fall factor for some older adults. The secondary hypothesis for this study is that this factor is modifiable.

A group of older adults without balance problems (n=18, mean 75 yrs), and a group with balance problems in circumstances such as standing up, turning a corner and walking on uneven surfaces (n=14, mean 76 years), were tested for their ability to minimize postural sway when sensory information was manipulated (Nashner, 1982). The symptomatic group demonstrated 5-7 percent more sway on each sensory condition. Symptomatic older adults are participating in an 8-week individualized training program which emphasizes central processing of different combinations of information from the balance senses. Preliminary pre/post test results (n=6) demonstrate a reduction in instances of loss of balance (LOB) from 8 to 0 when somatosensory information is altered, and a reduction in LOB from 13 to 7 when both somatosensory and visual information were altered.

It appears that impaired CNS processing of sensory information from the balance senses may be a modifiable fall factor for older adults.

360.5

POSTURAL RESPONSES DURING REACHES TO CONTRALATERAL TARGETS FOLLOWING STROKE.

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The successful execution of reaching movements while standing requires anticipatory responses to stabilize posture. Posture is maintained by counteracting centre of mass displacements of the reaching arm and reaction moments created by the focal musculature. Following stroke, patients demonstrate altered sensorimotor processing that impairs the integration and performance of postural and focal tasks.

This study investigated the relationships between posture and reaching to central and contralateral visually illuminated targets (n=4) at 2 velocities (ballistic and self-paced) in patients following stroke (n=5) and matched normals. The kinematics, kinetics and muscle activations associated with the task were studied using 3D body coordinates (Selspot II), ground reaction forces (GRFs) and multichannel EMG (n=13).

Analysis revealed postural responses that were target and velocity dependent in normals. Anticipatory muscle activation and subsequent GRF generation were related to the execution of the reach. Focal movements were initiated at the time of the peak vertical GRF and displayed stereotypical position/velocity profiles. Anticipatory muscle activations and GRFs were markedly delayed in patients following stroke. A loss or degradation of normal response patterns and large increases in variability across trials were evident. Decreased excursion of the centre of pressure (COP) in the anterior/posterior directions and towards the paretic side is indicative of poor weight transference during the execution of a dynamic upper extremity task.

360.6

CLINICAL CORRELATES OF PACED VOLUNTARY POSTURAL TASK IN PATIENTS WITH PARKINSON'S DISEASE (PD)

D.J. Beckley*, V.P. Panzer, M.P. Remler and L. Ilog, Dept. of Neurology, U.C. Davis CA 94553.

In this study we asked if movement amplitude abnormalities in PD correlate with direction of movement and which clinical features best reflect changes in amplitude scores. Ten PD patients (mean age 61.9, range 47-75) performed A-P and M-L weight-shifting at slow, medium and fast speeds. Movement amplitude scores were analyzed at 3 speeds (1s, 2s & 3 s), off-on medications, and in 4 directions (R, L, Fwd., Bwd.,). Changes in movement amplitude scores were correlated with modified UPDRS motor scores. We found that patients did better on right lateral weight shifts, while on medication, and that this correlated with improved postural instability and right-sided bradykinesia scores. There was a medication effect but no directional effect for A-P movements and improvement in A-P performance correlated only with improved postural instability scores. We conclude that paced voluntary movement abnormalities in PD exhibit a directional effect for lateral but not for A-P movements. Improvements in these abnormalities correlate with postural instability and bradykinesia scores but not with tremor or rigidity scores.

360.7

THE ONTOGENY OF MOVEMENT SEQUENCES IN JIMPY (MYELIN DEFICIENT) MICE.

V.J. Bolivar*, K. Manley and J.C. Fentress. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1.

The jimpy (jp) mutant mouse expresses severe hypomyelination in the CNS. This sex-linked mutation is due to a single base change within the myelin proteolipid protein (PLP) gene. Jimpy mice exhibit tremors, weak hind limbs and tonic-chronic seizures by weaning age. Numerous studies have examined the genetics, biochemistry, and physiology of these mice. However, the behavioral aspects of this genetic deficiency have not been well documented. Due to the progressive nature of the mutation it is important to examine the behavior ontogenetically. We selected supported swimming as a behavioral assay due to the difficulty these mice have with normal quadrupedal locomotion. Mutant jimpy males and littermate controls were videotaped (30 fps) at 2 day intervals from the 3rd to the 21st day postpartum. Videotapes were analyzed using two methods: 1) interlimb coordinations were determined by frame-by-frame analysis and 2) trajectories, accelerations and velocities of specific limb points were calculated using the PEAK Motion Measurement System. Our results indicate both subtle and gross differences between mutant and control mice and that mutants were not able to swim as efficiently as littermate controls. Our data confirm the importance of detailed behavioral documentation if we are to understand how gene expression affects motor development. (Supported by MRC grant MA7660 to J.C.F.).

360.8

COMPARISON OF THE SYNCHRONIZATION OF HUMAN MOTOR UNITS DURING THE VOLUNTARY CONTRACTION OF THE WRIST EXTENSOR MUSCLES IN THE PREFERRED OR THE NON-PREFERRED ARM.

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The synchronization of motor unit (MU) discharges can reveal the activity of branching axons shared by the motoneurons (short-term synchronization) and/or the activity of distinct inputs that are presynaptically synchronized (long-term synchronization). Milner-Brown, Stein and Lee (1975) showed that repeated muscle exercise could enhance MU synchronization, possibly as a result of a strengthening of corticomotoneuronal pathways. We documented the dependence of MU synchronization on muscle use with regard to the preferred arm of the subjects. MU synchronization was analyzed in the left arm of 9 right-handed and 6 left-handed subjects by cross-correlating the discharges of pairs of MUs recorded independently and differentiated as "slow" and "fast" on the basis of their force recruitment threshold and their twitch contraction time. The subjects tested with their preferred arm presented a significantly higher incidence of MU synchronization, affecting similarly the pairs of "slow" and "fast" MUs as well as the mixed pairs. The area of the narrow cross-correlogram peaks (≤7,5 ms) reflecting most likely MU short-term synchronization tend to be larger for all types of MUs pairs in the left arm of left-handed subjects. This suggests an increase in the efficiency of common inputs controlling "slow" as well as "fast" MUs in the preferred arm. Broader peaks likely to involved long-term synchronization components were significantly more numerous for pairs including one or two "fast" MUs. The dependence of MU synchronization on arm preference is confirmed by preliminary data obtained in the preferred and the non-preferred arm in the same subject.

360.9

MODELING DYSKINESIA: I - DEVELOPMENT OF A DYNAMIC MODEL. JB Lohr* and MP Caligiuri, Department of Psychiatry, UCSD Department of Psychiatry, and San Diego VA Medical Center, San Diego, CA 92161

Choreoathetoid dyskinesias have long been known to consist of random-appearing patterns of abnormal movement. Recently, DeLong and others (DeLong, 1990) have suggested that choreoathetoid dyskinesias may result from abnormal reduction in output from the internal segment of the globus pallidus and substantia nigra pars reticulata, in part related to an abnormal reduction in output from the subthalamic nucleus. We conjectured that, rather than a simple reduction in glutamatergic output from the subthalamic nucleus, there may in fact be erratic output, and that this erratic output could possibly be the result of chaotic behavior of the basal ganglia circuitry involved in dyskinesia. To follow-up on this idea, we modeled a modified version of the circuitry proposed by DeLong. The model presented gives evidence of chaotic behavior when certain critical parameters are varied. The results are consistent with the notion that chaotic dynamics may underlie the clinical appearance of abnormal movements induced by L-dopa and dyskinetic disorders such as neuroleptic withdrawal-emergent dyskinesia and tardive dyskinesia.

DeLong MR. Trends NeuroSci 13:281-285, 1990.

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360.10

MODELING DYSKINESIA: II - QUANTITATIVE CLINICAL STUDIES IN PARKINSON'S DISEASE. MP Caligiuri* and JB Lohr Motor Function Laboratory, VA Medical Center, UCSD Department of Psychiatry, San Diego, CA 92161

Nonlinear dynamic models are useful tools for examining the neural circuitry involved in abnormal motor behavior such as tremor (Beuter et al., 1991) and dyskinesia (Lohr and Caligiuri, 1992). These models suggest an interaction among tremor, akinesia, and dyskinesia as parameters of the model change. Purpose The aim of this study was to examine the interactions between tremor, akinesia, and dyskinesia in patients with l-dopa-induced dyskinesia with time post-drug as a critical parameter. Methods PD patients with peak dose dyskinesia were evaluated during six 5-minute segments 30 minutes prior to and following a single dose of Sinemet. Orofacial and hand dyskinesia and tremor were quantified using isometric procedures; bradykinesia was quantified by obtaining the peak velocity associated with rapid ballistic wrist flexion. Results Inspection of temporal interactions between dyskinesia and parkinsonism indicated that increased dyskinesia coincided with dopa-induced changes in movement velocity, but not tremor amplitude. Conclusion These findings contribute to ongoing efforts to model the time-dependent dynamic interactions between dyskinesia, bradykinesia, and tremor in PD patients exhibiting drug-induced dyskinesia.

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360.11

TREMOR AS A FACTOR IN DELAYED REACTION TIME
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[1]Bundeswehr University, Munich, Germany, [2]West Roxbury VA Medical Center & Harvard Medical School, Boston MA, [3]University of Bonn, Bonn, Germany.

We have recently shown (Soc. Neurosci. Abstr. 17:1032, 1991) the existence of a systematic phase relationship between resting tremor and the onset of rapid voluntary contraction in patients with Parkinson's disease (PD). The present study was undertaken to see whether this phase relationship contributes to the prolongation of reaction time in PD. Control subjects and PD patients produced fast index finger abductions to a visual target under a simple reaction time condition. Force/displacement and surface EMG from the first dorsal interosseus muscle were recorded. We used a new technique for on-line analysis of tremor to present the "GO" stimulus at certain phases of the actual tremor cycle. In PD significant differences were found in mean reaction times calculated separately for different locations of the "GO" stimulus within the tremor cycle in both isometric and isotonic conditions. Reactions with an expected onset during the first half of the EMG tremor cycle were delayed in comparison to trials with an expected onset during the second half of the EMG tremor cycle. Similar effects were also found for physiological tremor but they had less influence on reaction time because of the higher tremor frequency. We concluded that attraction of the onset of the voluntary motor response to the tremor oscillator contributes to a delay in reaction time which extent depends on tremor frequency.

(Supported by DFG grant)

360.12

OVERCOMING MOVEMENT RESTRICTIONS IMPOSED BY HYPERTONUS THROUGH ANTAGONIST MUSCLE ACTIVATION. S. Wolf*, P. Catlin, S. Blanton, J. Edelman, N. Lehrer, D. Schroeder. Dept. of Rehabilitation Medicine, Division of Physical Therapy, Emory University School of Medicine, Atlanta, GA 30322.

A traditional perspective on the rehabilitation of patients with spasticity presumes that hyperactive muscles must first be relaxed before antagonists are recruited if effective motion about a joint is to occur. To examine this concept, eight subjects with chronic stroke (greater than one year) received feedback to retrain the triceps in the presence of biceps spasticity. Eight additional subjects received conventional movement training. Ten training sessions were given using functional tasks of elbow extension. Measurements included range of motion, speed of movement, and triceps and biceps EMG during triceps contraction. Between group comparisons showed no significant differences. Within group comparisons showed significant increases in triceps EMG output during more measures in the feedback group than in the conventionally trained group. Passive and active range of motion in both groups also increased significantly, while biceps cocontraction persisted. This differentiation suggests that functional improvements at the elbow may have been due to biomechanical (peripheral) rather than neuromuscular (central) changes about the joint. Furthermore, subjects can be trained to increase movement without first specifically inhibiting activity in the spastic muscle group.

360.13

MODULATION OF CUTANEOUS INPUT DURING PREPARATION AND EXECUTION OF MOVEMENT IN THE ELDERLY. Arseneau C.A. and Forget R.* Hôpital Hôtel-Dieu de Montréal et Ecole de réadaptation, Univ. de Montréal, C.P 6128 succ. A, Montréal, Canada, H3C 3J7

Sensory input modulation and motor output were studied in 20 healthy elderly subjects (x= 64 ± 5 years). Responses to random electrical stimulation of the digits which were involved in a forewarned motor task were compared between 5 conditions: 1) 3250 ms and 2) 500 ms before the go signal during a 4 sec preparatory period, 3) during the holding period of an isometric force task, 4) during feedback processing and 5) at rest after the release of force. Recordings included: somatosensory evoked potentials (SEP) at 5 scalp locations (3 cm anterior and posterior to C3 and C4 and at Cz), EMG of finger flexors and neck muscles and the median neurogram at the wrist.

During movement execution, amplitudes of contralateral early SEP components were decreased by 35% (N20P30) and 50% (P22N30) at parietal and frontal locations respectively. Intermediate component P45N70 was also decreased by 20% during movement. These components also showed a significant gating, but to a lesser extent, at the end of the preparatory period (500 ms before the go signal). However, the late component P100N140, which had a bigger amplitude at Cz (over SMA area) was not affected during movement but was significantly increased before movement and during feedback processing after movement. Recording of the median n. and EEG subtraction of stimulated and non-stimulated trials showed that the modulation of SEP amplitudes was not due to a change in the afferent volley and/or contamination by cerebral motor activity (CNV, BP, MP). In some subjects the stimulation of the digits evoked a reflex contraction which was decreased during movement. It is concluded that in the elderly: 1) gating of early SEP components begins at the end of preparatory period and is strongest during execution of movement, 2) a dissociation between early and late SEP components is found in the preparatory period, 3) reflex activity is inhibited during execution of movement. (Supported by the FRSQ)

360.14

BRADYKINESIA IN ALZHEIMER'S DISEASE: STAGE-DEPENDENT OVERLAP WITH PARKINSON'S DISEASE JC Morris*, VD Buckles, SA Sahrmann, MC Smith, M Storandt, JD Baty, P Amrhein, Alzheimer's Disease Research Center, Washington University Sch Med., St. Louis, MO 63110; Dept Psychology, Univ New Mexico, Albuquerque, NM 87131

Advanced Alzheimer's disease (AD) is associated with extrapyramidal dysfunction resembling that of Parkinson's disease (PD). We studied subjects in milder stages of AD for presymptomatic parkinsonian dysfunction. There were 4 study groups: very mild AD (n=35; x age=72.7y); mild-moderate AD (n=19; x age=72.4y); PD (n=42; x age=69.5y); and controls (n=46; x age=73.5y). Mean Northwestern Disability composite score for PD subjects was 1.05 (equivalent to Hoehn and Yahr score of 2) and negligible for the other 3 groups (no clinical parkinsonism). Antiparkinsonian medications were withheld in PD subjects for 12 hours prior to motor testing. Subjects were assessed psychometrically (measuring aspects of memory, language, and psychomotor performance) and motorically (temporal measures of gait by motion analysis, finger-tapping, and paradigms for reaction time [RT] and movement time [MT]). Using age and education as covariates, the PD and control groups had generally similar psychometric performance profiles and performed significantly better than the AD groups. The mild-moderate AD group demonstrated bradykinesia comparable to the PD group on gait velocity, finger-tapping, and MT; both groups were impaired on these measures relative to the very mild AD and control groups. The mild-moderate AD subjects, but not the PD or other subjects, were impaired on RT. We conclude that preclinical bradykinesia is present in mild-moderate AD and is qualitatively similar to that of PD. However, it appears to have distinct pathophysiologic correlates: bradykinesia in AD is associated with both the presence and severity of cognitive dysfunction.

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360.15

MOTOR CONTROL DIFFERENCES IN ALZHEIMER'S AND PARKINSON'S DISEASES. V Diggles-Buckles*, JC Morris, and JD Baty. Dept. of Neurology, Jewish Hospital and Div. of Biostatistics, Washington Univ. Sch. of Medicine., St. Louis, MO 63110.

Motor control differences in simple, discrete movements were observed in patients with Parkinsons's Disease (PD, n=39) and Alzheimer's Disease (AD) as compared with controls (n=36). ADs were divided into 2 groups according to dementia severity: questionable, (n=24) and mild-moderate, (n=13). In a Fitts type task, subjects made discrete movements to a series of 4 targets of varying sizes at a distance of 60 cm from the start position, following an auditory 'go' signal. Index of Difficulty (ID in bits) for each target was calculated and individual, median reaction times (RT) and movement times (MT) were analyzed in repeated measures ANOVAs. **RT results** revealed a significant group main effect in which the mild-moderate ADs were significantly slower than all other groups. A surprising group x ID interaction was present in which mild-moderate ADs took longer to program low difficulty movements. A learning/familiarization effect may explain this result, since the larger targets (lower difficulty) were first. **MT results** revealed significant main effects of group and ID (MT increased with ID). MTs of PDs and ADs averaged 80 and 170 ms slower, respectively, than controls and questionable ADs. **Conclusions:** Questionable ADs performed essentially the same as controls on this task; slowing in the AD group ocurred only in the more severely demented. Mild-moderate ADs took longer for motor preparation (RT) than the other groups. PDs and mild-moderate ADs showed differences in motor system efficiency as indicated by baseline differences in MT. All groups produced the linear relationship between MT and ID that Fitts predicted, and slopes of the regression lines were not significantly different, indicating that the information processing rates (increase in time per bit of information) of the motor control system are comparable between all groups. Support: NIH Grant AG03991.

BRAIN METABOLISM AND BLOOD FLOW III

361.1

THE EFFECTS OF GLUCOSE DEPRIVATION AND ALTERNATIVE SUBSTRATES ON SYNAPTIC TRANSMISSION AND METABOLITE LEVELS IN HIPPOCAMPUS. K. A. Heldman*, N. A. Veyna, R. B. Marchase, and M. J. Friedlander. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham AL 35294.

Glucose is important in normal synaptic transmission in the mammalian brain. It is considered the primary energy source, although alternative substrates have been suggested (Cox & Bachelard, Exp. Brain Res. 69:368-372, 1988; Schurr et al., Science 24:1326-1328, 1988). In addition to producing energy, glucose supports pools of UDP-glucose and UDP-N-acetylglucosamine, which are necessary for the synthesis of complex glycoconjugates. In order to determine which roles of glucose are necessary for synaptic transmission, we investigated the 1) effects of glucose deprivation, 2) the adequacy of alternative energy substrates such as lactate and pyruvate, and 3) the routing of exogenous radiolabeled glucose during synaptic activity. The Schaffer collateral pathway was stimulated (0.05-0.10 Hz) in submerged, warmed hippocampal slices prepared from anesthetized guinea pigs. Normal synaptic efficacy (evaluated by ratios of rise time of the field EPSP to amplitude of the afferent volley) was maintained in glucose (5.5 or 11.0 mM, n=6), pyruvate (11 mM, n=4) and lactate (11 mM, n=4). Synaptic efficacy was greatly reduced in 0 mM glucose (n=5), 5 mM lactate (n=2) and 5 mM pyruvate (n=1), although the afferent volley was unaffected. In slices deprived of adequate substrate, UDP-glucose levels were more dramatically decreased than were ATP levels.

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361.2

STIMULATION OF THE DORSAL RAPHE NUCLEUS WITH KAINATE BUT NOT L-GLUTAMATE INCREASES CORTICAL CEREBRAL BLOOD FLOW IN RAT. M.J. Bakalian*, M.D. Underwood, V. Arango and J.J. Mann. Labs of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213

Electrical stimulation in the rostral portion of the dorsal raphe nucleus (DRN) elicits decreases in cortical blood flow (CBF), while stimulation in caudal DRN elicits increases in CBF. We sought to examine the effect of stimulation of DRN neurons, but not fibers of passage, by chemical stimulation with the excitatory amino acids L-glutamate (L-glu) and kainate (KA). Studies were performed in 23 adult male Sprague-Dawley rats anesthetized with isoflurane (1 - 5% in 100% O_2) and α-chloralose (30 - 40 mg/kg, s.c.). Animals were paralyzed and artificially ventilated to maintain arterial blood gases in physiological ranges. Parietal CBF (perfusion units, PU) was continuously monitored by laser-Doppler flowmetry. L-glu (0.1 - 10 nmol/100 nL) or KA (0.005 - 5 nmol/100 nL) were microinjected (15 - 100 nL) over 30 - 60 sec into the DRN using a stereotaxically-guided Hamilton syringe. Injection sites were marked with fast green dye and reconstructed from Nissl stained coronal sections. The DRN was mapped with L-glu (1 nmol) in 1 mm increments and no effect was observed from either the DRN (ΔCBF: 4 ± 3 PU; ΔMAP: -2 ± 2 mmHg) or 1 mm above the DRN (ΔCBF: 0 ± 3 PU; ΔMAP: 1 ± 4 mmHg)($p > 0.05$). Decreases in MAP (-12 ± 2 mmHg) and CBF (-6 ± 4 PU) were elicited from the median raphe nucleus located ventrally ($p < 0.05$). In the DRN, L-glu did not affect CBF or MAP at any dose examined ($p > 0.05$). The effect of microinjection of KA (0.005 nmol to 0.5 nmol) into the DRN on CBF or MAP did not differ from saline ($p > 0.05$). DRN microinjection of 5 nmol KA increased CBF (19% ± 10%) and MAP (8% ± 4%) 2 min following the onset of microinjection. The CBF elevation was sustained for 5 min, maximal (28% ± 10%; $p < 0.05$) at min 3 and returned to baseline levels 7 min following microinjection onset. The MAP increase was maximal at min 7 (Δ18 ± 7 mmHg) and remained elevated beyond the 10 min of observation. Decreases in CBF or MAP were not consistently observed. We conclude that stimulation of DRN neurons, but not fibers of passage, increases CBF in excess of increases of arterial pressure. Supported by NARSAD to MDU and MH46745.

361.3

DOSE-RELATED EFFECTS OF THE ANXIOLITIC BUSPIRONE ON REGIONAL CEREBRAL GLUCOSE METABOLISM (rCMRglc) IN AWAKE RATS. U. Freo*, M. Dam, G. Pizzolato, P. Pietrini#, A. Morico, S. Ruggero, L. Battistin. Neurology Dept., University of Padua Medical School, & # Psychiatry Dept., University of Pisa Medical School, Italy.

The relation to dose of rCMRglc was determined in awake 3-month-old male Fisher-344 rats after administration of the 5-HT1A receptor agonist buspirone, clinically used as an anxiolitic. Using the quantitative autoradiographic [14C]2deoxy-d-glucose technique, rCMRglc was measured in 47 brain regions 10 min after administration of buspirone 0.2, 4, and 20 mg/kg i.p., respectively.

Buspirone 0.2 mg/kg i.p. significantly ($p< 0.05$) reduced rCMRglc in 21 (42%) brain regions (average decline: - 21%), mostly in the limbic areas (dorsal and ventral hippocampus) and raphe nuclei (median and dorsal), which contain the highest 5-HT1A receptor densities. Buspirone 4 and 20 mg/kg i.p. reduced rCMRglc in 35 (74%) and 36 (76%) brain regions, respectively, including cortical, basal ganglia, diencephalic and cerebellar areas (average rCMRglc decline: - 30%) and increased rCMRglc in the lateral habenula (mean increase: + 55%). As we recently reported [Freo et al., Biol Psychiatry, 29 (11S): 204, 1991], rCMRglc is reduced in the raphe-hippocampal system also by administration of low-dose clomipramine, a clinical antidepressant. This is intriguing because buspirone is an atypical anxiolitic with no muscle relaxant and anticonvulsive activities, unlike benzodiazepines, and with a slow onset of action like tryciclic antidepressant. Both clomipramine and buspirone are clinically employed in the treatment of psychopathological conditions with coexistent anxiety and depression.

The pattern produced by high doses of buspirone (widespread rCMRglc reduction and isolated rCMRglc increase in the lateral habenula) is similar to those induced by dopaminergic antagonists such as haloperidol, and is in agreement with the reported antidopaminergic activity of buspirone.

361.4

THE CONSEQUENCES OF ESTRADIOL INCREASING CEREBRAL GLUCOSE UTILIZATION. J. Bishop and J.W. Simpkins*. Center for the Neurobiology of Aging, University of Florida, Gainesville, FL 32610.

We have previously shown that a single dose of estradiol increases glucose utilization in the CNS by as much as 220%. To assess the physiological consequence of this estradiol-modulated increase in cerebral glucose utilization, we evaluated two conditions in which estradiol may significantly affect glucose transport: (i) hypoglycemia, (ii) neuroglucopenia induced by morphine withdrawal. We measured ACTH as an index of the sympathetic response to changes in brain glucose availability. Rats were ovariectomized and treated with estradiol or oil. We provoked an insulin-induced hypoglycemia or a naloxone-induced increase in brain glucose utilization. There was no estradiol effect on plasma glucose levels as the animals responded to either insulin or naloxone. In both studies, however, estradiol significantly decreased basal ACTH levels. In response to the naloxone-induced hyperglycemia there was an ACTH response equivalent in the two groups for the first 30 minutes. In estradiol treated animals, we observed the expected return toward basal levels over the 120 minute period. In oil treated animals ACTH remained at peak levels for 2 hours. These results suggest (i) estradiol reduces the sympathetic response to neuroglucopenia and (ii) ovariectomized animals fail to respond to the enhanced availability of glucose with an appropriate termination of the stress response. (Supported by NIHAG 10485).

361.5

Dual-label autoradiographic comparison of glucose and deoxyglucose regional blood-brain transport kinetics.
J.L. Lear and R.F. Ackermann*. Nuclear Medicine, Health Sciences Center, Denver CO 08262; Nuclear Medicine, UCLA School of Medicine, Los Angeles CA 90024.

The deoxyglucose (DG) method for quantifying brain glucose metabolism assumes a fixed relationship (the "lumped constant" [LC]), between net DG and glucose (GLC) extraction. Although the LC can be affected by the rate of blood-to-brain DG transport relative to that of GLC, the regional uniformity of the DG vs. GLC transport-rate relationship is not well-studied. Here, we used dual-tracer autoradiography to directly compare the transport rate constant (K_1) of radiolabeled DG and GLC, in both normal and kainate-activated rat brains. Each rat received a 45 sec i.v. infusion containing both ^{18}F-fluorodeoxyglucose (FDG) and ^{14}C-GLC, then was immediately decapitated, and its brain sectioned for autoradiography. Two tracer concentration images, one representing FDG and the other GLC, were generated from each brain section. Each image was digitized and converted by computer into images of K_1 value. Overall K_1 values for FDG and GLC did not differ significantly, but certain areas such as hippocampus showed distinctly different patterns of FDG vs. GLC transport. Although kainate-activation changed the overall K_1 values, differences between hippocampal FDG and GLC transport pattern nevertheless persisted. Thus, the assumption of uniform DG vs. GLC transport throughout the brain is not entirely correct. Hence, the LC may differ substantially from a global norm in specific brain regions.

361.6

THERMAL MAPPING OF RAT CORTICAL FUNCTION
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Digital processing of images captured by infrared cameras has made it feasible to map small changes in cortical temperature that may be associated with altered functional activity[1]. We have sought to examine the capabilities of thermoencephaloscopy (TES) by studying thermal responses to direct intracortical electrical stimulation.

Infrared radiation at the dorsal skull surface of urethane anesthetized rats was detected with an Amber 4128 Focal Plane Array detector. Stimulation of forelimb sensorimotor cortex with 1 second train of 0.2 msec electrical pulses delivered at 500 HZ induced a focal thermal increase contralaterally. The thermal response started approximately 0.5 seconds after the end of the stimulus and lasted one to two seconds. This effect could be observed without digital averaging or subtraction.

These results suggest that TES could provide a powerful new method for studying rapid changes in cortical function.

[1]A.M.Gorbach et al., Thermology 3:108-111 1989.

361.7

FEASIBILITY OF IN-VIVO BRAIN CHEMILUMINESCENCE UNDER PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL CONDITIONS.
U.Dirnagl, U.Ködel, W.H.Örtel*, H.W.Pfister, L.Schleinkofer, A.Villringer. Dept.of Neurology, University of Munich, 8000 Munich 70, F.R.G.

The aim of this study was to test the feasiblity of counting photons generated by free oxygen radical species in brain cortex in vivo. Anesthetized and ventilated rats were equipped with a closed cranial window over the parietal cortex (0.25 cm^2 exposed). Intracranial pressure (ICP), mean arterial blood pressure (MABP), body temperature, endexspiratory pCO_2, and blood gases were monitored. The animals were housed in a dark box. The detector was a cooled photon counting camera (Hamamatsu Photonics). Chemiluminescence was enhanced with 10^{-2} M lucigenin i.v. The dark count was 234 ± 14 CPM (counts per minute ± SD, n=10 rats). 672 ± 196 CPM (n=10) from the brain were counted. I.v. infusion of lucigenin (no effect on MABP, ICP, cerebral blood flow, blood gases etc.) led to an enhancement to 969 ± 346 CPM (n=10). Hyperoxia (100 % O_2, n=2) increased the count from 1153 ± 652 to 1647 ± 716 CPM within 25 minutes. Treatment with the free radical scavengers superoxide-dismutase (Cu-Zn-SOD) or catalase decreased the hyperoxic count. Hypoxia (5% O_2, 95% N_2, n=2) for 5 minutes led to an immediate drop in the count rate from 1374 ± 56 CPM to 1014 ± 260 CPM, followed by a delayed increase to 1771 ± 130 CPM within 60 minutes. Global forebrain ischemia (n=2) for 10 minutes followed by reperfusion led to an increase in the count rate from 720 ± 12 CPM to 1238 ± 382 CPM. Pneumococcal meningitis (n=2) raised the count from 817 ± 160 to 1417 ± 143 CPM within 220 minutes, treatment with Cu-Zn-SOD decreased the count to 1238 ± 382 CPM. We conclude that it is feasible to monitor biophotons from the living, exposed brain of experimental animals, and that the system may enable continuous detection of oxygen free radical production in vivo.

361.8

NONINVASIVE MONITORING OF LOCAL CEREBRAL BLOOD VOLUME AND TISSUE OXYGENATION DURING COGNITIVE TASKS IN MAN USING NEAR INFRARED SPECTROSCOPY.
A.Villringer, K.Bötzel, C.Hock*, L.Schleinkofer, U.Dirnagl. Dept. of Neurology and Psychiatry, University of Munich, 8000 Munich 70, F.R.G.

The purpose of this study was to evaluate near infrared spectroscopy (NIRS) for the assessment of cerebral hemodynamic responses and alterations in tissue oxygenation during performance of cognitive tasks. 10 healthy human subjects (age 25 - 45) were examined. We used a Hamamatsu NIRO 500 system. The light from the Laser diodes (wavelengths 775, 825, 850, 904 nm) is guided through a fibre optic bundle, the end of which (the optode) is placed on the left forehead, another optode leading to the photomultiplier is placed at a distance of 3.5 to 5 cm. Depending on the absorption of the near infrared light at the various wave lengths in the brain tissue, changes in the local concentration of hemoglobin [Hb], oxygenated hemoglobin [HbO_2] blood volume (BV=Hb+HbO2) and oxygenated cytochrome oxydase [Cyt-O_2] are determined. The maximal temporal resolution is 0.5 seconds. The experimental protocol included periods of rest with eyes closed (6 min), eyes open (2 min), calculating (2 min), and rest with eye closed (6 min). Sufficient signal to noise was obtained in all subjects. Eye opening did not significantly affect the hemodynamic and tissue oxidation parameters. During calculating, [HbO_2] and BV increased and [Hb] decreased; Cyt-O_2 either increased or remained unchanged. We conclude that alterations in local brain tissue blood volume and oxygenation during cognitive tasks can be monitored noninvasively using NIRS. In combination with evoked potential techniques this method may become an important tool in stuying the interaction of cerebral function, hemodynamics, and energy metabolism.

361.9

COMPUTER-DIRECTED COREGISTRATION OF MULTISLICE FUNCTIONAL AND ANATOMIC BRAIN IMAGES WITH FIXED EXTERNAL FIDUCIALS.
R.T. Malison*, E.G. Miller, R. Greene, G. McCarthy, and R.B. Innis. Yale University School of Medicine and CORITechs, Inc., New Haven, CT 06510.

Using fixed external fiducials, we have developed and validated in a phantom a method of computer-assisted coregistration for multislice SPECT and MRI brain images. Reusable markers were fabricated from nylon-based plastic and consisted of two parts: a base which may be attached to the skin with adhesive; and a removable, spherical-cavity insert (5 mm i.d.). The markers can be filled with contrast agents appropriate for multiple imaging modalities. Skin dosimetry is reduced because the spherical insert can be removed between studies (and later exactly repositioned into the base). A set of markers internal and external to a 3D brain phantom provided a means of quantifying the accuracy of the coregistration. A computer algorithm, with precautions against local minima, was used to derive transformation matrices for image sets by minimizing the mean squared deviations obtained from differing permutations (n=10) of increasing numbers (range 3-11) of corresponding external SPECT/MRI point-pairs. As defined by the mean (±SEM) root mean square (RMS) values of non-coregistered internal fiducials, the minimal accuracy was 2.5±0.4 mm for three coregistration points. The absolute accuracy (likely greater than the RMS) was not significantly improved by using greater than 7-8 markers (RMS=2.3±0.3 mm), at which point internal and external RMS values asymptotically converged. This corresponded roughly to the largest SPECT (2.4 mm) and/or MRI (2.2 mm) voxel dimension(s). That is, the coregistration appeared nearly perfect and was confirmed by transformation of two MRI (or SPECT) phantom acquisitions obtained in deliberately different orientations. Using identical MRI (or SPECT) data sets, measures of test/retest- (RMS=0.5±0.1 and 0.5±0.1 mm, respectively) and interater-reliability (0.5±0.3 and 0.8±0.1 mm) were analogously obtained, establishing the method as highly reproducible and objective. Preliminary results in humans suggest its feasibility for clinical studies.

361.10

CORTICAL AND SUBCORTICAL METABOLIC PATTERN MAPPING BASED ON FUNCTION/ANATOMIC FUSION IMAGING FROM REGISTERED PET AND MR IMAGES
H.L. Loats*, E.A. Gastineau, T. Rippeon, M. McIver, S. Loats, H. Holcomb, C. A. Tamminga, Loats Associates, Inc., Westminster, MD 21157, University of Maryland, MPRC, Baltimore, MD 21228, Johns Hopkins Medical Institutes, Baltimore, MD 21205

The interpretation of functional images is generally impeded by the relatively limited anatomic information provided by the functional image. A rapid method for construction of cortical surfaces from registered MR images has been developed. This technique additionally permits construction of functional surfaces from registered PET data sets. Quantitative fusion of functional and anatomic images provides a tool for brain function mapping. Gyral and sulcal features are visually identified from the MRI cortical surface projections for right/left sagittal, anterior and posterior views. These serve as measurement templates for subsequent redirected sampling performed on a series of functional images. Cortical metabolic patterns are produced by projecting onto the cortical surface the average metabolic concentration measured from the surface to a depth of the cortex. Metabolic values for subcortical anatomic features are produced from volumetric shapes derived from volume MRI data sets. The change in cortical metabolic patterns with haloperidol have been analyzed in a series of eight on/off medicated patients. Volumetric metabolic activity and spatial correlation over specific cortical areas have been measured using the MRI cortical surface and the functional images. Accurate fusion of structure-function cortical surfaces provides a unique analytic method for resolving spatially complex, physiological changes.

361.11

DETECTION OF PHYSIOLOGICAL (PET) SIGNALS IN INDIVIDUAL SUBJECTS. J.T. Metz*, C.-T. Chen, M. Jiang, J.-S. Chou, M. Cooper. University of Chicago, Chicago, IL 60637.

Current methods for detecting metabolic or blood flow changes associated with behavioral or cognitive events ("signals") depend largely on averaging PET images across a number of subjects. Typically, some form of average image based on differences between activation and reference scans is used to define regions of increased activation. Although useful, this approach suffers from imprecision due to individual differences in brain anatomy and in spatial localization of activations. It also has limited usefulness about possible abnormalities in individual subjects.

We have identified five steps which can be added to the conventional methods to overcome these limitations. We begin by defining an appropriate group for across subject averaging. Each member of the group must meet behavioral criteria (obtained at the time of scanning) for inclusion in the group (1). All normalized PET images obtained from each subject are then correlated with the subject's MRI and other PET images (2) prior to pixel-by-pixel subtraction of activation and reference scans. Difference images are converted to stereotactic space and averaged across subjects. Thresholding on statistical criteria is used to define regions of increased activation. Each subject is then re-examined for evidence of an activation in close proximity to the group-defined average location (3). The likelihood of defined signals occurring in an individual subject, disregarding other activations which may also be present in the subject, is thus determined; this can be used to establish the importance of a lack of signal in some subjects. Repeated presentation of the same reference and activation conditions can improve sensitivity by minimizing spurious activations through within-subject averaging (4). Improved methods of interpolation and elastic warping can also reduce blurring when averaging across subjects (5).

The importance of each of these steps depends on the relative size of the physiological signal under study. Experiments using only methods 2 and 4 indicate that signals of about 3 standard deviations from the mean difference level can be detected in individual subjects.

361.12

INTER-SUBJECT VARIABILITY OF CEREBRAL ACTIVATION IN MOTOR LEARNING. G. Schlaug*, U. Knorr, R. J. Seitz. Department of Neurology, Heinrich-Heine-University, 4000 Düsseldorf 1, FRGermany.

Learning of a right hand finger movement sequence in healthy, right-handed volunteers has recently been shown on spatially standardized and averaged positron emission tomography (PET) images to be associated with a significant increase of the mean regional cerebral blood flow (rCBF) in left primary sensorimotor areas, premotor cortical areas, right cerebellum and with a significant augmentation of the mean rCBF in the left basal ganglia during learning (Seitz and Roland, Eur J Neurosci 4: 154-165, 1992). We applied now a new algorithm for the analysis of the individual task-specific cerebral activation patterns in each of the nine volunteers. As validated in a phantom study, task-specific activation areas are identified by exponential ranking of all activity changes in PET subtraction images according to size and peak value.

The individual analysis on integrated PET and high-resolution magnetic resonance images revealed a consistent task-specific activation in all nine subjects only in the contralateral sensorimotor cortex after learning. There was a positive correlation (r = 0.879) between the size of the activation area in the left motor and premotor cortex and the mean duration of the electromyographic bursts recorded from the right forearm muscles. Further task-specific activations in lateral and medial premotor, parietal, prefrontal, and cingular cortical areas, and subcortical structures of both cerebral hemispheres were present in all subjects but varied in distribution from case to case. Thus, apart from the motor cortical output area with small anatomical and functional inter-subject variability, single case analysis revealed a profound heterogeneity of the cerebral activation sites between the individual subjects which was obscured in the average PET images.

361.13

SINGLE CASE ANALYSIS OF CEREBRAL ACTIVATION IN POSITRON EMISSION TOMOGRAPHY (PET). R. J. Seitz[1]*, U. Knorr[1], G. Schlaug[1], Y. Huang[1], H. Steinmetz[1], H. Herzog[2], B. Nebeling[3]. [1]Department of Neurology, Heinrich-Heine-University, 4000 Düsseldorf 1, Institutes of [2]Medicine and [3]Radiochemistry, Research Center Jülich, FRGermany.

Areas of cerebral activation present as regions of interest (ROIs) in subtraction images calculated from PET images of a specific stimulation task and an appropriate control condition. We developed a new algorithm to identify task-specific signals in individual subjects. Our algorithm employs an exponential ranking of ROIs automatically drawn at a 30% isocontour of the maximal pixel value in subtraction images. The algorithm was validated by a cylindrical phantom with low activity in the background compartment and high activity in six spheres of different size. Noise was induced by subtraction of sequential PET-scans of the phantom utilizing the inhomogeneity of the PET images reconstructed with the back projection technique. Spheres with a diameter larger than 25 mm were identified as specific signals (threshold rank = 0.98) in subtraction images with a 100% noise using the SCX PC4096 PET-camera and a reconstruction filter of 6 mm (FWHM).

We applied this method on measurements of the regional cerebral blood flow (rCBF) with ^{15}O-butanol in five healthy subjects during execution of simple motor tasks in comparison to rest. After spatial alignment and integrated display of PET and high-resolution magnetic resonance (MR) images, the task-specific rCBF increases were identified accurately in the contralateral sensorimotor cortex and in additional cerebral motor structures of both hemispheres. Our results indicate that single case analysis of PET activation studies identifies the common essential areas of activity changes and other task-specific activation sites with individual distributions.

361.14

MAPPING INDIVIDUAL BRAIN FUNCTION TO INDIVIDUAL BRAIN ANATOMY. H. Steinmetz*, Y. Huang, U. Knorr, R.J. Seitz. Department of Neurology, Heinrich-Heine-University, W-4000 Düsseldorf 1, FRGermany.

Individual variability of brain anatomy and brain function are important factors in studies on structural-functional relationships. Using positron emission tomography (PET), these factors are underestimated systematically when image analysis is based on data averaged across subjects and mapped to 'standard' anatomy.

In a first step towards individual image analysis we have developed a head surface matching algorithm which allows the retrospective integration of PET and morphometric magnetic resonance (MR) datasets. Validation studies using an anthropomorphic phantom demonstrated high 3-D accuracy limited by spatial PET resolution only (XY-,XZ-,YZ-PET/MR misalignment: 2.2 to 2.3mm).

Employing this method in the localization of changes in regional cerebral blood flow (rCBF) induced by manual activity in 5 healthy subjects undergoing PET, the maxima of task-specific rCBF increases were precisely allocated to grey matter in primary sensorimotor and non-primary cortices identified individually by MR. The technique allows the topographical evaluation of individual patterns of functional activation.

361.15

BRAIN DEVELOPMENT IN VERVET MONKEYS: A PRELIMINARY PET STUDY. B. Jacobs*, H.T. Chugani, V. Allada, G.C. Harris, S. Chen, M.E. Phelps, D.B. Pollack, and M.J. Raleigh. UCLA School of Medicine, Los Angeles, CA 90024.

This positron emission tomography (PET) study examined age-related changes in local cerebral metabolic rates for glucose (LCMRglc) in 6 vervet monkeys (*Cercopithecus aethiops sabeaus*) ranging in age from 0.13 to 4.38 years. The 2-deoxy-2[^{18}F] fluoro-D-glucose (FDG) technique was performed on the CTI-713 tomograph (CTI/Siemens), which acquires 15 slices at 3.4mm spacing and has a practical in plane resolution of 4.5mm. Monkeys were anesthetized with ketamine (12 mg/kg/hr) and midazolam (0.17 mg/kg/hr) (animal approval #92-075). After FDG injection, arterial blood samples were obtained to determine the arterial input function. A total of 20 brain regions were drawn for each animal; LCMRglc were expressed in μmol/min/100gm.

Metabolic rates across all monkeys were highest in basal ganglia and frontal areas (e.g., anterior cingulate gyrus, sensorimotor and dorsolateral cortices). Lowest LCMRglc were found in the thalamus, cerebellum, brainstem and neocortical white matter. LCMRglc appeared to peak for most structures in the 0.25 year old monkey (Mean LCMRglc = 55.35). This peak in LCMRglc corresponds with the developmental spine density peaks observed in the primary visual cortex of *Macaca nemestrina* (Booth et al., 1979, *J. Comp. Neurol., 186*:473-490) and with synaptic overproduction in the sensorimotor, prefrontal and visual cortices of *Macaca mulatta* (Rakic et al., 1986, *Science, 232*:232-235). There was a steady age-related decline in LCMRglc in all regions after 0.25 years such that metabolic rates were an average of 57% lower in the oldest monkey (Mean LCMRglc = 23.79). Although preliminary, these findings are consistent with the metabolic changes reported in humans (Chugani et al., 1987, *Ann. Neurol., 22*:487-497), cats (Chugani et al., 1991, *J. Cereb. Blood Flow. Metab., 11*:35-47) and *Macaca mulatta* (Kennedy et al., 1982, *Ann. Neurol., 12*:333-340). (Supported by DOE #DE-FC03-87-ER-60615.)

361.16

DIFFERENCES IN THE DISTRIBUTION OF BRAIN ACTIVITY DURING HEARING AND IMAGINING AN AUDITORY EVENT AS MEASURED WITH [^{15}O]WATER AND POSITRON EMISSION TOMOGRAPHY (PET). H. Szechtman, J.M. Cleghorn*, S. List, C. Whelton, R. Kaplan, S. Ballagh, S. Franco, B. Szechtman, K. Bowers[1], E. Woody[1], G. Firnau, C. Nahmias and E.S. Garnett. Depts of Biomedical Sciences, Psychiatry, and Nuclear Medicine, McMaster Univ, Hamilton, Ontario, L8N 3Z5, and [1]Dept of Psychology, Univ of Waterloo, Waterloo, Ontario, CANADA.

The present study asks whether, in terms of the underlying neural mechanisms, imagining an event is equivalent to the experience of perceiving it. Cerebral blood flow (CBF) was measured in 8 normal volunteers under 3 conditions: at rest, listening to a taped message, and imagining hearing the taped message, followed by a repetition of the 3 conditions in reversed order. The subjects' eyes were covered throughout. Statistical analysis using the method of Friston et al (JCBFM, 11:690, 1990) revealed the expected activation of auditory cortex during hearing. During imagining, however, there was no activation of the auditory cortex but reduced CBF in the posterior temporal cortex and increased regional CBF in the visual cortex (compared to baseline), implicating different brain regions in the generation of auditory imagery. (Supported by OMHF. HS is a Research Associate of OMHF).

361.17

CORTICO-STRIATO-THALAMIC CIRCUITS AND BRAIN GLUCOSE METABOLIC ACTIVITY IN 70 UNMEDICATED MALE SCHIZOPHRENIC PATIENTS. B.V. Siegel, Jr.*, M.S. Buchsbaum, J.C. Wu, S.G. Potkin and W.E. Bunney, Jr. Dept. of Psychiatry, University of California, Irvine, CA 92717.

The cortico-striato-thalamic circuit modulates sensory processing and thus may be involved in the sensory dysfunction in schizophrenia. 18-fluoro-2-deoxyglucose positron emission tomography was used to measure glucose metabolic activity in the structures making up this circuit in seventy male schizophrenic patients after a period of at least four weeks off of neuroleptic medication and in thirty age-matched male normal controls. Analyses revealed decreased metabolism in medial frontal cortex, cingulate gyrus, medial temporal lobe, corpus callosum, and ventral caudate. Medial frontal cortical and thalamic activity correlated negatively with total Brief Psychiatric Rating Scale score and with positive and negative symptom scores assessed on the scan day, while lateral frontal cortical activity did not correlate with symptoms or show group effects.

Significant findings in medial frontal and temporal lobes, thalamus, and striatum are consistent with cortico-striato-thalamic circuit dysfunction in schizophrenia. These findings are also more suggestive of medial frontal than of dorsolateral prefrontal cortical dysfunction in schizophrenia.

361.18

SPECT IMAGING IN SCHIZOPHRENIC PATIENTS WITH SUBSTANCE ABUSE K. Bell* and E. DeMet. Long Beach VA Medical Center, Long Beach, CA 90822 and University of California, Irvine, Irvine, CA, 92717.

SPECT is a potential diagnostic tool in psychiatry. Enduring psychosis after drug abuse may represent unmasking of schizophrenia or a toxic effect of abused drugs on the brain. Sixteen patients were divided into 3 groups: 5 chronic schizophrenics with and 6 without history of polysubstance abuse antedating their illness; 5 substance abusers without psychosis at the time of scan who presented to the hospital with psychotic symptoms; and 6 controls. SPECT images were taken in the resting condition two weeks after hospitalization. The non-psychotic patient group had symptom-free intervals off medication and psychotic episodes after drug use. Patients were male, right-handed and of similar age ($p=NS$) to controls. All patients except 2 were on medication; Haldol(4), Phenothiazine(8), Moban(1), and Clozaril(1). Pilot data analysis restricted the number of regions of interest to left superior frontal, right parietal, left lateral temporal, and right superior frontal. MANOVA revealed a significant group ($N=4$) by region ($N=4$) interaction ($F=2.01$; $df=12,40$; $p<0.05$), due mostly to the left superior frontal ($F=4.45$; $df=3,18$; $p<0.02$) and right superior frontal ($F=2.7$; $df=3,18$; $p<0.08$) regions. BPRS scores showed a trend toward negative correlation with the flow to the left superior frontal ($rho=-.5$, $p<0.07$) and the left lateral temporal lobe ($rho=-.4$, $p<0.07$). SPECT imaging may distinguish a subgroup of schizophrenia.

361.19

NEURAL NETWORKS IN PATTERN RECOGNITION ANALYSIS OF FUNCTIONAL BRAIN IMAGES. J. D. Hidary*, J. Hsiao, R. Litman, W. Hong, D. Pickar. Experimental Therapeutics Branch, NIMH, NIH, Bethesda, MD 20892.

In this study we examine the utility of neural networks in the identification of functional patterns in dynamic PET and MRI data. We utilize both O15 and F18-deoxyglucose data of schizophrenic and normal subjects. In this approach, we first match the PET scans to their structural MRI. We then place each fused set into a canonical space so that group comparisons can be made without region of interest analysis. Next, we feed the scans into various types of neural networks. Once each network is trained, a number of scans are fed in to test it. Finally, we extract the hidden layer information from the network to determine precisely which patterns serve to discriminate pathological and non-pathological states.

LEARNING AND MEMORY: PHARMACOLOGY—OTHER I

362.1

GLUCOSE ADMINISTRATION ATTENUATES COLD-INDUCED IMPAIRMENT OF DELAYED MATCHING-TO-SAMPLE PERFORMANCE IN RATS. S.T. Ahlers*, D. Shurtleff, J.R. Thomas, and F. Paul-Emile. Naval Medical Res. Inst., Bethesda, MD 20889-5055.

Administration of glucose has been shown to enhance performance in a variety of test situations in which memory is impaired by some amnestic treatment. In the present study, the effects of glucose were examined on a working memory deficit produced by exposing rats to cold air stress using a delayed matching-to-sample (DMTS) task. In the DMTS task rats were required to respond on one of two levers cued by an illuminated light above the lever on the front wall of an operant chamber. Following a variable delay ranging from 1-16 sec, both lights were illuminated and rats were required to correctly respond on the lever previously cued for a food reward. Rats responded on the back wall lever during the delay interval to prevent position bias. Glucose (25-500 mg/kg) or saline, administered (ip) in a mixed sequence, were given one hour before a 75 minute session in which rats performed the DMTS task (180 trials). During test sessions the ambient air temperature was either 23°C or 2°C which varied unsystematically. Administration of glucose during exposure to 23°C did not effect matching accuracy across the delays. Exposure to 2°C produced a parallel downward shift in the delay gradient in that matching accuracy was impaired at all delay intervals. Glucose dose-dependently improved cold-induced impairment of matching accuracy at the short delays but was ineffective at the longer delay intervals. Selective modulation of cold-induced impairment of working memory at the short delays suggests that glucose preferentially enhanced stimulus acquisition.

362.2

THE CALCIUM CHANNEL BLOCKER AMLODIPINE ENHANCES MEMORY CONSOLIDATION IN MICE. A. Hawxhurst, D. Quartermain* and J. Puente. Lab. of Behavioral Neurology, New York Univ. Med. Ctr., New York, NY 10016 and Pfizer Inc., New York, NY.

Amlodipine (Am) is a calcium antagonist of the 1,4-dihydropyridine class, structurally related to nifedipine. The effects of this drug on memory were studied using passive avoidance and conditioned suppression paradigms.

In a single trial passive avoidance task, mice were injected s.c. with Am (0,3,5,7 and 9 mg/kg) pre-training (1 hr), post-training (immediate, 1,3 or 6 hr) or pre-testing (30 min, 1 hr or 3 hr). Memory enhancement (significant increase in step-through latency) occurred only when Am (5,7 and 9 mg/kg) was given immediate post-training.

In a conditioned emotional response lick suppression task, Am (7 mg/kg) given immediately after CS-UCS (tone-shock) training significantly enhanced conditioned suppression.

These findings suggest that Am may facilitate memory consolidation.

362.3

ENHANCED ACQUISITION OF REVERSAL TRAINING IN A SPATIAL LEARNING TASK IN RATS TREATED WITH CHRONIC NIMODIPINE. K. McMonagle-Strucko* and R.J. Fanelli. Inst. for Preclin. Pharmacol., Miles Inc., West Haven, CT 06516.

Nimodipine, a dihydropyridine calcium channel antagonist, has been shown to facilitate learning performance. The effects of chronic nimodipine, using 40 mg controlled timed-release pellets, on the performance of young, middle-aged and aged rats were studied in the Morris water maze. Animals were trained 3 sessions per day with a 60 sec ITI to locate a submerged platform. The latency to escape was recorded. Every 6th trial was a probe trial, during which the platform was removed and rats were placed in the maze for 30 sec. Time spent in each quadrant was recorded. After 6 days of initial training, the goal was moved to the opposite quadrant for an additional 6 days. Plasma levels of nimodipine measured following training were approximately 13 ng/ml.

During initial training, there were clear age-related acquisition deficits in place training, with no effects of chronic nimodipine. Nimodipine did, however, enhance performance during the probe trials of reversal training. Time spent in the goal quadrant by the nimodipine-treated rats was approximately 35% longer than that of the placebo-treated rats. The selective enhancement of performance by nimodipine suggests that nimodipine improves memory required to perform a complex discrimination, rather than altering simple reference memory.

362.4

POTENTIAL COGNITION-ENHANCING ACTIVITY OF THE ADENOSINE A_1 RECEPTOR ANTAGONIST MDL 102,234 IN SPATIAL LEARNING AND HIPPOCAMPAL LONG-TERM POTENTIATION MODELS. J.M. Hitchcock*, S.F. Chaney, J.M. Zwolshen, H.J. Ketteler, N.P. Peet, and S.M. Sorensen, Marion Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati, OH 45215.

Adenosine antagonists acting at the A_1 receptor enhance the release of various neurotransmitters, including acetylcholine, and may have potential for cognition-enhancing effects. MDL 102,234 [(R)-1,3-dipropyl-8-(1-phenylpropyl)xanthine], a potent and selective A_1 antagonist (Dudley et al., Soc. Neurosci. Abstr., 1992), was tested in *in vitro* and *in vivo* models of learning and memory.

Long-term potentiation (LTP) in the hippocampal slice has been suggested as a model for events that might occur at a cellular level during learning and memory processes. MDL 102,234 increased the basal population spike and augmented LTP recorded in hippocampal CA1 neurons. Similar results were found with another A_1 antagonist, KFM-19.

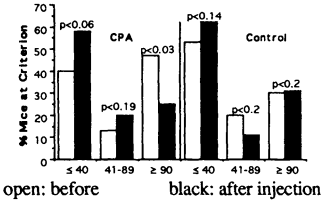
In order to assess central bioavailability, the compounds were tested for their ability to antagonize the reduction in spontaneous motor activity in mice induced by the A_1 agonist R-PIA. A water maze task was used to assess effects on learning. In this spatial learning model, rats are required to use distal spatial cues around a circular water tank to navigate to a hidden platform. The cholinergic antagonist scopolamine dose-dependently impaired acquisition of the water maze task. MDL 102,234 and KFM-19 significantly antagonized the scopolamine-induced learning impairment. These compounds had inverted U-shaped dose-response curves; intermediate doses were most effective, and low or high doses were ineffective, which appears to be a common pattern with potential cognition-enhancing compounds.

The present studies indicate that the adenosine A_1 antagonist MDL 102,234 has potential cognition-enhancing effects both *in vitro* (hippocampal LTP) and *in vivo* (spatial learning). MDL 102,234 thus may have potential as a treatment for cognitive deficits.

362.5

EFFECTS OF ADENOSINE AGONISTS AND ANTAGONISTS ON SPATIAL MEMORY IN MICE. Dag von Lubitz,[1] Jan A. Paul,[1] Raymond T. Bartus,[2] Kenneth A. Jacobson,[1]* [1]NIDDK, NIH, Bethesda MD 20892 and [2]Cortex Pharmaceuticals, Inc., Irvine CA.

The effect of adenosine agonists in spatial memory are poorly known. Therefore, we have examined the effect of treatment with N^6-cyclopentyladenosine (CPA) on spatial memory acquisition in the Morris water maze. Mice (C57/BL6 JAX strain) were trained daily for 120 sec in a 1 m diameter maze with an invisible target (8 cm dia.). After 10 trials, training was interrupted, and one group (N = 15) received i.p. injections of CPA (20 µg/kg, daily) for 7 days. At this dose there was no motor impairment, as tested on the roto-rod 30 min after dosing. Testing was resumed one day after the last injection and continued for the next 4 days. Based on prior studies with this apparatus and paradigm, the animals were divided into three categories: 1) good: those reaching target between 0 and 40 sec; 2) moderate: 41 to 89 sec; and 3) poor: 90 and above. Injections of CPA caused a significant ($p < 0.03$, Fisher's Exact Test) reduction in the number of poor performers and increase in good performers ($p < 0.06$). No changes were observed in the control group given vehicle (N = 15). The results indicate that prior treatment with an adenosine agonist may improve performance in a spatial memory test.



open: before black: after injection

362.6

EFFECTS OF REPEATED CAFFEINE TREATMENT ON HYPERLOCOMOTION AND CONDITIONED PLACE PREFERENCE. Ernest N. Damianopoulos* and Robert J. Carey. Res. and Devel. Serv. -151, VA Medical Center, Syracuse, NY 13210

Rats were administered 7 sessions of caffeine treatment either 10 or 50 mg/kg ip depending on group assignment once every four days interspersed with non drug trials. For one half of each dosage level, the animals were injected 20 min prior to test environment placement (paired group) while for the other half, injection occurred 30 min after removal from the test environment (unpaired group). Test environment placement during the drug and non drug state occurred in one of two flat black, clothlined chambers of equal area which were alike in every respect except for circular vs. square shape differentiation. The two chambers were connected by a pasageway which remained closed except during tests for conditioned place preference (CPP). Locomotor behavior was monitored by two separate computer-assisted camera tracking systems All animals on separate days were initially given two 20 min habituation trials on each chamber followed by a test for CPP. CPP was measured as percent of time spent in each chamber relative to the total time spent in the two chambers. This CPP baseline assessment phase was followed by 7 sessions of caffeine treatment. After a 7-day drug withdrawal period, all animals were given a single non drug test to evaluate CPP. Following 3 additional reacquisition trials with caffeine and after a 7-day drug withdrawal, all animals were given two 20 min trials on each test chamber on separate days in a counterbalanced sequence. After another caffeine reacquisitiion trial, the animals were tested once with 10 mg/kg cafeeine and once with 50 mg/kg caffeine on separate days in a counterbalanced sequence to assess sensitization/tolerance effects. The results showed a selective hyperlocomotion effect in the paired group and a complex interaction of drug dose and CPP.

362.7

EFFECTS OF GINKGO BILOBA EXTRACT EGb 761 ON LEARNING IN TWO INBRED STRAINS OF MICE. B. Martin†, G. Chapouthier†, F. Clostre‡ and Y. Christen‡*. † Génétique, Neurogénétique et Comportement, UFR Biomed., 45 rue Sts-Pères, 75006 Paris - FRANCE. ‡ Institut IPSEN, 30 rue Cambronne, 75015 Paris - FRANCE.

Ginkgo biloba extract EGb 761 (Tanakan®, Institut IPSEN) is known to have various different actions including learning/enhancing effects in mice. We have tested female mice of two different inbred strains (C57BL/6J and DBA/2J) at two ages (15±5 and 65±3 weeks) to investigate whether age or strain.could alter the effect of EGb 761. C57BL/6J is a strain known to retain good learning ability in old age whereas DBA/2J mice show learning-deficiencies in old age. The learning task was a discrimination between a lit and a dark alley in a T-maze with negative reinforcement (50 µA-2 sec footshock) in the lit side. Mice were submitted to 10 trials per day during 6 successive days, the 3 first days after *i.p.* administration of 40 mg/kg EGb 761 (treated) or saline (controls). Results show that in C57BL/6J mice, EGb 761 is devoid of any major effect. On the contrary, in DBA/2J mice, EGb 761 enhances learning, especially in learning-deficient older subjects.

362.8

EFFECTS OF INDELOXAZINE HYDROCHLORIDE, A CEREBRAL ACTIVATOR ON PASSIVE AVOIDANCE RESPONSE IN GERBILS SUBJECTED TO REPEATED CEREBRAL ISCHEMIA BY CAROTID ARTERY OCCLUSION. M. Yamamoto*, K. Takahashi, H. Takeuchi, S. Yatsugi. Applied Pharmacology & Development and Central Research Laboratories, Yamanouchi Pharmaceutical Co., Itabashi-ku, Tokyo 174, Japan.

Effects of indeloxazine hydrochloride, a cerebral activator on disturbance of passive avoidance learning in gerbils subjected to repeated brief episodes of cerebral ischemia were examined. Latency of step-through in passive avoidance learning was shortened for the entire 4-day period when gerbils were trained at 3 days after 3 episodes of carotid artery occlusion for 2 min each at 60-min intervals. Reacquisition was also impaired for 9 weeks when gerbils were retrained on day 14 after the repeated occlusion. Neuronal degeneration in the CA1 regions of the hippocampus was observed 4 and 17 days after occlusion. A close correlation was seen between latency and neuronal degeneration. Administration of indeloxazine was started from day 3 after carotid artery occlusion and repeated once a day for 12 days. Indeloxazine (10 mg/kg po) prolonged the shortened latency of step through without affecting spontaneous motor activity .and pain response. These results suggest that this cerebral ischemic gerbils model is useful for quantitatively measuring functional changes in the chronic phase of repeated cerebral ischemia and that indeloxazine can be used for treatment of stroke patients in the chronic phase.

362.9

THE COGNITION-ENHANCING AGENT, DM-9384, FACILITATES RATE AND LEVEL OF ACQUISITION OF CLASSICAL CONDITIONING IN OLDER RABBITS. D. S. Woodruff-Pak* & Y.-T. Li. Deptartment of Psychology, Temple University, Philadelphia, PA 19122.

The effects of the pyrrolidone derivative, DM-9384, were examined at three levels (1, 3, 10 mg/kg sc) in 40 older (21-40 mo.) New Zealand white rabbits classically conditioned for 90 trials/day for 10 days in the delay nictitating membrane (NM)/eyeblink conditioning paradigm with a 750 msec interval between the tone conditioned stimulus and corneal airpuff unconditioned stimulus. Older rabbits administered 10 mg/kg of DM-9384 took a mean of 463 trials to learning criterion, whereas the vehicle group attained criterion in 746 trials. Rabbits administered 1 and 3 mg/kg of DM-9384 reached criterion in 654 and 569 trials, respectively. These differences in learning rate were statistically significant as were the differences observed in percentage of conditioned responses (CRs), CR amplitude, and response latency. The motor unconditioned response was not affected by the compound. Rabbits tested at 10 mg/kg attained the highest learning rates we have ever observed for older animals (80% CRs). This rate is comparable to optimal performance in young rabbits. It was concluded that DM-9384 facilitates learning in NM/eyeblink classical conditioning, a behavior on which human patients with Alzheimer's disease are seriously impaired.

362.10

LINOPIRDINE (DuP996) FACILITATES THE RETENTION OF AVOIDANCE TRAINING AND IMPROVES PERFORMANCE OF SEPTAL-LESIONED RATS IN THE WATER MAZE. J.D. Brioni *, P. Curzon, M.J. Buckley, S.P. Arneric and M.W. Decker. Neuroscience Department, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064.

Linopirdine (DuP996) is a novel compound presently being evaluated in Phase III clinical trials for the treatment of Alzheimer's disease (Cook et al., Drug Dev. Res. 19:301 , 1990). Linopirdine releases ACh, DA and 5-HT from brain slices and at the behavioral level it facilitates the performance of rodents in memory tests. We investigated the behavioral effects of linopirdine on locomotor activity and on the inhibitory avoidance task in mice. Furthermore, we evaluated the effects of linopirdine on acquisition of spatial discrimination in the Morris water maze in septal-lesioned rats, a model of the cholinergic hypofunction characteristic of AD.

Linopirdine significantly enhanced retention of the inhibitory avoidance response in mice (0.026 μmol/kg, IP, n=12, $p<0.05$) and produced a small decrease in locomotor activity in an open field at a slighter higher dose. Septal lesions significantly impaired the performance of rats in the spatial discrimination version of the Morris water maze (errors: control=1.1 vs. lesioned=3.7), and linopirdine facilitated the performance of lesioned animals at 0.026 μmol/kg, IP (errors=1.5). At this dose, no effects were observed on septal-induced hyperactivity in an open field or in normals rats tested on the elevated plus-maze anxiolytic test. Our studies extend previous findings of facilitatory effects of linopirdine on memory by demonstrating improved spatial learning on septal-lesioned rats. Our results further demonstrate that this effect is not accompanied by a reduction of the hyperactivity characteristic of septal lesions suggesting a dissociation between the effects of linopirdine on the cognitive and non-cognitive effects of septal lesions. (Linopirdine was generously supplied by DuPont-Merck).

362.11

AIT-034 EFFECTS ON SOMATOSTATIN BINDING AND WORKING MEMORY, A. Glasky*, F. DeLeon-Jones, and R.F. Ritzmann. Advanced ImmunoTherapeutics, Irvine, CA, and Olive View Medical Center, Sylmar, CA

AIT-034, a derivative of the purine hypoxanthine, has been evaluated for its effect on working memory and on somatostatin binding to brain receptors. The neuropeptide somatostatin has been implicated in Alzheimer's disease (ALZ) since its depletion in the brain of ALZ patients is correlated with the degree of dementia. Somatostatin has been shown to stimulate the release of acetylcholine in the hippocampus, the area of the brain involved in working memory. It is of interest to develop a somatostatin-related therapy for ALZ. AIT-034 was evaluated in vitro as a modulator of somatostatin binding to brain membrane receptors. AIT-034 was demonstrated to be a potent non-peptide ligand for the somatostatin receptor, producing a 68% displacement at 10^{-7}M and 82% at 10^{-5}M. AIT-034 was evaluated in mice in the win-shift paradigm, a test for working memory employing the T-maze. In normal adult C57/BL mice, AIT-034 (30 mg/kg, i.p.) increased the duration of memory trace to over 210 seconds when comparable to 120 seconds in control subjects. AIT-034 increased the latency time to leave the start box in the T-maze and prolonged running time but had no effect on spontaneous locomotor activity. AIT-034 also increased the duration of the memory trace in Swiss Webster mice at 0.5 mg/kg. In summary, AIT-034 is one of the first small molecular non-peptide entities that inhibits somatostatin receptor binding in vitro and produces a working memory enhancing activity which is comparable to tacrine in two strains of mice.

Work supported by the National Institute of Mental Health.

362.12

REVERSAL BY SUBSTANCE P OF DEFICITS IN SPATIAL MAZE PERFORMANCE IN RATS WITH BASAL FOREBRAIN LESION. J.D. Stoehr, C.P. Cramer* and W.G. North. Depts of Physiology and Psychology*, Dartmouth Medical School and Dartmouth College*, Hanover, NH 03756, USA

Excitotoxic lesions of the nucleus basalis magnocellularis (NBM) and medial septum (MS) result in respective reductions in cortical and hippocampal cholinergic activity. Such animals have accompanying behavioral deficits in tasks designed to evaluate learning and memory. However, few studies have employed pharmacological manipulations aimed at reversing these behavioral deficiencies. Bilateral lesions of both NBM and MS in male Long-Evans rats were induced by 1 μl injections of 6μg/μl of ibotenic acid into each brain area. One week following surgery, rats were trained in the Morris Water Maze (place-learning task) for 5 consecutive days followed by paired reversal trials (goal-relocation task) and probe tests (goal-removal task). Lesioned animals showed significant performance deficits throughout place learning and in the first and second paired reversal and probe trials. For the last set of paired trials, lesioned animals received 50 μg/kg I.P. of substance P 30 min prior to testing. Lesioned rats subsequently showed no significant differences from controls in swim latencies or in probe trial performance. These preliminary studies support the notion that forebrain cholinergic functioning plays a role in spatial learning and that the peripheral administration of substance P may reverse behavioral deficits associated with hypocholinergic activity.

362.13

EFFECTS OF CONTINUOUS INFUSION OF TRH ON LEARNING AND MEMORY IMPAIRMENTS IN ANIMAL MODELS. M. Miyamoto*, Y. Kiyota, K. Hirai, H. Takahashi and A. Nagaoka. Biol. Res. Lab., Takeda Chem. Ind. Ltd., Osaka 532, Japan.

Effects of continuous infusion of TRH on learning and memory impairments in animal models were studied. Behavioral tasks were conducted between 3 to 12 days after subcutaneous administration of sustained-release dosage form of TRH. TRH (0.04 and 0.2 mg/kg/day) markedly ameliorated scopolamine-induced amnesia in mice. TRH improved the impairment of water maze learning in rats with AF64A-induced lesions. The effects of TRH on emotional disturbance and learning impairment in senescence-accelerated mouse (SAM) were also studied. In the elevated plus-maze test, SAM-P/8 mice showed low anxiety-like behavior as demonstrated by an increase in the number of entries into open arms and increase in time spent on open arms as compared with SAM-R/1 control mice. TRH (0.05 and 0.2 mg/kg/day) reversed the low anxiety-like behavior in SAM-P/8 mice. Continuous infusion of TRH ameliorated the impairment of the water maze task and shuttle avoidance task in SAM-P/8 mice, although a single administration of TRH had no significant effect. Furthermore, TRH (0.05 and 0.2 mg/kg/day) improved learning and memory impairments in aged rats, which were assessed by the step-down type passive avoidance and water maze tasks. These findings indicate that continuous infusion of TRH improves memory deficits in animal models at low doses, imply that TRH may be useful for therapy of dementia including Alzheimer's disease.

362.14

BEHAVIORAL EFFECTS OF A TRH ANALOGUE, RGH 2202, ON MOTOR AND COGNITIVE ALTERATIONS OF THE RAT F. Drago*, M. Grassi, A.A. Genazzani, G. Coppi** Institute of Pharmacology, University of Catania Medical School, and Poli Pharm. Company, Rozzano (Milan)**, Italy

TRH is known to improve cognitive and motor deficit in a number of behavioral models and to stimulate various endocrine systems. This study was undertaken to evaluate the effects of a TRH analogue that has no influence on TSH, L-6-ketopiperidine-2-carbonyl-leucyl-L-prolinamide (RGH 2202). This peptide restored learning and memory capacity altered in 24 month-old male rats or reduced by hypoxia, injection of scopolamine or prenatal exposure to methylazoxymethanol, both in active and passive avoidance tests. These effects were statistically significant when the drug was administered at the doses of 5 and 10 mg/kg. The ability to interact positively with cognitive processes was also shown in a radial maze, demonstrating that spatial memory is modulated as well. The TRH analogue was also able to augment motor performance of aged rats in the open field test and of adult rats in the rotorod test. These results suggest that RGH 2202 may be applied in the treatment of those diseases that result in motor and cognitive alterations.

362.15

INTRA-AMYGDALA POST-TRAINING INJECTIONS OF OXYTOCIN ENHANCE RETENTION OF AN INHIBITORY AVOIDANCE TASK

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There is evidence that the neurohypophyseal peptides vasopressin (VP) and oxytocin (OT) are involved in learning and memory. In general, when given either systemically or in the brain, post-training administration of VP has memory enhancing effects while OT has memory impairing effects. When OT is administered directly into limbic structures involved in memory, results are mixed. OT administered into the hippocampus or dorsal raphe nucleus following an aversive step-through task impairs memory. Yet, given in the dorsal septal nuclei, memory in this task is enhanced. Several lines of evidence implicate the amygdala as a primary candidate for the integration and modulation of memory. Although the central and basolateral nuclei of the amygdala are rich in OT receptors, OT has been reported to have no effect on memory when administered in the amygdala post-training at a dose of 25 pg. However, this dose has been reported to be effective in modulating memory in other brain regions. In this study, Sprague-Dawley male rats (250-300g) were implanted bilaterally with cannulae aimed at the central nucleus of the amygdala. Animals were trained in a step-through inhibitory avoidance task. After training, animals received intra-amygdala injections of saline or OT dissolved in saline, at doses of 1, 3, 10, 30, 100, or 300 pg. Retention was tested 48-h after training. The retention latencies of animals given 10 pg were significantly longer than those of all other groups. These results suggest that the amygdala is a site of memory modulation by OT.

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362.16

REVERSIBLE POSTTRAINING INACTIVATION OF THE NUCLEUS OF THE SOLITARY TRACT IMPAIRS RETENTION PERFORMANCE IN AN INHIBITORY AVOIDANCE TASK.

C. L. Williams* and J. L. McGaugh, Center for Neurobio. of Learning & Memory and Dept. of Psychobio., U. of Calif., Irvine, 92717.

Although it is well documented that peripheral administration of hormones or pharmacological agents which affect their release facilitate memory processes, it is not known how these substances influence the brain since they do not freely pass the blood-brain barrier. Visceral sensory information from peripheral glands and organs is carried by afferent vagal fibers to specific nuclei within the nucleus of the solitary tract (NTS). These neurons in turn, transmit information from the periphery to brain structures such as the amygdala which is involved in regulating memory formation. In view of the anatomical relationship between these two structures, it is possible that the NTS may mediate the effects of peripheral physiological influences on memory. To examine this hypothesis, male Sprague Dawley rats (175-200 g) were trained in a one-trial inhibitory avoidance task (0.35 mA footshock for 0.5 sec) and given immediate or delayed (2 hours) microinjections of buffer or 2% lidocaine into either the NTS, 4th ventricle, or the cerebellum. The effects of posttraining inactivation of these structures on retention of the avoidance training was assessed 48 hours later.

The retention latencies of rats receiving lidocaine into the NTS was significantly lower than those of animals given injections of a buffer solution, delayed injections of buffer or lidocaine, or injection of lidocaine into either the 4th ventricle, which is medial to the NTS, or into a region of the cerebellum just dorsal to this nucleus. These findings suggest that the impaired retention performance observed following lidocaine administration into the NTS is due to effects produced within this nucleus rather than to the spread of drug to adjacent brain regions, and further, indicate that the NTS plays an important role in mediating neural input from the periphery to limbic system structures that encode and store memory traces.

Supported by NSF (BNS-9006175) and UC Presidents Fellowship (CLW) and PHS MH12526 (NIMH and NIDA) and ONR N00014-90-J-1626 (JLM).

NEURAL PLASTICITY III

363.1

THE PROXIMITY OF AXOTOMY DETERMINES THE CHANGES OF DENDRITIC ARBORS OF ADULT RAT RUBROSPINAL NEURONS.

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The effect of axonal injury on adult mammalian CNS neurons remained to be investigated. Studies showed that distally axotomized corticospinal neurons were active electrophysiologically and maintained a pyramidal shape (Tseng and Prince, '89, '90, '91), but the model is inappropriate for examining the effect of distance of lesion since a proximal axotomy (PA) would have involved deafferentation. To explore this, we chose lumbar spinal cord-projecting rubrospinal neurons(*l*-NRs) since an upper cervical(C2-4) and lower thoracic(T8-10) tract lesion would represent PA and distal axotomy (DA) in this model.

Lesions were performed on 4 week old rats. A Golgi-aldehyde protocol (Berbel, '86) was used to study the soma-dendritic morphology of neurons. Retrograde labelling(Fast blue) showed that *l*-NRs were located mainly contralaterally in the ventrolateral caudal nucleus and in a thin ventral zone and lateral horn of the middle nucleus. Thus only neurons from these areas were analyzed. Impregnated neurons were reconstructed through serial sections using a camera lucida drawing tube. Dendritic arbors from such drawings were analyzed with the Sholl's method (Sholl, '55) with concentric circles drawn at 25 μm increment steps.

Like normal, both PA and DA neurons maintained a multipolar shape, but the number of dendritic branches were significantly higher($\alpha<0.05$) in PA($n=34$) than DA($n=27$) starting from 75 to 150 μm from the center of the soma. When compared to normal counterparts($n=45$), DA neurons have less (at 175μm) and PA neurons more dendritic branches(at 100μm, $\alpha<0.025$). The total dendritic length of the DA was shorter than both the normal and PA($\alpha<0.01$), while the latter two were comparable. The results show that, in contrast to the dendritic regression caused by DA, PA signals CNS neurons to increase, or the least maintain, their dendritic complexicity. The results suggest a differential induction of structural remodelling signals depending on the location of axonal lesion, which is in accord with recent findings on the increased expression of mRNAs for tubulin and GAP43 following only PA (Tezlaff et al., '91).(supported by NSC-81-0412-b002-03 from the National Science Council of R.O.C.)

363.2

ACOUSTIC IMPRINTING DOES NOT INFLUENCE SPINE FREQUENCY IN THE VISUAL ECTOSTRIATUM OF DOMESTIC CHICKS.

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In a previous study it was shown that spine frequency in a an area of the chicken forebrain (MNH) decreases substantially after acoustic imprinting (Wallhäußer & Scheich, Dev. Brain Res. 31, 1987). To test the specifity of this effect the morphology of the ectostriatum was investigated in Golgi-Cox impregnated tissue from animals of the MNH-study. The chicks differed in their ages (0-20 d posthatch) and in exposure to an acoustic imprinting stimulus (social, isolated-imprinted, isolated-unimprinted). In accordance with a study of Tömböl et al. (J. Hirnforsch. 29, 1988) two neuron types were found in the ectostriatal core. Both have intermediate to large somata, being mostly oval or triangular for the first and round for the second type. While type I has about 5 thick and spiny dendrites, type II has more, but thin and smooth dendrites. The most remarkable feature of type I neurons are numerous spine-like processes at the axon cone. A quantitative analysis was carried out at intermediate dendritic segments of type I neurons (48 brains, 960 neurons). There is a slight decrease in spine frequency within the first week posthatch. No difference in spine frequency was observed within the ectostriatum no matter whether the chicks were imprinted or not, kept isolated or not. This is in contrast to the significant influence of acoustic imprinting on MNH neurons.

363.3

BRAIN PLASTICITY AFTER CORTICAL LESION IN THE RAT.

M.Bajčetić, A.Milošević and N.Zečević*, Inst.biolog.res. University of Belgrade, Belgrade 11000, Yugoslavia.

Plasticity of dendritic arborization was examined after unilateral lesioning of rat somatosensory cortex at postnatal day 3 (P-3) or P-10. The P-3 lesioned animals were sacrificed at P-17 (group P3/17) or at P-60 (P3/60), while P-10 lesioned animals were sacrificed only at P-60 (P10/60). After Golgi impregnation morphometric measurements of total dendritic length (TDL) of II/III layers pyramidal neurons in ipsi- and contralateral somatosensory cortex were done. In the first group of the early lesioned and early sacrificed animals (P3/17) TDL did not differ between ipsi-and contralateral sides, but both had smaller values than control animals ($p<0.05$). In the group P3/60 neurons from the lesion side had TDL smaller than these from contralateral side ($p<0.05$) or controls ($p<0.01$). In the group P10/60 ipsilateral neurons had smaller TDL than neurons in both contralateral side and controls ($p<0.01$).

It seems that the early effect of the lesion was expressed on both ipsi- and contralateral sides. Two month later contralateral side recovered completely while ipsilateral side lag behind even more in TDL.

363.4

SEX AND ENVIRONMENT AFFECT BEHAVIOURAL RECOVERY AND DENDRITIC GROWTH AFTER NEONATAL FRONTAL LESIONS IN RATS.

B. Kolb*, M.Buday, G. Gorny, and R. Gibb, Dept of Psychology, University of Lethbridge, Lethbridge, Canada, T1K 3M4

Rats given prefrontal lesions at 7 days of age were raised in enriched environments along with littermate controls for 3 mo beginning at weaning. The animals were trained at paw reaching and at the Morris water task. The brains were processed for Golgi-Cox staining and the dendritic arborization and spine density measured in layer II/III pyramidal cells in Par 1 and Oc 1. Behaviourally, there were effects of lesion (operates were impaired), sex (male frontals and controls better than comparable female groups), and environment (enriched better in each case) on behavioural measures. Normal males had more dendritic branches than females and showed a greater enrichment effect than females. Frontal females had more dendritic arbor than female controls but equivalent spine density. In contrast, frontal males did not differ from controls in dendritic arbor but had an increase in spine density. Further, frontal males showed an enrichment effect, whereas females did not. The results show that males and females have different anatomical changes in response to neonatal cortical injury and environmental stimulation. These differences may explain the sex differences in behavioural outcome following neonatal cortical injury.

363.5

PHENOBARBITAL DELAYS RECOVERY FROM CORTEX DAMAGE. T.D. Hernandez* and L.C. Russell. Department of Psychology, University of Colorado, Boulder, CO 80309

The usefulness of anti-convulsant prophylaxis following brain injury has been the focus of much debate. Not only is there concern about how effective certain drugs are at preventing post-traumatic epilepsy, but also how these drugs might affect behavioral recovery from the brain damage itself. Results from the animal literature suggest that certain anti-convulsants, like diazepam, interfere with behavioral recovery, while mild, infrequent seizures (including partially kindled seizures) facilitate behavioral recovery following unilateral cortex damage. In addition, there are provocative data from a clinical study which suggest that the use of anti-convulsants following brain injury may not be beneficial for behavioral recovery, and may, indeed, be detrimental. The present studies were undertaken to investigate the effect of phenobarbital on behavioral recovery following unilateral frontal cortex damage in rats. Forty-eight hours after electrolytic lesion, a 7-day phenobarbital or saline regimen was initiated. Anti-convulsant doses of phenobarbital were administered twice daily. Recovery from somatosensory asymmetry (as measured by the bilateral tactile stimulation test) was significantly delayed in the phenobarbital group: saline treated animals recovered within 2 weeks, while the phenobarbital treated group did not recover until 8 weeks after surgery. Given the dramatic and opposing ways in which phenobarbital and kindled seizures individually affect behavioral recovery, it will be interesting to assess whether anti-convulsant administration interferes with seizure-associated changes in recovery patterns when the two treatments are combined, or if their effects balance each other out.

363.6

THE AMELIORATIVE EFFECT OF REPEATED INJECTIONS OF SCOPOLAMINE HYDROBROMIDE ON RECOVERY FROM BRAIN DAMAGE: AN ANALYSIS OF GENERALITY AND MECHANISM. A.M. Schneider*, E. Thomas, M. Jacobs, P. Oishi, R.J.Meagher, P. Asmann. Depts. of Psychology and Biology, Swarthmore College, Swarthmore, PA. 19081 and Bryn Mawr College, Bryn Mawr, PA. 19010

In an earlier study we found that septal lesions produce neophobia and that repeated injections of scopolamine hydrobromide, prior to surgery, ameliorate the neophobic effects of the lesions. The purpose of the present series of experiments was threefold: (a) to replicate the ameliorative effect using a more conventional test of neophobia, the elevated-plus maze; (b) to extend the generality of the effect to another cholinergic system--the nucleus basalis magnocellularis (NBM)--and to another type of behavioral loss--amnesia as measured by a standard passive-avoidance stepthrough procedure; (c) to characterize the underlying mechanism by correlating the decay of the ameliorative effect with the decay of upregulated muscarinic receptors in the hippocampus and cortex.

The results confirmed the generality of the effect and, indeed, implicated upregulated receptors as the underlying mechanism. The results were as follows: (a) repeated injections of scopolamine ameliorated the behavioral effects of subsequent lesions regardless of the site, septal area or NBM, or the type of behavioral loss, neophobia or amnesia; (b) both the ameliorative effect and the upregulation of muscarinic receptors decreased as the interval between drug and lesion and between drug and assay increased from 12 hrs. to 8 days.

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363.7

EARLY MK-801 TREATMENT PREVENTS THE REINSTATEMENT OF FORELIMB PLACING DEFICITS FOLLOWING UNILATERAL CORTICAL LESIONS IN THE RAT. S. Barbay*, T. D. Schmanke and T.M. Barth. Department of Psychology, Texas Christian University, Ft. Worth, TX 76129.

A previous experiment suggested that administration of MK-801 had both beneficial and detrimental effects on the recovery and maintenance of function following damage to the rat somatic sensorimotor cortex (SMC). For example, a regimen of MK-801 begininng 12-16 hr after the lesion accelerated recovery from somatosensory asymmetry, but had no effect on the rate of recovery from forelimb placing deficits. In contrast, when a single injection of MK-801 was given after recovery, there was a reinstatement of forelimb placing deficits that endured for up to 7 days. The facilitation of somatosensory recovery was linked to MK-801 preventing the degeneration of neurons in the striatum and substantia nigra pars reticulata following the cortical injury. In contrast, a potential anatomical correlate to the reinstatement effect has not yet been explored. The present experiment was designed to investigate whether the neuroprotective effects of early MK-801 treatment can prevent the reinstatement of forelimb placing deficits.

Rats received a regimen of MK-801 (1mg/kg) or saline beginning 16 hr after a unilateral lesion in the SMC. The animals were allowed to recover from somatosensory and forelimb placing deficits and then given two successive injections of MK-801. Rats in the Saline-MK-801 group showed a reinstatement of the contralateral placing deficits following MK-801 treatment. In contrast, treatment with MK-801 during the recovery period prevented the reinstatement effect. These data support the idea that the detrimental effects of MK-801 on the maintenance of function may be blocked by early treatment with the drug. Specifically, the reinstatement effect may be related to the transneuronal degeneration in subcortical structures that typically follows damage to the SMC.

363.8

TACTILE-EXTINCTION FOLLOWING UNILATERAL LESIONS IN THE RAT ANTEROMEDIAL CORTEX: THE EFFECTS OF A CONTRALATERAL CUE. T.M. Barth*, B. Marks and L.S. Young. Department of Psychology, Texas Christian University, Ft. Worth, TX 76129.

A previous experiment showed that restricted unilateral lesions in two subdivisions (i.e. anteromedial cortex [AMC]; caudal forelimb area [CFL]) of the rat somatic sensorimotor cortex (SMC) produce an ipsilateral asymmetry on a bilateral tactile stimulation test. The bias may be similar to the neglect or extinction phenomenon observed in people with right parietal lobe damage. Historically, the nature of extinction has been attributed to inattention, increased sensory thresholds or hypokinesia. The attentional hypothesis has been supported by the finding that a salient cue to the contralateral side may overcome the neglect. The present experiment was designed to investigate whether a contralateral cue presented before the simultaneous presentation of bilateral tactile stimuli, would neutralize the ipsilateral asymmetry observed after unilateral lesions in the AMC or CFL.

Rats received unilateral electrolytic lesions in either the AMC or CFL. One or two days after surgery, the animals were tested for an ipsilateral bias on the bilateral tactile stimulation test. Small adhesive patches were placed on the the radial aspect of each forepaw and the order of contact and removal (i.e. ipsi vs contra) was recorded. Animals in both groups contacted the patch ipsilateral to the stimulus before the contralateral patch (i.e. an ipsilateral bias). The rats were then cued by placing a patch on the contralateral forepaw and allowing them to contact and remove the stimulus. Following a cue-trial time interval (5-30s), the animals were again presented with bilateral patches. In animals with AMC lesions the contralateral cue neutralized the ipsilateral bias, whereas the cue had no effect on animals with CFL lesions. These data suggest that the ipsilateral bias observed in AMC damaged animals may reflect an impairment in attention.

363.9

LONG-LASTING ADAPTATION TO IMPOSED ACTIVITY IN REGENERATED CRAYFISH MOTONEURONS. B.A. Stewart, H.L. Atwood, H.C. Kwan.* Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada M5S 1A8

Can axonal terminal age affect the ability of a neuron to express activity-dependent changes in synaptic transmission? Imposing activity on the phasic motoneuron to the claw closer muscle leads to reduced initial transmitter release and greater fatigue resistance (FR). This long-term adaptation (LTA) is age dependent; it is expressed in young but not old animals. Since LTA is known to depend on somally-regulated mechanisms, we sought to separate the central and peripheral effects of age by examining LTA in animals with regenerated claws where the axonal terminals are younger than the centrally located cell body. We used a conditioning protocol of 5 Hz stimulation for 2 h/day for 3 consecutive days and measured excitatory postsynaptic potentials (EPSP) with standard intracellular techniques. Initial EPSP amplitude was reduced and FR increased in conditioned non-regenerated claws compared to non-conditioned contralateral controls in young, but not in old animals. Conditioned regenerated limbs of old animals exhibit increased initial EPSP amplitude and reduced FR compared to non-conditioned regenerated controls. Conditioning of regenerated limbs of intermediate sized animals led to less FR but no change in initial EPSP amplitude. We conclude that limb regeneration does not restore normal LTA in old animals; such limbs exhibit a novel form of long-lasting plasticity opposite to that observed in non-regenerated limbs.

363.10

ENDURANCE EXERCISE MODULATES NEUROMUSCULAR JUNCTION OF C57BL/NNIA AGING MICE. Mohamed A. Fahim* Faculty of Medicine, UAE University, Al Ain, United Arab Emirates.

Remodelling of neuromuscular junctions (NMJs) appears to be a lifelong process, as indicated by ongoing outgrowth, retraction, constriction and expansion. This morphological turnover of NMJs may be modulated by activity depending on age and muscle fiber type. To further study these phenomena the effect of age and endurance exercise on the structure and function of gluteus maximus NMJs from 10 and 28 month old C57BL/NNia mice were assessed under identical conditions. This muscle has mixed fibers in the mouse and is recruited to maintain posture and for locomotion. Animals were exercised at 28m/min for 60 min/day 5 days/wk for tweleve weeks. Camera lucida drawings made from ZIO-stained NMJs and conventional intracellular recordings for measuring spontaneous and evoked transmitter release of control and exercise gluteus maximus were assessed. Repeated measures multivariate analysis of covarience was used to test for differences in nerve terminal area, perimeter, extent length, fiber diameter, spontaneous and evoked transmitter release between ages and test conditions. There were significant increase in nerve terminal area, perimeter, length and quantal contents in old compared to young mice. Endurance exercise prevented physiological and morphological age - related changes. This suggests that the integrity of mouse NMJ during aging is well maintained through regular physical activity.

363.11

MUSTARD OIL APPLICATION INCREASES PERSISTING HIND LIMB FLEXION (SPINAL FIXATION) IN RATS. M.M. Patterson*, M.J. Bartelt, T.Jackson, & E.S. Johnson, Dept. of Psychology and College of Osteopathic Med., Ohio University, Athens, OH 45701.

Persisting hind limb flexion may be induced by applying approximately 40 mins of 2-4 mA electrical stimulation to spinalized rats' hind limbs, thus inducing a spinal fixation of the reflex alterations. Woofe and Wall *(J. Neurosci, 6*,1433-1442, 1986) reported that increased c-fiber input could increase spinal cord responsiveness for up to 3 hours. The present study examined the effects of mustard oil induced c-fiber activation on the production of spinal fixation.

We surgically anesthetized (50 mg/kg ip) 20 rats and randomly assigned them to mustard oil or saline control groups. We then transected the rats' spinal cords at T7 and placed them in the stimulation apparatus. Twenty min prior to stimulation, we applied mustard oil to the experimental rats' right hind feet and saline to the control rats' right hind feet. Immediately following 30 min of 1.5 mA (100pps) stimulation to the right hind limb, we assessed the spinal fixation by measuring the weight needed to remove the right hind limb flexion.

The mustard oil application significantly ($p<.0005$) increased the fixation, from 5 g in the control group to 19 g in the mustard oil group, thus indicating that c-fiber activation enhances fixation. We suggest that the enhancement of fixation by c-fiber activation is mechanistically similar to the sensory and motor changes which result from tissue injury. Tissue injury activates c-fibers which in turn enhance the spinal processes which store and maintain excitability alterations. These spinal processes may partially modulate pain persistence and could be implicated in chronic pain, phantom limb pain and other chronic syndromes. Supported by American Osteopathic Association grants 90-08-319 and 91-08-319.

363.12

ENVIRONMENTAL STRESSORS INCREASE PERSISTING HIND LEG FLEXION (SPINAL FIXATION) IN RATS. M.J. Bartelt*, E.S. Johnson, T. Jackson, & M.M. Patterson. Dept. of Psychology and College of Osteopathic Med., Ohio University, Athens, OH 45701

We have demonstrated that stimulating a spinalized rat's leg for approximately 40 min induces a flexion which persists for at least 3 days (Steinmetz, Cervenka, Robinson, Romano, & Patterson, *JCPP, 95*, 548-555, 1981). Because the excitability producing this leg flexion fixates in the spinal cord, it has been termed *spinal fixation*. In the present studies, we investigated the development of fixation following exposure to the stressors of swimming and noxious noise.

In the first study, we placed the experimental rats into a water bath and forced them to swim for 8 min. In the second study we exposed the experimental rats to white noise (approx 85 db) for 12 hours. Following exposure to either the control or the stress condition, we surgically anesthetized the animals (Nembutal 50 mg/kg ip), and performed spinal transections. Following the 30 min of right hind leg stimulation (3-4 mA, 100pps), we assessed the fixation by measuring the weight required to remove the right leg flexion.

In both studies the environmental stressors increased the fixation ($p<.0005$). The swimming stress group's (n=9) fixation was 36 g compared to their control group's (n=9) of 28 g, while the noise stress group's (n=16) fixation was 38 g compared to their control group's (n=16) of 32 g.

These findings confirm that generalized organismic stressors enhance the induction of spinal reflex excitability. Exposure to stressors increases responsiveness to pain and may also increase long-term spinal excitability. We suggest that these excitability alterations may be induced by somatic and visceral malfunctions and may in turn contribute to the development of chronic pain and other syndromes such as phantom limb pain. Supported by American Osteopathic Association grants 90-08-319 and 91-08-319.

363.13

TYROSINE HYDROXYLASE POSITIVE CELLS PROJECT FROM DORSAL AND MEDIAL RAPHE TO NUCLEUS ACCUMBENS AND VENTRAL FOREBRAIN SUGGESTING A ROLE FOR BRAINSTEM DOPAMINE IN SPARING ADULT REWARD FUNCTION IN NEONATAL 6-OHDA TREATED RATS. **K.S. Sidhu*, R. Sikes, and J.R.Stellar Departments of Psychology and Physical Therapy, Northeastern University, Boston, MA 02115.**

Three day old pups, pretreated with DMI (25mg/kg, s.c.), were given ICV administration of 6-OHDA (100ug) or it's vehicle. At 90-100 days postnatal, all animals received injections into the Nucleus Accumbens of anatomical retrograde tracers Fluoro-Gold or True Blue or both. After one week, brain sections were taken, processed for Tyrosine Hydroxylase (TH) immunoreactivity and examined with fluorescence microscopy for single and double-labeled cells.

In comparison to controls, neonatal 6-OHDA treated rats showed an expected severe reduction in the number of TH+ cells and double-labeled cells in the ventral tegmental area (VTA). However, an additional population of double labled cells located in the Raphe nuclei, paticularly the Median Raphe (MR), were less effected by 6-OHDA. These results raise the possibility that some of the residual dopamine known to exist in neonatally 6-OHDA treated rats may originate from the Raphe not the VTA.

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363.14

PLASTIC CHANGES IN RAT HYPOTHALAMO-NEUROHYPOPHYSIAL NEURONAL-GLIAL RELATIONS INDUCED BY RESTRAINT STRESS. G.H.Beagley* and G.I. Hatton, Alma College, Alma, MI 48801 and University of California, Riverside, CA 92521-0146.

Nerve terminal contact with the basal lamina (BL) of the posterior pituitary has been shown to increase, with a decrease in glial occupation, in response to stimuli that elevate neuropeptide release (e.g. parturition, lactation and dehydration by water deprivation or systemic injection of hypertonic NaCl). Increased contact with the BL by oxytocin (OX) and vasopressin (VP) terminals is thought to facilitate hormone release. Systemic adrenalin has been shown to play a crucial role in this phenomenon, suggesting stressful stimuli that cause sympathetic adrenalin release should lead to increased neural apposition with the BL. In the present study 100 day old male rats were confined in Plexiglass restrain tubes for 4 - 5 h and sacrificed immediately after release from confinement. Posterior pituitaries of confined rats (n = 8) were compared to those of unrestrained control rats (n = 6). Morphological analysis of electron micrographs revealed confined rats had greater neural apposition with the BL than control rats (51% vs. 35%, $p<.001$). These data suggest that, in rats, a stressful stimulus such as prolonged confinement can cause rapid pituicyte withdrawal from the BL as effectively as parturition or hypertonic saline induced dehydration. Supported in part by NS09140.

363.15

DIFFERENTIAL EXPRESSION OF IMMEDIATE EARLY GENES IN CEREBELLAR SLICES. K. Nakazawa, L. Karachot and T. Yamamori*. Lab. of Neural Networks, Frontier Research Program, RIKEN, Wako, 351-01, Japan

In order to investigate the molecular basis of cerebellar plasticity (specially for long-term depression: LTD), we have examined the induction of immediate early genes (IEGs) in rat cerebellar slices by application of α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA: as a substitute for parallel fiber stimulation) and 8-bromo cyclic guanosine monophosphate (Br-cGMP: substituting for climbing fiber activation). The sensitivity of cerebellar Purkinje cells to AMPA is persistently reduced for several hours when combined with Br-cGMP (M. Ito and L. Karachot, Neuroreport, 1, 129-132, 1991).

IEG induction has been detected with in situ hybridization and immunohistochemistry. In Purkinje cells, AMPA application induced c-*fos* expression, while induction of other IEGs was less prominent. Br-cGMP application alone had little effect. Other types of neuronal and glial cells in the cerebellum also displayed differential expression of IEGs in response to AMPA and/or Br-cGMP application although the patterns of expression differed from those observed in Purkinje cells. This study thus may reveal a dynamic pattern of IEG expression in neuronal circuitry of the cerebellum.

363.16

CHANGES IN PATTERNS OF C-FOS IMMUNOREACTIVE NEURONS IN THE RAT TEL- AND DI-ENCEPHALON FOLLOWING d-AMPHETAMINE TREATEMENT COMBINED WITH MOTOR TRAINING FOLLOWING UNILATERAL LESION OF THE SENSORY-MOTOR CORTEX.

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Clinical studies have demonstrated improvement of motor recovery in stoke patients given combined treatment of d-amphetamine with physical therapy. In order to understand the mechanisms involved in this therapeutic action of ,we studied how acute d-amphetamine treatment (2mg/kg i.p; 2h before sacrifice) alone or combined with motor training (2h), in a previously learned motor task (bar walking), affect the expression of c-fos immunoreactivity (IR) following unilateral mechanical lesions (24h earlier), of the sensory-motor cortex in adult rats In the dose used, d-amphetamine alone produced a wide spread appearence of c-fos IR nerve cell nuclei in the cingulate cortex, the sensory-motor cortex and in the dorsomedial 2/3 of the neostriatum. The lesion alone also produce a wide spread activation of c-fos IR nerve cell nuclei over the entire cortical hemisphere on the lesioned side. Acute treatment with d-amphetamine one day after the lesion, produced the paradoxal disappearance of c-fos IR neurons in the medial and lateral part of the cortex surrounding the lesion. Also d-amphetamine could no longer cause the appereance of c-fos IR neurons in the most dorsomedial-part of the neostriatum. The unique effect of motor training in the lesioned rats was that d-amphetamine could no longer induce the disappearance of c-fos (IR) nerve cell nuclei in the neocortical areas surrounding the lesion, so that the lesion induced increase in c-fos (IR) nerve cell nuclei was maintained. Furthermore, the motor training did not affecte the d-amphetamine induced changes in c-fos (IR) neuronal patterns in the neostriatum. However, the combined treatment led to the appereance of c-fos (IR) nerve cell nuclei within the rostral part of the nucleus accumbens. Such an interaction of the 2 treatments was also found in the non-lesioned rats given d-amphetamine and motor training. Since the proto-oncogenes mediate long term effects in gene expression in neurons, the present results indicate that the combination of motor training with d-amphetamine treatment may increase adaptive changes in the neuronal networks of the nucleus accumbens and neostriatum in combination with a mantainance of c-fos activation in the neocortical areas surrounding the lesion. The present findings suggest a structural correlate to the improvement of motor functions demonstrated in patients after such combined treatments. The role of catecholamines in neuroadaptive changes remains to be elucidated.

363.17

A HSV-1 VECTOR EXPRESSING AN UNREGULATED PROTEIN KINASE C FROM THE TYROSINE HYDROXYLASE PROMOTER CAUSES ROTATIONAL BEHAVIOR FOLLOWING STEREOTAXIC INJECTION INTO THE SUBSTANTIA NIGRA COMPACTA OF UNLESION RATS. Song Song*, Dean Hartley, John Bryan, Donna Ullrey, Oliver Ashe, Karen O'Malley, Rachael Neve, Alfred Geller, and Mathew During. Div. Endocrinol., Children Hosp., Boston MA. USA 02115; Yale U. Sch. Med., New Haven CT. USA 06510; Wash. U. Sch. Med., St. Louis MO. USA 63110; McClean Hosp. Belmont MA. USA.

We devised a genetic intervention strategy to determine if the protein kinase C (PKC) pathway in substantia nigra pars compacta (SNc) neurons controls dopamine release, and therefore rat motor behavior. We exploited our defective HSV-1 vector system (Science, 1988 241,166; TINS 1991, 14, 428) which can deliver genes into neurons. We have established that expression of the PKC catalytic domain, encoding an unregulated enzyme that is always active, causes an activity dependent increase in neurotransmitter release from cultured sympathetic and cortical neurons (Soc Neurosci Abs 1991 17, 603). Based on these results, we designed an approach to selectively increase dopamine release from SNc neurons in the brain: The vector pTHpkc places the rat PKC βII catalytic domain under the control of the rat tyrosine hydroxylase (TH) promoter; pTHpkc virus was unilaterally injected into the SNc of unlesioned adult rats. Our expectation was the TH promoter should limit expression of PKC to dopaminergic neurons of the SNc and the PKC catalytic domain should increase dopamine release from these neurons, perhaps affecting rat motor behavior. In initial experiments, pTHpkc was injected into the SNc of adult male Sprague Dawley rats. Starting two weeks after surgery, the rats were tested for rotational behavior following injection with apomorphine. Ten of 24 rats demonstrated long-term ipsilateral rotational behavior. Control rats injected with pTHlac (expresses *E. coli* ß-galactosidase), or PBS, did not show rotational behavior. One month after injection, rats were sacrificed and PKC expression from pTHpkc was demonstrated by immunohistochemistry; most PKC expression was localized to the SNc by staining adjacent sections for TH. Preliminary microdialysis data show an increase in dopamine levels in the striatum of rats injected with pTHpkc.

363.18

INDUCTION OF THE IMMEDIATE EARLY GENE NGFI-C IN CORTEX AFTER SEIZURES AND PHYSIOLOGICAL STIMULATION S. Chang, S.D. Yi, P. Mack, H.S. Schutta*, and K.J. Mack University of Wisconsin, Madison WI.

NGFI-C is an early response gene which encodes a Cys_2/His_2 zinc finger protein. NGFI-C has been demonstrated by Northern blotting to be inducible in PC12 cells after NGF stimulation, and in whole brain after a pentylenetetrazol induced seizure. This study sought to localize this gene in somatosensory cortex, and investigate its possible induction by seizure and physiological stimuli.

In situ analysis using digoxigenin labelled cDNA probes from non-homologous regions demonstrated NGFI-C containing neurons throughout the somatosensory cortex. Qualitatively, it appeared that staining was darker in post-seizure animals than controls.

To investigate the inducibility of NGFI-C, RT-PCR was performed on mRNA extracts from somatosensory cortex. Increased NGFI-C mRNA levels were seen after a single injection of pentylenetetrazol in a dose dependent manner. Similarly, kindled animals also showed an elevated expression of NGFI-C.

Physiological stimulation of the barrel cortex was also studied. In lightly anesthetized animals, vibrissae were stimulated by an artists paint brush. This resulted in an increased level of NGFI-C expression in the contralateral barrel cortex.

These studies indicate that NGFI-C is a neuronal zinc finger gene that is inducible in response to seizure or physiological stimuli. These findings delineate another pathway of transcriptional activity that may follow neuronal stimulation.

MOTIVATION AND EMOTION II

364.1

IS SCHIZOPHRENIA A RESULT OF EARLY DAMAGE TO THE HIPPOCAMPAL FORMATION? A BEHAVIORAL STUDY IN PRIMATES. M. Beauregard*, L. Malkova and J. Bachevalier. Dept. Neurobiology and Anatomy, Univ. Texas, Houston, TX 77225 and Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

We reevaluated the socioemotional behavior of adult monkeys with neonatal hippocampal lesions and compared it with previously reported analysis of their behavior at 2 and 6 months (Bachevalier et al., Innovations in autism, DeLong & Bauman Eds, 1992). Four animals had received hippocampal lesions (H) neonatally, 4 were normal controls (N/H) reared with monkeys from Group H, and six were normal controls reared together (N/N). At 7 years, each H animals was paired in a play cage with its age-matched control, and their behavior was observed for 2, 5-minutes intervals during 5 consecutive days. Similarly, each N/N was paired with another N/N. Monkeys in the N+H dyads displayed a lack of social interaction and an increase in locomotion and manipulation as compared to monkeys in the N+N dyads. In addition, H monkeys showed more motor stereotypies than either normal controls (N/H or N/N). Since only mild behavioral changes were seen in H monkeys at 2 and 6 months of age, the data indicate that early damage to the hippocampus and parahippocampal gyrus yields behavioral disturbances that become more severe when the animals reach maturity. The developmental time course and the nature of these disturbances closely resemble those seen in schizophrenia.

364.2

EFFECTS OF AMANTADINE ON PLAY AND OTHER SOCIAL EMOTIONAL PROCESSES. J. Panksepp*, W. Klimesch, E. Nelson, and C. Nocjar. Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403.

Brain dopamine (DA) release increases psychomotor activity and social play is characterized by such behavior. To analyze this relationship, we evaluated the effects of amantadine (AMANT), a DA releasing agent, on basic social processes, including rough-and-tumble play in rats and separation distress vocalizations (DVs) in domestic chicks. AMANT produced dose dependent reductions in play as measured by pins as well as overall rough-and-tumble motor activity (reductions of about 25%, 36% and 67% at 10, 20 & 40 mg/kg) lasting for about two hours. Solicitations (as measured by dorsal contacts) were reduced by 30% only at the highest dose. Repeated daily injections of 50mg/kg inhibited play completely with modest tolerance seen by day 4. Established play dominance was not modified when dominant rats received 20 mg/kg AMANT, but submissive rats receiving AMANT seemed more dominant. This was largely due to the dorsal contacts of control animals becoming less effective in producing pins. This pattern was even more evident if drug treatment was initiated at the very beginning of play testing. AMANT inhibition of play was not modified by pretreatment with .25 mg/kg haloperidol, which itself also reduced all aspects of play. The results suggest that vigorous play is sustained only in the presence of phasic DA activity, with both tonic release and blockade of DA being incompatible with rough and tumble ludic activities. Modifications in play and play dominanace appeared to be due to the ability of AMANT to decrease overall social responsivity, and social choice tests indicated normal animals prefered to socialize more with vehicle than AMANT treated animals. The decrease in social-emotional responsivity by AMANT was affirmed by the ability of the drug to reduce separation DVs in domestic chicks (at 50-100 mg/kg), suggesting that DA release in the brain may decrease social motivation and increase social dominance (a non-playful state) in animals.

364.3

TESTOSTERONE EFFECTS ON SOCIAL BEHAVIOR IN CHICKS. L.Normansell*, T. McCollister, C. Hélie, T. Bateson, and K. Gerecke. Department of Psychology, Muskingum College, New Concord, OH 43762.

Testosterone administration has long been known to affect morphological and behavioral development in chicks. The present series of studies investigated its effects on a variety of social behaviors. For a three week period, male and female chicks received daily injections of testosterone propionate (TP; 2.5mg) or oil control.

TP-treated chicks took longer to approach the flock than controls, with females generally having longer latencies than males. TP induced crowing, with males affected more than females. During the period of testing, no control chicks ever crowed. When like-treated mixed-sex groups were placed in a testing arena, TP chicks were more aggressive towards the other flock members, pecking the other birds over twice as often as controls. TP males were more aggressive than TP females but there were no gender differences in the control animals.

Effects of various drugs were assessed in some of these testing conditions. Morphine (1mg/kg) completely eliminated crowing, but had no effect on aggressive pecking. Diazepam (1mg/kg), the prototypical anxiolytic, modestly reduced both pecking and crowing. Scopolamine (1mg/kg), a muscarinic cholinergic antagonist, reduced aggression. Although scopolamine didn't affect the number of crows emitted, it disrupted many of the behaviors associated with crowing including neck and body extension and wing fluffing.

364.4

SOCIAL ENCOUNTERS WITH CONSPECIFICS ELICIT SELECTIVE HIGH-FREQUENCY (35-70 kHz) ULTRASONIC VOCALIZATIONS IN RATS. R.J.Blanchard*, S.M.Weiss, E.B.Yudko, H.K.Taukulis[1]. Bekesy Laboratory of Neurobiology and Dept. of Psychology, Univ. of Hawaii, Honolulu, HI 96822; Dept. of Psychology, [1]Univ. of New Brunswick-Saint John, New Brunswick, Canada E2L 4L5.

Colony-housed rats emit ultrasonic vocalizations in the 35-70 kHz range when one of the residents is reintroduced after a period of absence from the colony. The calls increase in number as a function of the length of the interval between removal and replacement. Singly-housed animals will also emit these calls when confronted with an anesthetized, same-sex conspecific, and females will produce a greater number than males. Reliably more vocalizations will occur when the animals are intruders into, rather than residents in, the test cage. Morphine, gepirone (1.0, 10.0 mg/kg), and diazepam (3.0 mg/kg) were found to attenuate this behavior, while ethanol (0.6, 1.2 g/kg) had no effect. This model presents a simple technique for eliciting high-frequency (35-70 kHz) ultrasounds unaccompanied by calls of the "22 kHz" variety. It may have potential utility as an index of social defense or anxiety.

364.5

PILOCARPINE REDUCES THE NOVEL INDUCED GROOMING BEHAVIOR IN TWO INBRED SUBLINES OF SPRAGUE-DAWLEY RATS. E. Doger*, A. Moyaho, A.M. Cajica and J.R. Eguibar, . Dept. Ciencias Fisiológicas, Instituto de Ciencias. UAP. México..

It is well known that ACTH and related peptides intraventricularly injected induce excessive grooming behavior and stretching-yawning syndrome in rats. These effects are blocked by systemic injection of muscarinic antagonists (atropine and scopolamine). We reported recently two inbred sublines of Sprague-Dawley rats which differ in their spontaneous yawning behavior (Behav., Brain Res. 40:29,1990). These animals also differ in their grooming scores under different experimental conditions. The purpose of these experiments was to explore the effects of pilocarpine, a cholinergic muscarinic agonist, in novelty and water immersion-induced grooming. Novelty-induced grooming was higher in high yawning rats (HY) than in low yawning (LY) animals ($p<0.002$, t test). The different grooming components: face washing (FW), body grooming (BG), genital grooming (GG), paw licking (PL) and scratching (S) showed also significant differences between both sublines (F/df 1,210; 15.09 ANOVA). The systemic administration of pilocarpine produced a dose-dependent inhibition on grooming scores in both sublines. The HY animals were more sensitive, with an ED_{50} of 2.14 mg/Kg than the LY with 3.39. In HY rats pilocarpine affected all the components, except S; on the other hand LY rats only FW was affected ($p< 0.01$ Duncan's test). Preliminary observations also demonstrated a clear reduction in the water immersion-induced grooming by this drug. These data suggest a direct participation of muscarinic receptors in the expression of grooming behavior.

364.6

REPEATED INJECTION OF THE GABA-A ANTAGONIST BMI INTO THE BASOLATERAL AMYGDALA (BLA) "KINDLES" EXPERIMENTAL ANXIETY IN RATS. S.K. Sanders* and A. Shekhar. Program in Medical Neurobiology and Dept. of Psychiatry, Indiana University Sch. of Medicine, Indianapolis, IN 46202.

We have previously reported that GABA blockade in the region of the anterior basolateral amygdala (BLA) elicits increases in heart rate (HR) and blood pressure (BP) as well as experimental anxiety. In addition, repeated injections of BMI into the BLA augments this cardiovascular response. In the present study, we have found that repeated injections of a subthreshold dose of BMI (6pmol/250 nl) into the BLA sensitizes the rats similar to kindling such that this same dose now increases HR and BP as well as experimental anxiety in the conflict and social interaction (S.I.) paradigms. Further, the BLA contains a high density of benzodiazepine (BDZ) receptors and may represent an important site of action for this anxiolytic class of drugs. We have found that the anxiolytic effects of chlorodiazepoxide (2.5 mg/ml, i.p.) may be reversed by injection of 6 pmol BMI into the BLA in the S.I. test and 6 pmol flumazenil in the conflict model of anxiety. These results suggest that the GABA/BDZ receptor complex in the BLA may be important in developing anxiety and mediating the anxiolytic effects of BDZ. (Supported by R 29 MH 45362-02 and the IUPUI Research Investment Fund)

364.7

VALIDATION OF THE DORSOMEDIAL HYPOTHALAMIC (DMH) GABA BLOCKADE MODEL OF "PANIC" WITH ANTI-PANIC DRUG TREATMENTS. A.Shekhar* and J.S. Katner Dept. of Psychiatry, Indiana Univ. Med.Ctr., Indianapolis, IN 46202.

Blockade of GABAA receptors with bicuculline methiodide(BMI) in the DMH of rats elicits a "panic" like response characterized by increases in heart rate(HR), blood pressure(BP), Respiration(RR) and measures of "anxiety" such as the conflict, elevated plus-maze and social interaction(SI) tests. The present study was aimed at testing the validity of this response as a model of panic drugs by chronically treating the animals with anti-panic attacks imipramine (5 & 15 mg/kg), clonazepam (5 mg/kg) and vehicle intra-periotoneally. Rats were implanted with bilateral microinjection cannulae in the DMH and arterial catheters. Rats were then randomized to chronic imipramine (7 days), clonazepam (3 days) and placebo injections. All treatments were double-blind and the HR, BP and SI responses to BMI injection (25 ng/250 Nl) into the DMH were recorded. Imipramine and clonazepam but not vehicle treated rats showed significant decreases in the effects of GABA blockade into the DMH. These results support the validity of GABA blockade in the DMH as a model of panic attacks. (Supported by R29 MH45362).

364.8

AUTONOMIC ORIGINS OF CARDIOVASCULAR RESPONSES TO NONSIGNAL STIMULI IN RATS TREATED WITH FG 7142. K.S. Quigley*, S. Faber, M. Sarter and G.G. Berntson. Dept. Psychology, Ohio State Univ., Columbus, OH 43210.

To assess the autonomic correlates of the putative anxiogenic effects of the benzodiazepine receptor partial inverse agonist FG 7142 (FG), heart rate and blood pressure were recorded in freely moving rats under both baseline and mildly evocative stimulus conditions. Effects on vagal and sympathetic activity were determined by co-administering antagonists of either input. FG only minimally affected baseline heart rate and blood pressure. However, FG significantly increased stimulus-induced acceleration of heart rate responses. This effect appeared to be based on a shift in the autonomic control of the heart. While the stimulus-induced autonomic response appeared to result from a coactivation of sympathetic and vagal innervation of the heart in control animals, the FG-induced augmentation of this response was due to a reciprocal pattern of sympathetic activation and vagal withdrawal. These results suggest that a profound shift in the autonomic control of cardiovascular responsivity represent a component of the putative anxiogenic effects of FG. Additionally, these findings indicate that the assessment of putative cardiovascular effects of inverse agonist-like drugs requires the analysis of autonomic responsivity to behaviorally significant stimuli.

364.9

EFFECTS OF REPEATED RESTRAINT ON THE TIME-DEPENDENT RESPONSES OF MESOLIMBIC DOPAMINE SYSTEM TO STRESS Stefano Puglisi-Allegra[1*], Assunta Imperato[2], and Simona Cabib[3], [1]dept of Psychology Univ. of Rome "La Sapienza", [2]dept of Neurosciences, B.B. Brodie, Univ. of Cagliari, [3]Isnt of Psicobiologia e Psicofarmacologia (C.N.R.), Roma , Italy

During exposure to either restraint or footshock stress a biphasic evolution of 3-Methoxytyramine (3-MT) concentrations (increase followed by decrease) is observable in the nucleus accumbens septi (NAS) of mice. Moreover, in vivo microdialysis data show biphasic changes in DA outflow (increase followed by decrease) in rats exposed to restraint. Taken together, these results support the hypothesis that biphasic alteration of DA transmission in the mesolimbic system is a general response to stress and suggest that the initial increase of DA release represents an arousal response while the subsequent decrease in DA release may be related to coping failure. Repeated exposure to restraint abolishes the increase in 3-MT concentrations in the NAS of mice as well as the increase in DA otflow in the NAS of rats. On the other hand, the decrease of DA outflow induced by prolonged restraint is anticipated in rats repeatedly exposed to the stressor. Finally, repeated restraint has strain-dependent effects on the decrease in 3-MT levels produced by stress in mice. In fact, while this response is unchanged in repeatedly stressed DBA/2 mice an anticipation of the response is evident in C57BL/6 mice.

These results point to a different effect of repeated stressful experiences on the two phases which characterize the response of the mesolimbic system to stress. While the phase of arousal characterized by increased DA release undergoes tolerance, the stress phase characterized by decreased DA release show sensitization possibly modulated by genotype-dependent factors.

364.10

ANTIPREDATOR DEFENSE, FEAR, AND ANXIETY IN THE LABORATORY MOUSE D.C.Blanchard*, S. Parmagiani, S.M. Weiss, J.K.Shepherd, and H.K. Taukulis. Bekesy Laboratory of Neurobiology and Department of Psychology, University of Hawaii, Honolulu, HI 96822.

The antipredator defense repertoire of the laboratory and wild rat is comprised of a number of behaviorally and neurologically distinct response patterns. We have described a similar defensive repertoire in the laboratory mouse when it is confronted with a predator or is exposed to situations associated with predatory threat.

Swiss-Webster mice show clear patterns of flight from and avoidance of an approaching human. When presented with an anesthetized rat, defensive vocalizations and bites at the rat's snout occur. When exposed to a cat for 15 minutes in visible burrow systems, the mice show behavioral changes that last for 22 hours. The reactions include initial patterns of flight, followed by risk assessment, with a prolonged (12-hour) period of freezing. The animals exhibit avoidance of the surface area and inhibition of non-defensive behaviors for the remainder of the 22-hour period.

We have devised two sets of procedures, a fear/defense test battery and an anxiety/defense test battery, designed to isolate and maximize these behaviors in contexts that are useful to the psychopharmacologist. To illustrate this, the effects of yohimbine, ethanol, and imipramine on the behaviors elicited by these procedures will be described. Yohimbine, for example, shows a clear potentiation of flight and avoidance with a reduction of freezing. These results support Deakin and Graeff's conceptions of flight behavior and its modulation by panicogenic compounds.

364.11

SEX DIFFERENCES AND DEVELOPMENTAL CHANGES IN PREDATOR-INDUCED ANALGESIA IN MEADOW VOLES.

L. Saksida, L.A.M. Galea, M. Kavaliers, K.-P. Ossenkopp and R. Shivers*. Dept. Psychology, Univ. British Columbia and Dept. Psychology, Univ. Western Ontario, London, Canada.

Although there is substantial evidence for sex differences in predator-induced responses of adult rodents, little is know about the responses of young animals. The present study examined developmental changes in predator-induced analgesia in male and female meadow voles, Microtus pennsylvanicus. The nociceptive responses of 8-18 day old (pre-weaning) and 20-30 day old (post-weaning) male and female voles exposed to a garter snake (a natural predator of young meadow voles) were examined. Brief (30 sec) exposures to the snake induced significant analgesic responses that were inhibited by the 5-HT_{1A} agonists 8-OH-DPAT and buspirone, and were insensitive to the opiate antagonist naloxone. Longer (15 min) exposures to the snake elicited lower amplitude opioid-mediated naloxone sensitive responses. Pre-weaning female voles displayed significantly greater 5-HT_{1A}-mediated analgesic responses than pre-weaning males, with no significant sex differences in the opioid mediated responses. In all cases, the levels of snake-induced analgesia declined during development, with post-weaning voles displaying lower amplitude responses than pre-weaning voles. These results show that there are sex differences in 5-HT_{1A} mediated predator-induced analgesia in young meadow voles. They also indicate that responses to predators can change developmentally according to the risk posed by the predator.

364.12

DOES MEDIATION OF AGGRESSIVE AROUSAL BY THE HAMSTER CORTICO MEDIAL AMYGDALA INVOLVE AN LTP-LIKE EFFECT? M.Potegal* Dept Med.Neurosci., Walter Reed Army Inst. Res., Wash. DC 20307

"Priming" a hamster by allowing it one attack on a conspecific increases its aggressive arousal, eg., reduces its attack latency on a subsequent trial. This arousal persists for at least 30 min. We previously reported two lines of evidence that the corticomedial amygdala (CMA) is part of the neural circuitry mediating the temporal persistence of aggression: 1) c-fos expression was increased in the CMA but not other brain regions of attack primed hamsters; 2) CMA lesions reduced aggression without affecting a number of other behaviors (Potegal et al, Soc. Neurosci., 1991,17, 877)

We now report a third line of evidence: Brief stimulation of the CMA through chronic electrodes with currents $\leq$100 uA reduces hamsters' latencies to attack a subsequently presented conspecific. Time course studies are now determining the optimal stimulation-test interval but it is already clear that this is a delayed effect consistent with the hypothesis that the CMA mediates aggression persistence.The optimal stimulation parameters appear similar to those which induce LTP. Studies of AMPA and NMDA receptor mediation of this effect are also underway. In so far as transient increases in aggressive arousal in rodents bear any relevance to affective state in humans, it can be conjectured that a non-associative, LTP or STP-like activation of the medial amygdala may underlie our experience of anger.

364.13

AXON-SPARING LESIONS OF THE MEDIAL NUCLEUS OF THE AMYGDALA SELECTIVELY DECREASE AFFILIATIVE BEHAVIOR IN PRAIRIE VOLES. B. Kirkpatrick*, C.S. Carter, T.R. Insel. Lab. Neurophysiology, NIMH, Poolesville, MD 20837.

Previous studies in several mammalian species have shown lesions of the amygdala decrease non-mating, non-maternal social behaviors; however, it is not clear which anatomical division of the amygdala might be involved, and whether these effects are due to the loss of amygdala neurons or damage to fibers of passage. In two studies, we used the axon-sparing neurotoxin n-methyl-d/l-asparate (NMA) to make lesions in the prairie vole, a gregarious, monogamous rodent that exhibits high levels of male parental care. Partial bilateral lesions of the medial nucleus of the amygdala were made in adult males. With no more than 50% volume loss in the anterior half of the nucleus, lesioned males exhibited a decrease in paternal behavior compared to controls. Lesioned animals showed no decrease in autogrooming, motor activity, sexual behavior, or social contact, and no increase in aggression. NMA lesions of the whole medial nucleus resulted in a decrease of both paternal behavior and social contact. Lesioned animals did not differ from control animals relative to level of motor activity, sniffing conspecifics, autogrooming, ability to find buried food, body temperature, or several other species-typical behaviors. These results and other studies implicate the medial nucleus in mammalian social behaviors.

364.14

MORPHINE AND AMPHETAMINE-INDUCED FEEDING DERIVED FROM THE NUCLEUS ACCUMBENS: SIMILARITIES AND DIFFERENCES. F.J. Vaccarino* and R. Gadzovski. Depts of Psychology and Psychiatry, University of Toronto, 100 St. George Street, Toronto, Ontario.

Morphine (MOR) and amphetamine (AMP)-induced feeding derived from the nucleus accumbens (N.Acc) are believed to be an expression of N.Acc opiate and dopamine reward functions, respectively. In an effort to behaviorally dissociate opiate and dopamine reward processes, the present study compared the behavioral profile of AMP- and MOR-induced feeding derived from the N.Acc.

METHOD. Male wistar rats implanted with cannulae aimed at the N.Acc were tested for their feeding/exploratory responses to intra-N.Acc microinjections of MOR (4.0 ug), AMP (1 ug) and saline.

RESULTS AND CONCLUSIONS: Consistent with previous results, intra-N.Acc injections of MOR and AMP stimulated sugar intake in rats. Further behavioral analyses indicated that AMP increased rate of eating while MOR increased time spent eating. In addition, MOR increased exploration, while AMP tended to decrease exploration during feeding. These results suggest that N.Acc. opiate and dopamine reward signals are behaviorally dissociable and demonstrate that analysis of natural behavior profiles may be an effective method for distinguishing the behavioral relevance of different reward signals. This research supported by NSERC grant 35036 to FJV.

364.15

EFFECTS OF LESIONS OF THE TEGMENTAL PEDUNCULO-PONTINE NUCLEUS (PPN) ON FEEDING INDUCED BY LATERAL HYPOTHALAMIC ELECTRICAL STIMULATION.

W. Trojniar and R. A. Wise*. Ctr. Stud. Behav. Neurobiol., Dept. Psychol., Concordia University., Montreal, Canada.

PPN receives inputs from nucleus accumbens septi (NAS) and appears to play a role in at least two behavioral functions associated with NAS circuitry: locomotor activity and the rewarding effects of such things as food, amphetamine, morphine, and lateral hypo-thalamic (LH) brain stimulation. NAS also appears to be involved in feeding induced by LH stimulation; the present study was designed to determine if PPN might play a role in this behavior as well.

Rats that fed in response to LH stimulation were given two-stage electrolytic lesions of PPN. The first lesion facilitated the behavior, decreasing the feeding threshold and increasing the speed of feeding with supra-threshold stimulation. Ipsi- and contra-lateral lesions were each effective; contralateral lesions were more consistent. The second-stage lesions impaired stimulation-induced feeding; the effects ranged from a slight increase in threshold to a complete cessation of feeding. These findings suggest a complex and bilateral interaction between PPN and mesolimbic motivational circuitry.

364.16

NEGATIVE CONTRAST ASSOCIATED WITH REINFORCING STIMULATION OF THE BRAIN. C. D. Anderson*, R. J. Ferland and M. D. Williams. Dept. of Psychology, Providence College, Providence, RI 02918.

When periods of behavior are being reinforced by events of a given quantity, quality, or temporal density, and alternate periods begin to occur that offer an improved reinforcement, work output for the original behavior is often decreased, a change called negative contrast. The present study attempted to maximize negative contrast by training rats on an FR-10 schedule for standard pellets of food, then introducing alternate periods of reinforcement by electrical stimulation of the lateral hypothalamus (0.1 s train of 50 Hz square wave pulses, 0.4 ms per pulse). Periods of reinforced behavior lasted 90 s and were signaled by a salient light. Reinforcement periods were separated by nonreinforcement intervals signaled by a 6000 Hz tone; pausing for 30 s was a contingency for shifting to the next reinforcement condition. Six rats trained on this procedure showed varied amounts of negative contrast, with drops to about 6% of the original response rate. Control conditions were used to discount satiation as an alternative hypothesis. The extremes of negative contrast observed here have not been seen with conventional reinforcers.

364.17

GENETIC INFLUENCE ON + MAZE PERFORMANCE IN MICE. Dudley Peeler.* Department of Neurosurgery, University of Mississippi Medical Center, Jackson, MS 39211.

Recombinant inbred (RI) mouse strains have been used to investigate possible major gene determinants of behaviors reflecting learning, memory and emotion. Major single gene influences have been described for various measures of activity and learning. In this study, male mice of the CXB set of 7 RI strains and their BALB/cBY and C57BL/6By progenitors were tested for exploration in a + maze. The crossed arms and center area of the maze were totally exposed except for opaque shelters (walls and top) positioned at the ends of two (opposed) arms. Mice were allowed to explore the maze for 5 min. in each of two sessions separated by 48 hrs. The first session was under bright illumination, the second under dim illumination. Approximately half the mice were tested in both sessions between 7:00 and 9:00, the other half between 13:00 and 15:00. Analysis of variance reveals a complex interaction of strain, illumination level and time of day (TOD). All strains exhibit greater movement out of the center area in dim than in bright illumination, but some (BALB/c, CXBD, CXBG & CXBH) as a function of TOD. All strains, when venturing out of the center area, spend less time in open than enclosed arms, but C57BL/6 consistently visit open arms more in dim than in bright light. BALB/c and CXBE show this illumination effect only in afternoon hours while CXBH do so only in the morning. There is no concordance between strain distribution patterns for these behaviors and CNS variations among these strains. Adequate assessment of genetic influences upon behavior and underlying neural substrates requires a multifactorial approach.

364.18

THE MOTIVATION PRODUCED BY FOOD AND MORPHINE ARE ISOMORPHIC: APPROACHES TO SPECIFIC MOTIVATIONAL STIMULI ARE LEARNED, K. Nader* and D. van der Kooy, Neurobiology Research Group, Dept. of Anatomy, Univ. of Toronto, Toronto, Ontario, M5S 1A8.

Using an unbiased place conditioning paradigm, we have identified two double dissociable motivational systems that generalize across different rewarding stimuli, but define a boundary between non-deprived and deprived motivational states. Lesions of the brainstem tegmental pedunculopontine nucleus (TPP) block only a non-deprived (food sated or opiate naive) rat's normal preference for an environment paired with either food or morphine, respectively. In contrast, neuroleptic pretreatment blocks only a deprived (food deprived or opiate withdrawn) animal's preference for an environment paired with food or morphine, respectively. The generalization of these motivational systems across stimulus boundaries begs the question of why food deprived animals specifically approach food and not other motivational stimuli in the environment. We asked if morphine can serve as a reward for a food deprived animal in the same way food does. We conditioned opiate naive rats such that one environment was paired with 22hr food deprivation, while the other environment was paired with 22hr food deprivation and morphine (20mg/kg i.p.). On the test day, rats showed a large preference for the side paired with morphine and hunger. This preference was uneffected by bilateral ibotenic acid lesions of the TPP, but was completely blocked by neuroleptic pretreatment with *cis*-flupentixol (0.8mg/kg i.p.) two and a half hours before each conditioning session. The failure of TPP lesions to attenuate the preference suggests that the rewarding effects of morphine occurring in opiate naive animals were not mediating the preference. Moreover, since the rats were opiate naive, the rewarding effects of morphine in opiate withdrawn animals could not be mediating the preferences. We conclude that morphine can substitute for food as a reward in food deprived animals. Thus, approaches to specific motivational stimuli are not an inherent property of motivational systems, but rather are learned during the ontogeny of the behaving animal.

364.19

MAUDSLEY REACTIVE (MR) AND NON-REACTIVE (MNRA) RATS: PERFORMANCE IN AN OPERANT CONFLICT PARADIGM. R.L. Commissaris*, L. Franklin, J.S. Verbanac and H.J. Altman. Depts. Pharmaceutical Sciences and Psychiatry, Wayne State University, Detroit, MI 48202.

As a result of selective breeding experiments in the 1960s, there exist two strains of Maudsley rats: Reactive (MR; "anxious") and NonReactive (MNRA; "non-anxious"). The present studies compared the behavior of these MR and MNRA rats in a modification of the Geller-Seifter multiple-schedule operant conflict paradigm. In the absence of a tone, every 30th lever press resulted in the delivery of a 45 mg food pellet. In the presence of a tone, each lever press resulted in both food and foot-shock (0.20 mA for 500 msec). Tone periods were 27 sec in duration and were presented on a VI-120 sec schedule (approx. 20 tones/40-min session). Conflict testing was conducted 5 d/wk for 35 wks. Initially, punished responding in the MR and MNRA rat strains did not differ dramatically. However, over the course of many weeks of conflict testing, rats of the MNRA strain came to accept significantly more shocks than did subjects of the MR strain. Additional experiments revealed that the difference in conflict behavior was not due to strain differences in shock sensitivity. The mechanism for this time-dependent difference in conflict behavior between the MR and MNRA rats remains undetermined. (MH #47181).

364.20

FOS-LIKE IMMUNOREACTIVITY IN BRAIN OF PRIMIPAROUS RATS AFTER MOTHER-LITTER INTERACTIONS. A. S. Fleming*, B. Rusak and E. J. Suh. Dept. of Psych., Erindale College, Univ. of Toronto, Mississauga, ON L5L 1C6 and Dept of Psych., Dalhousie University, Halifax, N.S.

The expression of the immediate-early gene *c-fos* in neurons is regulated with great anatomical specificity by a variety of pharmacological and environmental stimuli. The distribution of Fos protein in the brain can be used as a marker to help map functional neural systems involved in sensory processing and behavior. In this study, we examined the pattern of Fos-like immunoreactivity (Fos-lir) in the brains of primiparous female rats after their first sustained interaction with pups.

Pups were removed from their dams at parturition. On day 1 after parturition, one group of dams (n=9) were presented with six 1-3 day-old pups for 1 hr. A second group (n=6) were presented with a novel food (chocolate) for 1 hr. to control for olfactory stimulation and arousal, while another control group (n=11) were left undisturbed. All dams were sacrificed at the end of the 1 hr. period and their brains were fixed and prepared for immunohistochemical detection of Fos-lir in 40u frozen coronal sections.

Brain regions known to be involved in the processing of olfactory information and regulation of maternal behavior were studied. Exposure to pups significantly increased Fos-lir in the olfactory bulbs and bed nucleus of the stria terminals compared to the undisturbed control group but not the food exposed group. Compared to both control groups, pup-exposed dams showed increased Fos-lir in the MPOA, cortical and medial amygdala and piriform cortex. Fox-lir levels in other hypothalamic nuclei (SCN, VMN, SON), thalamic nuclei and the hippocampus did not differ significantly among the groups.

These results indicate that the sensory or behavioral changes associated with exposure of recently-parturient rats to pups increases nuclear Fos-lir in structures previously shown by other methods to mediate maternal behavior.

BIOLOGICAL RHYTHMS AND SLEEP III

365.1

DEVELOPMENT OF THE HUMAN RETINOHYPOTHALAMIC TRACT. S.T. Glotzbach*[1], P. Sollars[2], R.L. Ariagno[1] and G.E. Pickard[2]. Dept. of Pediatrics, Stanford Univ., Stanford, CA 94305 and Dept. of Psychiatry, Univ., Pennsylvania, Philadelphia, PA 19104.

It is presently unknown how and to what extent photoperiodic modulation may affect the development and coordination of circadian rhythms in premature or term infants. It is now possible to examine the development of the retinohypothalamic tract (RHT) in post-mortem tissue using the fluorescent tracer Di-I. Small crystals of Di-I were placed directly into the cut surface of the optic nerve in fixed tissue from preterm and full term infants of varying gestational and post-gestational ages. Sixteen-24 months after Di-I application, hypothalamic tissue was sectioned on a vibratome and examined for the presence of Di-I labeled retinal fibers in the region of the supra-chiasmatic nucleus (SCN). Tissue from a 36 week preterm infant had evidence of only a few labeled retinal fibers dorsal to the optic chiasm whereas tissue from a 5 month old full term infant had numerous labeled retinal fibers in the region of the SCN. Analysis of additional tissue at earlier gestational ages will supplement these preliminary results. Supported by NIH NS 21165 and HD 24315.

365.2

ORGANIZATION OF THE RAT SUPRACHIASMATIC NUCLEUS USING DiI INJECTIONS. I-H. Tang, D. M. Murakami*, and C. A. Fuller. Department of Animal Physiology, University of California, Davis, California 95616-8519.

This study examines the general organization of the SCN by making small injections of DiI into specific portions of the nucleus. Male Wistar rats were sacrificed and perfused with 4% paraformaldehyde, brains removed, and a specific part of the SCN injected with DiI with the aid of a dissecting microscope. Brains were stored for approximately 3 months, coronally sectioned at 100μ, and examined with an Olympus fluorescence microscope or BioRad confocal microscope. Injections of DiI into the dorsomedial SCN extensively labelled axons in the dorsal and medial portions of the SCN. However, very little labelling was found in the ventrolateral portion of the SCN. Densely labelled axons were found to course both dorsally along the wall of the third ventricle to the region ventral to the PVN, and laterally toward the SON, avoiding the ventrolateral SCN. Injections of DiI into the dorsolateral SCN also failed to densely label the ventrolateral SCN. Densely labelled axons project dorsally to the regions ventral to the PVN, but course lateral to the third ventricle. The lateral projection courses toward the SON. An injection into the ventrolateral SCN revealed extensive lateral projections, but light dorsal projections. These results suggest that the rat SCN exhibits an organization that may reflect distinct functional specialization within the nucleus. This research was supported in part by NASA Grant NAGW-2195.

365.3

GENERATION OF IMMORTAL ASTROCYTE CELL LINE FROM RAT SCN

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All mammalian cellular clock studies to date have of necessity used primary cell cultures. A cell line derived from the suprachiasmatic nucleus (SCN) that could be grown in culture indefinitely would be tremendously useful, as it would greatly enhance our ability to study the cellular and molecular basis of circadian clock function. Dispersed cells have already been used to restore circadian rhythms *in vivo* (Silver et al., 1991) and to demonstrate circadian rhythms *in vitro* (Murakami et al., 1991).

We introduced a temperature-sensitive (ts) oncogenic retrovirus (U19-tsA) into primary cultures of embryonic rat SCN cells using recombinant Moloney murine leukemia virus. This virus is replication deficient and has been manipulated to code for the ts SV40 large T antigen and neomycin (G418) resistance genes. Only cells that have incorporated the retrovirus will grow in the presence of the antibiotic G418. Dispersed cell cultures were prepared from E15 rat SCN. Cultures were infected with U19-tsA, and the resultant colonies were grown under G418 selection. Several colonies were isolated from each infection. One such G418-resistant colony showing multiple phenotypes was expanded and has been in continuous culture for over 4 months. Cell staining with antibodies to galactocerebroside, neurofilament, and GFAP has shown these cells to react only with GFAP, indicating they are astrocytes. When grown at a temperature non-permissive to oncogene function, the cells still stain only with GFAP antibody, although cell adhesion and growth seem to be enhanced in low levels of serum. The study of the role of these cells in expression of circadian rhythms may prove interesting.

365.4

SOMAL APPOSITIONS IN THE VENTROLATERAL SUPRACHIASMATIC NUCLEUS OF THE RAT. A.S. Elliott* S.M. Krauchunas and A.A. Nunez. Dept. of Psych., Neuroscience Program, Michigan State University, East Lansing, MI 48824.

It has been reported that cells in the dorsomedial portion of the suprachiasmatic nucleus (SCN) are often in apposition, forming "chains of neurons" (*J. Comp. Neurol.* 1980, **191**:661-702). The ventrolateral SCN (vlSCN) receives the bulk of retinal input to the nuclei and also contains peptide producing cells. Circadian fluctuations in the amount of direct neural membrane appositions may play a role in the pacemaker function of the SCN. These data are a preliminary report of the types of appositions in the vlSCN. They were collected from animals entrained to a 12:12 LD cycle and sampled at midday, a time when glucose utilization in the SCN is high (*Science* 1977, **197**:1089-1091). We measured the amount of somal membrane in apposition to glia (astrocytes and astrocytic processes), neural membrane (synapses, adjacent somas, unmylinated axons and dendrites), and other components of the neuropil (oligodendrocytes, myelinated axons) from electron micrographs. The initial data show that for cells in the ventrolateral SCN, 55% of somal membrane is in apposition to glia, 26% is in apposition to neural membrane, and 19% is in apposition to other aspects of the neuropil. In addition, every cell had an invaginated nucleus, with an average of 2.5 invaginations. Supported by grants BNS8908576 from NSF and NS 07279 from NIH.

365.5

PROJECTIONS OF THE SUPRACHIASMATIC NUCLEUS TO STRESS RELATED AREAS IN THE RAT HYPOTHALAMUS: A LIGHT AND ELECTRONMICROSCOPIC STUDY USING Pha-L TRACING AND FOS IMMUNOCYTOCHEMISTRY. R.M. Buijs*, M. Markman, S. Shinn and Y.X. Hou. Neurosciences Loeb Medical Research Institute, Ottawa, Canada.

A diurnal pattern for corticosteroid and ACTH release has been reported in a number of separate studies. This involves both a diurnal difference in basal levels as well as in the response to stressful stimuli. The structure responsible for the control of ACTH and corticosteroid release is the paraventricular nucleus of the hypothalamus (PVN) where the corticotrophin releasing factor (CRF) neurons are located. Since the suprachiasmatic nucleus (SCN) most likely is the sole source for all diurnal rhythms in mammals, it is to be expected that connections between the SCN and the PVN may explain this diurnal rhythm in ACTH secretion. However, previous anatomical studies in which the projections of the SCN were investigated could not provide evidence for such direct pathways. Therefore, the present study aimed to investigate the anatomical basis for the control of the SCN on ACTH and corticosteroid secretion. Pha-L tracings from the SCN were undertaken and revealed the sites of interaction between the SCN and Fos stained neurons involved in stress. LM and EM studies indicated a direct innervation of Pha-L labelled fibers with parvocellular neurons in the periventricular and caudal PVN, not in the part with putative CRF cellbodies. A high interaction was found also in the DMH, suggesting that interneurons are used to transmit the message of the SCN to CRF secreting neurons.

365.6

EFFERENT PROJECTIONS OF THE RAT INTERGENICULATE LEAFLET (IGL): A PHASEOLUS VULGARIS LEUCOAGGLUTININ (PHA-L) STUDY. R.P.Weis*, J.C.Speh and R.Y.Moore. Depts. of Psychiatry Univ. of Pittsburgh, Pittsburgh PA 15261.

The IGL is a distinctive anatomical and functional subdivision of the lateral geniculate complex that is a component of the circadian timing system. In this study, we examined the efferent projections of the IGL using PHA-L in the rat. PHA-L injections were localized to IGL, IGL and dorsal lateral geniculate (DLG) and IGL and ventral lateral geniculate (VLG). The major bundle of IGL projections runs rostrally and ventrally along the optic tract to form a dense terminal plexus in the ventrolateral suprachiasmatic nucleus (SCN). Other fibers extend through the SCN to terminate in sparse plexuses in the adjacent anterior hypothalamic area, subparaventricular zone, retrochiasmatic area and lateral hypothalamic area. These projections are bilateral but greater on the ipsilateral side. Another large group of fibers continues to run through the supraoptic commissure to terminate in the contralateral IGL. A smaller group of fibers extends medially from the IGL into the zona incerta and a small bundle of fibers runs rostrally along the lateral surface of the thalamus to terminate in the anterior nuclei. Another limited bundle, not entirely separate from VLG projections, runs over the dorsal thalamus into the pretectal area,periaqueductal gray and posterior hypothalamus. (Supported by NIH grant NS-16304.)

365.7

RETINAL AFFERENTS AS DEMONSTRATED WITH UNCONJUGATED CHOLERA TOXIN J. C. Speh* and R.Y. Moore Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15261

The retinal afferents to the hypothalamus and thalamus of the rat have been demonstrated with many neuroanatomical tract tracing techniques. Using the localization of anterograde transport of unconjugated cholera toxin (CT) with a sensitive immunocytochemical technique, we here demonstrate a more extensive array of projections to these areas than has been previously reported (Johnson et al, '88; Levine et al, '91).

Previously undescribed retinal projections to the organum vasculosum lamina terminalis, dorsal hypothalamic area, perifornical area, midline and ventral thalamic areas, subparaventricular zone of the hypothalamus, lateral dorsal thalamic nucleus, periaqueductal gray, reticular formation and an additional anterior accessory optic nucleus will be described. More extensive projections to the medial and lateral preoptic areas, lateral hypothalamic area, anterior hypothalamic and retrochiasmatic areas, the suprachiasmatic and intergeniculate leaflet nuclei, zona incerta and the ventral surface of the basal forebrain are demonstrated. In addition, a small number of retrogradely labeled cells are seen at the lateral border of the lateral habenula, in the medial pretectal area and the periaqueductal gray. This pattern of projections is more extensive than that reported for the hamster (Johnson, et al, '88). The functional significance of such a complex and extensive distribution of retinal afferents remains to be elucidated. (Supported by NIH grant #NS-16304)

365.8

EFFERENT CONNECTIONS OF THE RETROCHIASMATIC AREA IN THE RAT. M.M. Moga* and R.Y. Moore. Dept. Psychiatry, Univ. Pittsburgh Medical Center, Pittsburgh, PA 15261.

A major question in circadian biology is how the circadian rhythm of the suprachiasmatic nucleus (SCN) is transmitted throughout the brain when the efferent connections of the SCN are sparse and limited to only a few nuclei. The retrochiasmatic area (RCh) receives direct input from the SCN and may relay circadian information to nuclei not connected with the SCN. To examine this possibility, we placed iontophoretic injections of PHA-L into the RCh. After injections into the medial part of the RCh, located immediately caudal to the SCN, many labeled fibers with terminal boutons were observed in the bed nucleus of the stria terminalis (particularly in the sexually dimorphic posteromedial subnucleus), the medial preoptic area, the septohypothalamic nucleus, and the periventricular hypothalamic nucleus. After injections into the lateral part of the RCh, which extends laterally over the optic tract, labeled fibers were particularly numerous in the preoptic subnucleus of the bed nucleus of the stria terminalis, the paraventricular thalamic nucleus, the perifornical lateral hypothalamus and the periaqueductual gray. These results suggest that circadian information may reach additional nuclei via the retrochiasmatic area. (Supported by NIH grant # NS-16304)

365.9

ULTRASTRUCTURAL LOCALIZATION OF NERVE GROWTH FACTOR-RECEPTOR-LIKE IMMUNOREACTIVITY IN THE SUPRACHIASMATIC NUCLEUS OF THE RAT. K.G. Bina[1]*, T. Honda[2], B. Rusak[1] and K. Semba[2], Depts. of [1]Psychology and [2]Anatomy and Neurobiology, Dalhousie Univ., Halifax, N.S., Canada.

Heavy nerve growth factor receptor-like immunoreactivity (NGF-R-ir) has been localized in the suprachiasmatic nucleus (SCN) of the hypothalamus, a site responsible for the generation of circadian rhythms and for photic entrainment. At the light microscopic level, NGF-R-ir is located in the ventrolateral aspects of the SCN, an area that receives retinal afferents. Previous studies conducted in our laboratory revealed that NGF-R-containing neurons in the basal forebrain and the retina project to the SCN. Although no detectable levels of NGF-R mRNA have been reported in the SCN, at the light microscopic level some of the NGF-R-ir appears to be located on the surface of SCN neurons. This study was conducted to determine whether NGF-R-ir in the SCN was exclusively located on axon terminals or whether some of the NGF-R-ir is also located on SCN neurons. Both pre-embedding PAP-DAB and post-embedding immunogold staining techniques were used. NGF-R-ir was found to be located on axon terminals as well as on neuronal somata and dendrites within the SCN. In the neuronal somata and dendrites NGF-R-ir was associated with cell organelles such as microtubules and endoplasmic reticulum. These results suggest that NGF-R are not only transported anterogradely from the basal forebrain and retina but are also synthesized within the SCN.

365.10

NICOTINIC RESPONSES OF SUPRACHIASMATIC NEURONS IN INTACT AND OVARIECTOMIZED HAMSTERS RECORDED *IN VITRO*. L.R. Waldman and C.A. Fuller*, University of California, Department of Animal Physiology, Davis, CA 95616-8519.

The effects of nicotine and mecamylamine, a nicotinic antagonist, on neurons in the suprachiasmatic nucleus (SCN) of intact and ovariectomized (ov-x) female hamsters was examined. Three groups were studied: intact, ov-x and ov-x with estradiol implants. 500μ hypothalamic slices, including the SCN, were cut and incubated in artificial cerebrospinal fluid. As intact and ov-x animals with implants showed comparable blood levels of estradiol, they comprised a single control group; ov-x animals had non-significant blood levels. Rate vs. time histograms were constructed. Of the control groups, 63% (n=60) of the cells given 30 mmol nicotine showed a change in firing rate and the ratio of neurons that were stimulated to those that were inhibited was 1.3. The mean absolute value of 't' score for the changes in firing rate was high; t=6.864, p<.0001. In the ov-x group, 65% (n=51) of the cells exhibited changes in firing rate but the ratio of cells stimulated to those inhibited was 0.76. The mean absolute t score was also high, t=6.024, p< .0001. Forty three percent (n=26) of the cells given nicotine in the control groups were given mecamylamine (100 mmol mecamylamine with 30 mmol nicotine). Mecamylamine effectively blocked the nicotine response in 77% (n=20) of these cells. Of the ov-x group, 33% (n=17) received the mecamylamine/nicotine mixture. In this group, however, mecamylamine was effective in only 29% (n=5) of the cells. In conclusion, while nicotine effected both ov-x and intact hamsters, mecamylamine was not as effective in the ov-x group as it was in the control animals.

365.11

SINGLE UNIT RESPONSES OF RAT SUPRACHIASMATIC NUCLEUS NEURONS TO SUBSTANCE P AND GLUTAMATE IN VITRO. T. Shirakawa* and R. Y. Moore. Depts. of Psychiatry and Behavioral Neuroscience, Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261.

The suprachiasmatic nucleus (SCN) of the hypothalamus is a circadian pacemaker in the mammalian brain. It receives entraining input from the retina via the retinohypothalamic tract (RHT). Although RHT axons have been thought to produce glutamate (GLU), a recent study (Takatsuji et al. 1991) has shown that some RHT fibers contain substance P (SP). In this study we examined the responses of SCN neurons to SP and GLU using single unit recording from a hypothalamic slice preparation.

Neurons were recorded predominantly in the ventrolateral SCN. Approximately, 25% of neurons recorded showed an excitatory response (increased firing rate) to perfused SP (1,10,100,1000nM) and 6% showed an inhibitory response. In a number of instances, these acute effects were followed by long excitatory effects that persisted for minutes after washout of SP from the bath. GLU (10,100,1000μM) evoked excitatory responses from more than 80% of SCN neurons in a dose-dependent manner. There was no interactive effect of GLU and SP on SCN neurons. Supported by NIH grant NS-16304.

365.12

EFFECTS OF PREOPTIC-ANTERIOR HYPOTHALAMIC WARMING ON THE WAKING DISCHARGE OF POSTERIOR LATERAL HYPOTHALAMIC NEURONS. B.L. Krilowicz [1], R. Szymusiak[2,3]* and D. McGinty[2,4]. [1]Dept. Biol., Cal. State Univ., Los Angeles, CA 90032, [2]V.A. Medical Center, Sepulveda, CA 94343, [3]Dept. Anat. Cell Biol. and [4]Dept. Psychology, Univ. Calif., Los Angeles, CA 90024.

Interactions between sleep-wake regulation and the thermoregulatory system are well known, suggesting that preoptic/anterior hypothalamic (POAH) thermosensitive neurons may participate in the regulation of brain mechanisms of sleep and arousal. To test this hypothesis, we examined the effects of local POAH warming on spontaneous and evoked activity of neurons in the posterior lateral hypothalamus (PLH) of cats during waking and sleep. In response to POAH warming, 46% of 50 PLH cells decreased spontaneous discharge rates by 16-92%, 26% increased activity by 18-140%, and 28% exhibited minimal changes (-14-3%) in discharge rate. Cells that increased activity during POAH warming tended to have low firing rates during active wakefulness, while the other two classes of cells had high discharge rates when cats were active. Orthodromic responses evoked by brainstem or cortical stimulation were also examined. Cells that were most responsive to POAH warming (±25% change in spontaneous waking discharge rate) exhibited changes in waking evoked excitability during POAH warming that were similar to those evoked during normal slow-wave sleep. These results suggest that POAH thermosensitive neurons participate in the regulation of sleep and wakefulness via inhibition and/or disfacilitation of arousal systems.

365.13

CIRCADIAN RHYTHM OF NEURONAL TEMPERATURE SENSITIVITY IN RAT SUPRACHIASMATIC NUCLEUS. Philippe S. Derambure and Jack A. Boulant.* Dept. of Physiology, Ohio State University, Columbus, OH 43210.

The circadian pacemaker in the hypothalamic suprachiasmatic nucleus (SCN) affects several regulatory systems, including body temperature. To study SCN circadian activity and thermosensitivity, single units were recorded from dorsomedial and ventrolateral SCN in rat frontal hypothalamic slices during changes in tissue temperature. When analyzed according to circadian time, 350 neurons were characterized by firing rate, and 240 neurons were characterized by thermosensitivity. Dorsomedial SCN neurons displayed the greatest circadian rhythm in firing rate, while both SCN regions showed a strong circadian rhythm in neuronal thermosensitivity. At night, more than 30% of the neurons were warm sensitive; but during the day, only 5% were warm sensitive. These changes in neuronal thermosensitivity may reflect interactions between body temperature and circadian rhythm. (Supported by NIH grant NS14644 and by the Am. Heart Assoc., Ohio Affilitate.)

365.14

THE EFFECT OF NEUROPEPTIDE Y AND OPTIC NERVE STIMULATION ON SPONTANEOUS DISCHARGE RATE IN THE SUPRACHIASMATIC NUCLEI. T.E. Hermida and M.E. Harrington*, Dept. of Psychology, Smith College, Northampton, MA USA.

The circadian pacemaker in the suprachiasmatic nuclei (SCN) receives retinal input via the retino-hypothalamic tract (RHT). A second photic pathway, the geniculo-hypothalamic tract (GHT) arises from neuropeptide Y-immunoreactive neurons in the intergeniculate leaflet. We studied the role of the GHT by examining how neuropeptide Y and electrical stimulation of the optic nerves altered SCN cell discharge rate.

Extracellular single unit activity was recorded from the SCN in horizontal slice preparations with the optic nerves attached. Optic nerve stimulation was applied through a suction electrode. Neuropeptide Y was added to the bath in two minute intervals, and post-stimulus responses were recorded until the firing rate returned to baseline.

SCN cell responses to both neuropeptide Y and to optic nerve stimulation were both excitatory and inhibitory, with changes in firing rate of up to 200%. Some cells responded to neuropeptide Y with a long lasting (e.g., 2 h) oscillation in firing rate, while responses to optic nerve stimulation were of a much shorter duration. Application of neuropeptide Y could alter the response to optic nerve stimulation.

These results indicate that application of one putative neurotransmitter of the GHT can induce long-lasting oscillations in discharge rate of SCN neurons and can alter responses of SCN neurons to direct stimulation of the RHT.

This work was supported by NIH grant NS26496 (MEH) and Research Supplement for Minority Undergraduate Students grant NS26496-04S1 (TEH).

365.15

BRAINSTEM-PROJECTING NEURONS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS DECREASE THEIR SPONTANEOUS ACTIVITY FOLLOWING STIMULATION OF THE SUPRACHIASMATIC NUCLEUS. M.L.H.J. Hermes and L.P. Renaud*. Neuroscience Unit, Ottawa Civic Hospital, Ottawa, Canada K1Y 4E9.

Several lines of evidence indicate that in mammals the circadian rhythm in many behavioral, neuroendocrine and autonomic functions is generated by a pacemaker located within the suprachiasmatic nucleus (SCN). The efferent projections of this nucleus to the medial hypothalamus and thalamus are likely involved in the mediation of rhythmic information to the rest of the brain. Recently, applying extracellular electrophysiological techniques, we have observed that electrical stimulation in the SCN influences the excitability of identified neurosecretory neurons in the hypothalamic paraventricular nucleus (PVN). In view of the presence of caudally-projecting neurons in the PVN that are involved in the regulation of autonomic functions, we also studied the effect of electrical stimulation in the SCN on the excitability of PVN neurons that could be antidromically activated from the dorsal vagal complex (DVC).

In pentobarbital anesthetized male Long-Evans rats, PVN neurons projecting to the DVC showed a reduction in excitability after SCN stimulation. Mean latency of onset was 17.0 ± 2.7 ms; mean duration of effect was 132.2 ± 39.5 ms. These results support the idea that the SCN influences sympathetic and parasympathetic outflow through the PVN and may, via this pathway, induce rhythmicity in certain autonomic functions. This research was supported by the International Human Frontier Science Program Organization.

365.16

FUNCTIONAL ORGANIZATION OF THE HAMSTER SCN VISUALIZED WITH VOLTAGE-SENSITIVE DYES

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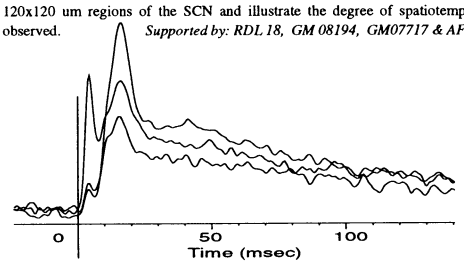
Division of Life Sciences, University of Texas at San Antonio, San Antonio TX, 78249 and Armstrong Laboratory, Brooks AFB, TX, 78235.

How afferent input from the retinohypothalamic tract (RHT) can phase shift the circadian oscillator is still largely unknown. Spatial differences in SCN field potentials have been observed in response to optic nerve stimulation (Shibata *et al.*, Brain Res. *12*: 377, 1984) suggesting a possibile functional organization to the SCN's intrinsic neural circuitry that might be related to the entrainment process. To investigate this possibility, we are using multiple-site optical recording techniques (Obaid *et al.*, Neurosci. Abstr. *16*: 1185, 1990) to visualize and map spatiotemporal differences in neuronal activity in the hamster SCN. The figure below shows three (of 124) optical traces recorded from a 400 um horizontal slice stained with the dye RH155 following electrical stimulation of the optic nerve. These traces were recorded from adjacent 120x120 um regions of the SCN and illustrate the degree of spatiotemporal variation observed. *Supported by: RDL 18, GM 08194, GM07717 & AFOSR 2312W6*



365.17

IRRADIANCE CODING BY SUPRACHIASMATIC NUCLEUS AND INTERGENICULATE LEAFLET NEURONS. M.E. Harrington, J. Meijer and B. Rusak*, Dept. of Psychology, Smith College, Northampton MA, USA, Physiology Dept., Leiden University, Leiden, THE NETHERLANDS, and Dept of Biomedical Sciences, McMaster University, Hamilton, Ontario, CANADA.

Cells in the suprachiasmatic nuclei (SCN) and intergeniculate leaflet (IGL) have been shown to alter their firing rates in response to changes in diffuse illumination. Firing rate changes are intensity-related and often sustained for the duration of retinal illumination. We examined whether responses of SCN and IGL neurons to manipulation of light irradiance and duration resemble phase-shifting responses.

Male golden hamsters were removed from a light:dark (14:10) cycle, anesthetized with urethane (2g/kg), and prepared for electrophysiological recording (see Brain Res., 554 (1991) 95-104). The photic stimulus was either a full spectrum light (tungsten halogen lamp) or the same light source filtered to produce monochromatic light (500nm, BW=25nm, Corion Corp.).

SCN cells showed irradiance coding with response thresholds at about 10^{14} photons/cm^{2}s. Cells in the IGL and anterior ventral lateral geniculate nucleus showed similar thresholds (10^{13} to 10^{15} photons/cm^{2}s). Response thresholds as measured by firing rate changes in anesthetized animals were several log units higher than those measured in behavioral experiments (J. Physiol., 439 (1991) 115-45), perhaps as a result of the urethane anesthesia.

For geniculate cells, irradiance-response curves for the first 5 s of a light stimulus were shifted toward lower irradiances as compared to responses over longer durations (e.g., 300 s). Irradiance-responses of SCN cells did not vary with light duration. These results suggest that the duration-dependence of phase-shift responses at CT19 might be mediated beyond the level of the photoreceptor.

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365.18

SPONTANEOUS FIRING PATTERN OF NEURONS IN THE CAUDO-LATERAL PERIBRACHIAL AREA. S.Datta*, J.Quattrochi and J.A.Hobson. Lab. of Neurophysiology, Dept. of Psychiatry, Harvard Medical School, Boston, MA 02115.

Cholinergic stimulation of the caudo-lateral peribrachial nucleus of the pons produces immediate state-independent ipsilateral LGB PGO waves and enhancement of SP and REM sleep lasting for seven days. These state-independent PGO waves persists for 3-4 days. In the present study neurons were recorded during the wake-sleeping cycles in normally behaving cats. About 79.5% of the cells increased or began firing (median around 40 Hz) during PGO related states (SP and REM). Of these PGO state-on cells 27% discharged high frequency (300-500 Hz) spike bursts 30-40 ms before each thalamic PGO wave, upon a background of tonically increased firing rates during the PGO related states. Another 9.5% of the cells fired (15 Hz)tonically during waking and slow wave sleep without PGO but stopped or decreased firing during the PGO related states. The remaining 11.0% cells fired (10 Hz) tonically but were unrelated to the wake-sleep state. This correlative evidence indicates that the caudo-lateral area is a candidate for PGO generation and driving element of the rostral peribrachial PGO-related transferring neurons. Supported by NIH grant MH-13923.

BIOLOGICAL RHYTHMS AND SLEEP IV

366.1

EFFECTS OF SHORT TERM EXPOSURE TO CONSTANT ILLUMINATION ON THE HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM (HNS). M.L. Weiss*, J.E. Smith, A. Sohanjan, P. Rusch and G.I. Hatton. Neuroscience Program, Michigan State University, E. Lansing, MI 48824-1117

It has been inferred that the suppression of 24 hr water intake induced by exposure to continuous environmental illumination (LL) is due to increased release of vasopressin from the posterior pituitary (e.g., Glantz, Physiol. Behav. 2: 49-54, 1967). We wanted to determine if LL could induce the same pattern of plastic changes produced in the HNS by other stimuli which activate the system, such as 10 d lactation. Twenty four male Sprague Dawley rats, housed in pairs, were randomly distributed into three groups: short term exposure to LL (24 hr), long term LL (48 hr) and control (12:12 L:D). Body weight and 24 hr water intake (for each pair) were recorded daily prior to the onset of the experiment. After exposure to experimental conditions for the appropriate time, half of the animals in each group were decapitated and trunk blood collected for plasma measurements of oxytocin (OX) and vasopressin (VP); assayed by J. Verbalis, U. Pittsburgh, osmolality, and sodium and potassium concentrations. The remaining animals were perfused with fixative and prepared for light and EM morphometric analysis of the HNS. We confirmed the suppression of growth curve and water intake for the first 24 hr of LL and found concomminent changes in the posterior pituitary (similar to what has been found with other stimuli of the HNS). There were no changes in cell or nuclear size of magnocellular neurons in the HNS. Plasma osmolality, ion concentrations, and OX and VP were not different between groups. Thus, the behavioral and morphological findings were supportive of an increase in VP release, but plasma hormone levels and cell size of supraoptic neurons were not affected by short term LL. We conclude that the suppression of water intake seen during short term LL is most likely due to other factors, possibly stress, and that long term LL may be needed to produce activation of HNS. Supported by NSF 8919898 and BRSG funds from Michigan State University.

366.2

PHOTOPERIOD-INDUCED DECREASES IN MEDIAN EMINENCE DOPAMINE CONCENTRATIONS IN MALE SYRIAN HAMSTERS ARE NOT DUE TO A CHANGE IN TYROSINE HYDROXYLASE ACTIVITY. K. Krajnak[1]*, J. Manzanares[2], K.J. Lookingland[2], and A.A. Nunez[1]. [1]Department of Psychology, [2]Department of Pharmacology/Toxicology, and Neuroscience Program, Michigan State University, East Lansing, Michigan 48824.

Steady-state stores of dopamine (DA) are maintained in tuberoinfundibular (TI) neurons by the coupling between neurotransmitter synthesis, release and metabolism. Exposure of male Syrian hamsters to a short photoperiod decreases median eminence (ME) DA concentrations (Biol. Reprod. 1982, 26:437), but has no effect on concentrations of its primary metabolite 3,4-dihydroxyphenyl-acetic acid (DOPAC; Krajnak et al., 1992, Soc. Biol. Rhythms). Thus, photoperiod-induced decreases in the amount of DA stored in TI neurons is not due to an increase in intraneuronal metabolism of DA. In the present study, the effect of photoperiod on the synthesis of DA in TIDA neurons was estimated by measuring the activity of tyrosine hydroxylase (accumulation of 3,4-dihydroxyphenylalanine [DOPA] in the ME following administration of the L-aromatic amino acid decarboxylase inhibitor NSD 1015) male hamsters. Animals were housed in either a long (LD; 16L:8D) or short photoperiod (SD; 6L:18D) for 12 weeks. On the day of sacrifice, each animal was injected with NSD-1015 (100 mg/kg; i.p.) 30 minutes prior to decapitation. The ME was microdissected from frozen brains and assayed for DOPA and DA by HPLC. Exposure to short photoperiod decreased DA concentrations in the median eminence, but had no effect on the accumulation of DOPA. These results reveal that photoperiod-induced decreases in DA stores in the ME in male Syrian hamsters are not due to an alteration in the synthesis of DA in TIDA neurons. (Supported by BNS 9008576 to A.A.N. and MH 42802 to K.J.L.)

366.3

DO PRESYNAPTIC MELATONIN HETERORECEPTORS MODULATE CATECHOLAMINE RELEASE IN AREAS OF RABBIT BRAIN POSSESSING HIGH LEVELS OF 2-[^{125}I]-IODOMELATONIN BINDING SITES? M.L. Dubocovich, S. Iacob, J.G. Hensler, F.B. Cutting, and M.I. Masana*. Dept. Pharmacol., Northwestern Univ. Med. School, Chicago, Illinois 60611.

2-[^{125}I]-Iodomelatonin (100 pM) binding sites were localized in coronal rabbit brain sections using in vitro autoradiography (50 mM Tris buffer, pH 7.4). Specific binding defined with 1 uM melatonin was unevenly distributed throughout the rabbit brain. The highest levels of specific binding were found in the dorsal hippocampal commissure (23 fmol/mg protein), followed by the retrosplenial cortex, dorsolateral thalamus, frontal, parietal and occipital cortex, cingulate girus, corpus callosum, suprachiasmatic nucleus, superior colliculus, caudate and ventral hypothalamus (4 fmol/mg protein). Brain slices prepared from hippocampus, frontal cortex, caudate or hypothalamus were labeled with either ^{3}H-dopamine (DA) or ^{3}H-norepinephrine (NE) and superfused with Krebs solution (1.3 mM calcium). In the caudate melatonin (1 pM - 10 uM) did not modify the calcium dependent release of DA elicited at 3 Hz (20 mA, 2 msec), in contrast to the rabbit retina where this hormone is a potent modulator of DA release (IC_{50} = 17 pM). In slices of frontal cortex and hippocampus, melatonin did not affect the release of NE, while in hypothalamic slices, melatonin inhibited release only at 1 uM. These results suggest that melatonin receptors in the rabbit brain do not function to modulate the release of DA or NE. Supported by USPHS Grant MH-42922 to MLD.

366.4

PHARMACOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF MELATONIN BINDING PROTEINS IN HAMSTER BRAIN. J.B. Hogenesch, R.C. Brown, M.L. Dubocovich*. Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

In Djungarian hamster brain 2-[^{125}I]-iodomelatonin (2-IMEL) labels a site (ML-2) with binding and pharmacological characteristics distinct from the well characterized ML-1 receptor. The specific binding of 2-IMEL to the ML-2 site shows rapid association at 0°C, with maximal binding at 5 min. It is reversible and saturable (Kd=1.5 ± 0.3 nM) (Duncan et al., Endoc. 125: 1011, 1989). The aim of this study was to identify putative melatonin binding proteins in hamster brain. Washed synaptosomal membranes were labelled with the irreversible ligand 2-[^{125}I]-bromomelatonin (Laudon and Zisapel, FEBS Lett. 288: 105, 1991) (BIM, 55 pM) by incubation at 0°C in 50 mM Tris (pH 7.4) with 10 mM $MgCl_2$. The association of BIM was time dependent (0 to 60 min), although specific binding (melatonin, 10 μM) was only observed up to two minutes. The concentration of melatonin (IC_{50}) necessary to protect the binding of BIM (55 pM) to hamster brain membranes was 115 nM. This value is similar to the value obtained when membranes were labeled with 2-IMEL (IC_{50} 90.7 ± 26.7 nM n=6). BIM-labeled membranes were processed by SDS-PAGE, and autoradiography showed five labeled proteins of approximate molecular weights of 40, 55, 58, 107, and >200 kD, whose binding was protected by melatonin (0.1-10 μM). Current pharmacological experiments are aimed at establishing the identity of these proteins with the 2-IMEL binding site (ML-2). Supported by USPHS grant MH 42922 (MLD) and training grant NS-07140 (RCB).

366.5

2-[^{125}I]-IODOMELATONIN BINDING IN LAYERS OF CHICK OPTIC TECTUM MEASURED OVER THE DIURNAL CYCLE. D.N. Krause*[1], F.B. Cutting[2] and M.L. Dubocovich[2], Depts. of Pharmacology, [1]Univ. of Calif., Irvine, CA 92717 and [2]Northwestern Univ. Med. Sch., Chicago, IL 60611.

We have shown specific, high affinity 2-[^{125}I]-iodomelatonin (2-IMEL) binding to be unevenly distributed throughout the major layers of chick optic tectum (Krause et al.,1992). Tectal 2-IMEL binding was further studied in coronal brain sections from chicks (3.5 week old) kept on a 14:10 light: dark cycle until sacrifice at either 07:00 or 18:00 hrs. Saturation analysis using quantitative autoradiography showed that 2-IMEL (1-500 pM) bound to the various tectal layers with similar picomolar affinities. The highest binding densities were found in the retinorecipient layer, stratum griseum et fibrosum superficiale (SGFS; Kd, 86 ±12 pM; Bmax, 201 ± 10 fmol/mg protein, n=3, determined at 18:00 hrs.), as compared to the underlying stratum griseum centrale (SGC; 123 ±16 fmol/mg) and the optic fiber layer, stratum opticum (SO; 26 ±2 fmol/mg). No significant differences between the two time points were found for tectal binding (Kd or Bmax) in any layer. Thus the sensitivity of tectal melatonin receptor binding sites appears to be the same at the beginning and end of the daily nocturnal peak in melatonin levels. These findings contrast with the dramatic decreases in 2-IMEL binding we have seen previously in SGFS and SO following optic nerve transection. (Supported by USPHS Grant MH-42922 to MLD.)

366.6

CIRCADIAN RHYTHM OF MELATONIN SECRETION FROM INDIVIDUAL PINEAL CELLS OF ANOLIS CAROLINENSIS DEMONSTRATED USING A REVERSE HEMOLYTIC PLAQUE ASSAY. G.E. Pickard* and W. Tang. Department of Psychiatry, Univ. Pennsylvania, Phila., PA 19104

It is not known whether individual vertebrate cells function as circadian oscillators or whether circadian rhythms occur as an emergent property of a large number of interacting cells. This issue was addressed by examining melatonin secretion from individual pineal cells from the lizard Anolis carolinensis using a modified reverse hemolytic plaque assay. Dispersed cells maintained in culture demonstrated rhythmic secretion of melatonin for up to 10 days while maintained under a 12:12 LD cycle at 32 °C. When chambers prepared from a single pineal gland were cultured in constant dark for successively longer periods before melatonin secretion was assayed, a circadian rhythm was detected. Mean plaque area from individual cells, assayed over a 12 hr interval, was greater during the subjective night than during the subjective day for at least 3 cycles (72 hrs). These data suggest that individual pineal cells function as circadian oscillators and rhythmically secrete melatonin. Supported by a grant from the Whitehall Foundation, Inc.

366.7

MELATONIN RESETS THE SCN CIRCADIAN CLOCK IN VITRO WITHIN A NARROW WINDOW OF SENSITIVITY NEAR DAWN. **A.J. McArthur* and M.U. Gillette. Dept. of Physiology & Biophysics and Dept. of Cell and Structural Biology, Univ. of Illinois, Urbana-Champaign, IL 61801.**

We have previously reported that melatonin (MEL) acts directly on the SCN *in vitro*, phase advancing the time-of-peak in neuronal activity only when applied near the day-night transition (McArthur *et al.*, 1991). Animal studies suggest that mammalian circadian systems have a second period of sensitivity to MEL just prior to dawn. MEL treatment at this time increases SCN metabolism (Cassone *et al.*, 1988) and induces c-Fos (Kilduff *et al.*, 1990). High affinity MEL receptors in the SCN demonstrate maximal density at late subjective night (Laitinen *et al.*, 1989).

Brain slices were prepared from 8 wk old male Long Evans rats housed in a 12L:12D cycle. Slices were treated with MEL (10^{-9} M) for 1 hr, and measurements of phase were made by determining the time-of-peak in neuronal activity. While MEL treatment at CT 22 has no effect on the SCN clock, the same treatment at CT 23 results in a near 4 hr phase advance (n=4) in the rhythm of electrical activity on days 2 and 3 *in vitro*. These results suggest 1) that MEL can regulate the SCN at both transition points in the environmental lighting cycle, and 2) that even in constant conditions *in vitro*, sensitivity to MEL is modulated over time. (Supported by NIH Grant 5T32GM07143 to A.J.M. and NINDS Grant NS-22155 to M.U.G.)

366.8

MEASUREMENT OF SPECIFIC 2-[^{125}I]-IODOMELATONIN (IMEL) BINDING SITES IN SIBERIAN HAMSTERS AFTER PINEALECTOMY OR SHORT PHOTOPERIOD EXPOSURE. M.J. Duncan*, C.C. Purvis and M.H. Stetson. Dept. of Anatomy & Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY and Dept. of Life and Health Sciences, Univ. of Delaware, Newark, DE.

These studies examined whether treatments which alter the secretion of melatonin affect specific IMEL binding sites in the pars tuberalis (PT) of Siberian hamsters. In Exper. 1, adults (N=8/group) were sacrificed 1 week after pinealectomy (PINX) or sham-surgery (SHAM). IMEL binding sites were measured by saturation studies ([FREE]= 8-370 pM IMEL) using quantitative autoradiography. No difference was found in the affinity (K_d) or density (B_{max}) of IMEL sites (SHAM: K_d=14.2±4.7 pM, B_{max}= 1.69±0.15 fmol/mg; PINX: K_d=17.4±4.4 pM, B_{max}= 1.99±0.12 fmol/mg). In Exper. 2, 18 d old hamsters were exposed to short days (SD, 10L:14D, N=11) or long days (LD, 16L:8D, N=9) for 2 weeks. SD inhibited testes and body weight but did not significantly affect the K_d (SD: 36.9±7.5 pM; LD: 17.3 ±2.04 pM) or B_{max} (SD: 2.46±0.19 fmol/mg; LD: 2.39 ±0.13 fmol/mg) of the IMEL sites. These results suggest that IMEL sites may not be regulated by chronic ligand exposure. (Supported by NIH DK42056 to M.J.D.)

366.9

DAILY PROFILES OF IN VIVO 5-HIAA AND GLUTAMATE RELEASE IN THE AMYGDALA OF THE FEMALE SYRIAN HAMSTER. U.E. Hauser, I. Van den Beukel and J.D. Glass* Dept. Biological Sciences, Kent State University, Kent, OH 44224.

The amygdaloid complex is involved in various cyclic functions, including behavior, sleep-wake patterns, and reproduction. The presence of extensive serotonergic and putative glutamatergic projections prompted us to examine the daily pattern of extraneuronal 5-hydroxyindolacetic acid (5-HIAA) and glutamate (GLU) in freely-behaving animals using microdialysis. Intact female hamsters were outfitted with microdialysis probes in or near the medial amygdaloid nucleus, and hourly samples were collected. A GLU release pattern was clearly visible, showing low levels during the day, and 3- to 5- fold increases soon after lights off. 5-HIAA release increased in anticipation of darkness, and reached peak levels (1.2- to 1.5-fold over daytime levels) about one hour before the GLU peak. This pattern appears to be location-specific. It is of interest that comparable GLU and 5-HIAA profiles are seen in the suprachiasmatic region, and therefore might be related to circadian function.

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366.10

DISCRETE INFLUENCES OF ACETYLCHOLINE ON THE CHOLINERGIC, NORADRENERGIC AND SEROTONERGIC NEURONS IN THE ANESTHETIZED RAT BRAINSTEM.

Y.Koyama and Y.Kayama*. Dept. of Physiology, Fukushima Med. Col., Fukushima 960-12, Japan.

Cholinergic neurons in the laterodorsal tegmental nucleus (LDT), noradrenergic neurons in the locus coeruleus (LC) and serotonergic neurons in the dorsal raphe (DR) are major components of the brainstem systems controlling activity of the upper brain. To clarify the cholinergic influences on these projection systems, acetylcholine (ACh) was applied ionophoretically to LDT, LC and DR neurons of urethane-anesthetized rats during single unit recording. ACh had an inhibitory effect on about half of the LDT and DR neurons generating broad spikes, which were postulated to be cholinergic or serotonergic, respectively. None of them were excited by ACh. Of the LC neurons, about one third were excited by ACh, while others were not influenced except only one neuron inhibited. These results suggest that the LDT neurons were inhibited via autoreceptors, and that neurons in the LC and DR, which were similar in that they cease firing during paradoxical sleep, are under the different control by cholinergic neurons. Influences of noradrenaline and serotonin on these neurons were also examined.

366.11

RESERPINE ABOLISHES THE PHASE-SHIFTING EFFECT OF TRIAZOLAM ON LOCOMOTOR ACTIVITY RHYTHMS OF GOLDEN HAMSTERS.

P.D.Penev*, P.C.Zee# and F.W. Turek. Dept. Neurobiology and Physiology, and #Dept. Neurology, Northwestern University, Evanston, IL 60208.

Injections of the short-acting benzodiazepine triazolam (Tz) 6 h before activity onset (CT6) produce large phase advances of locomotor activity circadian rhythms (CR) in young hamsters. The phase-shifting effect of the drug is absent or attenuated in old animals. Decreased monoamine transmitter levels, receptor densities and neuronal populations are associated with impairment of major integrative systems during senescence. The objective of this study was to determine if monoamine depletion with reserpine (Res) will change the phase-shifting effect of Tz on CR in young hamsters. Wheel running activity of 12 male golden hamsters (8 wks old) was continuously monitored in constant darkness. After a stable free-running circadian rhythm was established 6 animals were injected with Res (2.5 mg/kg s.c.), the rest received vehicle (Veh). Eight to ten days later all animals were injected with Tz (1 mg i.p.) at CT6 and the rhythm was monitored for 2 more weeks. Chi-square periodogram and ANOVA were used to evaluate data. Daily wheel-running activity was reduced by 83.3 +/- 8.4% after reserpine treatment ($p<0.001$). A free-running CR persisted (Chi-square, $p<0.05$) both after Res (period T=24.25 +/- 0.07h) and Veh (T=24.1 +/- 0.04h) treatment. Triazolam treatment at CT6 produced phase advances that were larger than 20 min in all vehicle-treated animals and in 1 reserpine treated hamster. The mean phase shifts for the control (83.3 +/- 18.4 min) and reserpine (12.3 +/- 25.6 min) groups were significantly different ($p<0.05$) from each other. Our results suggest that: 1. Free-running activity CR persists with reduced amplitude after Res treatment. 2. Reserpine significantly eliminates the phase shifting effect of Tz on activity CR. 3. Reserpine application to young hamsters produces changes in the circadian system similar to those observed with aging.

366.12

GABAERGIC AGENTS WHICH PREVENT LIGHT-INDUCED PHASE SHIFTS OF THE CIRCADIAN SYSTEM DO NOT INHIBIT THE PHOTIC REGULATION OF FOS-LIKE IMMUNO-REACTIVITY IN THE HAMSTER SUPRACHIASMATIC NUCLEUS. **C.M. Kaufman*, K. Shimomura, C.S. Colwell, and M. Menaker. NSF Center for Biological Timing, Dept. Biology, Univ. of Virginia, Charlottesville, VA 22901.**

Previous studies have shown that agents which alter GABA receptor function can prevent light-induced phase shifts of the circadian rhythm of locomotor activity in hamsters. The GABA antagonist bicuculline prevents light-induced phase delays, but not light-induced phase advances[1]. Conversely, the benzodiazepine diazepam, which can potentiate GABA activity, blocks only light-induced phase advances[2]. In the present study, we found that these agents did not interfere with photic induction of Fos-like immuno-reactivity (Fos-LI) in the suprachiasmatic nucleus (SCN). By themselves, these agents did not cause an induction of Fos-LI in the SCN. These results suggest either that Fos-induction occurs at a point in the light input pathway upstream from where these drugs are acting or that Fos-induction is not part of this light signal transduction cascade.

1. Ralph, M. and M. Menaker (1985) Brain Res. 325: 362-365.
2. Ralph, M. and M. Menaker (1985) Brain Res. 372: 405-408.

366.13

A CHOLINOCEPTIVE SITE IN THE BASAL FOREBRAIN IS INVOLVED IN CANINE NARCOLEPSY. S. Nishino, J. Shelton, M. S. Reid, J. M. Siegel, W. C. Dement* and E. Mignot. Psychiatry Dept., Stanford University Sch. of Med., Palo Alto, CA. Psychiatry Dept., UCLA Sch. of Med., Los Angeles, CA.

Narcolepsy is a REM sleep disorder may result from abnormalities in both the cholinergic and monoaminergic systems. Pharmacological studies on canine narcolepsy have shown that systemic administration of muscarinic agonists, α1 antagonists and D2/α2 agonists aggravate cataplexy, a pathological manifestation of REM sleep atonia in narcolepsy. A possible site of action for these effects might be the pontine reticular formation (PRF), a region where carbachol, a muscarinic agonist, induces muscle atonia in narcoleptic and control canines (M. S. Reid, personal communication). In this study, we have investigated the effects of locally injected monoaminergic and cholinergic compounds on cataplexy (by a behavioral assay) and polygraph recordings. Carbachol (1-10 nmol), prazosin, an α1 antagonist, (1-100 nmol) and BHT-920, a D2/α2 agonist, (1-10 nmol) were first administered to the narcoleptic Dobermans into the lateral ventricles, and only carbachol injections (10 nmol) induced status cataplecticus and a long-lasting muscle atonia with desynchronized EEG. This effect was completely antagonized by the administration of an α2 antagonist, yohimbine (96 μg/ kg i.v.), in direct contrast to the effect of carbachol injection (10 nmol) into the PRF (Nucleus reticularis pontis oralis), where yohimbine did not modify the carbachol-induced muscle atonia. These results suggest that cholinoceptive sites other than the PRF are involved in the regulation of cataplexy and/or REM sleep atonia in narcoleptic canines. We therefore injected carbachol into several brain structures and found that carbachol (10 nmol) injected unilaterally or bilaterally into the basal forebrain (diagonal bundle) induced status cataplecticus and a long-lasting muscle atonia with desynchronized EEG, while carbachol injections into the lateral preoptic area, lateral septum and amygdala had no clear effect. Yohimbine also antagonized this carbachol effect in the basal forebrain. Histological confirmation of the sites of drug injection will be performed when the animals are sacrificed. Our current results represent the discovery of the most rostral cholinoceptive site involved in REM sleep atonia in a pathological model.

366.14

THE INJECTION OF CHOLINERGIC DRUGS INTO THE SAME SITE IN THE BRAINSTEM RETICULAR FORMATION RESULTS IN DIFFERENT RESPONSES WHICH DEPEND ON THE ANIMAL'S PRIOR BEHAVIORAL STATE. F. López-Rodríguez*, K. Kohlmeier, F.R. Morales, and M.H. Chase. *Dept. of Physiology, Dept. of Anatomy and Cell Biology, and the Brain Research Institute. UCLA School of Medicine, Los Angeles, CA 90024.*

The microinjection of cholinergic drugs into the pontine reticular formation elicits active sleep-like states that are comprised of either some or all of the principal physiological patterns of activity that characterize spontaneous active sleep, i.e., PGO waves, EEG desynchronization, atonia and rapid eye movements. The different patterns of activity that occur have been attributed to the site of injection (Vanni-Mercier et al., *Arch. Ital. de Biol.*, 1989, *127:* 133-164). However, we have observed that different states arise even when cholinergic drugs are injected into the exact same location (*Sleep Research*, 1992, 21:8). The present study was conducted to explore the basis for the differences in the effect of the drug. A combination of acetylcholine (2M) and neostigmine (2M) was injected by microiontophoresis into the pontine reticular formation for three minutes with currents of 300-700 nA in four chronic, unanesthetized cats. Injections were applied into the site with the shortest latency of effect (*ibid.*), which corresponded to the dorsal region of the nucleus pontis oralis. Two injections into the exact same location were carried out on each experimental day; a minimum of four hours elapsed between injections. With identical injections on the same day, the state of the animal at the time of injection appeared to be the key factor in determining the response. When the animal was awake, cholinergic injections first resulted in a dissociated state in which the animal remained awake with a desynchronized EEG, even though atonia and occasionally PGO waves were present; sometimes this state was followed by slow waves in the EEG, with the animal still exhibiting atonia; subsequently, an active sleep-like state ensued. However, if the cat was in quiet sleep at the time of the injection, a typical active sleep-like state followed; on a few occasions there was a transitional period before active sleep which was characterized by a synchronized EEG, PGO waves and atonia. The results of this study indicate that the state of the animal at the time of drug injection is a critical variable that influences the responses which are induced by cholinergic stimulation of the pontine reticular formation. Supported by Grants NS09999 and MH43362.

366.15

VERAPAMIL INCREASES WAKEFULNESS AND SUPPRESSES REM SLEEP IN RATS.

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The dose-response effect of intraperitoneal administration of verapamil, a calcium channel blocker, on sleep in rats was examined. The 5mg/kg dose had no significant effect on sleep, but the 10mg/kg dose increased wakefulness during the first 3 hours following drug adminisration. This dose also increased REM sleep latency and suppressed REM sleep during the 6 hours of EEG recording. The findings suggest that doses of verapamil used for their cardiovascular effects in humans may also be responsible for deleterious effects on CNS.

366.16

INHIBITION OF NITRIC OXIDE (NO) SYNTHESIS SUPPRESSES SPONTANEOUS SLEEP AND TRANSIENTLY DELAYS INTERLEUKIN-1 (IL-1)-INDUCED SLEEP BUT NOT FEVER

Levente Kapás*, Masaaki Shibata, James M. Krueger Dept. Physiology, Univ. Tennessee, Memphis, TN 38163

The effects of a competitive NO synthase inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME), on sleep, and on sleep and febrile responses induced by IL-1 were studied. Male New-Zealand rabbits were injected with L-NAME iv (10, 100 mg/kg) or intracerebroventricularly (icv, 0.05-5 mg). In separate experiments L-NAME was injected icv (5 mg) or iv (100 mg/kg) 30 min before icv injection of 20 ng IL-1ß. Sleep-wake activity and brain temperature (T_{br}) were determined for 6 h after treatments. Iv and icv injections of L-NAME dose-dependently suppressed non-rapid-eye-movement-sleep (NREMS) and REMS without affecting T_{br}. IL-1ß induced NREMS and fever when injected alone. If animals were pretreated either iv or icv with L-NAME then given IL-1ß the somnogenic actions of IL-1ß were delayed 2-3 h but not the pyrogenic actions of IL-1. The suppression of sleep after L-NAME indicates that NO may contribute to sleep-promoting mechanisms. Since higher doses of L-NAME increase blood pressure and decrease cerebral blood flow it is also possible that the arousal response to L-NAME is due to hemodynamic changes. Current data suggest that NO has little to do with icv IL-1ß-induced fever.

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366.17

IDENTIFICATION OF THREE ADDITIONAL PUTATIVE OSCILLATOR PROTEINS (POPs) FROM THE EYE OF *APLYSIA* AS STRESS-RELATED PROTEINS. C. Koumenis*, M. Nunez-Regueiro and A. Eskin. Dept. of Biochem. and Biophys. Sci., Univ. of Houston, Houston, TX 77204.

Elucidation of a circadian oscillator requires the identification of its components followed by the determination of the biochemical processes by which these components interact with one another. Using 2-dimensional gel electrophoresis to find putative oscillator proteins (POPs), we searched for and found a number of proteins whose synthesis was altered by entraining agents and by other agents that perturb the circadian rhythm. To determine the function of POPs, we have begun to obtain their amino acid (aa) sequences. POP-1 (40-kD, pI 5.6), has already been identified as a member of the annexin family of proteins. Here we report the identification of 3 additional POPs.

POP-2 (36-kD, pI 5.7) has been identified as porin. Two non-overlapping sequences (30 aa and 25 aa) from this protein were found to have 60% and 72% identity with human porin. Porins are non-selective channels found in the outer membranes of mitochondria and plasma membranes of bacteria. In bacteria, the expression of this protein is regulated by osmotic pressure conditions.

POP-3 (70-kD, pI 5.1) has been identified as GRP78, which belongs to the 70-kD heat-shock family of proteins. Based on 2 non-overlapping sequences (40 aa each), the aa sequence of POP-3 was found to have a 90% sequence identity with the human GRP78 heat shock protein. GRP78 is in the endoplasmic reticulum and appears to function as a molecular chaperone. Heat shock experiments, in which eyes were exposed to 37°C for 30 min, provided additional evidence that POP-3, as well as another unsequenced POP (63-kD) are heat shock proteins.

POP-4 (31-kD, pI 5.6) yielded a 37 aa sequence with weak similarities to the *E. coli* stringent starvation protein (SSP) (40% identity) and the small heat shock protein HSP26 from soybean (30% identity). SSP may interact with RNA polymerase to negatively regulate the transcription of certain genes. The function of HSP26 is unknown. The synthesis of POP-4 also increased in response to a 37°C heat shock.

It is interesting that these three newly characterized POPs are "primitive" proteins in that they appear to be highly conserved and are similar to proteins found in bacteria. In addition, many environmental factors other than stress induce the synthesis of these proteins. Since it is likely that the circadian clock evolved from some long-lasting and profound effect of light on the biochemistry of a primitive organism, effects of light on these POPs could indicate such early steps. If so, then light may have similar effects on these proteins in *Aplysia* and some primitive organism. We are currently investigating this possibility.

366.18

NORDIHYDROGUAIARETIC ACID, AN INHIBITOR OF LIPOXYGENASE IN ARACHIDONIC ACID METABOLIC PATHWAY, PHASE-SHIFTS THE *APLYSIA* EYE RHYTHM. U. Raju and A. Eskin*. Dept. of Biochem. and Biophys. Sci., Univ. of Houston, Houston, TX 77204.

A number of proteins in the eye of *Aplysia* have been identified as putative oscillator proteins (POPs) based on their responses to light, cGMP, serotonin (5-HT) and cAMP. One of the POPs, POP-1, is a 40-kDa (pI 5.6) protein whose synthesis was decreased by light and cGMP and increased by 5-HT treatments. As a first step to investigate the function of this protein, we obtained a partial amino acid sequence of it. We discovered from this sequence and subsequent immunoblot analysis that the 40-kDa protein is an annexin (probably annexin-1). Annexins are a unique family of Ca^{2+}/phospholipid binding proteins. Annexins may modulate arachidonic acid metabolism by their ability to inhibit phospholipase A_2 activity. Some other proposed functions of annexins are regulation of some second meesengers, serving as Ca^{2+} channels, and controlling membrane protein-cytoskeletal linkages and exocytosis.

Identification of POP-1 as an annexin led us to hypothesize that arachidonic acid metabolism plays a role in the *Aplysia* eye circadian system. To test this hypothesis, we examined the ability of several inhibitors of enzymes in the arachidonic acid metabolic pathway to perturb the eye rhythm. Pulse treatments of isolated eyes with a lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA), phase-shifted the rhythm. The phase response curve (PRC) obtained using NDGA appears to be an "all-delay" PRC. The phase-shifting ability of NDGA suggests that arachidonic acid and some of its metabolites may play a role in the eye circadian system. Also, further evidence has been obtained for a role of POP-1 in the eye circadian system. With these results we have closed an "experimental loop" in which physiological properties of the circadian system were used to find POPs and now properties of POPs are beginning to yield new information about the nature of the circadian system. (Research supported by NIMH Grant 41979.)

366.19

Pharmacological heterogeneity of basal forebrain neurones,

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In a recent study, we demonstrated that cholinergic neurones within the substantia innominata, display a very homogeneous responsiveness to the major neurotransmitters contained in the afferent fibers that originate in the brainstem. Their responses include most notably a depolarization to noradrenaline and a hyperpolarization to muscarine and serotonin (Khateb et al., 1991). Another class of neurones within the same area, which displays 40 Hz oscillations and may represent non-cholinergic cells (Mühlethaler et al., this meeting), responds differently from the cholinergic neurones to the same neurotransmitters. This other class of cells can be further subdivided into two major subclasses according to their differential sensitivity to the neurotransmitters. One subclass of cells was hyperpolarized by noradrenaline and had very homogeneous responses to the other neurotransmitters, being consistently and strongly hyperpolarized by both muscarine and serotonin. In the second subclass, all the cells were depolarized by noradrenaline but showed variable responses to the other neurotransmitters. In particular, one subset of neurones was hyperpolarized by muscarine, while another subset was depolarized by muscarine. This pharmacological heterogeneity suggests that these presumed non-cholinergic neurones may possess heterogeneous receptors and perhaps also neurotransmitters, and may thus play different functional roles in determining the state modulated activity of their target structures. (Swiss NSF, Fondation Fyssen, Lyonnaise des banques and Canadian MRC).

366.20

Oscillatory bursts in basal forebrain cholinergic neurons induced by NMDA,

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We recently described the distinct electrophysiological properties of a major class of basal forebrain neurons recorded within the substantia innominata in guinea-pig brain slices. These cells were characterized by the presence of a strong A current and low threshold calcium spikes. When depolarized from rest, they fired at a slow tonic rate (maximum 10-15 Hz). When stimulated from a hyperpolarized level, they gave rise to a burst of 2-3 high frequency spikes (>100 Hz) followed by a long after-hyperpolarization (>200ms). Following intracellular filling with biocytin, the recorded cells displaying this burst activity were shown by immunohistochemistry to contain choline acetyl transferase (ChAT) and thus to be cholinergic. Glutamate agonists (NMDA, AMPA and trans-ACPD) were all found to depolarize cholinergic cells. Moreover in the case of NMDA, sustained slow oscillatory bursts (1-5 Hz) were elicited by the continuous application of the agonist when the membrane was held at or slightly below rest. These oscillations persisted in the presence of TTX, and their frequency was voltage dependent. They were blocked in a reversible manner by the application of the NMDA antagonist, D-AP5. These results suggest that glutamate, which could be released from putative glutamate containing neurons of the cerebral cortex and brainstem reticular formation that project to the basal forebrain, may stimulate an oscillatory burst firing mode in cholinergic basal forebrain neurons. In this mode, the cholinergic cells could in turn drive their cortical target neurons in a delta or theta rhythm of activity. (Swiss NSF and Canadian MRC).

367.1

COMPARISONS OF CALL AMPLITUDE AND AUDITORY SENSITIVITY IN ANURAN AMPHIBIANS. **J. H. Fox*. Dept. of Psychology, University of Texas, Austin, TX 78712.**

It is unclear how call amplitude and auditory sensitivity should interrelate interspecifically. Three possibilities were considered: (1) Species become specialized for longer or shorter range communication by increasing or decreasing both factors together. (2) One factor compensates the other, such that, for instance, species with low amplitude calls have enhanced auditory sensitivity. (3) The two factors are unrelated, evolving under dissimilar selection pressures. A database was compiled from published sources and analyzed, in order to address this and other issues. Low, nonsignificant correlations were found between call amplitude and estimated toral multiunit sensitivity for amphibian papilla ($r = 0.035$, $n = 28$, $p > 0.8$), basilar papilla ($r = 0.056$, $n = 28$, $p > 0.75$), and combined ($r = 0.060$, $n = 27$, $p > 0.75$) frequency ranges. These results suggest that call amplitude and auditory sensitivity evolve in response to dissimilar selection pressures. For example, no environmental predictors of auditory sensitivity are known, but certain habitat differences may affect call characteristics. Modeling results indicate that in heavily vegetated environments, benefits from higher amplitude calls are wasted, and lower amplitudes are more efficient. As predicted, call intensity and foliage density are negatively correlated ($r = -0.275$, $n = 43$, $p < 0.05$). Body size is the only factor known to predict both call amplitude ($r = 0.441$, $n = 64$, $p < 0.0002$) and auditory sensitivity (e.g. combined, $r = 0.737$, $n = 41$, $p << 0.0001$). (Supported by NSF grant BNS-9021185.)

367.2

CENTRAL AND PERIPHERAL SEX DIMORPHISMS IN MALE AND FEMALE CRICKET FROGS, *ACRIS CREPITANS*. B. E. McClelland* and W. Wilczynski, Dept. of Psychology, University of Texas, Austin, TX 78712.

Anuran reproductive behavior is highly sexually dimorphic, with vocalizations usually exhibited solely by males. We measured the volumes of laryngeal components, as well as the preoptic area (POA) and the ventral hypothalamus (VH) of male and female cricket frogs *(Acris crepitans)* to establish and compare sexual dimorphism in the peripheral and central areas associated with vocalization in these frogs. Following snout-vent length (SVL) and head width measurements, the heads of 7 males and 7 females were isolated and cut at 10 or 25 µm. The areas of the larynx, POA and VH were measured every 10 slices and the volumes and brain size were calculated. The females were larger than the males [all MALE vs FEMALE (SVL:2.204 vs 2.576; HEAD:.663 vs.793; all cm)], however, estimates of brain size derived from representative sections show a non-significant trend toward larger brain size in males. The volumes of the laryngeal components were significantly larger in males (ARYTENOID CARTILAGE:.612 vs .046; VOCAL CORDS:.045 vs .004; CONSTRICTOR MUSCLE:.516 vs .064; DILATOR MUSCLE:.194 vs .023; all mm^3). However, neither the POA nor the VH volumes were different between the sexes (POA:.066 vs .060; VH:.056 vs.062; all mm^3), even after correction for brain size differences. The sexual dimorphism in the laryngeal anatomy size is functionally related to vocalization behavior, although the POA and VH do not show a significant degree of difference in size between the sexes. In many vertebrate species, the POA and VH have separate functions in males and females and are sexually dimorphic. However, in amphibians the POA may be responsible for both male calling and female phonotaxis behavior. Our results show that an obvious sexual dimorphism in peripheral anatomy does not necessarily predict a concomitant degree of sexual dimorphism in forebrain areas responsible for the control of behaviors involving those peripheral structures. (Research supported by NSF BNS-9021185.)

367.3

METABOLIC MAPPING USING CYTOCHROME OXIDASE HISTOCHEMISTRY IN FROG BRAIN AREAS ASSOCIATED WITH AUDITORY PROCESSING AND REPRODUCTIVE BEHAVIOR. C. A. Marler*, W. Wilczynski and F. Gonzalez-Lima, Depts. of Zoology and Psychology, University of Texas, Austin, TX 78712.

Cytochrome oxidase stain was used to map metabolic activity in breeding gray treefrogs, Hyla chrysoscelis. Brainstem auditory areas, including the dorsolateral nucleus, superior olivary nucleus, and torus semicircularis, were darkly stained indicating high metabolic capacity. In contrast, two forebrain systems, receiving auditory input, showed differential staining. The interconnected areas of the central thalamic nucleus, striatum, and ventral hypothalamus were lightly stained, whereas the interconnected areas of the anterior nucleus, medial pallium, septal and preoptic areas were darkly stained. Furthermore, within the latter system, preliminary results indicate that there are sex differences. For example, the preoptic area, which is associated with male reproductive behavior, and the septal area stain more darkly in males. We are also currently examining whether experimental manipulations in testosterone levels can cause changes in areas of the brain where sex differences are found. (Supported by NIMH NRSA F32 MH10204 and NIMH grants T32 MH18837, MH43353)

367.4

TRIGEMINAL DEAFFERENTATION DISRUPTS BOTH THE LOCATION AND GRASPING COMPONENTS OF PREHENSION IN THE PIGEON. R. Bermejo* and H. P. Zeigler. Dept of Psychology, Hunter College (CUNY), New York, NY, 10021.

The pigeon's pecking response functions to transport a prehensile effector organ (the jaw) towards biologically significant objects such as food. During pecking at food, the transport component (head movement) is precisely localized and the grasp component (jaw movement) is scaled to the size of the object. To examine the kinematics of pecking in the pigeon, a head mounted accelerometer and a magnetosensitive jaw movement transducer were used in conjunction with a touch-sensitive device attached to the computer's monitor. Subjects were food reinforced for pecks made to a computer-generated circular stimulus. During testing, both the position and the size of the stimulus were randomly varied on successive trials. The locations of pecks made to the stimulus and the temporal organization of head and jaw movements during conditioned and ingestive pecks were monitored on each trial. Preoperatively subjects tracked the location of the stimulus on successive trials with great precision. Following trigeminal deafferentation, *which disrupted tactile but not proprioceptive inputs from the jaw*, both peck location and the grasping and manipulation of food pellets were significantly disrupted. The effects upon the head transport (localization) component are similar to the effects of limb deafferentation upon prehensile behaviors in primates.

(Supported by NSF Grant BNS88-10722 , NIMH Grant MH-08366. and Research Scientist Award MH-00320.)

367.5

JAW MUSCLE (EMG) ACTIVITY AND AMPLITUDE SCALING OF JAW MOVEMENTS DURING EATING IN THE PIGEON. R. G. Bout and H. P. Zeigler*. Organismal Zoology, Leiden Univ., Netherlands and Biopsychology Program, Hunter College (CUNY), New York, NY, 10021.

Amplitude scaling of gape size to object size is a prominent feature of eating in pigeon, and involves both visual and somatosensory inputs. Kinematic and neuromotor mechanisms of scaling were examined by correlating variations in interbeak distance (gape) with jaw muscle EMG activity during the ingestion (grasping, stationing, intraoral transport) of food pellets (3.9, 6.4, 8.7 mm). A magnetosensitive transducer was used to monitor variations in gape; conventional EMG techniques were used to record jaw muscle activity in the upper and lower jaw openers and several jaw closer muscles. Kinematic analysis indicates that the motor control strategy mediating amplitude scaling involves the modulation of both jaw opening velocity and jaw opening rise time, but their contribution differs for each phase. EMG analysis showed that, although both maxilla and mandible are moveable in pigeon, scaling involves primarily variations in the amplitude and duration of lower jaw-opener (depressor) activity. These are produced by modulating agonist burst duration and the onset of antagonist (pterygoid) activity. Scaling mechanisms are discussed in relation to task requirements and compared with mechanisms mediating amplitude scaling in humans.

(Supported by the Program in Neurobehavioral Morphology, Leiden University and by Grants (NSF) BNS88-10722; (NIMH) MH-08366;RSA MH-00320 to HPZ)

367.6

THE EFFECT OF ELEVATED DIETARY TRYPTOPHAN ON CENTRAL LEVELS OF SEROTONIN AND ITS METABOLITES IN BIRDS. M.M.S.Moore*, W.J. Kuenzel and J.A.Mench. Dept. of Poultry Sci., Univ. of Maryland, College Park, MD 20742.

Supplemental dietary tryptophan (TRP) has been shown to decrease aggression in developing and mature fowl(Gallus domesticus). To investigate whether this effect is mediated through central serotonergic transmission, cannulae were surgically implanted into the cisterna magna of six adult birds.Using a switch-back design, birds were fed high TRP(1.5%TRP) and control(.19%TRP) diets. Sample volumes of 50-200ul of cerebrospinal fluid were drawn from the cisterna magna of each bird. Samples were analyzed for 5-hydroxytryptophan, serotonin, and 5- hydroxyindoleacetic acid using high performance liquid chromatography. Results showed a significant difference ($p<.01$) in the rate of serotonin turnover in TRP treated birds while actual serotonin levels remained constant.

367.7

EFFECTS OF ECTOSTRIATAL LESIONS, WULST LESIONS AND HEMISPHERECTOMY UPON VISUAL CONCEPT OF FOOD IN PIGEONS. *S.Watanabe*.Dep. of Psychol.,Keio Univ.,Tokyo,Japan.*

Pigeons were trained on visual discrimination between four kinds of grains and four non-edible objects in an operant chamber. In Experiment I the birds received surgery of the ectostriatal lesions or Wulst lesions after they learned the discrimination. Either lesion did not impair the discrimination. In Experiment II pigeons were trained on the same task but received hemispherectomy after they learned the task. When they used the eye contralateral to the ablated hemisphere, they showed deficits in food discrimination. Whereas they maintained the discrimination with the eye ipsilateral to the ablated hemisphere. A feeding test with screws and hemp seeds also demonstrated a similar lateralized deficits in visual discrimination of food. These results suggest 1) either the ectostriatum or Wulst alone successfully process visual discrimination of food, and 2) damages to both structure impaired the discrimination.

367.8

MORPHOLOGICAL ALTERATIONS OF NEURONS DUE TO SOCIAL ENVIRONMENT CHANGES ARE RESTRICTED TO "AROUSAL" AREAS IN THE ZEBRA FINCH FOREBRAIN. A. Rollenhagen and H.-J. Bischof*. Universität Bielefeld, Fakultät Biologie, Postfach 8640 W-4800 Bielefeld 1, Germany.

2-Deoxyglucose studies (Bischof and Herrmann 1986) show that four areas of the forebrain of zebra finch males are selectively activated in arousing situations. We likewise showed that neuron morphology of these areas is affected by rearing conditions and by short social experience in adulthood (Rollenhagen and Bischof 1991). In this study we investigated whether these neuronal reactions to changing social conditions is a special feature of these "arousal" areas.

Rearing the birds in isolation decreases the density of spines, compared to social rearing, in the two "arousal" areas ANC (Archi-Neostriatum caudale) and the visual wulst. The acoustic field L and the visual ectostriatum showed only slightly decreased spine densities. Short social contact (7 days) after isolated rearing enhanced spine densities only in the "arousal" areas.

Our results clearly demonstrate that neurons of the "arousal" areas can react quickly to changes of the social environment, whereas the other areas can't. We suggest that these alterations are in some way linked to changes of activation by arousing stimuli.

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367.9

MEDIAL GENICULATE NEURONS IN THE SQUIRREL MONKEY SENSITIVE TO COMBINATIONS OF COMPONENTS IN A SPECIES-SPECIFIC VOCALIZATION. J.F. Olsen* and J.P. Rauschecker. Neuroethology Unit, LNP, NIMH, Poolesville, MD 20837.

We are investigating the neural mechanisms in the squirrel monkey for processing species-specific calls. The chuck is a stereotyped, broadband call (bandwidth > 19 kHz) that consists of an initial, upward frequency modulated component, followed immediately by a downward frequency modulated component and a constant frequency component (Winter et al., Exp. Brain Res. 1:359, 1966), respectively called the flag, mast, and cackle. Individually, the components of the chuck are acoustically similar to vocalizations produced in other contexts, thus presenting a discrimination problem to the monkey. As a possible neural mechanism for solving this problem, we sought neural sensitivity to combinations of the flag, mast, and cackle in the medial geniculate. The chuck was modeled on a computer and the components were presented, singly or in combination, from a free-field speaker to a halothane-anesthetized monkey while neural responses were recorded extracellularly. Thirty neurons were found that responded reliably to the model chuck presented at 70 dB SPL; of these, 8 responded only poorly to the three components presented individually. When two or more components were combined as found in the natural chuck, these 8 neurons were facilitated; gating the components separately or introducing more than 5 ms of delay between them abolished the facilitated responses. By contrast, we found 5 other neurons that responded well to the cackle alone and poorly to the cackle preceded by the mast, for cackle amplitudes up to 24 dB louder than the mast. The results suggest that combination-sensitivity may be a neural mechanism for categorizing acoustically related vocalizations that differ in their behavioral significance.

367.10

ENCODING SIGNAL REPETITION RATE AND DURATION BY INFERIOR COLLICULAR AND AUDITORY CORTICAL NEURONS OF THE FM BAT, *EPTESICUS FUSCUS*. M. Wu, T. T. Hou and P. H-S Jen*, Division of Biological Sciences, University of Missouri-Columbia, Mo 65211

Under free field stimulation conditions, we studied encoding of signal repetition rate and duration characteristic of different phases of hunting by the inferior collicular and auditory cortical neurons of the big brown bat. For each recorded neuron, the best frequency (BF) and the minimum threshold (MT) were first determined. The neuron's intensity rate function was then studied in order to determine its best intensity at which the neuron fired maximally. Train stimuli (150 ms duration delivered at 1-2/s) consisted of 2 pulses (10ms duration,100ms interpulse-interval = 10/s) or 6 pulses (4ms duration, 25ms interpulse-interval = 40/s) or 15 pulses (1.5 ms duration, 10ms interpulse-interval =100/s) were respectively delivered at the best intensity and the number of impulses of the neuron was recorded. Our data show that both inferior colliculi and auditory cortex contain different populations of neurons which responded maximally to one of the presented train stimuli. There were also neurons which responded equally to either two or all train stimuli. However, neurons in these two auditory centers encode these three train stimuli in different manners. While inferior collicular neurons generally responded in an one-to-one fashion to each pulse within a train stimulus, the cortical neurons only responded to 1-2 pulses within a train stimulus. Such responsive differences may be due to the different recovery cycles among neurons in these two auditory centers (supported by NIH DC 247).

367.11

FUNCTIONAL CONNECTIVITY WITHIN THE FM-FM AREA IN THE AUDITORY CORTEX OF THE MUSTACHED BAT. M. Kanou, T. Shimozawa, J. S. Kanwal and N. Suga*, Dept. of Biology, Washington Univ., St. Louis, MO 63130.

The FM-FM area in the auditory cortex of the mustached bat (*Pteronotus parnellii*) is 1.8 mm long along the rostro-caudal axis of the brain and consists of 3 subdivisions: FM_1-FM_2, FM_1-FM_3 and FM_1-FM_4. In each subdivision, FM-FM combination-sensitive neurons tuned to particular echo delays are systematically arranged for the representation of target distances from 7 to 310 cm (Suga & O'Neill 1979; O'Neill & Suga 1979). The aim of the present research is to explore (1) whether different types of FM-FM neurons with identical best delays interact through excitatory synapses to improve the representation of distance information, (2) whether FM-FM neurons with different best delays interact through inhibitory synapses to enhance the representation of distance information, and (3) whether FM-FM neurons show synchronized after-discharges which represent the integration of neural activities evoked by a single target.

Simultaneous recording of action potentials were made from two FM-FM neurons at different locations in the FM-FM area, and Joint peristimulus time histograms (JPSTHs) and predicted-JPSTHs (Aertsen et al. 1989) were plotted to examine the temporal relation in discharges between them. Out of 34 pairs of neurons (300-600 μm apart) studied, four pairs (12%) showed correlated activities which might be due to the common (shared) inputs. No direct interaction between paired neurons was observed even though the paired neurons were separated only by 300 μm. Unlike the cat's visual cortex, no oscillatory after-discharges were observed in the FM-FM area of the auditory cortex.

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367.12

RESPONSES OF CORTICAL AND THALAMIC FM-FM AND CF/CF NEURONS OF THE MUSTACHED BAT TO SPECIES-SPECIFIC COMMUNICATION SOUNDS. K.K. Ohlemiller*, J.S. Kanwal and N. Suga. Dept. Biol., Washington University, St. Louis, MO 63130.

The central auditory system of the mustached bat is specialized for echolocation. Over 60% of its auditory cortex is devoted to neurons sensitive to combinations of biosonar pulse and echo. For communicating with conspecifics in the dark, this species also uses a diverse array of calls. It is of interest whether biosonar-specialized neurons also process these calls, and thus serve a dual function. We examined this question by comparing the responses of FM-FM and CF/CF neurons in the auditory cortex and thalamus to biosonar sounds and to calls.

Calls were digitized at 250 kHz and classified based on their spectrograms. Eight call classes were presented. The 1-3 most effective calls were then used for further analysis. Minimum thresholds (MTs) and best amplitudes (BAs) in dB SPL, and maximum response (MR) were compared for calls and biosonar signals. For biosonar, MT and BA were taken to be that of the echo in the presence of the pulse. Since the temporal characteristics of the two stimulus types were different, MR was taken to be the largest sum of spikes in any 10 ms window.

Results appeared similar in cortex (n=57) and thalamus (n=10), and for FM-FM (64%) and CF/CF neurons: Neurons were typically not selective for call class, responding to an average of four classes. The pattern of selectivity varied from neuron to neuron. Biosonar sounds were clearly the preferred stimulus. MTs averaged 25.4±11.9 dB SPL for biosonar and 59.0±14.3 dB SPL for calls. That is, neurons were ~50 times more sensitive to biosonar than to calls. While responses to biosonar showed BAs averaging 49.9±12.1 dB SPL, calls responses had BAs of 77.9±8.8 dB SPL. 37% of the neurons did not show a BA to calls. The MR to biosonar averaged 2.13 times that to calls.

Compared to biosonar signals, responses of FM-FM and CF/CF neurons to species-specific calls appear modest. Thresholds of response to calls are reasonable, however, since calls are emitted at 100-110 dB SPL, and presumably mediate social interactions over short distances. These neurons may therefore function in call discrimination by graded responses of neurons partially overlapping in their call selectivity.

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367.13

INHIBITION SHAPES RESPONSE LATENCIES IN THE INFERIOR COLLICULUS OF THE MUSTACHE BAT

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Two types of combination sensitive neurons in the mustache bat's medial geniculate nucleus are though to be created by the convergence of projections from two frequency contours of the inferior colliculus. One type, the so called CF/CF neurons, discharge weakly to tone bursts but fire vigorously when two tone bursts having different frequencies are presented together. The other type, the delay tuned neurons, also require two frequencies, but in addition, they require that the two tone bursts be presented in a specific temporal order. The delay tuned properties are a consequence of the latency differential with which the inputs evoked by each of the two frequencies arrive at the geniculate neuron.

Here we report that there is an orderly arrangement of latencies in the 60 kHz isofrequency contour of the mustache bat's inferior colliculus. There were three salient features of the latency arrangement: 1) neurons in the dorsal colliculus had, on the average, longer latencies than neurons located ventrally; the average median latency in the more dorsal part of the colliculus was 15.4 ms which decreased to an average of about 9.8 ms in the more ventral region. 2) The latencies recorded dorsally, within 200-400 μ of the collicular surface, ranged from 30 to about 6 ms, whereas the latencies at depths from 1000-1300 μ ranged from 5 to 10 ms. 3) a similar distribution was seen in the monaural, E-I and E-E subregions of the contour.

We also investigated the effect of GABAergic inhibition on the latencies of collicular cells with iontophoretic application of bicuculline, an antagonist specific for $GABA_A$ receptors. Bicuculline caused a latency decrease in many cells, and latency changes ranged from 1-15 ms. These values are consistent with the range of latency differences among collicular neurons.

The ordering of latencies in collicular isofrequency contours could provide the topographical substrate for the generation of the combinatorial properties in geniculate neurons. Furthermore, the data suggest that GABAergic inhibition is an important mechanism for producing the wide range of latencies expressed by collicular neurons. Supported by NIH grant DC 00268.

367.14

SELECTIVE REPRESENTATION OF CALL-SYLLABLES IN THE DSCF AREA IN THE PRIMARY AUDITORY CORTEX OF THE MUSTACHED BAT.

J. S. Kanwal*, K. K. Ohlemiller, and N. Suga. Dept. of Biology, Washington University, St. Louis, MO 63130.

Mustached bats emit a diverse array of complex communication sounds (calls) in addition to the stereotypic biosonar pulses. In contrast to biosonar, however, nothing is known about the neural processing of calls. DSCF neurons in the primary auditory cortex of the mustached bat, *Pteronotus parnellii*, overrepresent 61 and 22-28 kHz frequencies and show facilitation to combinations of these non-harmonic frequency components. While the DSCF area is important for target detection and characterization, our recent findings suggest that neurons in this area may also process calls, which range between 7 and 90 kHz and contain either constant or modulated frequencies, or noise bursts, or their combinations.

To test this hypothesis we recorded single unit responses from the DSCF area of awake, restrained bats to CF tones, to broadband noise and to call-syllables. Bats' calls were tape-recorded, classified and digitized using SIGNAL (Engineering Design Inc.) prior to playback as stimuli. DSCF neurons responded either non-selectively ("generalists") to 6 or more of the 10 call-syllables presented or selectively ("specialists") to the 1 or 2 biosonar-like syllables that contained energy in the 61 kHz region in addition to the 25 kHz region. While best amplitudes were not generally observed, both the generalists and the specialists showed upper and lower thresholds to calls and did not respond to noise bursts. For paired CF tones, DSCF neurons exhibited a wide range in the width of facilitative tuning (200 Hz to 6 kHz at 30 dB SPL above threshold) that was flanked by inhibitory regions. For single CF tones, neurons were tuned either to the 61 kHz (Type I neurons) or 25 kHz (Type II neurons) or responded to both (Type III neurons) frequency components. The generalists (Types II or III) exhibited broad tuning in the 25 kHz region, while the specialists (Types I and III) exhibited relatively broad tuning in the 61 kHz region. Neurons with narrow tuning in the 61 kHz region did not show any response to calls. Thus, DSCF neurons process both biosonar signals and calls and call-selectivity is shaped by excitatory, faciltative and inhibitory tuning.

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367.15

RESPONSE PROPERTIES OF NEURONS IN THE PALLID BAT INFERIOR COLLICULUS THAT RESPOND SELECTIVELY TO MULTIPLE FEATURES OF THE BIOSONAR PULSE .

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The pallid bat relies on passive listening to capture ground-dwelling prey, and uses its biosonar system only for general orientation. Consequently, this bat must simultaneously process two different modes of auditory input while hunting. Perhaps in response to a need to partition these sensory channels, some inferior colliculus neurons tuned to the biosonar pulse are remarkably selective for the spectrum, FM sweep direction and the duration of the biosonar pulse, which sweeps downward from 55 to 30 kHz over a period of 3-6 msec. These cells are located within the 30-45 kHz layers of the tonotopically organized region of inferior colliculus. Within this neuronal population, some neurons respond to FM sweeps in both directions, others respond only to the normal downward FM sweep, but none respond selectively to upward FM sweeps. The most selective of these neurons respond only to FM sweeps, and cannot be driven by single tones within the sweep. Some FM sweep-selective neurons also act as band-pass filters for the normal sweep duration, and cease responding when the sweep duration exceeds 15 msec. While the mechanisms that underlie the response selectivity of these cells is not yet clear, this abstract presents evidence that suggests that these neurons may integrate acoustic information over periods of 5-10 msec before responding.

367.16

THE DEVELOPMENT OF MOTOR PATTERNS IN WEAVER MUTANT MICE.

J.C. Fentress*, V.J. Bolivar, W. Danilchuk and K. Manley. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1.

Although the weaver mouse has been extensively studied in terms of biochemistry and physiology, few detailed behavioral studies examining the development of these animals exist. This mutation results in deficiencies in cerebellar Purkinje, granule, and Bergmann glia cells, as well as a dopamine deficiency due to cell loss in the substantia nigra. Behavioral symptomology includes ataxia, tremors and poor limb coordination. We have examined the ontogeny of grooming in this neurological mutant mouse. Studies have documented that rodents perform a stereotypic, highly structured set of movements during grooming. Any deviation from this set of movements may indicate an effect of the weaver mutation. Double recessive and littermate control pups were videotaped (30 fps) in a warmed container at 2 day intervals from the 3rd to the 21st postnatal day. We report data obtained from frame-by-frame analysis of videotapes, including number and mean duration of grooming bouts and amount of time spent grooming. In addition, the PEAK Motion Measurement System was used to analyze trajectories, accelerations, velocities and angles of limb movements. Mutant animals displayed larger numbers of short duration bouts and in general spent less time grooming. The two groups also displayed differences in the general style of grooming. These observed differences between mutant and littermate controls indicate behavioral manifestations of the weaver mutation. (Supported by MRC grant MA7660 to J.C.F.).

367.17

ANTERIOR MEDIAL CORTICAL LESIONS IMPAIR PERFORMANCE IN A JUMPING TASK IN THE MONGOLIAN GERBIL.

C. Ellard*, Dept. of Psychology, University of Waterloo, Waterloo, Ont.

In a jumping task, gerbils can be trained to estimate the size of a gap with precision. Previous studies have suggested that one means by which gerbils compute distance is by using retinal motion information generated by vertical head movements. In the present experiment, we trained gerbils to jump across a gap varying randomly in size from 10 cm to 40 cm. Following this, animals were either subjected to a sham procedure or received aspiration lesions of anterior medial cortex (AMC). Following surgery and a short recovery period, gerbils were tested at randomly varying distances ranging from 10 to 34 cm. AMC animals not only produced fewer head movements prior to jumping, but also produced less accurate jumps, particularly at longer distances. These results suggest that a frontal cortical system is involved in the production of head movements that are used to generate retinal motion information.

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367.18

The role of the striatum in organizing complex behaviors as revealed by play in neonatally 6-OHDA depleted rats.

Sergio M. Pellis*, Ian Q. Whishaw, Mario M. McKenna and Eddie Castañeda. Dept. Psychology, Univ. Lethbridge, Lethbridge, AB T1K 3M4 and Dept. Psychology, Arizona State Univ., Tempe, AZ 85287.

What is the role of the striatum in complex actions? If decorticated neonatally juvenile rats can still perform complex sequences of playful behavior as juveniles, suggesting that both the behavioral elements and their sequencing are organized subcortically. Other complex behavioral sequences, such as grooming, have been shown to depend upon the striatum. In this study, neonatal ventricular injections of 6-OHDA were used to disrupt the development of striatal mechanisms. Although juvenile 6-OHDA rats are smaller in size, probably reflecting impairments in feeding, they appeared relatively normal in their exploratory and locomotor behaviors. Play, however, was severely impaired. All the behavioral elements were present and indeed were more frequent, but they were not correctly sequenced. For example, playful nape contact was more likely to lead to allogrooming or mounting and thrusting, rather than the leaping away of intact rats. That is, immediate stimuli were more likely to redirect their behavior away from completing the play sequence. These findings support the hypothesis that striatal mechanisms are involved in the organization of complex sequences of behavior.

368.1

MARKED FALL IN EXTRANEURONAL DOPAMINE DURING BEHAVIORAL DESPAIR IN RATS. Z.L. Rossetti*, Y. Hmaidan, M. Lai and G.L. Gessa. *Dept. of Neuroscience, Univ. of Cagliari, 09124 Cagliari, Italy.*

Accumulating evidence indicates that abstinence from drugs of abuse is accompanied by a marked inhibition of dopamine (DA) output in the mesolimbic system. Given the role of mesolimbic DA in reward mechanisms, the suppression of DA release has been hypothesized to constitute a common neurochemical correlate for the anhedonic and depressive state associated with abstinence syndromes. Prolonged, inescapable stress is also known to result in an anhedonic and depressive state. To study the effect of inescapable stress on DA neurotransmission, we measured by microdialysis the changes in extraneuronal DA concentrations in the ventral striatum of rats exposed to the forced swimming test ("behavioral despair test"). Rats were forced to swim into plexiglas cylinders containing water at 22-24 °C. Perfusate DA concentration was measured at time intervals during the test (1 hr) and 4 hr thereafter. Body temperature was monitored through a rectal catheter. Exposing the animals to the test resulted in a dramatic fall in extraneuronal DA to about 20% of pre-exposure (baseline) values. When animals were returned to their home cages DA output progressively returned to baseline values in about 4 hr. Manipulation of water level and temperature ruled out possible hypothermic effects on DA release. Furthermore, chronic treatment with the antidepressant imipramine (20 mg/kg/day for 3 weeks) significantly reduced (-40%) both the magnitude and the duration of the inhibition of DA output. These results support the hypothesis that an inhibition of DA release may constitute a neurochemical correlate for anhedonic and depressive states.

368.2

SKF 82958, A SELECTIVE DOPAMINE D_1 AGONIST, POTENTLY INDUCES ROTATION IN 6-OHDA-LESIONED RATS.

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Our current understanding of the relative involvement of dopamine D_1 receptor activation in motor control is based heavily on the use of the prototypical D_1 agonist, SKF 38393. While quite selective for the D_1 receptor, its behavioral actions often appear weak, suggesting a minor role for D_1 receptors. However, SKF 38393 is only 45% efficacious and it poorly penetrates the blood brain barrier. The purpose of this study was to evaluate the effects of a fully efficacious D_1 agonist SKF 82958 on rotation in rats with unilateral 6-OHDA lesions. Net contralateral rotations were recorded every 5 min during a 60-min test period. SKF 82958 (0.01-1.0 μmol/kg, s.c.) induced vigorous rotation which was completely antagonized by pretreatment with the D_1 antagonist SCH 23390 (0.3 mg/kg), but only partially antagonized by pretreatment with the D_2 antagonist eticlopride (0.1 mg/kg). SKF 38393 had no effect in the same dose range (0.1-1.0 μmol/kg), but did induce rotations at 17.1 μmol/kg. At this high dose, pretreatement with SCH 23390 completely suppressed rotations, whereas pretreatment with eticlopride had no effect. The D_2 agonist quinpirole (0.033-3.3 μmol/kg) induced turning behavior that was completely antagonized by eticlopride. However, SCH 23390, at a dose that completely antagonized SKF 82958, only partially blocked the effects of quinpirole. These results indicate that a full D_1 agonist potently induces rotation, and that both D_1 and D_2 dopamine receptors play an important role in motor performance.

368.3

UNILATERAL LESION IN BARREL CORTEX INDUCES ASYMMETRIES IN BEHAVIOR AND NIGROSTRIATAL DOPAMINE: CORTICAL INTERACTION WITH BASAL GANGLIA F.S. Adams, R.K.W. Schwarting, J.A. Nagel*, J.P. Huston Inst. Physiol. Psychol. I, Univ. of Düsseldorf, Germany

Previous experiments from our laboratory have shown time-dependent lateralized changes in behavior and nigrostriatal anatomy and neurochemistry following unilateral removal of the mystacial vibrissae of rats. The present experiment investigated the time-dependent effects of unilateral radiofrequency lesion of the cortical barrel fields in light of these results. We measured lateralized changes in behavior as well as the tissue content of dopamine and serotonin plus their metabolites, in neostriatum and substantia nigra, between 1 and 16 days post-lesion. Asymmetries in exploratory behavior (thigmotactic scanning) and neostriatal metabolite/serotonin ratios that lasted from 4 to 7 days were seen. In substantia nigra, time-related asymmetries in dopamine concentrations were found with higher ipsilateral values on day 3 and higher contralateral values on day 6. On day 6, the animals had recovered from these acute effects, and thereafter, neostriatal metabolite/dopamine ratios became asymmetrical. Also after day 6, an ipsiversive bias in spontaneous and apomorphine-induced turning was seen. Finally, neostriatal serotonin was bilaterally elevated on day 16. These results parallel some of the effects previously seen following unilateral removal of the vibrissae, indicating that functional interaction between the vibrissae and basal ganglia is routed via cortico-striatal connections.

368.4

D1 AND D2 RECEPTOR MEDIATION OF MOTOR BEHAVIOR IS ALTERED FOLLOWING DOPAMINE DEPLETIONS IN NEONATAL BUT NOT IN WEANLING RATS. D.Abrams*, E.McCone, and J.P.Bruno. Neuroscience Program & Dept. of Psychology, Ohio State Univ.,Columbus, OH 43210

Coactivation of D1 and D2 dopamine (DA) receptors appears necessary for the expression of certain motor behaviors in normal rats. This can be illustrated by the ability of a DA antagonist (i.e. D1) to block the behavioral effects of the counter DA agonist (i.e. D2). Our previous data indicate that D1 and D2 receptors can mediate motor behavior independently in adult rats depleted of DA as neonates. This study focused on the ability of DA antagonists to block agonist-induced locomotion and stereotypy in adult rats treated with 6-OHDA (100 μg) or its vehicle on postnatal Day 3 or 20. The D2 agonist quinpirole produced low levels of stereotypy and locomotion in all 3 groups. In vehicles and rats depleted on Day 20, these effects were blocked by either the D1 antagonist SCH 23390 or the D2 antagonist clebopride. However, in rats depleted on Day 3, these effects could only be blocked by clebopride. The D1 agonist SKF 38393 produced no obvious motor effects in controls or in rats depleted of DA on Day 20. This drug produced a moderate stereotypy and locomotion in rats depleted on Day 3. These effects were fully blocked by SCH 23390 but not by clebopride. Thus, the D1-D2 mediation of motor behavior is qualitatively changed following DA depletions during early but not later stages of development.

368.5

DEVELOPMENT OF ALTERED D1-D2 RECEPTOR INTERACTIONS FOLLOWING DA DEPLETIONS IN NEONATAL RATS. E.McCone and J.P.Bruno*. Neuroscience Program and Dept. of Psychology, Ohio State Univ., Columbus, OH 43210

Our recent data suggest that the normal ability of selective dopamine (DA) D1 or D2 antagonists to block behavior induced by the converse agonist is lost in adults depleted of DA as neonates. The present study determined the emergence of these altered receptor interactions as a function of time after the DA depletion. Rats received either 6-OHDA (100 μg) or its vehicle on postnatal Day 3 and were tested on postnatal Day 10. The D1 agonist SKF 38393 induced sniffing, grooming, licking, and modest locomotion whereas the D2 agonist quinpirole induced sniffing, mouthing, and locomotion. These profiles were similar in vehicle- and 6-OHDA-treated pups. Unexpectedly, vehicle- and 6-OHDA-treated animals also looked similar with respect to the ability of antagonists to block these effects. Only the D1 antagonist SCH 23390 blocked the SKF 38393-induced behaviors whereas only the D2 antagonist clebopride blocked the quinpirole-induced behaviors. These results suggest that the effect of the neonatal DA depletion may be to preserve an earlier developmental form of D1-D2 receptor interaction rather than to induce a new one.

368.6

D1 AND D2 RECEPTOR-MEDIATION OF STRIATAL ACETYLCHOLINE RELEASE IN RATS DEPLETED OF DOPAMINE AS NEONATES. B.Johnson* and J.P.Bruno. Dept. of Psychology and Neurosci. Program, Ohio State Univ., Columbus, OH 43210

We have reported that the D1 and D2 receptor-mediation of sensorimotor behavior is qualitatively changed in adult rats depleted of dopamine (DA) as neonates. Activation of either D1 or D2 receptors is sufficient for the expression of normal behavior in rats depleted as neonates, whereas coactivation of both receptor subtypes is necessary for these behaviors in intact animals. Given that striatal DA and acetylcholine (ACh) interactions are necessary for normal sensorimotor behavior, we compared the effect of D1 and D2 receptor ligands on striatal ACh release in adults treated with 6-OHDA (100 μg) or its vehicle on postnatal Day 3. Striatal ACh efflux was determined in freely moving animals using in vivo microdialysis. We unexpectedly found that the D1 and D2 mediation of striatal ACh release was similar in controls and rats depleted of DA as neonates. Systemic administration of D1 receptor agonists or D2 receptor antagonists stimulated ACh release, whereas D2 receptor agonists or D1 receptor antagonists inhibited release. These in vivo release data are in marked contrast to the behavioral data and suggest that the compensations underlying the sparing from deficits in rats depleted of DA as neonates are not at the level of striatal ACh neurons.

368.7

MICRODIALYSIS OF STRIATAL DOPAMINE IN FREELY MOVING RATS: EFFECTS OF COCAINE AND COCAINE METHIODIDE. I.A. Ivens, P.H. Janak, S.B. Rodriguez*, and J.L. Martinez, Jr. Dep. of Psychology, UC Berkeley, Berkeley CA 94720.

The effect of peripherally and centrally administered cocaine on striatal dopamine levels was investigated. Microdialysis probes were implanted in the caudate nucleus and microdialysis was performed in freely moving rats. Cocaine (5.5, 7.5, and 15.0 mg/kg IP) produced a dose-dependent increase in extracellular dopamine concentrations, while cocaine methiodide (19.6 mg/kg IP, equimolar to the 15.0 mg/kg dose of cocaine), a cocaine analog with limited ability to cross the blood-brain barrier, was without effect on central dopamine levels. Previous studies in rats indicated that 5.5 mg/kg IP of cocaine modulates both aversively- and appetitively-motivated conditioning, while an equimolar dose of cocaine methiodide is without effect. Dialysis of either cocaine (0.5 mg/ml cocaine in perfusate, 2 ul/min perfused) or cocaine methiodide (in equimolar concentration) directly into the caudate nucleus produced a marked elevation of dopamine levels. Thus changes in striatal dopamine levels could be associated with the changes in conditioned performance produced by peripherally administered cocaine, and cocaine methiodide's inability to alter conditioning may be associated with its failure to cross the blood-brain barrier.

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368.8

EVIDENCE FOR DOPAMINE-DEPENDENT BEHAVIORS IN MICE WITH LOW DENSITIES OF STRIATAL DOPAMINE RECEPTORS. E.Tirelli* and J.M.Witkin. Psychobiology Laboratory, NIDA Addiction Research Center, P.O. Box 5180, Baltimore, MD, 21224-5180.

Several reports indicate that DBA/2 mice may have a lower density of striatal dopamine (DA) receptors than C57Bl/6 mice and are unresponsive to postsynaptic DA agonists (e.g. apomorphine, APO). To evaluate whether this apparent insensivity is due to a peculiar expression of postsynaptic DA activity, we studied simultaneously several behaviors (full climbing, leaning against the wall, gnawing). Mice were tested in wire mesh cages and their behaviors scored using a time-sampling technique. We compared the effects of APO, and its modulation by cocaine (COC), in DBA/2 and C57Bl/6 mice. C57 mice showed a dose-dependent increase in APO-induced climbing, and little leaning. DBA mice however, showed sustained leaning, and only at a high dose of APO some climbing. Both strains showed strong gnawing, though DBA mice were less responsive at low doses. COC potentiated APO-induced climbing and gnawing at high APO doses in DBA and at all doses in C57 mice. APO-induced leaning was not substantially changed by COC in either strains. In addition, the potency of APO in inducing convulsions and lethality (at very high doses) were comparable in both strains. Thus, although showing a different drug potency for APO-induced climbing, DBA mice still respond behaviorally to APO, but in an idiosyncratic way.

368.9

INDIVIDUAL DIFFERENCES IN DOPAMINE BUT NOT SEROTONIN RECEPTORS ARE RELATED TO THE LOCOMOTOR RESPONSE TO NOVELTY. M. S. Hooks,* J. L. Juncos, and J. B. Justice, Jr., Departments of Chemistry and Neurology, Emory University, Atlanta GA, 30322.

High (HR) and low (LR) locomotor responding rats to a novel environment show differences in their vulnerability to drugs of abuse. In the current experiments, the nucleus accumbens (NACC), striatum (STR), and the medial frontal cortex (MFC) were removed 7-10 days after subjects were screened for novelty response. Both the density and affinity were determined for the dopamine uptake sites (DA-UP), D_1 receptors, D_2 receptors, serotonin uptake sites (5HT-UP), $5HT_2$ receptors, and $5HT_{1A}$ receptors with in vitro radioactive ligand binding in tissue homogenates. The B_{max} (fmol/mg protein) and K_D (nM) were determined from 3-6 pooled tissue homogenates run in duplicate using 9 concentrations of radioactive ligand.

There were fewer D_2 binding sites in HR rats in both the NACC ($B_{max}=268\pm26$, $p<0.05$) and STR ($B_{max}=412\pm42$, $p<0.005$) than in LR rats ($B_{max}=548\pm49$ and 711 ± 62 the NACC and STR, respectively). HR rats ($B_{max}=780\pm27$) showed a greater ($p<0.05$) number of D_1 binding sites in the NACC than LR rats ($B_{max}=660\pm26$). No differences in number of binding sites for D_1 receptors were observed between HR and LR rats in the STR ($B_{max}=1040\pm105$ and 1120 ± 98, respectively). HR rats ($B_{max}=1012\pm23$) showed a greater number ($p<0.05$) of DA-UP binding sites in the NACC than LR rats ($B_{max}=883\pm41$). However, HR rats ($B_{max}=2301\pm113$) had a lower number ($p<0.05$) of DA-UP sites in the STR than LR rats ($B_{max}=2771\pm114$). No differences in B_{max} for any of the dopamine receptors were observed in the MFC between HR and LR rats. There were no differences between HR and LR rats in affinity for any of the dopaminergic receptors in the regions examined. Furthermore, there did not exist any differences in either B_{max} or K_D for the 5HT-UP, $5HT_{1A}$, or $5HT_2$ receptors between HR and LR rats in the NACC, STR, or MFC. These results indicate that variation in the dopaminergic system in the STR and NACC, but not the serotonergic system are related to individual vulnerability to drugs of abuse.

368.10

THE EFFECTS OF THE DOPAMINE D2 AGONIST, QUINPIROLE, ON MEMORY, FINE MOTOR PERFORMANCE AND "HALLUCINATORY-LIKE" BEHAVIOR IN RHESUS MONKEYS. A.F.T. Arnsten* and P.S. Goldman-Rakic. Section of Neurobiology, Yale Medical School, 333 Cedar St., New Haven, CT 06510-8001.

Chronic, high dose amphetamine use can cause hallucinations in humans and "hallucinatory-like" behavior (HLB) in monkeys. These abnormal behaviors in monkeys include swatting and other organized responses to what appear to be imaginary or nonexistent objects. In the current study, monkeys were injected with the D2 agonist, quinpirole (0.00001-1.0 mg/kg), and tested on a working memory task, spatial delayed response, and a fine motor task; in separate experiments they were rated for behavioral changes in their home cage. An acute, high dose of quinpirole was found to produce HLB in young and aged monkeys; middle-aged monkeys required higher dose (1.0 mg/kg) treatment than younger or aged monkeys (about 0.05 mg/kg). Other behavioral changes included vacuous chewing, fear grimacing and agitation. Fine motor performance was impaired at the highest and lowest doses but not in the middle dose range; the low dose effect could result from preferential actions at presynaptic receptors. Unexpectedly, delayed response performance was unchanged or actually improved by high doses which induced HLB. As D1 stimulation has been implicated in cognitive enhancement but not in HLB, it is possible that the D2 family of receptors underlies the generation of HLB. Receptor mechanisms are currently being investigated. Furthermore, preserved delayed response performance in monkeys exhibiting HLB suggests that the prefrontal cortical circuits necessary for working memory may not play a central role in HLB.

368.11

A BEHAVIORAL ANALYSIS OF D1 and D2 RECEPTOR SYSTEMS. L.D. Middaugh,* B.P. Jackson, and G.D. Gentry. Dept. of Psychiatry, Medical University of South Carolina, Charleston, S.C. 29452

D1 and D2 receptor function was assessed by determining the discriminability of specific D1 (SKF-38393) and D2 (quinpirole) agonists and by determining their effects on motor activity of naive and habituated C57 mice. The mice learned to discriminate both of the agonists in an operant drug discrimination paradigm. D1 receptor activation appeared to be necessary for discrimination of the D1 agonist since its discriminative stimuli did not generalize to those of a D2 agonist, and its discrimination was blocked by a D1 antagonist. The D2 agonist produced a monotonic reduction in motor activity of nonhabituated mice relative to vehicle levels. The D1 agonist at low doses reduced the activity of nonhabituated mice relative to vehicle levels; however, activity increased with increasing dose. Activity levels of habituated and nonhabituated mice injected with the D1 agonist were similar. Because vehicle injected habituated mice were less active than nonhabituated mice, the D1 agonist elevated or reduced activity relative to basal activity levels of habituated and nonhabituated mice, respectively. (Supported by AA06611)

368.12

ACUTE ADMINISTRATION OF HALOPERIDOL AND RESERPINE INCREASES VACUOUS JAW MOVEMENTS IN YOUNG AND AGED RATS. R.E. Steinpreis* and J. D. Salamone. Psychology Dept., Univ. of Connecticut, Storrs, CT 06269-1020.

An experiment was conducted to examine the effects of two neuroleptics, haloperidol (HP) and reserpine (RES), on vacuous jaw movements in rats at three different age levels. Rats were given a single IP injection of either vehicle (VEH), 0.4 mg/kg HP, or 5.0 mg/kg RES. The age groups included young (3 mos), middle aged (6-9 mos) and aged (12-15 mos) rats. ANOVA revealed a significant elevation of vacuous jaw movements with increasing age. There was also a significant effect of drug treatment, with both HP and RES producing significant increases in vacuous jaw movements. The age x drug interaction was not significant, indicating that the combined effects of age and neuroleptic drugs were additive under the conditions tested. These results demonstrate the acute interference with dopamine systems by receptor blockade or terminal depletion can increase vacuous jaw movements. In addition, the present study indicates that age is an important variable in the production of vacuous jaw movements.

368.13

Variations in the Behavioral Responses Produced by Apomorphine in Different Strains of Rats. W. D. Essman*, P. McGonigle and I. Lucki. Depts. of Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA, 19104.

Experiments in inbred strains of rats and mice have suggested potential genetic contributions to the behavioral responses produced by drug administration. Dopamine systems associated with mechanisms of reward have provided one focus for these experiments in an attempt to identify genetic factors related to drug abuse. The present experiments compare the behavioral responses produced by the direct dopamine receptor agonist apomorphine (APO) in five inbred strains of rats; Brown Norway (BN), Lewis (LEW), Fisher (F-344), Buffalo (BUF) and DA. Examination of locomotor activity during repeated acclimation sessions and activity and stereotypy elicited by APO revealed strain-specific patterns of behavior. BN rats were most resistant to acclimation. Low doses of APO (0.1 and 0.32 mg/kg) produced a flat body posture in LEW animals which was generally not observed in the other strains. A higher dose of APO (1.0 mg/kg) markedly increased activity in F-344 rats compared to the other four strains. BUF animals showed licking during saline sessions, and also demonstrated the greatest increase in gnawing at higher doses of APO. DA rats were generally less responsive to the behavioral effects of APO. Autoradiographic analyses of dopamine receptors in the five strains of rats revealed region-specific differences in the density of D1 receptors labelled by ^{3}H-SCH 23390 and D2/D3 receptors labelled by ^{125}I-NCQ 298 in the caudate-putamen and nucleus accumbens. The results from these studies are discussed in terms of the neurochemical basis for differences in APO behavioral responses between strains. Supported by USPHS grant GM34781.

368.14

ENVIRONMENTAL ENRICHMENT ALTERS THE BEHAVIORAL RESPONSE TO AMPHETAMINE BUT NOT TO ELECTRICALLY-EVOKED DOPAMINE RELEASE. S.L. Bowling, J.K. Rowlett, M. Bradley, L.P. Dwoskin & M.T. Bardo*, Dept. Psychology & Coll. Pharmacy, University of Kentucky, Lexington, KY 40506.

Rats were raised from 21 days of age in either an enriched condition (EC) or isolated condition (IC). At age 50 days, locomotor activity and conditioned place preference (CPP) were assessed following amphetamine (0, 0.1, 0.3, or 1.0 mg/kg). Separate groups of EC and IC rats were also used to examine electrically-evoked release of dopamine in vitro in the presence of the D2 agonist pergolide. Only EC rats showed significant CPP. EC rats also showed a greater locomotor responsiveness to amphetamine than IC rats. In a separate experiment, amphetamine pre-exposure abolished these behavioral differences between EC and IC rats. The neurochemical results revealed no significant differences between EC and IC rats in dopamine release within the nucleus accumbens or striatum, suggesting that the altered behavioral response to amphetamine does not reflect a change in stores of dopamine which are releasable by electrical stimulation. (Supported by USPHS grant DA05312).

368.15

COMPARISON OF DOPAMINE RELATED BEHAVIORS AND DOPAMINE RECEPTORS IN MESOCORTICOLIMBIC REGIONS OF C57BL AND DBA MICE. D.E. Womer, V.G. Erwin* and A.D. Campbell. School of Pharmacy, University of Colorado Health Sciences Center, 4200 E. 9th Ave., Denver CO, 80262.

Previous studies have shown that C57BL/6Ibg (C57) and DBA/2Ibg (DBA) mice differ in cocaine and ethanol induced behaviors, e.g. locomotor activity and stereotypy. The present studies were undertaken to further characterize these strain differences in cocaine and ethanol related motor activities. Locomotor activation and stereotypy were more pronounced in DBA than C57 mice after administration of 15 mg/kg cocaine or 1.5 g/kg ethanol. The selective D2 antagonist, epidepride (.03 mg/kg), greatly attenuated cocaine and ethanol induced motor behaviors of DBA mice, but not of C57 mice. Dopamine receptors (D1 and D2) and the uptake transporter were characterized in specific brain regions of C57 and DBA strains. Values for KD or Bmax for the D1 receptor labelled with the antagonist, [3H]SCH 23390, were similar in C57 and DBA brain membranes. [125I]epidepride was used to characterize the D2 receptor and DBA caudate membranes had a 2 to 3 fold higher affinity for [125I]epidepride than C57; nucleus accumbens membranes from these strains showed similar KD values. Caudate and nucleus accumbens membranes from C57 mice had significantly higher binding capacities for [125I]epidepride than DBA membranes. No other significant differences by region between strain were noted for D2 receptors. [3H]CFT, a cocaine analogue, was used to identify the DA uptake transporter using GBR 12909, a specific DA uptake blocker, to determine nonspecific binding. [3H]CFT binding to DA uptake transporter showed no difference between strain by region, whereas, there were significant differences within strain by region. The results of these studies support the hypothesis that genetically determined differences in some cocaine and ethanol related behaviors may be mediated by variations in the dopamine D2 receptor in specific brain regions. This work was supported, in part, by USPHS grants DA 01717 and AA 00079.

368.16

THE EFFECTS OF SEPARATE AND COMBINED ADMINISTRATION OF DOPAMINE D-1 AND D-2 AGONISTS ON KEY-PECKING IN THE DEVELOPING CHICK. J. M. Dose* and J. F. Zolman. Physiology and Biophysics, Univ. of Kentucky, College of Medicine, Lexington, KY 40536.

In rodents, stimulation of either the dopamine D-1 or D-2 receptor elicit receptor-specific behavioral profiles yet these receptors are often functionally coupled (see Clark & White, Synapse, 1, 1987). A coactivation effect of these two dopamine receptors has also been reported in 7-day and older chicks (Zarrindast & Amin, Psychopharmacology, 106, 1992). We have studied the ontogeny of this functional coupling using the developing chick. One- and 4-day-old chicks of two different strains were rewarded with heat in a key-peck autoshape task of 24 discrete trials. Twenty minutes prior to testing, reserpine pretreated (5mg/kg, sc, 18 hr prior) chicks were given ip injections of either distilled water, 10mg/kg SKF 38393, 2mg/kg quinpirole, or a combined dose of SKF 38393 and quinpirole. Chicks of both ages responded on more trials when given the combined administration of both drugs compared with chicks given either distilled water or single drug injections. Hence, functional coupling D-1 and D-2 receptors is evident within 1-day after hatching. This coactivation effect was found in both strains of chicks indicating that the effect is not strain specific.

368.17

PROFILE OF BEHAVIOURAL CHANGES DURING THE COURSE OF CHRONIC TREATMENT WITH D-2 AGONIST QUINPIROLE. D. Eilam*, H. Talangbayan, H. Dai, G. Canaran and H. Szechtman. Dept. Biomedical Sciences, McMaster Univ, Hamilton, Ont. CANADA L8N 3Z5.

This report extends our initial observation that chronic treatment with quinpirole produces profound sensitization of activity with compulsive and rigid locomotion along few paths, confined to a small region of a large open field [1,2]. The number of rats tested has been increased to 22 and the number of repeated quinpirole administrations to as many as 90. A separate group of animals (N=18) received an equivalent number of drug administrations in their home cage but were not tested in the open field (160 x 160 cm) until the final test day. Results indicate that the most profound sensitization of activity in the open field is produced when treatment with quinpirole is administered in the test environment.

Moreover, the profile of response to quinpirole appears related to the animal's baseline reaction to the open field. (Supported by MRC. DE is an Allon Fellow and HS is a Research Associate of the Ontario Mental Health Foundation).

1. Eilam et al. (1989) Soc Neurosc Abstr, 15:1157
2. Szechtman et al. (1989) Soc Neurosc Abstr, 15:1156

368.18

RATS SENSITIZED TO D-2 AGONIST QUINPIROLE SHOW PERSEVERATION IN A SPATIAL LEARNING TASK. H. Einat and H. Szechtman*. Dept. Biomedical Sciences, McMaster Univ, Hamilton, Ont. Canada L8N 3Z5.

The present study examines whether behavioral sensitization induced by quinpirole produces longterm changes in undrugged behavior. Rats received 10 injections of quinpirole (0.5 mg/kg; N=12) or saline (N=12) spaced at 4 day intervals, in Omnitech activity cages. Quinpirole induced a 4-6 fold increase in various measures of locomotion and rearing. Following the sensitization phase, both groups of rats were trained on a spatial learning task in the Morris water maze, without the injection of any drugs. During acquisition, sensitized animals showed better retention from training day 1 to day 2, but both groups performed equally well by the 3rd day of training. During extinction, sensitized rats stayed significantly longer in the (empty) training quadrant. This perseveration in spatial bias continued even into the initial trials of reversal learning. These results suggest that the process of sensitization to quinpirole has longterm consequences for undrugged behavior.

(Supported by MRC. HS is a Research Associate of the Ontario Mental Health Foundation).

368.19

DISCRIMINATIVE STIMULUS PROPERTIES OF THE D_1 DOPAMINE AGONIST, DIHYDREXIDINE. Q.D. Walker*[1], M.H. Lewis[1,2,3], A.M. Perkins[3], D.A. Eckerman[3], D.E. Nichols[5] and R.B. Mailman[1,2,4]. Curr. in Tox.[1], Depts. of Psychiatry[2], Psychology[3] and Pharmacol[4]. UNC, Chapel Hill 27599, NC and Dept. of Med. Chem., Purdue University[5], West Lafayette, IN 47907

Dihydrexidine (trans-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo-[a]phenanthridine) is a high potency full efficacy D_1 dopamine agonist with some D_2 affinity (10-fold less than D_1). Because dihydrexidine (DHX) is a useful neuropharmacological probe, as well as a potential antiparkinsonian drug, we have studied its actions in vivo using the drug discrimination paradigm. The hypotheses tested were that DHX would function as a discriminable stimulus, and that the cue would be mediated specifically by D_1 receptor stimulation. Using a two-lever milk reinforced operant task (FR20), rats were trained to discriminate 2.0 mg/kg DHX (15 minutes prior to testing, sc) from its vehicle. Rats acquired this discrimination in an average of 67 test sessions. The training dose engendered 98% drug lever responding. Substitution of 0.5 and 1.0 mg/kg in animals trained to 2.0 mg/kg produced only 10 and 55% drug appropriate responding, respectively. The time course of substitution of the training dose indicated that rats selected the vehicle lever 2.5 and 240 min after dosing, but the drug lever 5-120 min after dosing. The receptors involved in this cue were investigated by administering dopamine antagonists. All rats pretreated with the D_1 antagonist SCH23390 (5 μg/kg, sc) responded exclusively on the vehicle appropriate lever in the first trial, with only a 10% decrease in response rate. Conversely, the D_2 antagonist remoxipride (0.2 or 0.45 mg/kg, sc) did not attenuate DHX lever selection (>80% DHX responding), and decreased response rates 30%. Agonist substitution experiments demonstrated that only high doses of the mixed agonist apomorphine (50 μg/kg, sc) produced partial (60%) generalization. Initial observations suggest that 8.0 mg/kg SKF 38393 (a partial D_1 agonist) substitutes completely, and that the selective D_3/D_2 agonist, quinpirole, does not substitute for DHX. Together these data indicate that DHX produces a robust interoceptive cue mediated selectively by D_1 receptor stimulation. Animals trained to recognize DHX in this behavioral paradigm will be used to study functional interactions of dopamine receptor subtypes and to identify other D_1 agonists.

368.20

INTRACEREBRAL INJECTIONS OF THE FULL EFFICACY D_1 AGONIST DIHYDREXIDINE: BEHAVIORAL EFFECTS IN THE RAT. T. J. Schmitt[1], S. B. Southerland[2], R.B. Mailman[1,2,3,4,*], D.E. Nichols[5], and M. H. Lewis[1,2,3]. Toxicology Curriculum[1], Brain and Development Research Center[2], Departments of Psychiatry[3] and Pharmacology.[4], University of North Carolina, Chapel Hill, NC, 27599 and School of Pharmacy[5], Purdue University, West Lafayette, IN, 47907.

Dihydrexidine (DHX) has been characterized as a potent, full efficacy D_1 agonist that has a ten-fold selectivity for D_1 vs. D_2 binding sites. We recently reported significant increases in grooming, locomotion and sniffing in rats systemically injected with DHX. The purpose of the present study was to identify specific brain regions important in the mediation of these DHX-induced behaviors. Male Sprague-Dawley rats had cannulae implanted stereotaxically in the caudate nucleus or nucleus accumbens, and were allowed to recover at least seven days prior to behavioral testing. DHX was intracerebrally injected (1.0, 3.0, and 10.0 μg). Discrete behavioral topographies (e.g., rearing, grooming, locomotion, etc.) were quantified by direct observation. The frequency of grooming was increased by 1 and 3 μg DHX in both brain regions, with a decline toward control values observed at the highest dose. DHX significantly increased vacuous chewing at all doses when injected into the nucleus accumbens, but had no effect when injected into caudate nucleus. The frequency of locomotion was significantly increased by all three doses of DHX in the caudate nucleus, whereas in the nucleus accumbens, locomotion was not increased at any dose. Sniffing behavior was not significantly different from control values in either brain region, at any of the DHX doses tested. Particularly when injected into the nucleus accumbens, DHX was observed to induce a marked hyperreactivity to both auditory and tactile stimuli, despite extensive pre- and post-operative handling. These data provide new information concerning the functional roles of select populations of dopamine receptors in the mediation of behavior, and suggest an important role for D_1 receptors in mediating reactivity. (Supported, in part, by PHS Grants MH40537 and MH42705).

HORMONAL CONTROL OF REPRODUCTIVE BEHAVIOR I

369.1

INFLUENCE OF VOMERONASAL ORGAN ON LORDOSIS BEHAVIOR AND LUTEINIZING HORMONE-RELEASING HORMONE NEURONAL SYSTEM IN FEMALE RATS. G. Rajendren*, C.A. Dudley and R.L. Moss. Dept. Physiol., Univ. Texas Southwestern Med. Ctr., Dallas, TX 75235-9040.

The vomeronasal system influences reproduction in mammals. In the present experiments the role of the vomeronasal organ (VNO) in mediating the effects of conspecific males on lordosis behavior and on the luteinizing hormone-releasing hormone (LHRH) neuronal system in female rats was studied. Ovariectomized rats were subjected to VNO removal (VNX) (n=5) or sham (VN-Sham) surgery (n=5). Forty eight hours after estrogen priming, the females were subjected to repeated mating (15 min mating followed by 15 min resting) for 180 min. For each female, the increase in lordosis quotient (LQ) was calculated by subtracting LQ during the first test (0-15 min) from that of the last test (150-165 min). At 180 min, the brains were fixed and were double labelled for Fos protein and LHRH. LHRH neurons with or without Fos immunoreactivity were counted in sections obtained from a block of brain encompassing AP bregma +0.2 mm through bregma -0.8 mm (*cf.* Paxinos and Watson, 1986). The enhancement of lordosis following repeated mating was significantly reduced in the VNX females as compared with the controls (p=0.006). Similarly, the proportion of LHRH neurons exhibiting Fos-like immunoreactivity was significantly lower in the VNX females than in the VN-Sham controls (p=0.049). There was a significant positive correlation between LQ and the proportion of Fos positive LHRH neurons (r=0.859; p<0.01). The present results demonstrate a close link between lordosis behavior and the accessory olfactory-LHRH systems in rats. Supported by MH41784.

369.2

HIPPOCAMPAL ELECTRICAL ACTIVITY IN THE FEMALE RAT: THE ESTROUS CYCLE, COPULATION, PARTURITION, AND PUP RETRIEVAL. L.A. Mead* and C.H. Vanderwolf. Dept. of Psychology, University of Western Ontario, London, Ontario, CANADA N6A 5C2.

Hippocampal electrical activity was examined in female rats across the four phases of the estrous cycle, as well as during copulation, parturition, and pup retrieval. Possible hormonal effects on hippocampal activity were examined by recording daily during struggling and immobility throughout five estrous cycles. No differences in the characteristics of rhythmical slow activity (RSA), large-amplitude irregular activity (LIA), or their relation to behavior were found between the phases of the estrous cycle.

Behaviors examined during copulation were lordosis, hopping, and ear wiggling. Lordosis and ear wiggling were both accompanied by irregular waves, with some low frequency RSA also present during lordosis. Hopping was accompanied by RSA which was higher in amplitude and frequency than RSA during walking. Pre-parturition behaviors examined included body extensions, genital or pup licking, and "body flattening". Body extensions were found to be associated with irregular activity, and appeared to be a Type 2 behavior. Interestingly, sudden suppressions of the hippocampal record lasting 1-5 s often preceded body extensions. Irregular hippocampal activity was present at all times during genital and pup licking. Body flattening was typically accompanied by very high amplitude irregular waves which, although the rats were awake, resembled a sleeping hippocampal pattern. Pup retrieval involved walking and was thus always accompanied by RSA.

These experiments provide additional information on the relationship between hippocampal activity and behavior in rats by documenting the hippocampal electrical activity accompanying several exclusively female conditions and behaviors.

369.3

PROGESTERONE - OXYTOCIN INTERACTIONS: BEHAVIOR AND PHYSIOLOGY. D. S. Janowsky*, J. D. Caldwell, J. M. Johns, and C.A. Pedersen; BDRC & Dept. of Psychiatry, School of Medicine, Univ. of North Carolina, Chapel Hill, NC 27599-7250

Oxytocin (OXT) and progesterone (P) interact at several levels to achieve sexual receptivity. We have found that infusions of the OXT antagonist PM-OXT into the medial preoptic area (MPOA) of rats immediately before injection of 500 μg P (4 hr before testing) results in increased frequency and duration of punch/kicks and vocalizations. Giving PM-OXT immediately before testing also increased rejection behaviors such as punch/kicks, vocalizations and mounts without lordosis. Microdialysis found that while estrogen pretreatment increased OXT release in surges in the MPOA, peripheral injection of 500 μg P initially suppressed these surges but resulted in a larger peak of OXT recovered about four hours after P injection. Recoveries of OXT using push-pull cannulas found that P linked to albumin (P-BSA) when perfused through the MPOA-AH, VMH or near the PVN significantly increased OXT recoveries (levels increasing from 1-3 pg/μl to 40-50 pg/μl over a 50 μl collection). OXT recoveries increase immediately and return to basal levels for subsequent perfusions, indicating an immediate and transient effect of P-BSA. Push-pull perfusions with equimolar BSA had no effect on OXT recoveries. Since P-BSA is not believed to penetrate the cell membrane, it appears that P releases OXT by an extracellular mechanism. Such an effect might explain the ability of P to increase OXT receptor number in the MPOA and move OXT receptors in the VMH as well as its ability to augment the capacity of OXT to facilitate female sexual receptivity.

369.4

5,7-DHT INFUSIONS INTO THE MEDIAL HYPOTHALAMUS FACILITATE LORDOSIS IN PREWEANLING RATS. G. S. Benedict* and C. L. Williams. Departments of Psychology, Columbia University and Barnard College, New York, NY 10027.

During the first week of life, lordosis and ear wiggling are displayed by both male and female rats without need for prior hormone priming (Williams, Behav. Neurosci., 1987). Work from our lab has shown that lordosis and ear wiggling become sexually dimorphic and more dependent on ovarian hormones for elicitation by postnatal days (PD) 10-18. Defeminization ultimately results from the presence or absence of perinatal testicular androgen, although behavioral consequences are not manifested until one week later perhaps following the maturation of inhibitory neural system(s). Serotonergic innervation of the ventromedial hypothalamus (VMH), established by PD 9 (Ugrumov et al., Dev. Brain Res., 1983), may contribute to this developing suppression. We infused 5 μg of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) into a site dorsolateral to the VMH of 9-day-old male and female Sprague-Dawley rats and tested them for lordosis 10 days later. Although 5,7-DHT did not alter ear wiggling frequencies, the infusions significantly increased lordosis frequencies of both males and females even without hormone priming. Furthermore, females generally displayed lordosis more frequently than males, and priming with estradiol benzoate (1 or 100 μg) and progesterone (0.5 mg) increased lordosis and ear wiggling frequencies of males and females. The behavioral data suggest that serotonergic afferents to the VMH may contribute to the defeminization and hormonal dependence of lordosis.

369.5

3α-OH-DHP AND 5α-THDOC IMPLANTS TO THE VTA FACILITATE SEXUAL RECEPTIVITY IN HAMSTERS AFTER PROGESTERONE PRIMING TO THE VMH. C.A. Frye and J.F. DeBold*. Psychology Department, Tufts University, Medford, MA 02155.

Progesterone (P) stimulation to both the ventral medial hypothalamus (VMH) and the ventral tegmental area (VTA) is necessary to facilitate sexual receptivity in the female hamster, in spite of the sparse population of estrogen (E) induced P receptors found in the VTA. Recently we have found that P may act instead at neuronal membranes in the VTA. These P effects may be mediated by progestin actions on the $GABA_A$-benzodiazepine receptor complex (GBR). Many progestin metabolites have a greater effect on benzodiazepine binding and Cl^- flux than P. If P's actions are due to metabolism to a progestin more potent at the GBR, then applying one of those progestin metabolites directly to the VTA should facilitate receptivity, if coupled with P to the VMH. Similarly, progestin metabolites with greater effectiveness on GBR might have an enhanced effect on behavior, independent of affinity for the P receptor.

To test this hypothesis three P metabolites, in decreasing order of activity at the GBR, were tested: 5α-pregnane-3α-ol-20-one (3α-OH-DHP), 5α-pregnane-3α, 21-diol-20-one (5α-THDOC) and 5β-pregnane-3α, 21-diol-20-one (5β-THDOC). Ovariectomized hamsters were implanted with chronic cannula, one aimed about the VMH and the other over the contralateral VTA. Animals were E primed and tested for sexual receptivity 4 hours after a P containing insert was applied to the VMH and a progestin containing insert was applied to the VTA. The following week the contents of the tubes were reversed. Facilitation of receptivity occurred only when P was applied to the VMH and either P or a metabolite was applied to the VTA. The reversed treatment was not effective. The progestin metabolites in order of behavioral effectiveness were: 3α-OH-DHP, 5α-THDOC, 5β-THDOC, this rank order is consistent with efficacy at the $GABA_A$-benzodiazepine receptor complex.

369.6

OPIATE RECEPTOR BLOCKADE ENHANCES THE DISPLAY OF PROGESTERONE-FACILITATED LORDOSIS IN JUVENILE FEMALE GUINEA PIGS. D.H. Olster*. Department of Psychology, University of California, Santa Barbara, CA 93106.

In adult female guinea pigs, administration of morphine, an exogenous opiate, inhibits the display of steroid-induced lordosis; however, treatment with naloxone, an opiate receptor antagonist, does not facilitate receptivity, suggesting the absence of tonic inhibition of lordosis by endogenous opiate peptides in adult females (Nock and Cicero, 1991). In contrast to adults, juvenile female guinea pigs are not behaviorally responsive to estradiol and progesterone (Olster and Blaustein, 1989). In light of the reported opiate effects in adults, the present study was designed to test the hypothesis that endogenous opiates tonically inhibit the display of steroid-induced lordosis in juvenile females. Hartley guinea pigs were ovariectomized at 9-10 days of age and treated with estradiol benzoate (EB, 10 μg) at 15 days of age. Approximately 40 hours later, animals received naloxone (2 mg/kg) or vehicle, or progesterone (P, 0.5 mg). The EB+P-treated females then received naloxone (2 mg/kg) or vehicle 4.5 h after the P injection. None of the animals which received naloxone or vehicle 40 h after EB displayed lordosis (n=6/group). Among the groups that received EB and P, 73% displayed lordosis following naloxone treatment, while only 23% were sexually receptive following vehicle injection ($p < 0.05$, n=11-13/group). These data suggest that endogenous opiates do tonically inhibit the display of progesterone-facilitated lordosis in juvenile female guinea pigs and are consistent with the theory that pubertal maturation may include a release from endogenous opiate inhibition of steroid-induced sexual behavior. (Supported by NIH HD 23483.)

369.7

SEPTAL REGION AND PARAVENTRICULAR NUCLEUS: TWO NEUROANATOMICAL SUBSTRATES CONTROLLING MATERNAL BEHAVIOUR IN SHEEP. A.Da Costa, K.Broad, R.Guevara* and K.M.Kendrick Dept Neurobiology, AFRC, IAPGR, Babraham, Cambridge CB2 4AT, UK; *UNAM

In order to identify the neuroanatomical substrates involved in controlling the induction of maternal behaviour in sheep, and the formation of the selective ewe/lamb bond, the changes in the expression of the immediate early gene, c-fos, were mapped. In addition, *in vivo* neurochemical changes were monitored, using microdialysis, in two areas found to express high levels of c-fos expression during the induction of maternal behaviour and the formation of the selective bond.

Changes in the expression of c-fos mRNA in the brains of Clun Forest ewes were investigated using *in situ* hybridization histochemistry using a specific ^{35}S labelled, 45 mer oligonucleotide probe. High levels of expression were found in both the septal region(SR) and paraventricular nucleus(PVN) in maternal but not control ewes.

Microdialysis sampling used CMA microdialysis probes (Sweden, CMA-10) inserted into 18 gauge guide tubes which were surgically implanted (under halothane anaesthesia) in the skull over the septal region and PVN. Ringer was pumped through these probes at 5μl/min using battery driven syringe pumps attached to the animal's backs and samples collected at 5 min intervals during parturition.

Aspartate, glutamate and GABA concentrations were measured by HPLC with fluorescence detection. In the SR, aspartate,glutamate and GABA increased around parturition and a second peak of release occured at around 2h post-partum whereas in the PVN, these neurotransmitters showed identical increases around parturition but only glutamate demonstrated a second peak of release at around 2h post-partum.

The results suggest that the increase in the neurotransmitters measured around parturition may be related with the induction of maternal behaviour whereas the increase at around 2h post-partum may be related with the formation of the selective olfactory memory between the ewe and its lamb.

This work was in part financed by JNICT, Portugal.

369.8

NEURONS LOCATED IN THE LATERAL HABENULA MEDIATE THE HORMONAL ONSET OF MATERNAL BEHAVIOR IN RATS. K.P. Corodimas*, J.S. Rosenblatt, M.E.Canfield and J.I. Morrell. Rutgers University, Institute of Animal Behavior, Newark, NJ 07102.

Previously we demonstrated that radiofrequency lesions of the entire habenular complex made on day 12 of pregnancy severely disrupted the hormonal onset of maternal behavior (MB). This study explored the importance of neurons in the lateral habenula (Lhb) for the onset of MB. On day 12 of pregnancy bilateral axon-sparing lesions were produced by injecting kainic acid (1.0ug) into the Lhb, or as a control, just dorsal in the medial hippocampus. Animals were hysterectomized-ovariectomized and given 20ug/kg estradiol benzoate on day 16 of pregnancy, then tested 48h later with pups for MB. Following 10d of behavioral testing, the retrograde tracer Fluoro-Gold (FG) (0.2ul) was injected into the ventral midbrain (where many Lhb and medial habenula (Mhb) neurons project). FG labeling demonstrated that Lhb cells were lesioned, whereas Mhb cells were intact. We are examining the number of FG labeled neurons in limbic areas that project through the Mhb to verify intact axons-of-passage. Complete Lhb lesions (>95% cell loss) severely disrupted all components of MB without any recovery. Activity and body weight changes were similar between groups. These results suggest that Lhb neurons are important for the onset of MB. (HD22893 to JIM)

369.9

SEXUAL BEHAVIOR OF MALE RATS IS AFFECTED BY CELL BODY LESIONS OF THE VENTRAL PREMAMMILLARY NUCLEUS. S.G. Truitt*, C. Ulibarri, and T. R. Akesson. Dept of Vet & Comp Anatomy, Pharmacology, & Physiology, Washington State Univ, Pullman, WA 99164.

Through its afferent and efferent connections, the ventral premammillary nucleus (PMv) interacts with more-extensively studied nuclei that make up a system that controls sexual behavior. The nucleus accumulates some estrogen, but mainly androgen; it contains cells that produce peptides that are known to influence sexual behavior; and although the size of the nucleus is similar in males and females, there is a sexually dimorphic density of neurons immunoreactive for substance P. Taken together, these findings suggested to us that the PMv may have a role in the regulation of sexual behavior.

Castrated, testosterone-supplemented adult male Sprague-Dawley rats were tested with ovariectomized estrogen- and progesterone-primed receptive female rats through two ejaculations to establish baseline sexual behavior. Bilateral cell body lesions were generated by infusing ibotenic acid into the PMv in one group of rats (n = 13). The control group (n = 11) received sham surgeries using only vehicle infusate. Behavior testing was repeated every 4 days following surgery for 4 test periods. Post-testing, lesions placement was verified histologically. Data were analyzed using univariate ANOVA for a block design with repeated measures with a 2 x 2 factorial treatment.

In rats that received bilateral lesions of the PMv, mount number and intromission number increased and intromission latency and ejaculation latency increased as compared to control rats. There were no significant differences in mount latency, postejaculatory interval, copulatory rate, or copulatory efficiency between treatment and control groups. Thus the PMv may be important in the completion of copulatory behavior. Supported by HD22869 (TRA) and BSN9112097 (CU).

369.10

EPIGENETIC FACTORS IN PERSISTENT MATING BY CASTRATED MICE. A. Coquelin*. Dept. of Cell Biology & Anatomy, Texas Tech University Health Sciences Center, Lubbock, Texas 79430.

Sexual activity of male mice ordinarily depends on the presence of their testes, yet B6D2F1 hybrid males may perform the entire copulatory sequence indefinitely after castration. Two experiments examined whether sexual experience or presence of the testes during puberty is required for persistent mating by B6D2F1 males. In the first, sexually naive males (n=14) were castrated at 2.5 months of age; behavioral testing began 5 months later. The males were tested 3 times at weekly intervals, then they cohabited with 3 different, mature females for 5 days apiece, and finally they were tested 3 more times. Half the males mounted, 29% intromitted, and 14% "ejaculated" before cohabitation; 64% mounted, 50% intromitted, and 29% "ejaculated" afterwards. In the second experiment, juvenile males were castrated or received sham surgeries at 1 month of age, before their pubertal rise of androgens (n=10 per group). At 3 months of age the males cohabited with 3 females as above. Subsequent behavioral tests revealed no sexual activity in the castrated males, whereas all the intact males mounted and intromitted, and 80% ejaculated. Together these results demonstrate that sexual experience prior to castration is unnecessary but the testes must be present during pubertal maturation for B6D2F1 males to initiate or maintain copulation.

(Supported by NSF BNS-9104425.)

369.11

BEHAVIORAL RESPONSES TO ANABOLIC STEROIDS.

A. S. Clark* and D. M. Barber. Dept. of Psychology, Dartmouth College, Hanover, NH 03755

Anabolic steroids are synthetic compounds which are often taken in massive quantities by athletes with the intention of enhancing muscular appearance and/or physical strength. Although it has been speculated that these compounds have significant neurobehavioral effects, few studies have systematically examined the influence of anabolic steroids on the brain or behavior. The present studies focus on the impact of anabolic steroids on reproductive behavior and aggression.

Sexual behavior was tested weekly in castrated adult male rats receiving daily injections of anabolic steroids or vehicle. Testosterone cypionate and methyltestosterone were as effective in maintaining male sexual behavior after castration as testosterone propionate. Treatment with either stanozolol or fluoxymesterone was ineffective in maintaining male sexual behavior in castrated males. Intermale aggression was assessed in anabolic steroid-treated and control, castrated male rats which were housed with intact female rats. Aggressive responses of the resident male to "intruder" male rats were examined weekly for 3 weeks. Rats treated with methyltestosterone and testosterone propionate had elevated levels of intermale aggression relative to controls. In contrast, stanozolol treatment failed to stimulate intermale aggression. Variations in the chemical structure, metabolic fate and/or neural receptor potency of anabolic steroids may account for their distinct behavioral profiles. Supported by NS 9109687 and the Hitchcock Foundation.

369.12

ABSENCE OF MORPHOLOGICAL CHANGES IN PERINEAL MOTONEURONS WITH INTRATHECAL TESTOSTERONE.

L.M. Freeman*, E.P. Monaghan, J.W. Hershey & S.M. Breedlove, Psychology Department, Univ. California, Berkeley, CA 94720.

Motoneurons of the spinal nucleus of the bulbocavernosus (SNB) mediate "cup-like" erections in the male rat, while the motoneurons of the dorsolateral nucleus (DLN) mediate penile flips. Castration eliminates penile reflexes and also causes shrinkage of SNB cells. Last year we reported that testosterone (T) administered (14.4 μg/day, 2 weeks) to the lumbosacral spinal cord via osmotic minipumps attached to intrathecal catheters (ITT) maintained low levels of penile flips in castrated adult rats. However, giving the same dose of hormone peripherally in combination with intrathecal vehicle (ITveh) was equally effective in maintaining the reflexes. We measured soma size of both SNB and DLN cells of the rats in the behavioral study to see if cell morphology was influenced by the hormone treatment. Cross-sectional soma areas are given in $\mu m^2 \pm$ SEM.

	ITT (n=7)	ITveh (n=6)	GNX (n=5)
SNB	1050±55	1001±41	1104±41
DLN	751±39	724±40	731±47

There were no significant differences between the treatment groups. This suggests that either T does not act in the spinal cord to alter motoneuron size or that we failed to provide sufficient localized T treatment. Supported by NIH #NS28421 and an NSF predoctoral fellowship.

369.13

ALTERATION OF SNB MOTONEURON SOMA SIZE AFTER TWO WEEKS OF ANDROGEN TREATMENT.

Barbara A. Padgett, Louise M. Freeman and S. M. Breedlove,* Depts. of Neurobiology and Psychology, Univ. California, Berkeley, CA 94720.

Androgen treatment increases soma sizes of motoneurons in the rat spinal nucleus of the bulbocavernosus (SNB). A decrease in soma size has been demonstrated in males four weeks after castration. We examined whether such an effect can be seen as early as two weeks following castration. We castrated adult male rats and subcutaneously implanted either blank or testosterone propionate (TP) filled Silastic capsules, which were left in place for two weeks. Animals were overdosed and perfused with saline and buffered formalin. Spinal cords were cross-sectioned at 50 μm and stained with thionin. At least 25 SNB cells were drawn from each animal using a camera lucida and computer digitizing tablet. As expected, the difference in cross-sectional soma area between the two groups was smaller than has been reported after four weeks, but still marginally significant ($p<.05$, one tailed). The TP treated animals' cells had a mean area of 1509.1 $\pm$ 55.2 μm^2 (SEM, N=8), while the cells of the animals with blank capsules had a mean of 1388.9 $\pm$ 32.6 μm^2 (N=7). These results demonstrate that an androgen regimen as short as two weeks can alter SNB soma size.

Supported by NIH Grant NS28421.

369.14

ULTRASTRUCTURAL CHANGES IN GNRH CELLS AS A FUNCTION OF AGE AND OVARIECTOMY.

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Synaptic input to GnRH perikarya is significantly increased with age in virgin male rats (Witkin, Neurosci. 22: 1003, 1987). We addressed the question of whether similar changes occur in female rats and whether ovariectomy (ovx) had any added effects. Virgin female rats aged 3 (young) or 20 (old) months were divided into 4 groups: 1. Young intact, 2. Young short term ovx (4 weeks), 3. Old short term ovx, and 4. Old long term ovx (9 months). Coronal sections of preoptic area were immunostained for GnRH and subsequently processed for electron microscopy. Total plasma membrane length and that percent of membrane with synaptic modifications were quantified using a morphometric program. The volume fraction of various organelles was estimated by point counting. Preliminary data indicate that synaptic input to the soma of GnRH cells does not change significantly as a function of age or gonadal steroid milieu. The volume of rough endoplasmic reticulum (RER) was significantly decreased in young females following ovx and in old females after long term ovx (Mann-Whitney U, $p<.01$). This later finding may indicate a general decline in protein synthesis. However, short term ovx of old females had no effect on the volume of RER when compared to young intact females. A group of old intact females is presently under study to determine whether the differences observed are due to steroid hormonal milieu or to age. Supported by 5T32AG00189 and AG05366

HORMONAL CONTROL OF REPRODUCTIVE BEHAVIOR II

370.1

GONADAL STEROIDS REGULATE IMMUNOREACTIVE TACHYKININ IN THE VENTROMEDIAL NUCLEUS OF THE FEMALE BUT NOT THE MALE RAT.

T.R. Akesson*, Dept of VCAPP, Wash State Univ., Pullman, WA.

Tachykinin-immunoreactive (TACir) neurons are found in all subdivisions of the ventromedial nucleus of the hypothalamus and in the ventrolateral subdivision (VMHvl), many of them concentrate estrogen. A substance P-specific projection to the midbrain periaqueductal gray may represent a component of circuitry essential to lordosis since injections in the periaqueductal gray facilitate this behavior. SP injections in the preoptic area (POA) facilitate male sexual behavior and the POA is filled with TACir fibers, however, the VMH and its connection with the POA is not considered to be an important participant in circuitry mediating male sexual behavior.

Sex	N	Treatment	# TACir cells in the VMHvl
♂	5	Intact	1174 ± 45
♂	4	Castrate - 3 wk	1139 ± 39
♀	5	Ovex - 2 wk	1079 ± 28
♀	4	Ovex - single injection of EB	1334 ± 83
♀	4	Ovex - E capsule, 2 wk	1557 ± 63

Significant differences in numbers of tachykinin immunoreactive cells were found in the VMHvl such that: ♀cap > ♀inj > ♂, ♂, ♀. Tachykinin cells in the anterior 1/3 of the VMH, as well as the central and medial subdivisions of the posterior 2/3 did not respond to changes in steroid levels. In the posterior ¼ of the VMH (VMHp), staining intensity of TACir fibers varied with treatment. In intact males, castrated males, and ovex females, fibers were lightly stained. In ovex-injection rats they were moderately intense, and in ovex-capsule females, they formed a darkly stained plexus. Thus TACir cells in the VMHvl and fibers in the VMHp respond to gonadal steriods in a sexually differentiated manor. Supported by HD22869.

370.2

SEX STEROID REGULATION OF PEPTIDERGIC TRANSMISSION IN THE FEMALE RAT.

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The medial amygdala modifies the output of the hippocampus using a peptide transmitter similar to vasopressin and oxytocin in structure (Brain Res., 1990, 511:7 and 15). The synthesis of the peptide is steroid-dependent and the system probably plays a role in the generation of sexual behavior in males. The function of the system in females is unclear. Here we report our preliminary studies of the peptidergic network in female rats.

The strength of the peptidergic signal in the hippocampus was measured with field potential recording techniques in six males and six females. No difference in signal strength could be detected between the genders. Then 25 males and 22 females were castrated and the intensity of the signal evaluated in groups of 3 to 7 individuals at 3-week intervals. The signal remained strong in males for 6 weeks and then declined quickly, approaching zero at 15 weeks. The signal in females remained strong for 3 weeks and then declined towards zero at 15 weeks. Estradiol replacement (for females) and testosterone replacement (for males) restored the signal to the baseline condition.

The estrous cycle of rats is only four days long. The fact that total removal of gonadal steroids has no effect until after 3 weeks strongly suggests that steroid fluctuations during the estrus cycle should have no impact on the peptidergic transmission. We tested this by recording the signal in 37 females and subsequently determining stage of estrus with vaginal smears. No difference was found between the proestrus, estrus, metestrus and diestrus conditions. Thus, physiological investigation of the system in females can proceed without concern for stage of estrus.

370.3

SITE SPECIFIC EFFECTS OF NEUROKININS ON MALE RAT COPULATORY BEHAVIOR. Krista Vink, William M. Struthers, Christopher Barrett, and Wayne A Dornan*. Dept. of Psychology, Illinois Wesleyan University, Bloomington, IL 61701.

In this study, a four part experiment was done. Experiment 1a was conducted in an attempt to replicate the neurokinin K (NKK) induced inhibition of male copulatory behavior following bilateral injections into the Medial Preoptic Area (MPOA) observed previously in this lab. In experiment 1b, the effects of another neurokinin, neurokinin y (NKy), on male copulatory behavior was assessed. Experiment 1c examined the question of whether the effects observed previously are specific to the MPOA by assessing the effects of bilateral injections of NKK into the Caudate Putamen (CP) and Bed Nucleus of the Stria Terminalis. Lastly, in order to ascertain whether the effects on male copulatory behavior were mediated via a neurokinin receptor (NK-2), animals were pre-treated with a selective NK-2 antagonist ([Try^{6}, D-Trp^{6,8,9}, Arg^{10}]-Neurokinin A (4-10)) followed by bilateral injections of saline, NKy, or NKK into the MPOA (experiment 1d). In support of a previous study, our results indicated that bilateral injections of NKK inhibited the expression of male copulatory behavior when compared to injections of saline ($p < 0.001$). In contrast, bilateral injections of NKy failed to effect the expression of male copulatory behavior of sexually vigorous male rats when compared to control injections. Bilateral injections of NKy did, however, produce marked increases in intromission and mount latencies, although only intromission latencies were statistically significant. No other parameter of male copulatory behavior was affected. Injections of NKK into the CP failed to affect male copulatory behavior. Injections of NK-2 receptor antagonist failed to block the effect of injections of NKK into the MPOA, while antagonist injections were successful in blocking effects of NKy injections.

370.4

LESIONS OF THE ACCESSORY OLFACTORY BULB (AOB) REDUCE LORDOTIC RESPONSIVENESS AND DECREASE C-FOS IMMUNOREACTIVITY IN THE ACCESSORY OLFACTORY SYSTEM (AOS) OF FEMALE RATS. R.L. Moss, C.A. Dudley, and R.N. Rosenberg*, Depts. Physiology and Neurology, UT Southwestern Med. Ctr., Dallas TX 75235-9040

The gradual increase in the lordosis-to-mount (L/M) ratio observed in ovariectomized (OVX), estrogen(E)-primed female rats as a consequence of repetitive mating is thought to be partially due to pheromonal cues processed through the AOS. Repetitive mating-induced increases in L/M can be blunted by removal of the vomeronasal organ (Rajendren et al., 1990) and are associated with induction of fos-like immunoreactivity (fosIR) in several AOS sites (Dudley et al., 1992). The present study examined the contribution of the AOB to these effects. OVX, E-primed rats were repeatedly mated before and 10 and 20 days after AOB lesion or SHAM surgery. At 10 days post-surgery, the L/M scores in both groups were significantly lower than their pre-surgery values. By 20 days, lordotic responsiveness in SHAM animals had returned to pre-surgery levels while the L/M ratios in AOB-lesioned animals remained significantly depressed. Some SHAMS and AOB-lesioned rats underwent a third mating test 2 to 3 months after surgery. Immediately after 3 hr of repetitive mating, they were sacrificed and their brains were processed for fosIR. The number of fosIR neurons in several AOS structures was significantly higher in SHAM animals than in AOB lesioned animals. The results suggest that the AOB contributes to increased lordotic responding observed during repetitive mating and that centrifugal projections of the AOB also participate. Supported by MH41784.

370.5

MATING BEHAVIOR AND AGGRESSION RESULT IN A DIFFERENTIAL PATTERN OF FOS INDUCTION IN THE MALE SYRIAN HAMSTER BRAIN. S.S. Kollack* and S.W. Newman. Department of Anatomy & Cell Biology, University of Michigan, Ann Arbor, MI 48109-0616.

Mating behavior results in a selective pattern of fos expression within the medial nucleus of the amygdala (Me), the bed nucleus of the stria terminalis (BNST), and the medial preoptic area (MPOA) (*Soc. Neurosci. Abstr.*, 17: 1059). The specificity of the mating-induced pattern of fos expression was determined by comparing fos induction after mating behavior with fos induction after aggressive behavior. Both of these behaviors are dependent upon chemosensory cues and gonadal steroids. Adult male hamsters were divided into three groups: (1) 10 min mating, (2) 10 min aggression against a male intruder, and (3) isolated controls. All hamsters were perfused with 4% paraformaldehyde; brains were cut into 40 um sections and immunostained for fos protein (Ab-2; Oncogene Sciences). Fos expression was increased within Me and posteromedial BNST in males following mating behavior or aggression, relative to isolated males. In contrast, only mated hamsters showed increased fos immunostaining in medial preoptic nucleus (MPN) and magnocellular MPN. These data demonstrate that two social behaviors, both dependent upon chemosensory cues and gonadal steroids, result in distinctly different patterns of neuronal activation. (Supported by NIH NS 20629 to SWN)

370.6

C-*FOS* PROTO-ONCOGENE ACTIVITY INCREASES IN THE AMYGDALA AND PREOPTIC AREA OF THE FEMALE RAT AFTER CERVICAL STIMULATION: ROLE OF AFFERENT INPUT VIA THE PELVIC NERVE. M.S. Erskine* and D.W. Rowe. Department of Biology, Boston University, Boston, MA 02215

Cervical stimulation (CS) received during mating in the female rat is required for induction of the twice-daily prolactin (PRL) surges of early pregnancy. In order to identify brain areas which receive afferent genitosensory input and may be important for induction of PRL surges, we compared FOS immunoreactivity (FOS-IR) in brains of estrous females who received CS (15 intromissions from males, INTR) or mounts-without-intromission (MNTS); these mating treatments induced PRL surges in 100% and 0%, respectively, of females in a separate experiment. Brain FOS-IR was also examined after bilateral pelvic n. transection in separate INTR and MNTS groups. Brains taken 1 hr after mating onset were cut and processed for FOS-IR. Darkly-stained FOS-positive cells were counted at 50X using a camera lucida. In intact animals, significantly greater numbers of FOS-IR cells were seen in the POA and AMYG of INTR animals than of MNTS animals. Pelvic n. transection completely eliminated the response to CS in these two areas, and FOS-IR levels in nerve-transected INTR and MNTS animals were similar to those seen in intact MNTS females. FOS-IR did not differ between groups in the paraventricular nucleus of the hypothalamus or in the central tegmental field/peripeduncular area. These data demonstrate that afferent input via the pelvic nerve activates cell groups within the POA and AMYG, and suggest that these areas may be involved in initiation of CS-induced PRL surges. (HD21802)

370.7

INDUCTION OF FOS-IMMUNOREACTIVITY IN THE BRAIN FOLLOWING MATING IN FEMALE RATS. M.J. Tetel*, D.C. Celentano and J.D. Blaustein, Neurosci. and Behav. Program, Univ. of Massachusetts, Amherst, MA 01003.

To determine the neuroanatomical areas which respond to mating, we visualized Fos-immunoreactivity (Fos-IR) in three groups of ovariectomized, estradiol and progesterone-treated rats: 1)"Mated" animals were mated with three males, consecutively, for a total of 30-36 intromissions, 2)"AIM-exposed" were exposed to an anesthetized, intact male and 3)"Home Cage" remained in their cage and were not exposed to males or the testing arena.

Mating resulted in a dramatic increase in Fos-IR cells in the medial preoptic area, bed nucleus of the stria terminalis, posterodorsal medial amygdaloid nucleus and midbrain central gray within 1 hour of exposure to the males when compared with AIM-exposed and Home Cage animals. There were no differences between AIM-exposed and Home Cage groups, suggesting no effect in these areas of stimuli associated with exposure to an anesthetized, intact male. In an estrogen-receptor rich area ventrolateral to the ventromedial nucleus of the hypothalamus, AIM-exposed animals had an increase in Fos-IR cells when contrasted with Home Cage animals, while mating did not potentiate this effect. Although Fos-IR cells were observed in some other areas, such as the lateral habenular nucleus, no consistent differences among the three groups were detected.

All the areas discussed above, which respond to mating stimuli, contain estrogen-sensitive neurons as well. Thus, this technique may allow investigation of the possible integration among somatosensory and hormonal factors involved in female sexual behavior.

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370.8

SIMILAR INCREMENTS IN LIMBIC AND PREOPTIC *C-FOS* IMMUNOREACTIVITY AFTER MATING IN MALE AND FEMALE RATS. S.R. Wersinger*, M.J. Baum and M.S. Erskine. Department of Biology, Boston University, Boston, MA 02215

Significant increments in FOS immunoreactivity (IR) are induced in the medial preoptic area (mPOA), medial amygdala (MA), bed nucleus of the stria terminalis (BNST), and midbrain central tegmental field (CTF) of male rats after mating (Baum and Everitt, 1992). We compared nuclear FOS-IR in these same brain regions from mated and unmated male and female rats in order to determine if this genetic response to mating is sexually dimorphic. Rats of both sexes were gonadectomized and treated with estradiol benzoate for 5 days followed by progesterone 4h prior to testing. Males and females were either placed alone in a testing chamber for 10 minutes or were paired until one ejaculation was observed. Animals were killed 1 hour after the test ended. Significant, mating-induced increments in FOS-IR occurred in the mPOA, MA, BNST, and CTF of both sexes, suggesting that the neural processing of sensory stimuli (olfactory and genital/somatosensory) derived from mating is similar in male and female rats. (supported by HD21094; HD02971 and MH00392)

370.9

EQUIVALENT LEVELS OF MATING-INDUCED NEURAL *C-FOS* IMMUNOREACTIVITY IN CASTRATED MALE RATS GIVEN ANDROGEN, ESTROGEN, OR NO STEROID REPLACEMENT. M.J. Baum*, S.R. Wersinger, and J.C. Alvarez. Dept. of Biology, Boston University, Boston, MA 02115.

Previous research (Baum & Everitt, 1992) showed that *c-fos* immunoreactivity is augmented in neurons of the medial preoptic area (mPOA), medial amygdala (MA), and midbrain central tegmental field (CTF) of male rats following mating. An experiment was conducted to determine whether circulating testosterone or either of its reduced or estrogenic neural metabolites play a role in this process. Equivalent numbers of FOS immunoreactive neurons were counted in the medial preoptic area, medial amygdala, and central tegmental field of sexually experienced, castrated male rats which were injected for 7 days with either testosterone propionate, dihydrotestosterone propionate, estradiol benzoate, or vehicle and then were killed 1h after achieving 8 intromissions with an estrous female. Sex steroids appear to contribute little to the ability of sensory stimuli (olfactory and genital/somatosensory) associated with mating to promote *c-fos* expression in the male rat brain. (supported by HD21094 and MH00392)

370.10

EXPRESSION OF FOS PROTEIN IN PERIAQUEDUCTAL GRAY (PAG) NEURONS BY ELECTRICAL STIMULATION OF THE MEDIAL PREOPTIC AREA (MPO). M.T. Shipley*, T.A. Rizvi, M Jiang, M.N. Lehman and M Ennis. Dept. Anatomy, University of Cincinnati, College of Medicine, Cincinnati, OH 45267.

We have recently shown that MPO robustly innervates discrete, longtudinally-organized columns running through the rostrocaudal axis of PAG (Rizvi et al., 92). The neurons targeted by these MPO inputs are unknown. Intense synaptic stimulation can induce the expression of the immediate early gene, c-fos, in activated target neurons. Thus, we used immunocytochemistry to map the distribution of PAG neurons that express FOS protein following stimulation of the MPO–>PAG pathway.

Focal electrical stimulation (30 Hz; 5 sec on-3 sec off; 500 uA; 1 hr) of MPO in anesthetized male rats dramatically increased the number of PAG cells staining for FOS compared to sham controls. Neurons expressing FOS were organized into 2-3 longitudinal columns extending along the rostrocaudal axis of PAG. Columns of FOS+ cells corresponded remarkably well to the afferent terminal columns from MPO. Double labeling studies show that some FOS+ PAG neurons are immunopositive for estrogen receptors (Mab H222). Additional studies indicate that some FOS+ cells are retrogradely labeled by injections of Fluorogold in the ventral medulla (VM).

MPO, which is strongly modulated by olfactory stimuli, is critical to sexual behavior and neuroendocrine function. Activation of PAG induces analgesia, autonomic changes and the expression of specific sexual postures. The present results suggest that the MPO–>PAG–>VM circuit reported here, may coordinately regulate neuroendocrine, motor and autonomic components necessary to the elaboration of sexual behaviors. *(Supported by: NIH DC00347, NS20643, NS24698, NS29218 & NS29635).*

370.11

FOS AND JUN EXPRESSION IN THE FEMALE RAT FOREBRAIN FOLLOWING HORMONE TREATMENT AND SEXUAL STIMULATION. J.G. Pfaus*, S.P. Kleopoulos, C.V. Mobbs, R.B. Gibbs, & D.W. Pfaff, The Rockefeller University, New York, NY 10021.

Regions of the brain that concentrate estrogen and progesterone are thought to regulate feminine sexual behavior by altering gene expression and neural sensitivity to afferent stimulation. We used immunocytochemistry and in situ hybridization to examine the expression of Fos and Jun within estrogen-concentrating regions of the forebrain as a function of various types of sexual stimulation with or without hormone treatment. Ovariectomized rats (n=20) received injections of estradiol benzoate (10 µg) 48 hr and progesterone (500 µg) 4 hr before testing. Control rats (n=20) that had been ovariectomized at least 5 months before testing did not receive hormone treatment. Rats were then either placed into bilevel chambers with sexually vigorous males (n=5), received manual stimulation of the flanks (n=5), received vaginocervical stimulation with a glass rod (n=5), or were left in their home cages (n=5) for one hour prior to sacrifice. A dramatic induction of c-fos mRNA and Fos-like immunoreactivity (IR) was detected in estrogen-concentrating regions of the medial preoptic area, bed nucleus of the stria terminalis, ventromedial hypothalamus, and medial amygdala, as well as regions in the neocortex, lateral septum, paraventricular nucleus of the hypothalamus, and lateral habenula, following copulation with intromission and ejaculation in hormone-treated rats or stimulation of the vaginal cervix in both hormone-treated and control rats. Mechanical stimulation of the flanks produced a much smaller induction in these rats, whereas hormone treatment alone had no effect on Fos expression (consistent with Gibbs et al. (1991) Mol & Cell Neurosci, 1, 29). In contrast, Jun-like IR was detected in these regions but did not appear to be altered by sexual stimulation. These data demonstrate that afferent sensory stimulation, but not estrogen or progesterone, regulates c-fos gene expression selectively within estrogen-concentrating regions of the female rat forebrain. The effect of hormone treatment on Jun is being investigated and will be reported.

370.12

MATERNAL BEHAVIOR IN FEMALE RATS IS ASSOCIATED WITH INCREASED NUMBERS OF FOS CONTAINING NEURONS IN THE MEDIAL PREOPTIC AREA. M. Numan* and M.J. Numan. Dept. Psychology, Boston College, Chestnut Hill, MA 02167.

Lesion and hormone implant studies have suggested a central role for the medial preoptic area (MPOA) in the neural circuitry of maternal behavior. In this study we used Fos immunocytochemistry to support the view that MPOA neurons are activated during maternal behavior, and to localize the anatomical distribution of such neurons. The behavioral method used took advantage of the fact that virgin female rats can be induced to show maternal behavior after several days of cohabitation with pups. Therefore, virgin female rats were exposed to pups on a daily basis until maternal behavior was displayed for 2 hr. Each of these maternally behaving females was yoked to a control female that was concurrently exposed to pups but had not yet displayed maternal behavior. Using standard immunocytochemical procedures it was found that the MPOA of maternal females had significantly more Fos labeled neurons than did the MPOA of control females. This difference was especially evident in the more lateral aspects of the MPOA. The Fos mapping procedure will also be used to uncover novel regions in the maternal behavior circuit. We are currently investigating the location of brainstem neurons that may be activated during maternal behavior.

Supported by a grant from the Whitehall Foundation.

HORMONAL CONTROL OF REPRODUCTIVE BEHAVIOR III

371.1

TYROSINE HYDROXYLASE NEURONS IN THE MALE HAMSTER FOREBRAIN CONTAIN ANDROGEN RECEPTORS. S.E. Asmus* and S.W. Newman, Dept. of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109.

The male Syrian hamster relies on chemosensory and hormonal signals for mating. Chemosensory signals are relayed through the medial amygdaloid nucleus (Me), bed nucleus of the stria terminalis (BNST), and medial preoptic area (MPOA), regions that accumulate gonadal steroids. Neurons immunoreactive for tyrosine hydroxylase (TH-IR), a catecholamine biosynthetic enzyme, are located in Me (*Brain Research* 575:199-207), posteromedial BNST (BNSTpm) and the lateral part of caudal MPOA in colchicine-treated hamsters. The present study investigates whether TH-IR neurons in these areas also produce androgen receptors (AR). Colchicine-treated hamsters (n=3) were perfused with 4% paraformaldehyde, and the brains were cut at 40 um. Sections were processed for immunocytochemistry using an AR antiserum (G.S. Prins) with $NiCl_2$-intensified DAB and subsequently with a TH antibody (Incstar) visualized with unintensified DAB. Colocalization of TH and AR was most abundant in subdivisions of Me and BNST containing the highest number of steroid-concentrating neurons. In posterior Me and BNSTpm, approximately 80% of the TH-IR neurons contained AR-IR nuclei, whereas a smaller percentage of TH-IR neurons contained AR in anterior Me (30%) and MPOA (5%). (Supported by NIH NS 20629 to SWN)

371.2

RESTRICTION OF METABOLIC FUEL AVAILABILITY DECREASES ESTROGEN RECEPTOR-IMMUNOREACTIVITY IN VENTROMEDIAL HYPOTHALAMUS OF FEMALE SYRIAN HAMSTERS. H.-Y. Li* and G. N. Wade. Neuroscience and Behavior Program and Department of Psychology, University of Massachusetts, Amherst, MA 01003.

Metabolic fuel availability is essential for initiation and maintenance of reproductive function in female Syrian hamsters. Decreased fuel availability induced by food restriction, metabolic inhibitors, or redirection of fuels into storage interrupts estrous cycles. Steroid-induced lordosis is suppressed by 48h of food deprivation or treatment with metabolic inhibitors. The neuronal changes responsible for deprivation-induced suppression of estrous behavior are unknown, but a reduction in neural steroid binding is one possibility. Since ventromedial hypothalamus (VMH) is an important site of estradiol action on sexual behavior in female rodents, immunocytochemistry for estrogen receptor was used to examine the response of estrogen receptor-immunoreactive (ER-IR) cells in the VMH to changes in the fuel supply.

We have developed an immunocytochemical technique to localize ER-IR cells in female Syrian hamster brain. ER-IR cells were found in the medial preoptic area, bed nucleus of the stria terminalis, amygdala, periventricular hypothalamus, paraventricular hypothalamus, VMH, and the arcuate area. ER-IR cells were also detected in the midbrain central gray and the nucleus of the solitary tract (NTS) in the hindbrain.

The number of ER-IR cells in the VMH was reduced following 48h of food deprivation or treatment with inhibitors of glucose and fatty acid oxidation compared to *ad lib.*-fed animals. However, no significant effect of these metabolic manipulations on ER-IR cells was observed in the NTS, which relays visceral information from the periphery to the central nervous system. These results are consistent with the hypothesis that metabolic fuel availability affects estrous behavior by altering VMH estrogen receptor content. (Supported by NS10873, DK32976, and MH00321)

371.3

DOWN-REGULATION OF ESTROGEN RECEPTOR-LIKE IMMUNOREACTIVITY IN THE FEMALE BRAZILIAN OPOSSUM BRAIN. P.L. Pearson*, L.R. Ross, and C.D. Jacobson. Molecular, Cellular, and Developmental Biology Program & Dept. of Veterinary Anatomy, Iowa State University, Ames, IA 50011

Previous studies in this laboratory have described estrogen binding and estrogen receptor-like immunoreactivity (ER-LI) in the brain of the Brazilian opossum, *Monodelphis domestica*. This study focused on regulation of ER-LI by estrogen in the adult female opossum lateral septal nucleus, ventral part (LSV); medial preoptic area (MPA); bed nucleus of stria terminalis, medial division (BSTM); and the medial amygdaloid nucleus (MeA). Female opossums were gonadectomized and two weeks later given subcutaneous silastic implants containing estradiol benzoate (EB) mixed with cholesterol (EB group), or cholesterol (control group). Another group contained intact female opossums in estrus (estrus group). Estrogen receptors (ER) were detected by an indirect immunohistochemical technique, using the H222 monoclonal antibody (a gift of Abbott Laboratories). Image analysis was used to quantify the percent area displaying ER-LI in each of the four brain regions. Prolonged EB treatment reduced ER-LI levels when compared to the control group. In addition, ER-LI was lower in the LSV, MPA, and MeA, in the EB group vs. the estrus group. In the estrus group, ER-LI was lower in the MPA and BSTM when compared to the control group. These results support a down-regulatory role of estrogen in relation to expression of ER. Also, these results indicate that ER may be differentially regulated in the brain, allowing for sensitive modulation of estrogen responsiveness.

371.4

EFFECTS OF HYPOTHALAMIC ADMINISTRATION OF ANTISENSE DNA FOR PROGESTERONE RECEPTOR mRNA ON LORDOSIS BEHAVIOR AND PROGESTERONE RECEPTOR IMMUNOREACTIVITY.
Sonoko Ogawa, Ursula E. Olazábal & Donald W. Pfaff.* The Rockefeller University, New York, NY 10021.

Reproductive behavior of female rats can be correlated with estrogen (E)-induced increase of progestin binding in hypothalamic neurons (Parsons & Pfaff, Current Topics in Neuroendo., 5:103, 1985). E increases progesterone receptor (PR) mRNA in the ventromedial nucleus (VMN) of hypothalamus of genetic females (Romano et al., Mol. Endo., 3:1295, 1989). If PR mRNA increases help cause facilitation of reproductive behavior, then effective PR antisense sequences might decrease this behavior and PR immunoreactivity. Antisense (AS) oligonucleotides (15 bases) were chosen to span the translation start site. Control (SC) oligomers were composed of the same nucleotide bases in scrambled order. For behavioral studies, E was injected s.c. at hour 0 to ovariectomized female rats implanted with bilateral cannulae directed at VMN; then AS or SC oligomers were microinjected at hour 12 or 24; finally progesterone (P) was given s.c. at hour 44, four hours before behavioral test. Antisense effects were specific as to time of administration and strongest for a specific P-dependent behavior. When given 12hr, but not 24hr after E, AS (vs. SC) significantly reduced lordosis behavior measured as a reflex (44%↓, $p<0.05$) or in a mating behavior test (73%↓, $p<0.01$). Occurrence of proceptive behavior, known to be P-dependent, was greatly reduced (80%↓, $p<0.01$) by AS oligomers (vs. SC). For immunocytochemical studies, E was injected s.c. to ovariectomized female rats. 12hr later, AS oligomers were administered near VMN on one side of the brain, and the other side received SC or vehicle control. 48-50hr after E, PR-immunoreactive cells on AS side were decreased (27-42%↓, $p<0.005$). Interrupting gene expression for PR, a transcription factor, can have behavioral and immunocytochemical effects.

371.5

NEONATAL INTRACEREBRAL INFUSION OF ANTISENSE DNA TO ESTROGEN RECEPTOR mRNA ALTERS ESTROGEN-DEPENDENT PARAMETERS IN ADULT RATS. M.M. McCarthy*, E. Schlenker and D.W. Pfaff, Rockefeller University, 1230 York Ave, New York, NY 10021

Androgen treatment of neonatal females produces masculinization via estrogen (E) derived from aromatized testosterone binding to neuronal E-receptors, a process also neccessary for masculization in males. Normal feminization also depends on gondal steroids. Therefore, we tested the hypothesis that hypothalamic E-receptor gene expression is involved in sexual differentiation. On day 3 of life, Sprague-Dawley pups were cold anesthesized before bilateral hypothalamic infusion (1µl/side; 2µg DNA) with either antisense DNA (AS) spanning the translation start codon for the E-receptor, scrambled sequence control DNA (SC) or vehicle (VE; oil). Groups were females (FE), females androgenized with 50 µg TP (AND), and males (MA).

Daily vaginal smears indicated trends toward disruption of estrous cyclicity in FE-AS, and a protection against persistent estrus in AND-AS, with ovaries significantly heavier in AND-AS females vs controls ($p<.05$). After ovariectomy and treatment with EB+P (10µg, 2 days, P 4 hr prior), 77% of AND-AS exhibited lordosis, vs. 23% and 15% for AND-SC and AND-VE ($p<.05$). Lordosis quotient also differed significantly (33.1% vs. 9.2% and 9.2% for controls, $p<.05$). Open field testing of EB-treated females (5µg 4-8 hrs prior) indicated a significant increase in # of crossings by FE-AS vs controls($p<.05$).

Intact males given three tests for sexual behavior on alternating days exhibited behavioral differences as a result of treatment. Mount latency was significantly greater and the mount to intromission ratio was significantly less in MA-AS compared to the other two groups on the first test, with this pattern reversing by the third test so that MA-AS had a significantly shorter latency and higher mount to intromission ratio ($p<.05$).

Preoptic area analyses showed total volume of the SDN/POA to be significantly smaller by 26% in AND-AS vs AND-SC and AND-VE ($p<.05$). Results from other groups and brain nuclei will be reported on.

371.6

DISTRIBUTION OF ANDROGEN RECEPTOR-LIKE IMMUNOREACTIVITY IN THE BRAIN OF MALE SYRIAN HAMSTERS. D.C. WHITMAN*, A.N. CLANCY, AND H.E. ALBERS. Lab. of Neuroendocrinol. & Behav., Depts. Biol. & Psychol., Georgia State Univ. Atlanta, Ga. 30303; Dept. of Psychiat., Emory Univ. School of Med., Atl., Ga. 30322.

The immunocytochemical distribution of androgen receptors (AR) was examined in male hamster brain using a rabbit poly-clonal anti-androgen receptor antibody raised against a fragment of the human AR (Affinity Bioreagents PA1-110) (Chang, et al, Endocrinol. 125, 1097, 1989). Coronal sections (40 μM) from castrated and gonadally intact males were compared after processing by the peroxidase-avidin-biotin complex method. AR-like immunoreactivity (AR-ir) in both castrate and intact males was observed in the septum, bed nucleus of stria terminalis, amygdala, hippocampus, thalamus, preoptic area, and several hypothalamic nuclei including the paraventricular, supraoptic, and ventromedial nuclei. The distribution of AR-ir was found to be similar to the pattern of androgen binding previously reported with [H^3] dihydrotestosterone autoradiography in male hamster brain (Doherty, & Sheridan, Brain Res. 219, 327, 1981). Supported by NSF BNS-8910863 and The Emory University Research Committee.

371.7

MATING ACTIVATES ANDROGEN RECEPTOR-CONTAINING NEURONS IN THE BRAIN OF THE MALE SYRIAN HAMSTER. R.I. Wood* and S.W. Newman. Reproductive Sciences Prog., Dep't of Anatomy & Cell Biology, Univ. of Michigan, Ann Arbor, MI 48109.

Mating in the male Syrian hamster activates a specific population of neurons in the medial amygdaloid nucleus (Me), bed nucleus of the stria terminalis (BNST), and medial preoptic area (MPOA) as determined by the pattern of fos immunoreactivity (*Soc Neurosci Abstr 17:1059, 1991*). Fos-immunoreactive neurons are present in subnuclei that relay chemosensory information and contain receptors for gonadal steroid hormones, as determined by lesion studies and steroid autoradiography. Both hormonal and chemosensory stimuli are essential for copulation in the male hamster. The present study determined if neurons activated during mating behavior contain androgen receptors. Adult male hamsters (n=6) were allowed to mate with a sexually receptive female for 30 min. They were perfused one h later with 4% paraformaldehyde and 40 um frozen sections were processed for immunocytochemistry using antibodies against c-fos (Cambridge Research Biochemicals) and the androgen receptor (G.S. Prins). Three males were perfused as non-mated controls. Mating dramatically increased the number of fos immunoreactive neurons in Me, BNST, and MPOA relative to controls. These nuclei contain abundant androgen receptors, as determined by immuno-cytochemistry. A subpopulation of fos-immunoreactive neurons in each of these regions also contained androgen receptors. This suggests that chemosensory input or changing steroid concentrations during mating in the male hamster activate neurons that contain gonadal steroid hormone receptors. (Supported by NIH NS 20629 to SWN and HD 070514 to RIW)

371.8

INCREASES IN ESTROGEN RECEPTOR mRNA IN THE MEDIAL PREOPTIC AREA OF THE MALE RAT FOLLOWING LONG-TERM CASTRATION. R.F. McGivern*, M.R. Bollnow, J.A. O'Keefe, and R.J. Handa. Dept. of Cell Biology, Neurobiology and Anatomy, Loyola Univ., Stritch School of Medicine, Maywood, IL and Dept. Psychology, San Diego St. Univ., San Diego CA.

To explore the role of estrogen in neural function of the male, we have begun studies examining the hypothesis that estrogen receptor (ER) synthesis is autologously regulated in the male rat brain. Male Sprague/Dawley rats (Charles River, Portage, MI) were castrated at 25-28 days of age and sacrificed at 5-6 mos. of age. Littermate controls were left intact. Animals were group housed from weaning and were experimentally naive at the time of sacrifice. Following sacrifice, brains were removed, frozen in isopentane and sectioned at 16 u for subsequent analysis of steady state ER mRNA density using In Situ hybridization (ISH) histochemistry. ISH was performed using a 171 base ^{35}S labelled cRNA probe corresponding to the 3'end of the ligand binding domain of rat ER mRNA. Northern blot analysis showed that this probe hybridizes to a single 6.2 kb species of mRNA in rat brain. Film autoradiograms and emulsion coated slide autoradiograms were analyzed for hybridization density using NIH image analysis software. ER mRNA density was increased by over 150% in the medial preoptic area and bed nucleus of the stria terminalis of castrated males as compared to intact controls ($n=7$; $p<0.01$). These data suggest that testosterone or an estrogenic metabolite of testosterone are involved in the regulation of estrogen receptor synthesis in the male rat brain. Supported by NSF BNS9109226 and NIAAA AA06478

372.1

GnRH cDNA SEQUENCE FROM THE SEXUALLY POLYMORPHIC TELEOST FISH, *PORICHTHYS NOTATUS*. M.S. Grober*, A. Bass and D. Myers†. Section of Neurobiology and Behavior, Cornell University; and †Laboratory for Pregnancy and Newborn Research, New York State College of Veterinary Medicine, Ithaca, New York, 14853.

The plainfin midshipman, *Porichthys notatus*, exhibits two alternative male reproductive morphs (TINS 15:139, 1992). Since gonadotropin releasing hormone (GnRH) regulates the onset of sexual maturation, we are examining GnRH gene expression in this sexually polymorphic species. Using PCR technology utilizing degenerative oligonucleotide (21 mer) primers derived from the GnRH sequence of salmon and cichlid fish, we have identified two amplification products of 200 and 300 bp hybridizing with an oligonucleotide probe that is complementary to the base sequence for amino acid positions 1-7 of the GnRH decapeptide. The presence of two distinct PCR products suggests a molecular basis (e.g. alternate splicing or different GnRH genes) for the existence of multiple forms of GnRH within species.

We have cloned and partially sequenced the 200 base pair cDNA. There is a high degree of homology for amino acid (AA) and cDNA sequences (NA) for GnRH (AA:100%; NA:83%) and GnRH-associated peptide (GAP; AA&NA:>81%) within the fishes. Between fish and mammals there is a high degree of homology for GnRH (AA:80%; NA:73-80%), but less homology for GAP (AA:6-13%; NA:28-33%). It has been proposed that GAP functions as a biologically active peptide and/or dictates secondary structure necessary for processing of GnRH. As with AA and NA sequence data, hydrophobicity profiles based on GAP AA sequences suggest conservation of secondary structure within, but not between mammal and fish GAP. Thus, unlike the GnRH decapeptide, GAP may have different biological roles across the vertebrate taxa, but conserved functions within taxa.

372.2

SYNERGISM BETWEEN ANDROGEN AND ESTROGEN IN THE INDUCTION OF AROMATASE IN THE QUAIL BRAIN: IMMUNOCYTOCHEMICAL AND RT-PCR STUDIES J. Balthazart*, A. Foidart, R. Loeffen, S. Dohmae, and N. Harada Lab. Biochemistry, Univ. Liège, B-4020 Liège, Belgium and Molec. Genetics, Fujita Health Univ., Toyoake, Aichi 470-11, Japan.

It is established that testosterone (T) increases aromatase activity (AA) in the quail brain and that this induction of AA represents a limiting factor in the activation of male copulatory behavior (Balthazart et al, 1990, 47:83-94). This action of T presumably results from an induction of aromatase synthesis since the number of aromatase-immunoreactive (ARO-ir) cells increases (Balthazart et al, 1990, Brain Res. 514:327-333) and in parallel, there is an increase in aromatase mRNA as measured by reverse transcriptase-polymerase chain reaction (RT-PCR) technology (Harada et al, 1992, Molec. Brain Res., in press). The specific role of androgenic and estrogenic metabolites of T in this induction is not yet clear but product-formation assays suggest that both types of compounds synergize to increase AA (Schumacher et al, 1987, C.R.Acad. Sci. 305:569-574). We have analyzed this question by studying both the aromatase protein by immunocytochemistry and the aromatase (ARO) mRNA by RT-PCR in castrated quail that had been treated with T or its androgenic metabolite, 5α-dihydrotestosterone (DHT) or its estrogenic metabolite, estradiol-17β (E2) or both DHT and E2 simultaneously. A specific quantitative PCR technique using a modified aromatase as internal standard was developped for this purpose. T increased the number of ARO-ir cells both in the preoptic area (POA) and in the tuberal hypothalamus and increased the concentration of ARO mRNA in the POA-anterior hypothalamus (POA-aHYP) and in the posterior hypothalamus (pHYP). E2-treated birds had more ARO-ir cells in the POA and tuber, their ARO mRNA concentration was significantly increased in the POA-aHYP but this effect did not reach significance in the pHYP. DHT by itself had no effect on either the number ARO-ir cells or the concentration of ARO mRNA. DHT however synergized with E2 both in inducing ARO-ir neurons and in increasing ARO mRNA concentration. This synergism was observed in all brain areas that were studied. These data demonstrate that both androgens and estrogens regulate aromatase at the pretranslational level. Because the percentage increase in the number of ARO-ir cells was in general very similar to the increase in ARO mRNA concentration, these data also suggest that steroids regulate aromatase only by changing its mRNA secretion or catabolism.

372.3

AROMATASE-IMMUNOREACTIVE CELLS IN THE QUAIL PREOPTIC AREA: SEX DIMORPHISM AND EFFECTS OF TESTOSTERONE A. Foidart*, A. De Clerck, N. Harada, and J. Balthazart Lab. Biochemistry, Univ. Liège, B-4020 Liège, Belgium and Molec. Genetics, Fujita Health Univ., Toyoake, Aichi 470-11, Japan.

We previously demonstrated that testosterone (T) increases aromatase activity (AA) and that AA is sexually dimorphic (males>females) in the quail preoptic area (POA). The precise anatomical localization of these effects is however impossible to obtain by the product-formation assay even when samples are dissected by the Palkovits punch technique. We were recently able to set up an immunocytochemical (ICC) procedure that permits visualization of aromatase-immunoreactive (ARO-ir) cells in the quail brain. This showed that the ARO-ir cells of the quail POA are all located in the sexually dimorphic medial preoptic nucleus (POM) which is actually outlined by this neurochemical marker. This ICC technique was used here to analyze at a cellular level of resolution the sex dimorphism of the quail preoptic aromatase and the localization of T effects on ARO-ir cells. In experiment 1, the number of ARO-ir cells was counted in one section every 100 µm throughout the rostral to caudal extent of the POM in 6 pairs of males and females. This showed that the POM of males contains more stained cells than the POM of females but only in a restricted region located just rostral to the anterior commissure (AC). In experiment 2, the same procedure was used to study the effect of increasing doses of T (silastic implants of 5, 10 or 20 mm) on the number of ARO-ir cells. These T-treatments produced a dose-related increase in the sexual behavior of the birds and they increased the number of ARO-ir cells in POM, especially in the region rostral to the AC. In experiment 3, the number of ARO-ir cells was determined in the POM of males and females that had been gonadectomized and treated with a same dose of T (40 mm). No sex difference in the number of ARO-ir cells could be detected in these conditions. This suggests that the sex difference in AA that had been previously observed in T-treated birds results either from a difference in aromatase concentration or activity in a similar number of positive cells or from a difference in the number of ARO-ir cells that is very discrete from the anatomical point of view. These experiments also indicate that ARO-ir cells in the region of POM located just rostral to AC are sexually dimorphic and T-sensitive which suggests their involvement in the control of male sexual behavior. This conclusion is also supported but our recent studies based on electrolytic lesion and stereotaxic implantation of T in POM.

372.4

HORMONAL CONTROL OF SEX DIFFERENCES IN THE BRAIN, BEHAVIOR AND ACCESSORY SEX STRUCTURES OF WHIPTAIL LIZARDS. J. Wade[1*] and D. Crews[1,2] Departments of [1]Psychology and [2]Zoology, and the Institute of Reproductive Biology, University of Texas, Austin, TX 78712.

The effects of steroid hormones on sexual dimorphisms in the accessory sex structures, behavior and brain were investigated in two species of whiptail lizards. The studies were conducted both in adults and hatchlings of a sexually reproducing species, *Cnemidophorus inornatus*, and an all-female species, *C. uniparens*, which displays "sexual" behaviors typical of males and females. Androgens and estrogen stimulated somatic structures in all animals at both time periods. The hormones also stimulated courtship and copulatory behaviors in many of the adult animals. However, testosterone in adult males of the sexual species was the only treatment which produced parallel effects on the volume of the anterior hypothalamus-preoptic area (AH-POA) and the ventromedial hypothalamus (VMH) and the behaviors which the areas control. Steroid hormones did not elicit parallel effects on the volume of the two brain areas and behaviors in female *C. inornatus* or parthenogenetic *C. uniparens* in adulthood. Further, hormones did not affect the volume of either brain area in hatchlings of either species. The data on males add *C. inornatus* to the list of species in which steroid hormones alter behavior and the volume of brain regions in adulthood. However, the data from females and parthenogens indicate that the size of brain regions and the frequency with which behaviors are produced may not be directly linked.

This work supported by NIMH grant 41770 and Research Scientist Award 00135 to D.C. and NIMH NRSA 09878 to J.W.

372.5

ARGININE VASOTOCIN (AVT) EFFECTS ON VOCAL BEHAVIOR IN CRICKET FROGS (ACRIS CREPITANS). J. Chu, C. A. Marler, and W. Wilczynski*. Depts. of Psychology and Zoology, University of Texas, Austin, Texas. 78712.

AVT induces courtship and clasping behavior in male urodeles (Moore & Miller, *Peptides*, 4:97-102, 1983). We investigated whether AVT similarly increases male reproductive behavior in frogs, where courtship displays take the form of advertisement calls. After their calls were recorded, male cricket frogs in a breeding chorus were injected with AVT (10µg/g BW, IP) or saline, or handled without injection, and replaced on their call sites. After 30 min., advertisement calls were recorded for 5 min. AVT injected frogs were significantly more likely to call than saline injected or handled frogs (χ^2 test, $p<0.025$), indicating that as in newts AVT induces male courtship behavior in frogs. Spectral and temporal call characteristics change in a graded fashion when male cricket frogs engage in aggressive interactions with conspecific males (Wagner, *Ethol.*, 82:27-45, 1989). Preliminary results suggest that AVT treated frogs produce calls with temporal features indicative of higher aggression levels than control frogs, including a lower rate of calls within call groups, an increase in call group duration, and a decrease in call group rate. These preliminary results suggest that AVT not only increases the likelihood of calling, but may also increase the aggressive nature of those calls that are produced. (Supported by NIMH R01 MH45350 and NIMH NRSA F32 MH10204).

372.6

SOCIAL FACILITATION OF SPHINCTER COLOACAE MUSCLE MOVEMENT IN MALE JAPANESE QUAIL. C.M. Seiwert* and E. Adkins-Regan, Department of Psychology, Cornell University, Ithaca, NY 14853.

Male Japanese quail (*Coturnix japonica*) produce a stiff meringue-like foam that is transferred to the female during copulation. This foam may be produced by rhythmic "spontaneous" movements (SMs) of the sphincter cloacae muscle (mSC), which overlies, and is interdigitated with, the proctodeal gland. As a complement to anatomical and physiological studies of mSC and the motoneurons which innervate it, this experiment investigates the possibility that social stimulation enhances expression of SM, and that response to females is greater than that to males. On each of 2 days, mSC movement was monitored in reproductively naive males when the subject was isolated (baseline), when a conspecific was present in the arena but separated from the subject by wire mesh (screen), and when the barrier between subject and stimulus was removed (contact). Subjects were presented with either male or female stimuli on a given day, and order of presentation was counterbalanced [male Day 1 (n=12), female Day 1 (n=12)]. Contact with conspecifics of either sex facilitates expression of SM. Presentation of a stimulus animal significantly increases time exhibiting SM, number of SMs, and number of SM bouts, while latency to begin SM significantly decreases. The level of SM in screen and contact conditions is significantly greater than in baseline, and screen and contact conditions are equally effective in eliciting the behavior. Females are a more potent stimulus than are males. Furthermore, presentation of females enhances subsequent response to males. [Supported by NSF # BNS 88-09441.]

372.7

NEUROPHYSIOLOGICAL EFFECTS OF CORTICOSTERONE RELATED TO BEHAVIOR AND REPRODUCTION IN AN AMPHIBIAN. J. D. Rose* and F. L. Moore Dept. of Psychology, Univ. Wyoming, Laramie, WY 82071 and Dept. of Zoology., Oregon State Univ., Corvallis, OR 97331.

Stress-induced corticosterone (CORT) release blocks courtship clasping by male rough-skinned newts (Taricha granulosa) by an effect involving a neuronal membrane receptor. We studied CORT effects on brain activity in male newts by recording through fine-wire microelectrodes chronically-implanted in forebrain regions rich in CORT receptors (e.g. hippocampal primordium) and in rostral brainstem sites. Neural activity consisted mainly of small spikes, with large spikes occurring during strong sensory stimuli or pronounced movements. Systemic CORT in behaviorally-effective doses elevated forebrain activity in 2-5 min. This effect lasted for several min and was followed by a sustained activity decline. During these activity changes, forebrain neural responses to food or other newts and activity associated with reflexive clasping in response to cloacal stimuli were reduced or abolished. Thus, CORT attenuated forebrain neural responses to stimuli significant for reproductive behaviors and neuroendocrine function. Supported by NIH grant NS13748 (J.R.) and NSF grant BNS8909173 (F.M.).

372.8

DISTINCT HIGH-AFFINITY BINDING SITES FOR ALDOSTERONE AND CORTICOSTERONE ON NEURONAL MEMBRANES. F.L. Moore*, C.S. Bradford, and M. Orchinik. Dept. of Zoology, Oregon State Univ., Corvallis, OR 97331.

Rapid, inhibitory effects of stress on reproductive behaviors in the urodele amphibian *Taricha granulosa* appear to involve membrane-bound corticosteroid receptors that are coupled to G proteins (Orchinik et al. *Science* 252: 1848-1851,'91; *Proc. Natl. Acad Sci.* in press,'92). We recently conducted equilibrium saturation binding studies with radiolabeled aldosterone ([^{3}H]ALDO) or corticosterone ([^{3}H]CORT) and synaptic membranes from *Xenopus laevis*. Synaptic membranes from *Xenopus* were found to contain high-affinity and saturable binding sites for [^{3}H]CORT (K_d = 0.2 nM; B_{max} = 85 fmol mg^{-1} protein), as found previously in *Taricha*, and for [^{3}H]ALDO (K_d = 4.1 nM; B_{max} = 46 fmol mg^{-1} protein). These findings suggest that in distantly related amphibians there are distinct mineralocorticoid and glucocorticoid receptors in neuronal membranes. Supported by NSF grant BNS8909173.

NEUROPEPTIDES AND BEHAVIOR II

373.1

ANXIOLYTIC-LIKE ACTION OF NEUROPEPTIDE Y (NPY) IN THE RAT: MEDIATION BY THE AMYGDALA. K.T. Britton* (1), G.F. Koob (2) and M. Heilig (2). 1: Dept. of Psychiatry, San Diego VAMC and UCSD 2: Dept. of Neuropharmacology, The Scripps Res. Inst., 10666 N. Torrey Pines Rd., La Jolla, CA 92037.

Disturbed NPY transmission has been suggested in depression. Anxiety is a core symptom of the depressive syndrome. NPY concentrations in CSF of depressed patients have been reported to correlate inversely with anxiety scores. In experimental animals, previous results have suggested that centrally administered NPY at low doses may have anxiolytic properties, while higher doses in addition produce sedation.

We have used an established, pharmacologically validated animal model of anxiety, the Geller-Seifter test, to further examine the anxiolytic properties of NPY. Animals were trained to press a lever for food reward. Drug effects were then tested in sessions consisting of an unpunished component (pressing the lever followed by reward only), and a punished component (reward accompanied by mild electric footshock, incremented with consecutive responses). Unpunished responding reflects effects unrelated to anxiety, e.g. on appetite or locomotor performance. Punished responding is selectively increased by anxiolytic drugs. Intracerebroventricular injection of NPY in freely moving rats (0.2 - 5.0 nmol) dose-dependently increased punished responding after 60 min, by up to 100%. A minor increase was seen in unpunished responding, probably reflecting the orexigenic action of NPY. Bilateral injection of NPY into the amygdala also increased punished responding by up to 100%, required only 50 pmol/side for maximum effect, and was effective within 15 min. No effect on unpunished responding or food intake was present. Current data are in agreement with previous observations suggesting NPY to be an endogenous anxiolytic. Further, the anxiolytic-like and orexigenic actions of NPY seem to be anatomically dissociated.

373.2

EFFECTS OF NPY AND NPY$_{2-36}$ ON BODY TEMPERATURE AND FOOD INTAKE FOLLOWING ADMINISTRATION INTO DISCRETE HYPOTALAMIC NUCLEI . S. M. Bouali*, A. Fournier[1] and F. B. Jolicoeur. Depts of Psychiatry and Pharmacology, , University of Sherbrooke, Sherbrooke, Québec. Canada, J1H 5N4 and [1]INRS-Santé, Pointe Claire, Qué, Canada.

Our previous in vivo structure activity studies suggested that the putative receptors mediating the effects of NPY and NPY$_{2-36}$ on food intake and body temperature following ICV administration are pharmacologically different (Jolicoeur et al.1991b, Brain Res. Bul 26: 309). In the present study, we examined and compared dose related effects of NPY and NPY$_{2-36}$ on ad libitum food intake and rectal temperature after administration into discrete hypothalamic nuclei in the rat. Results indicate that NPY and NPY$_{2-36}$ have opposite effects on body temperature when injected into the lateral preoptic area, hypothermia and hyperthermia respectively, and into the anterior lateral hypothalamus, hyperthermia and hypothermia respectively. However, when administered into the paraventricular nucleus, both increased body temperature. Into the third ventricle , NPY produced a biphasic effect, hypothermia at low doses and hyperthermia at high doses. Similar effects were obtained with NPY$_{2-36}$ but in an inverse dose related fashion. In the arcuate nucleus, NPY induced significant hypothermia whereas NPY$_{2-36}$ had no effect. Finally, neither peptide affected body temperature when injected into the ventromedial and perifornical sites. For feeding, both NPY and NPY$_{2-36}$ increased food intake after injection in all regions examined. In general, NPY was more potent and efficacious than NPY$_{2-36}$. The present results clearly dissociate the effects of NPY on food intake and body temperature. Furthermore, data suggest that the putative receptors underlying the effects of NPY and NPY$_{2-36}$ on food intake are similar, whereas those mediating effects on body temperature are pharmacologically different.

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373.3

SCN/MPOA MICROINJECTION OF GRF AND NPY PREFERENTIALLY INCREASE THE CONSUMPTION OF SUGAR-SWEETENED CHOW IN RATS: SIMILARITY TO MORPHINE EFFECT SUGGESTS COMMON UNDERLYING REWARD MECHANISM. S.A. Josselyn* and F.J. Vaccarino, Dept Psychology, University of Toronto, Toronto, Canada, M5S 1A1

Previously, this lab has demonstrated growth hormone releasing-factor (GRF) stimulates chow intake when microinjected into the suprachiasmatic nuclear or medial preoptic (SCN/MPOA) areas of non-deprived male rats. In addition, data suggest GRF's orexigenic action may be mediated by opiates and reflect an enhancement of the food's reward value. The present experiment was designed to explore the consequence of GRF administration on the intake of foods that differed in hedonic value [sugar/chow (10% sugar), saccharin/chow (0.018% saccharin) or unsweetened chow] and further, to compare with the effect of a similar administration of neuropeptide Y (NPY). Non-deprived male rats with SCN/MPOA cannulae were given free access to the food choices. The quantities of foods eaten, microstructure of meal patterns and general locomotor activity were measured following establishment of a stable baseline and microinjections of vehicle (0.01% ascorbic acid, 0.5 ul), GRF (1.0 pmol) and NPY (2.0 nmol). Preliminary results indicate that both GRF and NPY, when microinjected into the SCN/MPOA, produce an augmentation in sugar/chow consumption without accompanying changes in locomotor activity. As baseline measures revealed rats initially preferred the sugar/chow alternative, GRF and NPY preferentially increased intake of the preferred food choice. This pattern is consistent with that obtained following systemic morphine in a similar choice paradigm and implicates a shared underlying reward mechanism.

373.4

TESTS OF AGONIST EFFECTS OF NPY ANALOGS. S. Sheriff, A. Balasubramaniam, J. Krstenansky and W. Chance*. Dept. Surgery, Univ. Cincinnati Med. Ctr., Merrel-Dow & VA Med. Ctr., Cincinnati, OH.

Neuropeptide Y (NPY) is an extremely potent stimulator of feeding. This feeding effect is mediated by Y1 receptors, requiring nearly the whole sequence of NPY for biological activity. To develop agonists to NPY, we have synthesized several analogs modified at the C-terminal. These analogs were tested for NPY binding and adenylate cyclase activity in rat hypothalamic membranes as well as for feeding effects following their injection into the perifornical hypothalamic area of adult, male, SD rats. The potencies of the analogs on these tests were: NPY > NPY 32-PRO > NPY CYS CYS > NPY 36-D-TRP > NPY 36- D-TRP(CHO) > NPY 34-D-TRP. Although the order of potencies for adenylate cyclase and feeding were similar, the feeding response was lost as adenylate cyclase activity was reduced to 50% (NPY 36 D-TRP(CHO)) of NPY. None of the centrally-modified analogs (AOC-8-17-NPY, C2, C5) obtained from Merrel-Dow stimulated feeding significantly. These results indicate that although partial agonist effects remain for adyenlate cyclase following C-terminal modifications of NPY, feeding behavior is lost.

373.5

INTRAHYPOTHALAMIC INJECTION OF NEUROPEPTIDE Y REDUCES METABOLIC RATE AND INCREASES BEHAVORIAL THERMOREGULATION. C.L. Bivens and H.J.Carlisle* Dept. of Psychology, University of California, Santa Barabara, CA 93106

Neuropeptide Y, a potent orexigenic neurochemical, has been observed to decrease core temperature in rats when administered centrally. We investigated the hypothesis that NPY may alter the "set point" for body temperature. Rat-Human neuropeptide Y was injected in various doses (0, 78, 117, 235 pmoles) into the perifornical and preoptic areas of the hypothalamus of male Sprague-Dawley rats while metabolic rate, behavioral thermoregulation, and food intake were measured. In the perifornical hypothalamus, metabolic rate decreased significantly in a dose-dependent manner from 12% to 25% after injection of rNPY in the absence of food. Metabolic rate increased if food was available, with the increase being related to the amount of food consumed (r=.99 for both control and rNPY treated rats). Behavioral thermoregulation increased by 30% to 80% in a dose-dependent manner after injections into the perifornical hypothalamus. Thermoregulatory behavior also increased following preoptic administration of rNPY.

These results indicate that NPY reduces energy expenditure, but not by lowering the set point since thermoregulatory behavior increased so as to oppose the fall in temperature.

373.6

A NEUROENDOCRINE SYSTEM REGULATING EGG-LAYING BEHAVIOR IN THE NUDIBRANCH *ARCHIDORIS MONTEREYENSIS*. B.L. Wiens* and P.H. Brownell, Oregon State University, Corvallis, OR. 97331

In the gastropod molluscs *Aplysia californica* and *Lymnaea stagnalis* egg-laying is a long-lasting behavior triggered by discrete populations of peptide-secreting neurons, the bag cells (BCs) and the caudo-dorsal cells (CDCs), respectively. We describe here a group of neurons in the cerebral ganglia of the nudibranch mollusc *Archidoris montereyensis* that exhibit morphological, immunological, and behavioral similarities to the BCs and CDCs. These intercerebral white cells (ICWCs) are distributed in bilateral clusters each comprised of approximately 50 small (20 μm) cells that appear white under epi-illumination. The location of these clusters, immediately posterior to the cerebral commissure and adjacent to vascular spaces, is identical to that of the CDCs. The ICWCs and their processes in the intercerebral commissure and cerebral ganglia are immunoreactive for alpha bag cell peptide (αBCP) but not alpha caudo-dorsal cell peptide. Each pleural ganglion contains an additional immunoreactive neuron (60 μm) reminiscent of the ectopic BCs and CDCs. These cells appear to be electrically silent, with resting potentials of approximately -60mV, and refractory to discharge of action potentials when focally stimulated. Like the BCs and CDCs, the ICWCs appear to contain hormones that stimulate egg-laying. Injection of cerebral ganglia (6 trials, $p<.01$; Fisher exact probability test) or ICWC homogenates (3 trials, $p<.05$) cause resting animals to lay eggs within 4 hours. Thus, the ICWCs of *Archidoris* have properties similar to the neurons that trigger egg-laying behavior in *Aplysia* and *Lymnaea*, providing further support for the hypothesis that the neuroendocrine mechanism controlling this behavior is common to all gastropods. (Supported by NIH pre-doctoral fellowship #MH09818, and Sigma Xi)

373.7

BLOCKADE OF FATTY ACID OXIDATION INCREASES THE NUMBER OF VAGAL SENSORY NEURONS CONTAINING GALANIN mRNA AND hnRNA. N.Y. Calingasan*, S. Ritter and P.W. Kalivas. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

Mercaptoacetate (MA)-induced lipoprivic feeding is mediated by vagal sensory neurons. Since we previously showed that these neurons synthesize galanin (GAL), a peptide which stimulates feeding when injected intracranially, vagal GAL may participate in lipoprivic feeding. Therefore, we examined the effect of MA-induced lipoprivation on GAL gene expression in the nodose ganglion using in situ hybridization with a 45-base oligonucleotide probe complementary to nucleotides 259-303 of rat preproGAL mRNA. Rats maintained on a fat-supplemented diet were injected with 600 μmol/kg MA, 200 mg/kg 2-deoxy-D-glucose (2DG), 4 μg/kg cholecystokinin (CCK) or saline (n=4/group) and killed 2 hr later. We found that MA significantly increased the percentage (mean ± SE) of GAL mRNA-labeled cells to 75.9 ± 5% compared to saline (16.8 ± 6%), 2DG (21.3 ± 5%) or CCK (16.5 ± 4%). The number of hnRNA- labeled cells was also significantly increased by MA (79.2 ± 2%) but not saline (24.9 ± 7%), 2DG (25 ± 2%) or CCK (19.4 ± 4%). The absence of significant effect of 2DG which, like MA stimulates feeding, and CCK which, like MA acts through vagal sensory neurons, suggests specificity of MA effect. Thus, GAL is a potential neurochemical participant for vagal responses to lipoprivation.

373.8

INCREASED PROENKEPHALIN mRNA IN SPECIFIC HYPOTHALAMIC AREAS DURING LACTATION. M.A. Ottinger[1]*, K.T. Margaretten, K.L. Rosewell, and P.M. Wise, [1]Dept. Poultry Sci, U. of Maryland, College Park, MD and Dept. of Physiology, U. of Maryland School of Medicine, Baltimore, MD 21201.

Enkephalin appears to modulate several aspects of reproductive function in rats and steroids influence the level of gene expression (Romano et al 1989 Mol Brain Res 5:51). In addition, ß endorphin content changes in response to lactation (Hammer & Bridges 1987 Brain Res 420:48). The purpose of this experiment was to determine whether lactation influences proenkephalin mRNA levels in specific brain regions known to be involved in maternal behavior and prolactin secretion. Rats were sacrificed on diestrous day 1 (D1), day 3 (Lac3) or day 10 (Lac10) of lactation. Eight micron sections were subjected to in situ hybridization using an ^{35}S-labelled riboprobe complementary to rat proenkephalin (gift of James Douglass). mRNA levels per cell were analyzed in rostral and mid-arcuate nucleus, magnocellular and parvocellular aspects of paraventricular nucleus and ventromedial nucleus. We observed a highly significant ($p<.0001$) increase in proenkephalin mRNA levels in the mid-arcuate nucleus in both Lac3 and Lac10. mRNA levels increased ($p<.05$) in the rostral arcuate nucleus in Lac10 compared to D1, Lac3 was intermediate. We are currently analyzing mRNA levels in the other brain regions, including the medial preoptic nucleus and corpus striatum. These data provide evidence that enkephalins may play a role in the endocrine and behavioral changes that characterize the lactational state.

373.9

PRESSOR RESPONSES TO MICROINFUSION OF AMINOPEPTIDASE M INTO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS IN NORMOTENSIVE AND HYPERTENSIVE RATS. C.M. Batt*, L.L. Jensen, J.W. Harding and J.W. Wright, Departments of Psychology and Veterinary and Comparative Anatomy, Physiology and Pharmacology, Washington State University, Pullman, Washington 99164.

Experiments were designed to address the potential role of leucine aminopeptidase M (APM) as a therapeutic hypotensive agent in normotensive and hypertensive rat models. In earlier reports from this laboratory blood pressure was reduced in normotensive Wistar-Kyoto (WKY) rats and to a greater degree in spontaneously hypertensive rats (SHR) when APM was microinfused into the lateral ventricles of anesthetized animals (Jensen *et al*, 1992). It has also been shown that microinjections of angiotensin II (AII) into the paraventricular nucleus (PVN) of the hypothalamus results in pressor responses in anesthetized (Brosnihan *et al*, 1987; Diz *et al*, 1985) and alert (Jensen *et al*, in press) rats.

A dose response relationship between APM and hypotension was established in both WKY and SHR rats, with WKY showing maximum mean (±SEM) pressor changes of -38.4±5.3 mmHg in anesthetized and -36.8±2.6 mmHg in alert, free-moving subjects. SHR animals showed changes of -71.2±6.6 mmHg in anesthetized and -76.5±6.0 mmHg in alert animals. Pretreatment with the specific angiotensin receptor antagonist Sar^{1}Thr^8-AII (sarthran) greatly diminished the APM-induced depressor responses, strongly suggesting the responses are angiotensin-dependent.

373.10

ENDOGENOUS OPIOIDS AUGMENT LOCOMOTION VIA A MU1 OPIOID RECEPTOR AFTER DOPAMINE DEPLETION IN THE NUCLEUS ACCUMBENS. L. Churchill* and P.W. Kalivas. Dept. of Veterinary & Comparative Anatomy, Pharmacology, & Physiology, Washington State University, Pullman, WA 99164-6520.

Kelatorphan, a mixed peptidase inhibitor that blocks enkephalin degradation, stimulates locomotion when microinjected into the nucleus accumbens (NA). After bilateral lesion of the dopaminergic innervation to the NA with 6-hydroxydopamine (4 μg/μl free base in 0.25 mg/ml ascorbic acid in sterile saline), kelatorphan augments the horizontal and vertical locomotor activity in a dose-dependent fashion relative to sham-lesioned rats. A specific μ1 antagonist, naloxonazine (10 mg/kg, i.p., 10-12 hrs prior to the microinjections in the NA), blocks the kelatorphan-induced augmentation over the sham-lesioned controls without affecting the kelatorphan-induced responses in the sham-lesioned rats. Similarly, naloxonazine blocks the locomotor augmentation induced by microinjection of the μ-selective opioid peptide, Tyr-D-Ala-Gly-NmePhe-Gly-OH (DAMGO) at 1 nmol/0.5 μl after dopamine depletion in the NA. These results indicate that endogenous opioids also produce an augmented locomotor response and that the μ1 opioid receptor is involved. The molecular mechanism that underlies this behavioral augmentation has not been elucidated yet. Although [^{3}H]phorbol ester binding to protein kinase C has been increased in the NA after dopamine depletion, an inhibitor of protein kinase C activity, 1-(5-isoquinolinesulfonyl-2-methylpiperazine dihydrochloride (H7), does not block the behavioral augmentation to DAMGO, suggesting that the translocation of protein kinase C to the membranes is not a necessary step in this augmentation.

373.11

MEDIATION OF OPIOID EFFECTS IN THE MD AND PPN BY GABA AND MESOLIMBIC DOPAMINE. M.A. Klitenick* and P.W. Kalivas. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

The results from a number of recent studies provide evidence suggesting a role for the dorsomedial nucleus of the thalamus (MD) and the pedunculopontine nucleus (PPN) in the expression of locomotor activity induced by enhanced transmission within the mesocorticolimbic system. The present experiments examined the role of the ventral tegmental area (VTA) in the mediation of the motor stimulant effects of opioid injections into either the MD or PPN. In the first experiment it was found that injection of the μ-opioid agonist DAMGO into either the MD or PPN elicited a dose-dependent increase in locomotor activity. Peripheral administration of haloperidol, at a dose that attenuated amphetamine- but not caffeine-induced locomotion, blocked the motor stimulant effects of DAMGO in the MD as well as in the PPN. However, whereas intra-VTA injection of the $GABA_B$ agonist baclofen attenuated the motor response elicited by DAMGO in the PPN, baclofen did not block the locomotion elicited from the MD following DAMGO. These data suggest that although the opioid-induced behavioral response elicited from the MD and PPN are dopamine-dependent, only the activity elicited from the PPN appears to be mediated, in part, by the VTA. Neurochemical experiments utilizing *in vivo* microdialysis are currently in progress to further assess the involvement of the VTA in the motor stimulant response to opioids in the MD and PPN.

373.12

THE ACTH(4-9) ANALOG ORG2766 MODULATES NMDA RECEPTOR ACTIVITY. Berry Spruijt*. University of Utrecht, IMB, Padualaan 8, 3584 CH, Utrecht, The Netherlands.

In studies on senescent and fimbria fornix lesioned rats the ACTH(4-9) analog ORG2766 has been demonstrated to improve hippocampal functioning. These results have suggested that functional recovery is due to protection against consequences of damage or stimulation of compensatory processes. It is hypothesized that these beneficial effects are due to modulation of NMDA receptor activity which is known to be involved in neuronal protection as well. The modulatory action on NMDA receptor activation is addressed by studying the interaction between ORG2766 and NMDA or its antagonist AP5, on locomotor activity and spatial orientation in a Morris maze. Intracerebroventricularly (icv) injected NMDA (300 ng) induced a dramatic increase in locomotor activity. This enhanced locomotion could be antagonized by AP5 (3μg icv) and equally potent by ORG2766 (10 μg sc). ORG2766 also counteracted the NMDA antagonist-induced impairment in performance in the Morris water maze test. ACTH(4-9) alone in these tasks did not result in behavioral changes. The present results indicate a very potent indirect modulation of NMDA receptor activity by the ACTH(4-9) analog.

373.13

THE EFFECT OF ADRENALECTOMY AND FOOD DEPRIVATION IN THE PORSOLT SWIM TEST. D. Jefferys* and J.W. Funder. Baker Medical Research Institute, Prahran, Victoria, Australia 3181.

In the Porsolt swim test intact animals become progressively immobile over a 15 min test period, and on retest 24h later remain immobile for ~70% of the 5 min retest period. Adrenalectomy (ADRX) blocks retention of the response, with animals immobile for ~35% of the retest period, an effect reversed by dexamethasone (6μg) or ketocyclazocine (1mg/kg). When the antiglucocorticoid RU38486 (3.75mg) is administered alone to intact animals it is without effect, as is MR2266 (1mg/kg) a kappa opioid antagonist, administered alone. In contrast, when RU38486 and MR2266 are simultaneously administered to the intact animals they behave like ADRX on retest.

Recently we have shown that glucose (100mg/kg) reverses the effect of adrenalectomy. Secondly, animals food deprived for 24h behave like ADRX animals on retest, an effect not seen with 48h food deprived animals. In the present study we show that simultaneous administration of RU38486 and MR2266 reverses the effect of 48h food deprivation; administered alone, neither agent is effective. We have also shown that glucose reverses the effect of the simultaneous blocking of glucocorticoid and kappa opioid action in 48h food deprived animals. The results from the present studies provide additional support for the role of metabolic factors in modulating retention of the behavioral responses. They also suggest that in the 48h food deprived animal both adrenal signals facilitating retention are restored.

373.14

TONIC NEURONAL INHIBITION BY ANGIOTENSIN II REVEALED BY IONTOPHORETIC APPLICATION OF A SPECIFIC ANGIOTENSIN II TYPE I RECEPTOR ANTAGONIST. S.N. Thornton*, F.P. Martial and S. Nicolaïdis. CNRS URA 637, Neurobiologie des Régulations, Collège de France, 11 pl. Marcelin Berthelot, 75231 Paris CEDEX 05, France.

Deoxycorticosterone acetate (DOCA) pretreatment enhances neuronal activity and sensitivity to angiotensin II (AII) in the septum and medial preoptic regions of rats. Using 7 barrelled micro-iontophoretic (Io) electrodes sealed to a recording electrode we have investigated extracellularly neuronal responsiveness to Io application of AII, of the specific AII type 1 receptor non-peptidergic antagonist losartan potassium (DUP-753), of atrial natriuretic peptide (ANP) and of aldosterone in two groups of urethane anaesthetised male Wistar rats. The animals in each group, controls (non-pretreated) or experimentals (pretreated, DOCA 0.5 mg/kg s.c.) also had their femoral arteries catheterised for blood pressure recording. Unit activity was also recorded down through the stria terminalis to the central nucleus of the amygdala.

In the above mentioned regions losartan could block the excitatory responses to AII but not those to ANP nor to aldosterone. However, not all the neurons had their responsiveness to AII blocked by losartan. Similar results were found in the stria terminalis but not in the amygdala. Losartan also showed an effect by itself since it could induce an activation lasting as long as the Io application in DOCA pretreated rats, and a prolonged and dramatic activiation in the non-pretreated rats.

Therefore, it could be proposed that in these regions AII exerts a tonic inhibitory action on an as yet unidentified population of neurons via a type 1 receptor. However, not all neurons in these areas appear to have type I AII receptors.

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373.15

THE EFFECTS OF ANGIOTENSIN II IN WATER ABSORPTION RESPONSE BEHAVIOR IN THE SPADEFOOT TOAD, SCAPHIOPUS COUCHII. C.R. Propper, W.E. Johnson, and D.J. Prior *, Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011.

In vertebrates, angiotensin II (A-II) is a potent inducer of drinking behavior in many species. Instead of oral drinking, anuran amphibians obtain water by placing their ventral pelvic region on a moist substrate. The purpose of this investigation was to determine whether A-II induces water absorption response behavior in the spadefoot toad, Scaphiopus couchii. We determined 1) the dehydration rate, 2) whether dehydration induces a behavioral water absorption response, 3) whether A-II activates a behavioral water absorption response in fully hydrated animals, and 4) whether A-II antagonists or angiotensin converting enzyme inhibitors block the water absorption response in dehydrated animals. It took toads 3 hours to dehydrate to 90% of their ad libitum weight. Dehydrated animals spent significantly more time displaying water absorption response behavior than did fully hydrated toads ($P < 0.01$). Angiotensin II at 10 μg/ 100g of toad induced water absorption behaviors in fully hydrated toads compared to saline treated controls ($P < 0.01$). However, saralasin, an A-II antagonist, and captopril, an angiotensin converting enzyme inhibitor, had no significant effect on blocking the water absorption response behavior in dehydrated animals. These results suggest that exogenous, but possibly not endogenous, A-II may influence water absorption response behavior in amphibians.

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373.16

WATER ABSORPTION RESPONSE BEHAVIOR IN THE GREAT PLAINS TOAD, BUFO COGNATUS: EFFECTS OF ANGIOTENSIN II, SARALASIN, AND CAPTOPRIL. W.E. Johnson* and C.R. Propper, Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011.

Angiotensin II (A-II) induces rehydration behavior in vertebrates. Desert amphibians such as Bufo cognatus display water absorption response behaviors in order to rehydrate after exposure to xeric conditions. The following experiments examined the putative role of A-II in water absorption response behavior of B. cognatus. We determined 1) the dehydration rate over five hours, 2) water absorption response in dehydrated toads, 3) whether A-II induces water absorption response in hydrated animals, and 4) whether an A-II antagonist, saralasin, or an angiotensin converting enzyme inhibitor, captopril, blocks water absorption response in dehydrated toads. Bufo cognatus lost 90% of their ad libitum weight in 5 hours via dehydration. When presented with water, dehydrated toads exhibited significantly more water absorption response behavior than hydrated toads ($p < 0.02$). Hydrated toads injected with 100 ug A-II/100g.-toad showed more water absorption response behavior than saline injected controls ($p < 0.02$). Neither saralasin or captopril induced significant blockade of water absorption response behavior in dehydrated toads. These results suggest that exogenous A-II promotes water absorption response. The role of endogenous A-II in water absorption behavior is not clearly defined for B. cognatus.

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374.1

The Effect of Alpha-MSH and MCH on Auditory Sensory Gating

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Auditory gating refers to the decrease in amplitude of tone-evoked CNS potentials that can be measured when two identical tones are presented in rapid succession to the ear. Studies of auditory gating have been carried out in scalp EEG recordings of humans and have been extended to depth recordings in a rat model. Within approximately 50 msec after a tone, an auditory evoked potential occurs in both humans (50 +/- 10 msec) and rats (40 +/- 10 msec). The ratio of the response evoked by the 2nd tone (presented 500 msec after the 1st) to that evoked by the 1st is termed the testing/conditioning (T/C) ratio and is a measure of the effectiveness of gating. Using the rat model, the peptides alpha-MSH and MCH (rat and salmon sequence) were administered icv in a continuous flow system to chloral hydrate (400 mg/kg ip) anaesthetized rats. Depth recordings were carried out in the dorsal hippocampus and T/C ratios were collected both during peptide administration and during infusion with the vehicle alone, both pre- and post-peptide. The effect of alpha-MSH administered alone was to improve auditory gating via an increase in the response to the 1st tone. The effect of MCH was to worsen auditory gating via a decrease in the response to the 1st tone. The maximally effective response was attained at 200 uM for alpha-MSH and 125 uM for MCH. When MCH (125 uM) was administered prior to alpha-MSH (200 uM), the effect of alpha-MSH was blocked. Thus, the two peptides appear to be functional antagonists. These results may be relevant to behavioral disorders characterized by impaired attention to and defective processing of environmental stimuli, specifically schizophrenia, for which numerous studies have now demonstrated a deficit in auditory sensory gating.

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374.2

MELANIN-CONCENTRATING HORMONE AND α-NEOENDORPHIN IMMUNOREACTIVE NEURONS IN THE LATERAL HYPOTHALAMUS PROJECT TO THE PARABRACHIAL AREA OF THE RAT.

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In previous studies we showed that the ibotenic acid lesion of the neurons of the lateral hypothalamus (LH) 1) suppressed self-stimulation in the medial part of the parabrachial area (PBm), the second gustatory relay station, 2) increased the preference threshold for saccharin, 3) altered the modulation of taste by morphine.

In the present study, by coupling retrograde transport and immunohistochemistry, we attempted to identify some of the neuropeptides implicated in the LH-PBm projection. We found that 55-60% of the LH neurons projecting to the PBm are immunoreactive to the melanin-concentrating hormone antiserum. These neurons are localized in the middle and posterior parts of the LH, mainly in juxtacapsular position. In the perifornical area, 28% of the neurons projecting to the PBm are labelled by α-neoendorphin antiserum. In the PBm, a great number of terminals immunoreactive to one or the other antisera was observed.

The colocalization of the two immunoreactivities in the same LH neuron projecting to the PBm was examined.

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374.3

Opiate antagonist effects of the Tyr-MIF-1 family of peptides and their analogs in lizards.

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Previous work suggests that Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) and MIF-1 (Pro-Leu-Gly-NH_2) act as opiate antagonists. Based on the structure of the newly isolated peptide, Tyr-Pro-Trp-Gly-NH_2, new analogs were created by substituting Trp for Leu. All compounds were then combined factorially with a free acid (OH) or a amide (NH_2) at the C-terminus and injected IP in 450 lizards at six doses: 0.0001, 0.001, 0.01, 0.1, 1.0 and 10.0 mg/kg, each with a constant dose of 2.0 mg/kg of morphine. A diluent control group and a morphine control group were also tested. Tail-flick latenices were obtained 15 min. after injection. ANCOVA performed on the data yielded a triple interaction for Peptide x Analog x C-terminus, $p<.05$, as MIF-1 with Leu was more effective with an amide while with Trp it was more effective with a free acid; the opposite was true for Tyr-MIF-1. Peptide x Dose x C-terminus was also reliable, $p<.05$, with the relationship varying as a function of dose. Finally, Dunnett's test indicated that four treatments significantly reduced tail-flick latencies below those associated with morphine: Pro-Leu-Gly-NH_2 (0.0001 mg/kg), Pro-Trp-Gly-OH (0.0001 and 1.0 mg/kg), and Tyr-Pro-Trp-Gly-NH_2 (0.1 mg/kg). Under optimal conditions, some compounds may function as opiate antagonists.

374.4

THE ANORECTIC EFFET OF FENFLURAMINE INJECTED IN PVN IS MEDIATED BY CRF NEURONS.

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Centrally administered d-fenfluramine (dff) decreases food intake when injected into the hypothalamic paraventricular nuclei (PVN). This diminution is specifically due to the reduction (34±3.2% $p<0.001$) of the carbohydrate and the fat (9±0.4%) intake, the protein one being slightly increased when Long Evans rats have free access to pure macronutrients. At the same time the release of CRF increases (J. P. Max et al. Int. J. Obes. 14 sup. 2, p. 164 1990) suggesting the involvement of this neuropeptide in the anorectic effect of dff after PVN injection. We have previously demonstrated that intracerebral administration of a monoclonal antibody to neuropeptide together with ricin A chain and monensin resulted in a long term inhibition of the biological function of the peptidergic neuron (A. Burlet et al Neuroendocrine Res. Methods1991)

We tested the effects of dff PVN injections on feeding behaviour of Long Evans rats after immunolesion of CRF neurons. The lesion was induced by injection of a mixture of toxin [(ricin A chain (230 µg/ml) and monensin (50 nM)] and CRF-monoclonal antibody (generously given by F. G. H. Tilders and J. W. A. M. Van Oers) above PVN (0.25 µl/nucleus). 300 n moles of dff in 0.5 µl of synthetic CSF were injected at the end of the light period, macronutrient selection was measured 24 hours after injection. In these conditions, compared to CSF, dff did not modify food intake and body weight evolution.

These results indicate that fenfluramine needs intact CRF neurons to promote anorectic effect after PVN injection.The ability of rats to select macronutrients is complex and may involve neurotransmitters but also neuropeptides. Our results are in favour of the necessary involvement of CRF in the anorectic effects of dextrofenfluramine when centrally injected.

374.5

ANTAGONISM OF CRF RECEPTORS IN THE CENTRAL NUCLEUS OF THE AMYGDALA ATTENUATES SHOCK-INDUCED FREEZING BEHAVIOR IN RATS.

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Extrahypothalamic corticotropin releasing factor (CRF) is involved in mediating stress-induced behavioral responses. Studies conducted in this laboratory demonstrated that intracranial administration of CRF antagonist attenuated shock-induced freezing behavior in rats. The amygdala has a high density of CRF receptors and is integral to the expression of fear-related behavioral responses.

The present study examines the effect of administration of the CRF antagonist into the central nucleus of the amygdala on shock-induced freezing. The CRF antagonist, alpha-helical CRF_{9-41}, was infused bilaterally into the central nucleus of the amygdala of adult male Sprague-Dawley rats. The duration of electric foot-shock induced freezing was measured in a 15 min test.The antagonist at a dose of 200 ng per animal decreased duration of freezing by 51% ($p < 0.01$).These results suggest that CRF receptors in the central nucleus of the amygdala participate in the expression of fear-related behavioral patterns.

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374.6

SUPPRESSION OF CORTICOTROPIN-RELEASING FACTOR ACTIVITY IN THE PARAVENTRICULAR NUCLEUS ENHANCES FEEDING INDUCED BY NEUROPEPTIDE Y.

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Brain feeding circuits which control meal initiation or rate of food intake may balance the strength of stimulatory signals arising from Neuropeptide Y (NPY) containing pathways and inhibitory input produced by Corticotropin-Releasing Factor (CRF) systems. Since the anatomical and physiological correlation of these two neuropeptides is apparent in the paraventricular nucleus of the hypothalamus (PVN), the present studies examined the effect of both intracerebroventricular (ICV) and intra-PVN administration of a CRF antagonist, α-helical CRF_{9-41}, on NPY-induced hyperphagia. Administration of 1 µg, but not 5 or 25 µg, ICV doses of the CRF antagonist enhanced significantly the hyperphagia induced by a 5 µg ICV dose of NPY. Intra-PVN pretreatment with 250 ng, but not 125 or 500 ng, doses of α-hel CRF potentiated significantly the feeding induced by a 500 ng intra-PVN dose of NPY. No effect of either peptide or their interactive effect was observed following administration into the central nucleus of the amygdala. Systemic pretreatment with the synthetic glucocorticoid, dexamethasone (100 µg/kg), also enhanced appetite stimulated by intra-PVN administration of NPY. These results suggest that endogenous, hypothalamic CRF pathways inhibit feeding evoked by NPY and that both direct neurotropic and peripheral steroid feedback onto CRF neurons serve to modulate short term food intake.

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374.7

PHENELZINE POTENTIATES THE ANXIOGENIC EFFECTS OF INTRACEREBROVENTRICULAR CORTICOTROPIN RELEASING FACTOR IN THE RAT. H. Ward, D. Cottrell, I. Goodman, A. Azzaro* Depts. of Behavioral Med./Psych., Neurology, and Pharmacology/Tox., West Virginia University School of Medicine, Morgantown, WV 26506

There is growing evidence for the role of extrahypothalamic corticotropin releasing factor (CRF) in the neurobiology of anxiety. A defensive withdrawal assay of rat behavior is often used as an animal model that supports the anxiogenic effects of this neuropeptide, and can be antagonized by benzodiazepines. We used a modified form of this assay to confirm these anxiogenic effects and compared 1.0 microgram intracerebroventricular (i.c.v.) CRF to i.c.v. injection of artificial cerebrospinal fluid. CRF treated rats reared less than controls ($p<.001$), spent more time in a withdrawal chamber ($p<.001$), and made fewer passages into a lighted open field ($p<.01$). (N=11) Phenelzine (Nardil) is effective in the treatment of various pathologic anxiety states including panic disorder and social phobia. It was anticipated that chronic pretreatment with this agent would "protect" the animals from the effects of i.c.v. CRF. However, 3.0mg/kg/day subcutaneous administration of this nonselective monoamine oxidase inhibitor for up to six weeks augmented these behavioral findings, thought to represent anxiety in this model. These surprizing results may be explained by a pertubation of normal monoaminergic transmission by phenelzine as opposed to a corrective action of this agent in clinical states of anxiety.

374.8

EFFECTS OF ELECTROCONVULSIVE (ECS) AND KINDLING (KIN) SHOCK ON TRH-PEPTIDES & FORCED-SWIM TEST (FST). A. Sattin* & A.E. Pekary. Endocrinol. Rsrch, Wadsworth-UCLA & *Antidepressant Neuropharm. Rsrch, Sepulveda-UCLA, Sepulveda, CA 91343-2099.

Both ECS and invasive electrical KIN induce synthesis of TRH and its mRNA in rat limbic forebrain regions (rev. MJ Kubek *et al*, **Ann NY Acad Sci, 553**: 286, '89). To examine possible TRH-peptide roles in the antidepressant (AD) effects of seizures (SZ), we now include RIA of related tetrapeptide, TRH-Gly & FST as one measure of AD efficacy. Age 60da male S-D rats (n=19) got sham ECS, corneal ECS or corneal KIN (Skeen *et al*, **Soc Neurosci**'90); alternate days x 3. Sequential ECS current: 50, 100 & 167 mA x 0.6s; for each KIN: 8mA x 4.0s. All ECS gave max. GM SZ. KIN rats all reached Racine stage 3/4. The 15 min pre-swim in 30cm depth, 36°, occurred 24h before 1st Rx; post-swim (5 min; 15cm): at 48h & sacrifice at 72h after last Rx. FST immobility times [Mean(sec)±SEM(N)]: Sham, **223±11(6)**; KIN, **267±5(5)**; ECS, **181±35(6)** (KIN > Sham, $p < 0.05$, 2-tail t test). *Increased* immobility supports expected *pro-depressant* effect of KIN. Predicted anti-immobil. effect of ECS was too variable. This is the first report of TRH-GlyLI in hippocampus (HC): **8.3±1.1(7)** & pyriform/olfactory ctx. (PYR): **28.9±9.6(7)**pg/mg tissue. Like earlier reports of TRH in HC, TRH-Gly increased to **12.7±3.5(6)** after KIN; to **24.1±3.1(6)** after ECS. Both Rx's increased TRH-Gly to ca. **50(6)** in PYR (not signif.). In present results, TRH-Gly exceeded TRH > 10-fold in PYR & > 3-7-fold in HC. Both steady-state & SZ-related processing of TRH-peptides appears to differ in these two limbic regions. Supported by VA Research Service.

374.9

EFFECTS OF CCK_A AND CCK_B ANTAGONISTS ON AMPHETAMINE (AMP)-INDUCED BEHAVIOR IN RATS. G.A. Higgins*, D.M. Tomkins, T.L. Sills+, E.M. Sellers and F.J. Vaccarino+. Departments of Pharmacology, Medicine and Psychology+, University of Toronto and Clinical Research and Treatment Institute, Addiction Research Foundation, Toronto, Ontario M5S 2S1, Canada

The neuropeptide CCK is closely associated with the dopamine (DA) mesolimbic system. In order to examine any functional interaction between these neurotransmitter systems, we examined the effects of a CCK_A antagonist (devazepide) and a CCK_B antagonist (L365-260) against AMP-induced locomotor activation (LMA), stereotypy, place conditioning and palatability-induced feeding. Devazepide (0.001-0.1 mg/kg) failed to modify any of the behaviors elicited by AMP pretreatment. L365-260 (0.01-0.1) produced a mild but non-significant increase in AMP-induced LMA. However, previous research has shown marked individual differences between rats with respect to AMP-induced behavior (Piazza et al. Science 245: 1511-1513, 1989; Sills and Vaccarino. Psychopharmacol 105: 329-334, 1991). L365-260 produced a significant potentiation of AMP-induced LMA in low AMP responders (veh = 611 ± 52; L365-260 0.1 = 1182 ± 223; $p < 0.05$) but not in high AMP responders (veh = 1032 ± 44; L365-260 0.1 = 1062 ± 95). L365-260 was also found to potentiate sugar intake in low but not high baseline feeders. Furthermore, L365-260 potentiated a sub-effective dose of AMP to produce a conditioned place preference, but had only inconsistent effects against AMP-induced stereotypy. Taken together, these studies suggest that L365-260 may potentiate certain DA-dependent behaviors, at least under conditions of low baseline responding.

374.10

THE CCK_B RECEPTOR ANTAGONIST, L-365,260 DOES NOT SUBSTITUTE FOR COCAINE OR MORPHINE IN RAT MODELS OF DRUG ABUSE. F.D. Tattersall, A. Jackson, G. Bentley and M.D. Tricklebank* Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex CM20 2QR, U.K.

The CCK_B receptor antagonist, L-365,260 has been reported to enhance the analgesic effect of morphine in rodents. In order to assess the ability of L-365,260 to enhance the stimulus properties of drugs of abuse, we have examined the effects of the compound in rats trained to (a) discriminate morphine from saline and (b) self-administer the stimulant, cocaine.

Male Sprague-Dawley rats were trained to discriminate morphine (5 mg/kg, i.p.) from saline using a food reinforced operant technique. In substitution tests, L-365,260 (0.063 - 4 mg/kg, i.p., suspended in ethanol/carboxymethylcellulose) failed to induce morphine-appropriate responding. When given immediately before morphine (0.63 - 5 mg/kg, i.p.), L-365,260 (1 mg/kg, i.p.) also did not alter the potency of morphine to induce drug-lever responding.

For self administration studies, male Wistar rats were implanted with a chronically indwelling jugular catheter. During daily 2 h training sessions, the pressing of a lever in an operant chamber elicited a 0.1 ml injection of cocaine (0.25 mg per injection). When response rates had stabilised on an FR5 schedule of reinforcement, L-365,260 (0.3 - 10 mg/kg, dissolved in polyethylene glycol 300) was administered via the catheter immediately before the test session. Response rates were not altered by L-365,260.

Thus, the results suggest that L-365,260 is unlikely to have the stimulus properties often associated with drugs of abuse.

374.11

MODULATION OF 8-OHDPAT-INDUCED HYPERPHAGIA AND ANXIOLYSIS BY CCK-4 AND CCK-8S. H. Fink, A. Rex, Th. Barth and C. A. Marsden# (sponsor: Brain Research Association), Inst. Pharmacol. Toxicol., Charité, Humboldt University, Berlin 1040, Germany. # Dept of Physiology & Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK.

Cholecystokinin (CCK) receptors can be divided into CCK-A and CCK-B/gastrin receptors. CCK-A receptors show an enhanced sensitivity to CCK-8s, while those of CCK-B type display a high affinity for CCK-4 and CCK-8s. The aim of the present study was to investigate the influence of these CCK fragments on $5\text{-}HT_{1A}$ receptor-mediated behavioural effects in rats. $5\text{-}HT_{1A}$ agonists such as 8-OHDPAT induce hyperphagia and anxiolytic effects in suitable rat models. In contrast CCK-A receptor stimulation is known to induce hypophagia while CCK-4 has been shown to produce a pro-conflict effect in a test based on novelty-induced suppression of feeding in rats.

In the present study, both CCK-4 (2 - 50 µg/kg i.p.) and CCK-8s (1 - 25 µg/kg i.p.) suppressed hyperphagia induced by 8-OHDPAT (300 µg/kg s.c.) in freely feeding rats. The anti-conflict effect of 8-OHDPAT (30 µg/kg/i.p.) was also abolished by CCK-4 (2 - 100 µg/kg i.p.) and CCK-8s (0.4 - 100 µg/kg i.p.). These data indicate that CCK-4 and CCK-8s modulate $5\text{-}HT_{1A}$ receptor-mediated hyperphagia and anxiolysis, respectively, and suggest a complex interaction between $5\text{-}HT_{1A}$ and CCK mechanisms.

374.12

BLOCKING CCK-ELICITED HYPOACTIVITY IN HAMSTERS. P. Schnur*, M. Espinoza, R. Flores. Dept. of Psychology, Univ. So. Colo., Pueblo, CO 81001.

Previous research has demonstrated that cholecystokinin (CCK) inhibits locomotor activity in hamsters and that CCK is co-localized in dopaminergic neurons. The present work was designed to test the effects of selective CCK and dopamine receptor antagonists on CCK elicited hypoactivity. In Experiment 1, the effects of devazepide, a CCK_A receptor antagonist, and of L365,260, a CCK_B receptor antagonist, on CCK elicited inhibition of running wheel activity were investigated. Group CCK (n=16) received an IP injection of CCK (25 ug/kg). Group SAL (n=16) received an equivolume IP injection of saline. On four test days, half of the animals in each group received an IP injection of devazepide (0, 0.1, 0.5, 1 mg/kg) and half received an IP injection of L365,260 (0, 0.1, 0.5, 1 mg/kg) 10 min before the injection of CCK or saline. Results indicated that devazepide attenuated CCK elicited hypoactivity in a dose related manner, but that L365,260 had no effect. Neither receptor antagonist alone affected locomotor activity. The same design was used in Experiment 2 to test the effects of SCH23390 (0, 0.1, 0.2, 0.5 mg/kg), a D_1 receptor antagonist, and of sulpiride (0, 5, 10, 20 mg/kg), a D_2 receptor antagonist, on CCK elicited hypoactivity. The results indicated that sulpiride, at a dose of 5 mg/kg, attenuated CCK elicited hypoactivity. At other doses, however, sulpiride had no effect. Sulpiride alone had no effect on locomotor activity. SCH23390 had no effect on CCK elicited hypoactivity at any dose tested, but alone, SCH23390 suppressed locomotor activity. These results indicate that CCK's inhibitory effect on locomotor activity is mediated, in part, by CCK_A receptor subtypes. (Supported by NIGMS/MBRS Grant, #08197-10).

375.1

EFFECTS OF CHRONIC EXPOSURE TO AMPHETAMINE AND NICOTINE ON THE RESPONSE OF THE MESOLIMBIC DOPAMINE SYSTEM TO COCAINE: AN IN VIVO MICRODIALYSIS STUDY B.A. Horger*, A. Valadez and S. Schenk, Dept. Psychology, Texas A&M Univ., College Station, TX 77843.

We have previously reported that preexposure to the psychostimulants amphetamine or nicotine facilitated the acquisition of cocaine self-administration by rats. In the present study we investigated whether this behavioral sensitization could be explained by a neurochemical sensitization in the mesolimbic dopamine system. Rats were preexposed with 9 daily injections of d-amphetamine SO4 (1.0 mg/kg, IP), nicotine bitartrate (0.6 mg/kg, base weight, SC) or the saline vehicle. On the tenth day a microdialysis probe was inserted into the ventral striatum and following a number of baseline dopamine determinations (1-3 pg/35 ul) the rats received an injection of cocaine HCl (15 mg/kg, IP). Samples were collected at 20 min intervals for 60 min post injection. Dopamine levels in saline pretreated rats rose to about 200% of baseline values. Amphetamine pretreatment potentiated this effect. For these rats, cocaine -induced increases in synaptic dopamine levels peaked at about 450% of baseline values. In contrast, nicotine preexposure failed to potentiate the neurochemical correlate of cocaine. Rather, these rats were slightly less responsive to cocaine's ability to increase dopamine in the ventral striatum. Thus, this neurochemical correlate of cocaine cannot explain the sensitized response to cocaine's reinforcing effect produced by preexposure to nicotine.

375.2

CHRONIC COCAINE TREATMENT DECREASES COCAINE-INDUCED EXTRACELLULAR DOPAMINE OVERFLOW IN THE VENTRAL TEGMENTAL AREA: IN VIVO MICRODIALYSIS STUDIES. R. Marmur, J. Chen, A. Pulles, W. Paredes, P.A. Vincent* and E.L. Gardner, Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, New York, NY 10461

We previously reported that cocaine sensitization, as manifested by enhanced extracellular nucleus accumbens (Acc) dopamine (DA) content to acute cocaine challenge, does not appear to involve local neural mechanisms within the Acc (Chen et al., *Soc. Neurosci. Absrt.* 17:323, 1991). The present work sought to assess possible involvement of somatodendritic DA efflux in the ventral tegmental area (VTA) in the development of cocaine-induced sensitization. We treated rats with cocaine (20 mg/kg/day, i.p.) for 16 days followed by 7 days wash-out. On the 24th day rats were challenged with 40 mg/kg cocaine i.p. and extracellular DA in the VTA was measured by *in vivo* microdialysis. Rats treated with chronic cocaine for 16 days showed significantly decreased somatodendritic VTA DA overflow when challenged with acute cocaine as compared to drug-naive rats similarly challenged with acute cocaine. Since it has been previously suggested that increased VTA DA levels mediate the cocaine-induced inhibition of the electrophysiological activity of mesoaccumbens DA neurons (Einhorn et al., *J. Neurosci.* 8:100-112, 1988), the present data may indicate that cocaine sensitization is partially mediated by attenuation of recurrent inhibition produced by somatodendritic DA release in the VTA. (Supported by a research grant from the Aaron Diamond Foundation).

375.3

CHRONIC COCAINE (COC) ADMINISTRATION (7 DAYS) INCREASES TYROSINE HYDROXYLASE (TH) mRNA AND ACTIVITY IN THE RAT NIGROSTRIATAL AND MESOLIMBIC DOPAMINE PATHWAYS. S.L. Vrana*, K.E. Vrana, J.E. Smith and S.I. Dworkin. Center for Neurobiology of Drug Abuse, Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

Cocaine (COC) is a potent catecholamine reuptake blocker and has dramatic effects on neurochemical, neurophysiological and neurobehavioral characteristics of dopamine (DA) pathways. Since TH catalyzes the rate-limiting step in DA synthesis, we have investigated the consequences of COC administration on TH gene expression. Fischer 344 rats were implanted with chronic, indwelling jugular catheters through which they received saline or COC (1 mg/inf) every 8 min for 6 hr daily. This schedule of drug delivery is similar to those seen during self-administration studies. Following 7 days of treatment, TH activity was significantly increased (75%) in the ventral tegmental area (VTA; mesolimbic) and 40% in the substantia nigra (SN; nigrostriatal) by cocaine treatment. Activity levels were not significantly changed in the terminal fields (nucleus accumbens and corpus striatum) by COC. Messenger RNA levels (assessed by northern blot analysis) were increased in both the VTA (65%) and SN (60%) by COC administration. These results indicate that COC can increase TH expression (mRNA and protein) in the cell bodies of the mesolimbic and nigrostriatal pathways, however, this increase is not reflected in the terminal field regions. Since the nigrostriatal pathway has not previously been implicated in COC reinforcement, these effects (in the VTA and SN) may reflect physiological responses indirectly related to the abuse potential of COC. (Supported by NIHGM Grant 38391 (K.E.V.) and DA-03628, P50-DA06634 (J.E.S., S.I.D.).

375.4

CHRONIC COCAINE INCREASES TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN THE VENTRAL TEGMENTAL AREA S.Y. Chen and P.W. Kalivas*. Department of VCAPP, Washington State University, Pullman, WA 99164-6520

We examined the levels of tyrosine hydroxylase (TH) in the ventral tegmental area (VTA), substantia nigra (SN), nucleus accumbens (NA), striatum (STR) and medial prefrontal cortex (mPFC) in cocaine-sensitized rats. In the first experiment, the control group received 7 days of saline, and the remaining groups received either 6 days of saline or 6 days of cocaine (15 or 30 mg/kg, i.p.) followed by cocaine on day 7 (15mg/kg, i.p.). Animals were sacrificed at 2 or 24 hr after the last injection. In an additional experiment rats were given the same pretreatments but the cocaine challenge was given 3 weeks after the last daily injection and animals sacrificed 24 hr later. Locomotor activity measured on day 1 and the challenge day showed an augmented behavioral response to the cocaine challenge in all chronic cocaine treated groups. In the first experiment, Western blot analysis of TH levels showed no changes 2 hr after either acute or chronic cocaine treatment in the brain areas tested, including the VTA and NA. However, at 24 hr, both chronic cocaine-treated groups showed a 25% elevation of TH levels in the VTA . This change was seen only in the VTA and not in the SN, NA, STR or mPFC. In the late withdrawal group, there were no differences between treatment groups in TH levels in the VTA or NA. These results demonstrate that rats sensitized to cocaine show increased TH levels in the VTA 24 hr after the last injection but that levels return to normal after a 3 week withdrawal period.

375.5

ACTIONS OF DRUGS OF ABUSE ON REWARD-RELATED ACTIVITY IN NEURONS OF THE VENTRAL TEGMENTAL AREA AND PREFRONTAL CORTEX IN THE RAT. Kosobud, A.E.*, Harris, G.C. and Chapin, J.K. Hahnemann University, Philadelphia, PA. 19102, USA.

Using chronically implanted microwire electrodes, the activity of multiple single neurons can be recorded simultaneously in awake, behaving animals. In the present experiments, multiple bundles containing 4-6 microwire electrodes were chronically implanted in the ventral tegmental area (VTA) and prefrontal cortex (PFC) of male rats, 450-500g. Following recovery from surgery, recordings were obtained from rats pressing a lever for sucrose reward on a fixed-ratio schedule of reinforcement. In both the VTA and PFC, units were found that showed activity changes around the time of reward, consistent with the involvement of these brain regions in reward processes. Cocaine (10 mg/kg i.p.) produced a significant increase ($p<.01$) in baseline firing rates in cells recorded in both VTA (18%, n=5) and PFC (53%, n=2). These increased firing rates did not seem to be directly related to enhanced dopamine (DA), since acute injections of apomorphine (1 mg/kg i.p.) produced a significant ($p<.01$) decrease (36%, n=6) in baseline firing rates (VTA) and no change in PFC. Cocaine enhanced both excitation and inhibition related to reward in both brain areas (n=8, $p>.01$), while apomorphine enhanced activity just prior to reward onset, in VTA only (n=6, $p<.01$). Ethanol (.5 g/kg i.p.), like cocaine, sharpened reward-related activity, but this effect was less pronounced. Smaller changes in basal rates and patterns of reward-related activity were also observed in the VTA (23%, $p<.01$, n=4) during control sessions with or without saline injections, suggesting that endogenously released DA had effects similar to those seen following drug-mediated DA release. Consistent with this interpretation, haloperidol (30 µg/kg i.p.) blocked these changes. Supported by grants NS26722, AA06965, K02-AA00089 and AFOSR 90-0266.

375.6

THE EFFECTS OF COCAINE AND MORPHINE ON MESOLIMBIC NEURONS IN THE RAT. H. Zhang* and E.A. Stein, Depts of Pharmacology and Psychiatry, Medical College of Wisconsin, Milwaukee, WI 53226.

The mesolimbic system has been ascribed a critical role in the reinforcing properties produced by both psychomotor stimulant and opiate drugs. A common neural substrate (eg, nucleus accumbens (NAS) and olfactory tubercle (olf tub) has been suggested. However, while NAS plays an important role in both cocaine and opiate reinforcement, the critical opiate receptor(s) mediating reinforcment may be postsynaptic to the dopamine (DA) terminal. Thus, a neural circuitry of reward beyond the DA terminal appears warranted. Significant efferent projections from both the NAS and olf tub to the ventral pallidum (VP) have been described and investigations into this output have established the VP as a critical component in the expression of behaviors produced by stimulants. Thus, we examined the effects of IV cocaine (1.0 mg/kg) and morphine (0.5 mg/kg) on the spontaneous discharge rate of neurons in 2 ventral tegmental (VTA) terminal fields: NAS and olf tub, and their major efferent projection field, VP in urethane anesthetized rats. The ability of VTA stimulation to drive recorded mesolimbic neurons allowed subsequent subgroup division. In all 3 regions, neurons both excited and inhibited by VTA stimulation were observed. After systemic cocaine, excitatory and inhibitory responses seen in the NAS apparently corresponded to the VTA stimulation response direction. In olf tub, while both excitatory and inhibitory responses were seen to VTA stimulation, most of these cells showed no response to either systemic cocaine or morphine. Finally, VP neuron responses to both drugs were predominantly excitatory. Supported in part by DA 06485 to EAS.

375.7

LISURIDE REDUCES COCAINE SELF-ADMINISTRATION AND AMPHETAMINE WITHDRAWAL IN RATS *L. Pulvirenti* and G.F. Koob Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA.*

The maintainance of intravenous (IV) self-administration of psychostimulant drugs in rats seems to depend upon activation of dopamine (DA) receptors within mesolimbic areas, while the withdrawal phase is thought to be associated with a state of relative DA hypofunction. The effects of administration of lisuride (a direct DA D2 receptor agonist) were therefore studied in rats trained to lever press for IV cocaine self-administration (0.75 mg/kg/inj.; 3hr/day) and during withdrawal from self-administration of high doses of amphetamine. Acute pretreatment with lisuride (0; 0.025; 0.1; 0.2; 0.4 mg/kg IP) produced a dose-dependent decrease in cocaine intake. Lisuride also prevented the increase in responses observed when saline was substituted for cocaine. Rats withdrawing from chronic exposure to high doses of amphetamine (15hr/day x 10 days; average intake 7.37 mg/kg/day) experienced a state of psychomotor retardation as measured by reduction of spontaneous locomotor activity and increased catalepsy 48 and 96 hrs after discontinuation of access to the drug. Subchronic administration of lisuride during the withdrawal phase (0.2 mg/kg IP twice daily) prevented the occurrence of such changes. These results suggest that lisuride may reduce cocaine-seeking behavior and some behavioral signs of amphetamine withdrawal and warrant further studies on the effect of lisuride in psychostimulant addiction in humans.

375.8

MODELING COCAINE SELF-ADMINISTRATION IN RATS WITH REWARDING MFB STIMULATION: EFFECTS OF RACLOPRIDE AND SCH23390. E. McCaskill*, A. Foster, and J.R Stellar, Dept. of Psychology, Northeastern University, Boston, MA. 02115.

Recently it has been established that self-administration behaviors can be modeled with rewarding medial forebrain bundle (MFB) stimulation administered in an unconventional cocaine like waveform (Franklin et al. NS Abstracts:15:1092,1989 and McCaskill et al. NSAbstracts: 17:489.11,1991). We challenged rats working in this paradigm under various stimulation parameters (current, waveform duration) with D1 and D2 blocking drugs (SCH23390, .002-.025 mg/kg; Raclopride, .003-.08 mg/kg). In comparison to rats running on the standard rate-frequency curve-shift method, rats engaging in self-administration of self-stimulation appear to be more sensitive to either D1 or D2 receptor blockade. Evidence for the classic self-administration response enhancing effects of dopamine receptor blockade was found and is related to changes in stimulation current which was also previously reported to increase response rate and therefore average stimulation density. The effects of dopamine receptor blockade are also discussed in terms of other changes in response pattern and in relation to aversive side-effects of MFB stimulation.

375.9

THE EFFECT OF HALOPERIDOL DECANOATE AND ALPHA-FLUPENTHIXOL DECANOATE ON COCAINE SELF-ADMINISTRATION IN RATS. N.R. Richardson*, A.M. Smith and D.C.S. Roberts. Department of Psychology, Carleton University, Ottawa, Canada, K1S 5B6

The effect of decanoate forms of haloperidol (HAL) and alpha-flupenthixol (α-FLU) on cocaine self-administration were evaluated using FR 1 and progessive ratio (PR) schedules. Rats were implanted with chronically indwelling IV cannulae and trained to self-administer cocaine (0.6 mg/inj) during daily 5 h sessions until a stable baseline was established. A single IM injection of either HAL (2.5 mg) or α-FLU (2.0 mg) produced an increase in cocaine intake on an FR 1 which was maximal after 3-4 days and diminished slowly over the next several days. Separate groups of animals showed a decrease in breaking points on the PR schedule with a similar time course.

These data demonstrate that one injection of the decanoate form of either HAL or α-FLU can reduce the reinforcing effects of cocaine for up to a week. While the use of neuroleptics in the treatment of cocaine abuse remains controversial, the use of a depot administration circumvents one issue, that of patient compliance. (Supported by NIDA contract no. 271-90-7401)

375.10

ROLE OF DOPAMINE RECEPTOR SUBTYPES IN THE DEVELOPMENT OF COCAINE-INDUCED BEHAVIORAL SENSITIZATION. B. A. Mattingly*, T.Hart, K. Lim, and C. Perkins. Department of Psychology, Morehead State University, Morehead, KY 40351.

Previous research indicates that the development of behavioral sensitization to amphetamine and apomorphine may be prevented by concurrent treatments with dopamine D_1, but not D_2, antagonists. The objective of the present study was to extend this receptor analysis to include the development of behavioral sensitization to cocaine. In two experiments, male Wistar rats (250-350g) were injected daily for 4 to 7 days with either cocaine (15 mg/kg, IP) or vehicle in combination with either the D_1 dopamine antagonist, SCH 23390 (0.3 or 0.5 mg/kg) or the D_2 dopamine antagonist, sulpiride (100 mg/kg, IP). After the daily injections, the rats were tested for locomotor activity in photocell arenas. At the conclusion of this sub-chronic pretreatment phase, all rats were tested for locomotor activity following a challenge injection of cocaine (15 mg/kg) alone. Consistent with previous results, cocaine produced a progressively greater increase in activity over the first few treatment days. Moreover, this acute cocaine-induced increase in activity was blocked by concurrent treatments with either SCH 23390 or sulpiride. In contrast to previous work with amphetamine and apomorphine, however, concurrent SCH23390 treatments did not prevent the development of behavioral sensitization. That is, rats pretreated with SCH 23390 and cocaine did not differ from rats pretreated with only cocaine for 4-7 days when given a cocaine challenge injection. These results suggest that behavioral sensitization to cocaine may develop through either dopamine D_1 or D_2 receptor stimulation. (Supported by the Kentucky EPSCoR committee and Morehead State University).

AGING AND BEHAVIOR I

376.1

DO DEVELOPMENTAL BRAIN LESIONS HASTEN AGING IN BRAIN FUNCTION? M.H. Lee* and A. Rabe. New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

In experimental animals, perturbations during prenatal or early postnatal brain development by teratogenic agents can lead to increased functional impairment in old age. The age-associated decline may be either premature appearance of functional decline typical of normal aging, or recurrence of specific symptoms that appeared after treatment but from which the animal recovered.

(1) Rats with micrencephaly, resulting from prenatal exposure to methylazoxymethanol, showed exaggerated decline in spatial navigation during the second half of their lives. This age-associated decline could be partially prevented by normal neocortical tissue transplants given in infancy. (2) C57BL/6 mice, exposed to a single dose of ethanol in utero, exhibited an augmented retention deficit of spatial location at middle-age (Rabe & Dumas, *Soc. Neurosci. Abstr.* 1992). (3) Wallace et al. (*Dev. Psychobiol.* 5:35, 1972) reported recurrence in old age of abnormalities in motor and emotional behavior which had appeared following neonatal cerebellar irradiation, but from which the rats had recovered. These phenomena appear to be related and suggest that developmental lesions reduce "redundancy" in the brain (Glassman, *Neurosci. Biobehav. Rev.* 11:275, 1987), and thus hasten functional aging. Structural disorganization resulting from the early treatment may play an additional role in the accelerated functional decline.

376.2

ANALYSIS OF AGE-RELATED MOTOR ACTIVITY IN SQUIRREL MONKEYS. L.E.DeLanney[1]*, B.Tabrizi[2], J.W.Langston[1], [1]California Parkinson's Foundation, San Jose, CA 95128; [2]Dept. Indust. Engineering, Stanford University, CA 94045.

MPTP-treated squirrel monkeys provide a model strikingly similar to idiopathic Parkinson's disease, an age-associated affliction. However, development of parkinsonian behavioral traits is more subtle in squirrel monkeys than in humans and some Old World monkeys. Therefore a principal objective was to assess motoric behavior in untreated and treated monkeys of various ages. To achieve this objective two devices were designed and built. The first was an infra-red beam, computerized activity cage to record movements per minute (mpm). The second device was a grasp-and-pull treat retrieval apparatus (tr).

The results obtained through the use of these two techniques to date are: a) Each animal has its own characteristic mpm, unvarying with the time of day or year. b) Caged MPTP-treated animals that might not show overt parkinsonism, show significant and persistent drops in their mpm; c) Mpms of naive animals can be correlated with their ages, when known, and may become predictive of ages of feral animals. d) Young animals (3 yr) master the tr task in as short a time as 2 hours of training and retain memory, on re-challenge, for as long as 3 months to date. e) Each animal's technique to retrieve is unique. f) MPTP-treated animals without prior training have been refractory to tr learning. g) A future objective is to assess tr in older monkeys.

We conclude that from these two systems we obtain quantifiable data to permit correlations of movements with age, with MPTP treatment and with neurochemistries and neuropathologies, and permit analysis of refined motor performance achieved after mastery of the treat retrieval.

376.3

COGNITIVE FUNCTION AND AGING IN THE DOG E. Head, E. Weiner, T. Yearwood, C. Reid, E. Thomas and N.W. Milgram*
Life Sciences Division, Scarborough Campus, U. Toronto, Scarborough, Ontario Canada, M1C 1A4

Although it is widely believed that cognitive function deteriorates during aging in the dog, no previous studies have been addressed to this topic. We first compared old (> 8 years), middle aged (5-8), and young (< 5) dogs on tests of object discrimination learning, reversal learning and recognition memory using a delayed non-matching-to-sample (DNMS) paradigm. As has been found with other species, there were no significant age differences in discrimination learning - but there was considerably greater variability in the oldest group of animals, and the correlation between chronological age and errors to criterion was also significant. Again, consistent with work on other species, we did find significant differences between old and young animals in reversal learning. In DNMS testing there were more marked differences, and none of the oldest group (n=7) were able to learn the task when the delay was set at 10 seconds. In contrast, 7 out of 8 young dogs and 6 out of 8 middle aged dogs successfully achieved criterion levels of accuracy at a 10 second delay. Examination of the response patterns revealed that dogs typically tested position strategies in their attempts to solve the problems presented. The aged dogs were often much more unwilling to discard this strategy and this accounted for some of the age differences.

We have also tested dogs on a spatial version of the DNMS test involving presentation of a sample stimulus, followed by a test in which the correct response is to approach the side opposite to that of the sample. Overall, this proved to be a much easier task for dogs, and some of the oldest animals have already achieved a high degree of accuracy (>80% correct) at delays as high as 90 seconds. For the dog, in contrast to the primate, the spatial memory task appears to be much easier than the object recognition task.

376.4

DISCRIMINATION AND EXPECTANCY IN ADULT AND OLD RATS. A. Caprioli*, O. Ghirardi, A. Pietromarchi, M.T. Ramacci, #L. Angelucci, Institute for Research on Senescence, Sigma Tau, Pomezia; #Pharmacology II, La Sapienza Univ. of Rome, Italy

The role of aging in discrimination and expectancy was measured in an independent two-choice reaction time test in Fischer-344 adult (5-7 months) and old (22-24 months) rats. Each rat discriminated between two stimuli, light and tone (Exp. 1), and between 4000Hz Tone 1 (T1) and 12000Hz Tone 2 (T2) (Exp. 2) by releasing one of the two correspondent levers, left or right. Expectancy to each stimulus was modified by varying the probability of stimulus occurrence (10%, 50%, 90%, 100%). Reaction time (RT), percent of correct responses (CRs), discriminability (D) and response bias criterion based on CRs were measured. In Exp. 1, between two different stimulus modalities, the auditory RT in old rats was slower than in adult rats, but there was no difference in the other measures between the two age groups. In Exp. 2, within the tone modalities, the CRs and D were lower in old rats than in adult rats. Stimulus probability influenced RTs and CRs in both experiments and in both age groups. In fact, RTs and CRs to the more probable stimulus were faster and higher, respectively, than those to the less probable stimulus for both visual and auditory stimuli and T1 and T2 stimuli. The results demonstrated that rats developed an expectancy about the probability of future events. Rats shifted their response bias and decreased RT to the more probable stimulus. Expectancy was not remarkably different between the two age groups.

376.5

BRAIN CHOLINERGIC, NORADRENERGIC AND OPIOID CORRELATES OF SPATIAL MEMORY IMPAIRMENT IN AGED RAT. T.D. Smith*[1], M. Gallagher[2] and F.M. Leslie[1].
[1]Dept. of Pharmacology, University of California, Irvine, CA 92717 and [2]Dept. of Psychology, University of North Carolina at Chapel Hill, NC 27599.

Age-related alterations in the density of cholinergic, noradrenergic and opioid receptor (sub)types and/or reuptake binding sites were simultaneously examined in discrete forebrain regions of Long-Evans rats using quantitative autoradiography. Spatial memory performance was assessed in the Morris water maze. Age-associated neurochemical changes were distinguished from specific memory-dependent alterations by correlating density of binding sites with behavioral performance of aged rats. Regionally widespread age-related changes were observed in density of muscarinic and nicotinic cholinergic binding sites, while significant differences in noradrenergic markers were limited to limbic structures. Although the density of some binding sites decreased, others remained constant or even increased. With respect to the opioid receptors, the only significant change observed was a decrease in the density of mu binding sites in the basal ganglia. Thus, these data argue against a hypothesis that a generalized decline in neurochemical integrity occurs with advancing age. Furthermore, an increased density of cholinergic M_1 and noradrenergic β_2 binding sites in hippocampal subfields and piriform cortex, respectively, was correlated with impaired spatial memory. These results indicate that synergistic changes in cholinergic and noradrenergic systems may, in part, lead to impairments in mnemonic processes.

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376.6

AGE-RELATED CHANGES IN THE PHARMACOLOGICAL IMPROVEMENT OF RETENTION IN SENESCENCE ACCELERATED MOUSE (SAM). J.F. Flood*and J.E. Morley, Geriatric Research Education and Clinical Center (GRECC), VA Medical Center and Department of Internal Medicine, Division of Geriatric Medicine, Louis University School of Medicine, St. Louis, MO 63106.

The P/8 line of the senescence accelerated mouse (SAM) model exhibits characteristics of aging early in its lifespan including an early onset of impaired learning and memory which becomes progressively worse with age. Age-matched controls of the R/1 line do not show impaired learning and memory. We now report age-related changes in the drug dosage needed to improve one week retention in the P/8 but not R/1 line. The results indicate that 8 month old P/8 mice show a reduced sensitivity to memory enhancing doses of cholinomimetics and an increased sensitivity to a serotonin antagonist compared to 4 month old mice. By 12 months of age, improvement of retention required higher doses of cholinomimetics and lower doses of the serotonin antagonist. Higher doses of an opioid antagonist and a dopamine agonist were needed to improve retention in 12 month old mice. A GABA antagonist and an alpha noradrenergic agonist improved retention at the same dose in mice 4, 8 and 12 months of age.

376.7

CORRELATION BETWEEN MORPHOLOGICAL AND BEHAVIORAL DEFICITS IN AN ADULT RAT MODEL FOR AGING. T. Kadar*, I. Arbel[1], S. Dachir, M. Silbermann[1], and A. Levy [1]Faculty of Medicine, Technion, Haifa. Israel Inst. for Biol. Res., Ness-Ziona, ISRAEL.

The use of old rats in the study of age related cognitive deficits does not enable a follow up of the aging changes as they evolve, and is impaired by physical incapacitation of part of the subjects. Using the radial arm maze, we have shown recently high correlation between cognitive impairments and morphological alterations at the hippocampal formation, in Wistar rats aging 3 to 24 months. In an attempt to establish the adult rat as an animal model for the study of progressive aging processes, the present study monitored the acquisition of the Morris water maze in a large group of 12 months old Fischer rats, in comparison to a group of 3 months old rats. Two distinguished populations of impaired vs unimpaired rats were found among adult rats. The degree of behavioral impairment was correlated in individual animals with the severity of hippocampal damage, based on quantitative morphometric analysis in the hippocampus. It is suggested that the adult 12-months old rat may serve as a preferred animal model for the study of the mechanism of age-related cognitive and morphological deficits.

376.8

COMPLEX ENVIRONMENTAL CUES AND EXTENDED TRAINING AMELIORATE AGE-RELATED SPATIAL LEARNING DEFICITS IN F-344 RATS. C.F. Maciutus*, H.L. Black and R.M. Booze, College of Pharmacy, Tobacco & Health Res. Inst. & University of Kentucky Medical Center, Lexington, KY 40546-0236.

Age-related deficits in spatial learning are often interpreted as reflecting a decline in the capacity to acquire and/or utilize spatial information. Conflicting reports on the influence of simplification of extramaze environmental cues on spatial learning prompted us to examine the role of visual disability as a contributing factor. The demonstration of an improvement in spatial learning as a function of complex and/or redundant environmental stimuli would strongly argue against a major role of visual decline in cognitive processing. Aged Fischer-344 male and female rats, were trained in the Morris water maze task, beginning at 28 months of age. Animals were trained under enriched and standard environmental cue conditions in a cross-over design to permit assessment of the factors of cue complexity, extended training, and their interaction. For each training condition one male and one female from each litter (*ns*=8) received 8, 8, and 4 training trials on three consecutive days followed by a probe test trial at the end of every day. Cue complexity condition did not differentially affect the decrease in training trial latency, although the decline in latency was primarily linear, without any quadratic component typical of young adult animals. Quadrant preference scores, although not reflecting an effect of cue complexity during an animals' initial training cue condition, demonstrated an enhancement as a function of cue complexity and continued training. Annulus crossings demonstrated a similar, though not statistically significant, pattern. In sum, although neither cue complexity or extended training increased performance to that expected of young adult animals, the influence of cue complexity is counter to a functional role of visual impairment in the aged-related decline in spatial learning capacity. (Supported by UKMCRF and AG 10747).

376.9

AIT-082 MODULATES WORKING MEMORY IN NORMAL MICE AND MICE WITH AGE-INDUCED MEMORY DEFICITS, R.F. Ritzmann*, B. Pirzadeh, C. Melchior and A. Glasky. Advanced Immuno-Therapeutics, Irvine, CA, and Olive View Medical Center, Sylmar, CA

Since working memory has been shown to be impaired in early and middle stages of Alzheimer's disease, it is of therapeutic interest to develop a drug that can restore working memory. The win-shift foraging paradigm is a single trial T-maze learning model of working memory. By increasing the interval between successive trials, the length of time a mouse can remember can be determined (i.e. duration of memory trace). AIT-082 is a derivative of the purine hypoxanthine that has been evaluated for its effects on working memory in the win-shift model.

In young adult Swiss Webster mice, AIT-082 increased the duration of the memory trace from 60 seconds to 120 seconds. AIT-082 is active over a dose range from 0.5 to 60.0 mg/kg with the optimal effect at 20 mg/kg. The onset of action is rapid (1 hour) and lasts for more than 7 days after a single administration of 60 mg/kg. Tolerance does not develop after 18 days of treatment and the memory enhancing effects are blocked by atropine, a known cholinergic antagonist. AIT-082 also increased the duration of the memory trace in young adult C57Bl/6 mice

In 11 month old Swiss Webster mice, we found (a) 70% of these mice had a moderate deficit, (b) 15% had a mild deficit and (c) 15% could not remember at 10 seconds. AIT-082 increased the duration of the memory trace in subjects with a mild and moderate working memory deficits.

In summary, the win-shift foraging model can detect strain and age-induced differences in working memory which are reversed by AIT-082.

Work supported by grant from the NIA-AG09911and NIAAA-AA08709.

376.10

EMBRYONIC ALCOHOL EXPOSURE AND AGING: A DELETERIOUS FUNCTIONAL INTERACTION. A.Rabe* and R.Dumas. NY State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

An embryonic exposure to alcohol produced a deficit in long-term memory that became prominent already at middle-age. No readily obvious brain abnormalities accompanied the functional defect. Young adult (3 mo), middle-aged (12 mo) and old (24 mo) C57Bl/6J mice of both sexes were trained on a place learning task in a cross-shaped water maze. The alcohol groups (n=62) of these three ages were offspring of dams who had been exposed to a single teratogenic (5.8 g/kg) dose of ethyl alcohol by gavage on gestation day 9 (plug = GD 1). The 3 control groups (n=54) came from dams exposed to dextrose. Testing consisted of (a) 6 acquisition trials a day for 6 consecutive days, and (b) 6 retention trials on a single day 24 days later. The middle-aged and old alcohol-exposed mice displayed a marked retention deficit on 3 different measures of performance, whereas the young alcohol animals showed only a modest loss on one measure. No alcohol-exposed group was inferior to controls on the acquisition trials, nor did they show a retention loss after a 24-hour interval.

376.11

INCREASED SENSITIVITY TO AVERSIVE MOTIVATION IN MICE FOLLOWING SHORT- AND LONG-TERM DIETARY RESTRICTION. M. J. Forster* and H. Lal. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

Male C57BL/6NNia, DBA/2NNia and B6D2F$_1$ mice fed ad libitum or reared under dietary restriction (60% of ad libitum beginning at 4 months) were tested for sensitivity to aversive motivation when approximately 9, 19, or 27 months old. In order to estimate the contributions of short- and long-term diet restriction, half of the mice within the ad libitum (A) and chronic restricted (R) conditions were switched to the opposite condition one month prior to the test (yielding RR, RA, AA, and AR groups). Aversive motivation was measured according to a spatial preference procedure [*Biomed. Environ. Sci.*, **4**:144, 1991] in which seven intensities of scrambled, constant current shock (from 0 to 0.24 mA) were presented through the grid floor of a small chamber in durations of up to 20 s. The mouse could minimize shock in the chamber by moving a short distance (8 cm or less) to the opposite side and remaining there for the rest of each shock period. Comparison of aversion functions (% safe time x current intensity) failed to suggest remarkable changes with age for any of the genotypes or diet groups. However, there was a profound (2-5-fold) increase in shock aversion for RR groups relative to AA groups within all age x genotype combinations. Comparison to AR and RA groups suggested that both long- and short-term restriction of diet contributed to the overall differences. These findings indicate that diet restriction may substantially modify neural processing of aversive stimuli and, thereby, influence the outcome of studies of learning and an memory processes using aversively-motivated behavioral paradigms. Supported by NIA grants AG07695 (HL) and AG06182 (MJF).

376.12

INTERACTIONS BETWEEN THE EFFECTS OF AGE AND OF BENZODIAZEPINE RECEPTOR LIGANDS ON ATTENTION: TAXING PROCESSING CAPACITY. J. McGaughy* and M. Sarter. Dept. Psychology, Ohio State Univ., Columbus, OH 43210.

We have previously demonstrated that the performance of differently aged rats in simple and choice reaction time tasks shows considerable face validity in terms of modelling the effects of human aging and practice on vigilance performance. However, in contrast to the human situation, performance in the choice reaction time task did not more powerfully separate age-related differences, and the effects of age did not reliably interact with the effects of compounds known to augment the age-related attentional deficits in humans. As it is assumed that inadequate task demands on processing capacity formed the basis of these limitations, differently aged rats were trained and tested in a signal detection paradigm that required the animals to detect differently salient signals and, in addition, to process propositional response rules. Performance in this task varied with age, treatment with benzodiazepine receptor ligands affected performance, and these effects interacted with age. These results suggest that by increasing the demands on processing capacity, more valid behavioral paradigms of the age-related impairments in attentional abilities can be developed. Such paradigms are required for the examination of hypotheses about the neuronal basis of age-related impairments in cognitive abilities.

376.13

AGE RELATED CHANGES OF SOCIAL MEMORY/RECOGNITION IN MALE FISCHER 344 RATS. X.-B. Guan* and D.E. Dluzen. Department of Anatomy, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Two different habituation-dishabituation test paradigms were used to evaluate differences in social memory/recognition among 3, 15 and 22 month old male Fischer 344 rats (N=8/group). In Experiment I, males received three 2 minute exposures to the same stimulus ovariectomized female, followed by three 2 minute exposures to a different stimulus female with an inter-trial interval of 6 minutes. Investigation (mean±SEM in seconds) of the stimulus animal upon first exposure (trial 1) was not significantly different among the three groups: 19.56±4.04, 22.93±4.02 and 28.14±2.02 for the 3, 15 and 22 month old animals, respectively. All groups showed a habituation response with investigation times decreasing on trials 2 and 3. Investigation times were not significantly different among the 3 groups on trial 3: 9.47±3.34, 5.66±2.88 and 3.30±1.11, respectively. Introduction of a different stimulus female on trial 4 resulted in significant differences with investigation times of the 3 month animals (22.83±4.09) being significantly greater than both the 15(12.77±2.40) and 22(5.27±1.09) month animals. The 15 and 22 month animals failed to differ statistically. In Experiment II, all animals received two trials with different stimulus females used in each trial. Respective investigation times for the 3, 15 and 22 month animals on trial 1 vs 2 were 14.02±2.71 vs 22.76±4.00, 19.91±2.10 vs 11.33±1.75 and 24.79±1.55 vs 5.65±0.94. Taken together, these results demonstrate that the 22 month old rats show habituation, but markedly defecient dishabituation responses compared to the 3 month old animals, while the performance of the 15 month animals was intermediate. These results suggest an age dependent decrement in social memory/recognition processes in the male Fischer 344 rat.

376.14

CHRONIC INFUSIONS OF GABA INTO THE MEDIAL PREFRONTAL CORTEX INDUCE SPATIAL ALTERNATION DEFICITS IN AGED RATS. S. Meneses [1], Galicia, O., Hernández, E. and S. Brailowsky* Instituto de Fisiología Celular and [1] Facultad de Psicología, U.N.A.M., 04510 México D.F., MEXICO.

Lesions of the prefrontal cortex (PFCx) may produce severe deficits in spatial delayed alternation tasks (DAT). Previous experiments have demonstrated that GABA infusions into the PFCx of young rats affect the execution of a spatial DAT learned before the infusion (Behav. Brain Res., 40:81, 1990). It has been proposed that functions associated with the PFCx could change as a consequence of aging. In the present study we investigate wether chronic infusions of GABA into the PFCx of aged rats induce deficits similar to that observed in young rats in a spatial DAT.

Fifteen 26-month old rats were trained to alternate the arms in a T-maze (win-shift paradigm); after the criterion for acquisition was reached, cortical infusions of GABA (50 µg/µl/hr) or saline were applied through osmotic minipumps for 7 days. Rats were tested on the T-maze during this period.

The results indicate that old rats need more sessions to acquire the task than young rats. GABA infusion into the PFCx produce severe deficits in spatial alternation tasks similar to that observed in young rats. After the infusion period the performance rapidly recovered.

The results can be taken to suggest that the PFCx in aged rats is important for the retention of previously learned spatial delayed alternation tasks.

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376.15

THE EFFECT OF LONG TERM DEPRENYL ADMINISTRATION ON BEHAVIOR AND BRAIN FUNCTION IN AGED RATS P. C. Bickford*, C. Heron, G.M. Rose, N. Miniclier, K. Poth, A.M-Y. Lin, M. Friedemann, and G.A. Gerhardt Medical Research Service, VAMC; Depts. of Pharmacology and Psychiatry, and Neurosciences Training Program UCHSC, Denver, CO 80220.

Deprenyl has been shown to increase the longevity of rats. This study was undertaken to examine the effects of deprenyl on behavior and brain function. Twenty F344 rats were treated with l-deprenyl (0.5 mg/kg/day) via the drinking water starting at 12 months of age, twenty age-matched rats served as controls. The mean lifespan was extended in the deprenyl group from 51.5 ± 3.7 months to 59.0 ± 1.4 months ($p < 0.05$ two-tailed Student's t-test). At 18 months of age, the rats were tested on a series of motor coordination tasks and a motor learning task that involves learning to negotiate unevenly spaced pegs on a runway. Performance on these motor coordination and learning tasks was not different between l-deprenyl and control aged rats. There was also no difference when the rats were tested for spatial learning in the Morris water maze at 24 months of age. At 28 months of age the remaining rats (6 deprenyl and 4 controls) were studied with *in vivo* electrochemistry using carbon fiber electrodes coated with nafion. The clearance of exogenously applied norepinephrine (NE) from the extracellular space of the cerebellar cortex was not different between the two aged groups. The ability of nomifensine to increase the $t_{1/2}$ of the NE signal, however, which is normally lost in aged controls was intact in the deprenyl treated group. (Supported by Vet.Admin. Med. Res. Service, USPHS grants AG04418, AG10755, AG06434, AG00441 and AFAR).

376.16

BEHAVIORAL DEFICITS AND MORPHOLOGICAL CHANGES IN FRONTOPARIETAL CORTEX AFTER BASAL FOREBRAIN LESIONS IN ADULT AND AGED RATS. C.L. Wellman*, S.F. Logue, and D.R. Sengelaub. Program in Neural Science and Department of Psychology, Indiana University, Bloomington, IN 47405.

Regressive changes in aging may interact with other degenerative processes to produce unique neuropathologies. We are using basal forebrain lesions in adult and aged rats to determine the contribution and potential interaction of age and depletion of cholinergic input on frontoparietal morphology and function. Six-, 13-, and 21-month-old rats received sham or ibotenic acid lesions of the basal forebrain. One month after surgery, rats were trained in a radial arm maze task, which has been shown to be sensitive to alterations in the forebrain cholinergic system, until 4 of their first 5 visits were to baited arms on 4 of 5 consecutive trials. Number of trials to criterion and average between-trials errors did not vary with age. However, lesioned rats took significantly longer to reach criterion and made more between-trials errors than did sham-lesioned rats. Lesioned aged rats entered significantly fewer arms per trial and were significantly slower than were the other groups. Average within-trials errors were unrelated to either age or treatment. Three months after surgery, cellular changes in frontoparietal cortex (Frontal I and Parietal I) were assessed in frozen sectioned, cresylecht violet stained material. Neuronal soma area in lamina II-III increased from 9 to 16 months of age, but decreased from 16 to 24 months of age. Across groups, basal forebrain lesions differentially affected smaller neurons. Lesions increased soma size in lamina IV of both cortical areas of 9-month-old rats, and decreased soma size in lamina II-III in both areas and lamina IV of parietal cortex in 16-month-old rats. In 24-month-old lesioned rats, soma size increased in lamina IV in both areas and decreased in lamina VI of parietal cortex. Thus, while changes in soma size across ages are unrelated to maze performance, basal forebrain lesions produce correlated changes in cortical morphology and behavior.

376.17

AGE-ASSOCIATED CHANGES IN PROPERTIES OF BASAL FOREBRAIN NEURONS PROJECTING TO THE FRONTAL CORTEX IN THE RAT. J. Tanaka* M. Nomura, J. Nishimura and F. Kimura. Dept. of Physiol., Saitama Med. Sch., Saitama 350-04, *Dept of Physiol., Yokohama City Univ. Sch. of Med., Yokohama 236, Japan

We recorded extracellular single-unit activities of neurons that were antidromically activated by electrical stimulation of the frontal cortex, in the nucleus basalis area of urethane-anesthetized young (4-5 months old) and aged (30 months old) male rats, and compared them in an attempt to find changes with age in various electrophysiological properties of these neurons. Post-antidromical orthodromic excitatory responses were found, and the number of neurons showing such an orthodromic excitation was significantly smaller in aged rats. There was no significant difference in the threshold, latency or the conduction velocity of antidromic activation, or in the spontaneous firing rate of driven cells between the two groups of subjects. The absolute refractory period was significantly longer and the incidence of successful antidromic propergation into the somatodendritic complex was significantly lower in aged rats. The data provide evidence that cortically projecting putative cholinergic neurons in the nucleus basalis area undergo dramatic changes with age in their physiological properties.

376.18

EEG CORRELATES OF AGE RELATED BEHAVIORAL IMPAIRMENTS. P. Curzon*, M.W.Decker, R.J. Radek, Neuroscience, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL. 60064-3500

EEG changes related to senesence have been identified in humans and in animal models of aging. Increased incidence of a spike and wave pattern termed high voltage spindling (HVS) has been observed in the EEG record of aged rats. Aged rats have impairments in a variety of learning and memory tasks, but as yet there is no established link between increases of HVS and these impairments. The purpose of this study was to determine whether HVS in 8 (mature) and 22 (aged) month old rats is associated with deficits on a spatial discrimination version of the Morris water maze.

Wistar rats were surgically implanted with recording electrodes over the frontal and parietal cortices. After recovery, the rats were trained for 5 days in the water maze to distinguish between a visible escape platform and a platform that sank under the weight of the rat. Contacts with the incorrect (sinking) platform were scored as errors. After completion of the water maze experiments, 60 minute EEG recording sessions were conducted during which HVS durations (in sec.) were determined for each rat. Spindles were identified as regular 6-10 hz burst complexes.

Mean errors on days 4 and 5 of water maze training in mature (n=9) and aged (n=10) rats were 2.0±0.3 and 3.7±0.6, respectively ($p<0.05$). Mean HVS times of mature and aged rats were 50.3±25.9 and 58.4±13.5, respectively ($p>0.05$). Two rats in the mature group expressed high levels of spindling which substantially increased the mean for this group. Correlation coefficient (r) from linear regression analysis between HVS and day 4/5 water maze errors was 0.73 ($p<0.05$) for aged rats, and 0.43 ($p>0.05$) for mature rats. The 2 mature rats with high spindling times had the poorest training performance among rats in the mature group. These results suggest the existence of a correlation between increased incidence of HVS in aged rats and behavioral impairments in a spatial discrimination water maze task.

376.19

NICOTINIC MODULATION OF RAT HIGH VOLTAGE SPINDLING AND EEG. R.J. Radek*, S. Ryu, Neuroscience, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Il. 60064-3500

Increased incidence of a spike and wave pattern termed high voltage spindling (HVS) has been observed from the EEG of 8 and 22 month old rats and may be associated with cognitive deficits of aged rats. Since nicotine improves cognitive performance in rats, this study sought to determine whether nicotinic agents modulate HVS activity in a way consistent with effects on learning and memory tasks.

Wistar (10 month) rats were surgically implanted with recording electrodes over the frontal and parietal cortices. HVS were identified from EEG recordings as regular 6-10 Hz burst patterns. Duration of HVS (in sec.) for each rat was determined from 60 minute recording sessions. Rats were administered (i.p.) either nicotinine, cotinine, mecamylamine or dimethylpiperazinium (DMPP) immediately before recordings commenced.

Nicotine desynchronized the EEG in all rats. Nicotine at 0.62 and 1.9 µMol /kg lowered HVS time 83% and 94% from baseline ($p<0.05$, n=9), respectively. Cotinine (0.62, 1.9 µMol/kg), a major metabolite of nicotine, had no effect on spindling (n=9). Mecamylamine (15.0 µMol/kg), a nicotinic antagonist which impairs rat water maze performance (Decker and Majchrzak, Psychopharm., in press), increased HVS time 76% from baseline ($p<0.05$, n=6). A lower dose of mecamylamine (5.0 µMol/kg) did not significantly increase HVS but blocked nicotine (0.62 µMol/kg) induced attenuation of HVS. DMPP (0.62, 1.9, and 6.2 µMol/kg), a nicotinic agent which does not cross the blood brain barrier, had no effect on HVS (n=6). Cotinine, Mecamylamine, and DMPP had no pronounced effect on EEG amplitudes during non-spindling EEG. These results suggest that HVS are sensitive to those centrally active nicotinic agonists and antagonists that possess specific cognitive enhancing or impairing properties.

377.1

MAGNETIC SOURCE IMAGING (MSI): DIRECT INTRA-OPERATIVE VERIFICATION OF NEUROMAGNETIC LOCALIZATIONS OF SOMATOSENSORY EVOKED AND SLOW WAVE FIELDS USING A LARGE ARRAY BIOMAGNETOMETER. C.C. Gallen*, B.J. Schwartz, T. Waltz, B. Copeland, D.S. Sobel, M. Aung and F.E. Bloom The Scripps Research Institute (TSRI), La Jolla, CA 92037

Presurgical localization of critical brain functional and dysfunctional regions potentially allows more effective planning to avoid important regions in the surgical approach to pathology. Noninvasive neuromagnetic localization of the postcentral gyrus in humans was accomplished using a large array (37-channel) biomagnetometer in a rapid (60-120 min) procedure allowing localization of the large 45-70 evoked field generated by a painless tactile stimulus. These localizations computed in a 3-dimensional Cartesian coordinate system were then merged with Magnetic Resonance Imaging (MRI) scans of the subjects via common fiducial points to allow visualization of functional and dysfunctional activity relative to brain structures and lesions in a procedure referred to as Magnetic Source Imaging (MSI). Five subjects were subsequently taken to surgery and intraoperative corticography was used for localization of the central sulcus, median nerve somatosensory or motor stimulation and slow wave activities. In all cases neuromagnetic and corticographic methods agreed completely on the localization of the central sulcus relative to lesion. The MSI and corticographic localizations of the median nerve area agreed within 3-15 mm. Areas implicated as producing slow wave activity on MSI were verified intraoperatively. These combined data proved useful in presurgical planning.

377.2

THE HUMAN EPILEPTIC FOCUS STUDIED BY SIMULTANEOUS LASER DOPPLER FLOWMETRY, MICRODIALYSIS AND ELECTROCORTICOGRAPHY.

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The aim of this study was to monitor pathophysiological changes in the human epileptic focus by the combination of laser doppler flowmetry (LDF), intracerebral microdialysis (MD) and electrocorticography (ECoG).

Five patients with medically intractable epilepsy, undergoing resection of the epileptic focus, were included in the study. The preoperative localization of the focus was confirmed intraoperatively by ECoG together with electrical stimulation of the cortex. A combined subdural electrode and LDF probe was placed at the focus. During the ECoG and blood flow measurements MD from the epileptogenic area was performed.

Electrical stimulation elicited epileptiform activity with a duration of 12-120 sec in all the patients. Epileptiform activity was associated with changes in the regional cortical blood flow of 0,7-7 times the basal leves. In four patients there were increases and in one patient a decrease of blood flow. The blood flow returned to preseizure levels within 2-6 min. Heart rate and systemic blood pressure remained constant. MD was performed in cortex of the temporal lobe in one patient, hippocampus in three patients and in both cortex and hippocampus in one patient. Epileptiform activity with a duration of 40 sec or longer, was associated with an increase in aspartate, glutamate and serine, with a maximum of 2.6, 2.1 and 2.5 times the basal level, respectively.

In conclusion, the combination of LDF, MD and ECoG appears to be a powerful tool for studying temporal relationships between changes of blood flow, transmitter release and electrophysiological events in the human epileptic focus.

377.3

P300 ACTIVATION TIME: A NON-INVASIVE TOOL FOR PRE-SURGICAL EVALUATION IN TEMPORAL LOBE EPILEPSY

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Evidence suggests that the P300 long-latency auditory evoked response is related to activity in the temporal and medial-temporal structures (McCarthy and Wood, 1987; Okada, Kaufman, & Williamson, 1983). These areas are often the source of focal abnormalities in epileptic patients. This study describes a new measure, "P300 Activation Time" (AT), that varies according to the hemisphere of epileptic pathology, and identifies left temporal lobe patients with better accuracy than absolute P300 latency.

P300 AT was calculated for each hemisphere in 20 normal subjects, 19 left temporal lobectomy (LTLs), and 8 right temporal lobectomy (RTLs) patients prior to surgery. Absolute latencies at 18 electrode sites did not differ between groups at any site. However, the difference in AT between the hemispheres varied significantly between groups ($df=2$, $p<.0005$). Eleven (58%) of the LTLs had a left hemispheric AT that extended more than 10 ms over the right hemispheric AT. None of the normal subjects, and only 1 (12%) of the RTLs exhibited this pattern. These findings suggest that the non-invasive measure, P300 AT, is potentially more useful in the pre-surgical evaluation of epilepsy patients than absolute latency.

377.4

REGIONAL CEREBRAL BLOOD FLOW MAPPING IN EPILEPTICS USING TECHNETIUM-99M-HMPAO SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY. J. S. Cheon*, Department of Neuropsychiatry, Kosin Medical College and Gospel Hospital, Pusan 602-702, South Korea.

The cerebral blood flow in the epilepsy was reported to be increased during ictal phase and decreased during interictal period, while others insisted of interictal hyperperfusion due to subictal neuronal hyperactivity.

In this study, the cerebral blood flows of seventeen idiopathic epileptics were compared with those of twelve neurotic controls measured by 99m Tc-HMPAO SPECT to observe the perfusion changes in the interictal phase.

As compared to the controls whose activity counts per frame were decreased to $5.5\pm3.3\%$(mean$\pm$S.D.), 29.4% of idiopathic epileptics showed hyperperfusion to $10.9\pm7.2\%$ ($P<0.05$), and 70.6% showed hypoperfusion to $10.8\pm3.3\%$($P<0.001$). Most foci of maximal blood flow change were located at the right hemisphere and posterior part. The foci in the anterior part could be found more in controls (33.3%), while posterior foci in epileptics(88.2%). The degree of EEG abnormalities was not correlated with the activity counts per frame, while the abnormal findings mostly composed of high voltage(88.9%) spike or slow waves were notified more in the hyperperfusion group(80.8%) than in the hypoperfusion group(41.7%).

377.5

CORPUS STRIATUM ABNORMALITY IN MAGNETIC RESONANCE IMAGING (MRI) AND N-ACETYL-ASPARTATE (NAA) IN THE URINE AS A CAUSE OF CONVULSIONS IN A 2 YEAR OLD BOY. P.B.Toft, O.Pryds, O.Kiehn*, E.Christensen, H.Lou, O.Henriksen. Danish Research Center of Magnetic Resonance, KKHH, DK-2650 Hvidovre.

A nine mo old boy presented with febrile, generalized, tonic-clonic convulsions. Unprovoked convulsions from the age of 15 mo. He sat alone 9 mo old and walked 18 mo old. Electroencephalogram was normal and seizures could be controlled with valproat. Urine examination at the age of two y showed large amounts of NAA (6.1 g/L urine). He was suspected to have a mild form of Canavan disease and MRI and 1H-MR-spectroscopy (MRS) were done to reveal the white matter changes typical for this disease. MRI showed perfectly normal white matter, but the globus pallidus, putamen and caudate nucleus gave high-signal intensity in the T2 weighted images. MRS of 8 mL occipital lobe gave [NAA]=11.9 (9.5), [creatine]=5.4 (6.3), [choline]=0.9 (1.6) mmol/L brain tissue, results from a four year old boy with non-metabolic convulsions in (). The elevated NAA/Cho is consistent with earlier findings in Canavan disease but the classical form is excluded by the normal white matter pattern in MRI. This case suggests that the corpus striatum may have an influence on seizure susceptibility. Urine examination for NAA should be done in children with seizures.

377.6

DIFFERENCES IN LONG-TERM RECOGNITION BETWEEN LEFT AND RIGHT TEMPORAL EPILEPTICS AFTER SODIUM AMYTAL INJECTION. J. Robidoux*, I. Rouleau, & R. Labrecque. Neurology Service, Notre-Dame Hospital, Montréal and Université du Québec à Montréal.

The intracarotid sodium amytal test (ISA) was used in 51 patients with intractable unilateral temporal lobe epilepsy (LT=30, RT=21) prior to surgery. To bypass the problem of aphasia and to enable comparison of performance after left and right injection, common objects were presented visually (color pictures) and tactually, and their naming was prevented. Memory was assessed by yes/no recognition. Two measures were obtained for each patient: (1) short-term recognition (STR) of stimuli presented and recognized during the effect of the drug; (2) long-term recognition (LTR) of the same stimuli when normal brain function has resumed (20min after the injection). Statistical analysis revealed that, although both LT and RT patients' scores improved from STR to LTR after the injection ipsilateral to the epileptic focus and worsened after contralateral injection, both effects were more pronounced in LT patients. The present findings suggest that the use of color pictures and concrete objects presented tactually, although theoretically amenable to dual coding, is more discriminative for LT than for RT patients. The hemispheric processing of these stimuli is discussed as a function of the sensory modality of presentation, language dominance and methodological factors such as the duration of stimulus presentation and the level of sedation at the begining of memory testing assessed by the percentage of delta waves on EEG.

377.7

COHERENCE IN HUMAN SUBDURAL EEG HAS FINE STRUCTURE IN SPACE AND TIME. T.H. Bullock*, J.Z. Achimowicz and M.C. McClune. Dept. Neurosci. & Neurobiology Unit, Scripps Inst. Oceanog., U.C.S.D., La Jolla, CA 92093-0201.

Analyzing EEGs from subdural strips of 8 electrodes 5-10 mm apart in epileptic patients, power, phase and coherence (C) spectra were computed in alert, sleep and seizure states, none with sharp peaks in power. C was averaged for bands 2-5, 5-8, 8-13, 13-20, 20-35, 35-50 Hz. Spatial fine structure is suggested by pooling C values for all pairs of the same electrode distance. C vs distance falls from 1.0 within a small distance to 0.5-0.7 at 5 mm, 0.3-0.5 at 10 mm, 0.2-0.4 at 20 mm, approaching at 50-70 mm the level for 2 independent noise sources. Variance is high in 5 s samples but means are reproducible in 30-120 s samples. Different bands are remarkably similar. Higher frequency (F) bands tend to show slightly lower C values at a given distance. Slow wave sleep has slightly higher values in all F bands but the visual impression of synchronization is due mainly to a difference in power spectrum, not to true population synchrony. Seizures show markedly higher C values at all Fs. The highly local nature of C is also shown by maps of values for adjacent electrode pairs 10 mm apart in strips of 8: values may be quite different for contiguous pairs but consistent over time. Remarkably, the profile of values along a strip is similar across bands. Steepness of fall with distance is also locally different and similar between bands. Temporal fine structure was studied by computing C with autoregressive models in 1 s epochs and examining the time series of values over 100-200 s for given pairs and states. Histograms of values in sleep, alertness and seizure are unimodal and quasi-normal. Variation of C over time is high but often departs from the null hypothesis of being a totally random sequence. By first lag auto-correlation and by comparing the power spectrum of the time series with shuffled versions, we find most 10 mm pairs have significant power at one, two or three periods between 7 s and 60 s, less often in time series from higher bands. Maps show some adjacent pairs have peaks at the same periods, but most do not. Low-passed time series of C values from different EEG bands show high cross-correlation; from adjacent pairs some do, many do not. *C can be quite local and change slowly over many seconds. The similarity in C across bands in several respects does not support an EEG model of discrete oscillators.*

377.8

INFORMATION PROCESSING DURING ABSENCE EPILEPSY IN RATS. W.H.I.M. Drinkenburg, A.M.L. Coenen, J. Duysens*, and E.L.J.M. van Luijtelaar. Dept. of Psychology, University of Nijmegen, Nijmegen, The Netherlands.

A characteristic of absence epilepsy is the temporary change in consciousness. Processes controlling these changes are largely unknown: however, visual evoked potentials, registered during spike-wave discharges (SWD), showed that sensory transmission, the first part of information processing, is altered.

The present study investigates a second part: stimulus evaluation, which requires also some memory retrieval. For this purpose, 10 male, adult rats of the WAG/Rij strain were permanently implanted with fronto-cortical EEG electrodes and mildly deprived of food. WAG/Rij rats frequently show spontaneously occurring SWD and are considered as a valid animal model for absence epilepsy.

Firstly, all rats were habituated to auditory stimuli of 8kHz with a 20 dB low (L) and a 45 dB high (H) intensity by repeatedly presenting these tones without any contingencies. Next, these neutral stimuli were presented during SWD and effects on epileptic activity were analyzed. In the second part, conditioning of these tones took place with a classical procedure: for one group (n=5) each presentation of the high intensity tone was followed by a food reward (H^+), while presentation of the low intensity tone was never rewarded (L^-). For the other group rewarding was reversed (H^- and L^+). Tones were then presented during SWD and effects on ongoing epileptic activity were again analyzed.

Only in about 10 % of the presentations of the neutral tones (both H and L) SWD were influenced. After conditioning 77.7% of the H^+ and 60.7% of the L^+ presentations totally stopped ongoing SWD, whereas the previously not rewarded stimuli H^- and L^- stopped SWD in only 37.2 and 36.5%.

It is therefore concluded that, notwithstanding the fact that disturbances in information processing during SWD exist, evaluation of external stimuli to a certain degree still takes place.

377.9

Kindling Characteristics and Susceptibility to Status Epilepticus of Various Temporal Lobe Structures. D. C. McIntyre*, C. Dufresne, M. E. Kelly and P. Mohapel. Dept. of Psychology, Carleton Univ., Ottawa, Canada, K1S 5B6.

Previously Le Gal La Salle (Kindling 2, 1981) demonstrated heterogeneity between various amygdala nuclei in their rates of kindling. We extend this observation by comparing the kindling profiles of 3 amygdala nuclei (basolateral, central, medial), and the piriform (Pir) and perirhinal (Prh) cortex. Thirty Sprague-Dawley rats were stimulated daily from one site until 6 generalized stage-5 convulsions were triggered. The different sites were compared for local afterdischarge (AD) thresholds, kindling rates and convulsion characteristics, including latency and duration of clonus and AD duration. Susceptibility to status epilepticus (SE) was then assessed for each of the 5 structures by subjecting the kindled site to 60 min of electrical stimulation at an intensity 10% above the AD threshold.

The rate of kindling in the Prh was significantly faster than either the basolateral or medial n., and only slightly faster than the central n. and Pir. In addition, the latency to onset of forelimb clonus was significantly shorter in the Prh, suggesting it may be functionally closer to the mechanisms of secondary generalization than the amygdala nuclei or the Pir. Despite being slower to kindle than the other structures, provocation of the basolateral n. most easily triggered SE. These data suggest that the mechanisms and circuits associated with development of kindling and SE likely are not the same.

377.10

TWO TYPES OF NEUROPLASTICITIES IN THE KINDLING PHENOMENON I, EFFECTS OF STIMULUS-INTERVAL. K. EMORI* and Y. MINABE. Tanino Gozan Hospital and Toyama Med. & Pharm. Univ., Toyama 930-01, Japan.

Recently we reported that pulse-number threshold (PNT), the number of stimulating pulses required for the triggering of epileptic afterdischarge (AD) and the indicator of seizure threshold in the low-frequency hippocampus kindling, dropped profoundly in the initial stage of kindling and the behavioral seizure stage (BSS) developed in the later stage (Brain Res. 509: 355-357, 1990). We assume that there is a possibility that there are two types of neuroplasticities in the kindling phenomenon which are independent of each other, i.e. the early type involving the decrease of seizure threshold and the late type involving the development of BSS. The advantage of our procedure is that we get a quantitative seizure threshold measure during a single stimulation train, whereas the conventional train-intensity procedure requires the delivery of at least several trains and can not neglect the influence of repetitive sub-threshold testing stimuli. In this study we further provided the dissociation of seizure threshold (PNT) changes and the increases in strength in HP kindling.

During the kindling process, PNT showed a sudden decrease at the early stage; in contrast, BSS increased in the later stage. After the completion of kindling, a 4week interval elevated PNT significantly compared to the value at the completion of kindling, whereas BSS showed no regression. A 2-week interval after the first 5 AD-triggering stimulations elevated PNT of the 6th stimulation significantly without affecting BSS and AD duration compared to the values of the no-interval group and PNT dropped again by the following a few stimulations. A 2-week interval after 10 AD-triggering stimulations failed to elevate PNT.

377.11

TWO TYPES OF NEUROPLASTICITIES IN THE KINDLING PHENOMENON II, PHARMACOLOGICAL STUDY. Y. MINABE* and K. EMORI. Toyama Med. & Pharma. Univ. and Tanino Gozan Hospital, Toyama 930-01, Japan.

Using the low-frequency kindling technique, we studied the effects of chronic MK-801 (0.05 and 0.1 mg/kg/day i.p.), scopolamine (SCOP, 0.5 and 1.0 mg) and methamphetamine (MAP, 6 mg) administration on hippocampal kindling seizure development. The number of stimulating pulses required for the triggering of epileptic afterdischarge (pulse-number threshold, PNT) was used as an indicator of the seizure threshold. PNT, afterdischarge duration (ADD) and behavioral seizure stage (BSS) of each induced seizure in the initial stage of kindling, kindling rate, seizure parameters at the completion of kindling were recorded and compared to the values of each saline-treated control group.

MK-801 administration prior to each electrical stimulation selectively and significantly increased PNT in the initial stage of kindling without affecting other seizure parameters. Although SCOP 1.0 mg/kg increased PNT at the 5th stimulation compared to control, no other significant changes of the seizure parameters were found by SCOP. Chronic pretreatment of MAP caused a selective and significant decrease of PNT of the first two stimulations in the kindling process. Taken together with the previous studies, these results support our findings that physiological and pharmacological characteristics of the reduction of seizure threshold, but not of the development of induced seizures in the hippocampal kindling process are quite similar to those of long-term potentiation.

377.12

ABSENCE OF NEURONAL DEATH IN KINDLING: A STUDY WITH THE CUPRIC SILVER DEGENERATION STAIN. M. Khurgel[1]*, R.C. Switzer[2], G.C. Teskey[3], A.E. Spiller[3], R.J. Racine[3] and G.O. Ivy[1]. [1]Dept. Anatomy and Cell Biology, Univ. of Toronto, Scarborough, ON M1C 1A4, [2]Neurosci. Assoc., Knoxville, TN 37922 & [3]Dept. Psychology, McMaster Univ, Hamilton, ON L8S 1B9.

It is controversial whether or not kindling results in neuronal degeneration. We have studied this question with a sensitive cupric silver degeneration stain. Rats were implanted with electrodes in the right amygdala and, after 4 weeks, kindled until the first stage 5 seizure. Rats were subsequently sacrificed at 3, 12, 24, 48, 72 hr, and 7 d. Brain sections were stained with the cupric silver method and were processed for GFAP and vimentin (Vim) immunoreactivity (IR). The silver stain revealed a pattern of degenerative changes which was similar for all animals, including electrode implanted controls. Traces of degeneration were, in most instances, unilateral to the implantation site and were confined to fiber tracts: dorsomedial part of corpus callosum, internal capsule and fimbria. There was no evidence of degenerating neurons or their immediate processes anywhere along the neuraxis. Nevertheless, a prominent increase in GFAP and Vim IR was present in astrocytes in the amygdala/piriform region bilaterally. Taken together, the evidence suggests that kindling does not result in neuronal death and that in this preparation astrocytes react with dramatic cytoskeletal changes to signals other than neuronal degeneration. Supported by NSERC.

377.13

NORADRENERGIC REGULATION OF ELECTROSHOCK-INDUCED FOREBRAIN AND BRAINSTEM SEIZURES IN RATS. C. Wang*, P. K. Mishra[1], P. C. Jobe[1] and R. A. Browning, Dept. of Physiol. Southern Illinois Univ. Sch. of Med., Carbondale, IL 62901 and [1]Dept. of Basic Sci. Univ. of Illinois Coll. of Med. Peoria, IL 61656.

Previous studies have shown that facial and forelimb (F&F) clonic convulsions emanate from the forebrain, while tonic hindlimb extensor (THE) convulsions emanate from the brainstem. Inasmuch as norepinephrine (NE) has been shown to exert seizure attenuating effects in a wide variety of animal models of epilepsy, it was of interest to determine if the noradrenergic terminal fields of the forebrain and of the brainstem exert differential effects on these two types of convulsions. In the present study we examined this question by evaluating electroshock-induced F&F clonus and THE convulsions in Sprague-Dawley and Genetically Epilepsy Prone rats 2 weeks to 2 months after one of the following 6-hydroxydopamine (6-OHDA) treatments: (1) neonatal intracisternal (depleting forebrain and spinal cord NE); (2) dorsal noradrenergic bundle (depleting forebrain and midbrain NE); (3) medial forebrain bundle (MFB) (depleting forebrain NE only); (4) A-1 medullary (depleting brainstem and spinal cord NE only) and (5) intrathecal (depleting spinal cord NE only). Neonatal intracisternal 6-OHDA significantly increased the incidence of THE convulsions in adult rats. Treatments that depleted forebrain NE predominantly (i.e. dorsal bundle and MFB 6-OHDA) were found to alter neither F&F clonus nor THE convulsions. Depletion of brainstem and/or spinal cord NE (A-1 or intrathecal 6-OHDA treatments) facilitated THE convulsions. These results suggest that forebrain NE regulates neither electroshock-induced F&F clonus nor THE convulsions, while brainstem and/or spinal cord NE regulates THE convulsions. Inasmuch as whole brain NE depletion has been shown to facilitate eletroshock-induced F&F clonus in other studies, it is possible that brainstem NE regulates both forebrain and brainstem seizures. Such a possibility is currently under investigation (Supported by NIH grant NS 22672).

377.14

EFFECT OF 5,7-DIHYDROXYTRYPTAMINE (5,7-DHT) ON AUDIOGENIC SEIZURES IN GENETICALLY EPILEPSY-PRONE RATS (GEPRs). M.A. Statnick*, C. Wang, M. Maring, J.W. Dailey[1], P.C. Jobe[1], and R.A. Browning. Dept. of Physiol., Southern Illinois Univ. Sch. of Med., Carbondale, IL 62901 and [1]Dept. of Basic Sci., Univ. of Illinois Coll. of Med., Peoria, IL 61656.

Previous studies have suggested an inverse relationship between serotonin (5-HT) neurotransmission and audiogenic seizure severity (AGS) exists in the GEPR. Thus, pharmacological treatments that elevate central 5-HT were found to decrease AGS, while treatments that decrease central 5-HT increase AGS. To further assess the role of 5-HT in the regulation of AGS in the GEPR, changes in AGS following destruction of central 5-HT neurons by intracerebroventricular (i.c.v.) administration of 5,7-DHT in protriptyline-pretreated, male, moderate seizure GEPRs (GEPR-3s) were examined. Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and injected i.c.v. with either 200 µg 5,7-DHT in saline-ascorbate (30 µl) or vehicle only (30 µl). A significant increase in seizure severity was observed at 2, 3 and 4 weeks following i.c.v. 5,7-DHT (mean scores of 5.1, 4.6 and 4.6, respectively) as compared to vehicle-injected controls (mean scores of 2.9, 2.8 and 2.9, respectively). The increase in severity was reflected by a significant increase in the incidence of tonic convulsions in 5,7-DHT treated animals over the testing period (average incidence= 53% in treated animals compared to 0% in controls). Although the latency to the running seizure was prolonged ($p<.05$), no difference was observed in the latency to the clonic-tonic convulsion. The significance of the effect of 5,7-DHT on running latency remains to be determined. Neurochemical analysis revealed significant depletion of 5-HT in structures of the telencephalon (>90%), diencephalon (>60%), midbrain (>75%) and in the pons-medulla (45%). However, no significant reductions in norepinephrine content were observed in any of the regions assayed. The present study provides further evidence that 5-HT exerts inhibitory control of AGS in the GEPR.

377.15

SEIZURE EXPERIENCE ALTERS THE DEVELOPMENT OF SEIZURE SEVERITY IN THE GENETICALLY EPILEPSY-PRONE RAT (GEPR). S.M. Lasley*, M.C. Green and P.C. Jobe. Dept. Basic Sciences, U. Illinois Coll. of Medicine, Peoria, IL 61656.

Past work (Reigel *et al.*, *Epilepsy Res.*, 1989) has shown that the ontogeny of sound-induced seizure activity in GEPRs develops primarily within the 3rd and 4th postnatal weeks when audiogenic stimulation is applied to previously untested (UT) animals. The current study was conducted to determine the effect of repetitive audiogenic seizure (AGS) induction on seizure ontogeny in moderately severe (clonic) seizure GEPRs (GEPR-3s). The moderate nature of GEPR-3 seizures permits a ready discrimination of the enhancing or diminishing effects of repetitive induction. These animals also exhibit a transient susceptibility to more severe tonic seizures during early development. Male GEPR-3s were exposed once daily to sound stimulation to induce AGS beginning at day 18 and continuing through day 25 with behavior quantified by Audiogenic Response Score (ARS) and latencies to wild running or convulsions. Male littermates of animals receiving daily sound stimulation were exposed to seizure induction once during this age interval to quantify behavior in UT animals. In rats receiving daily seizure experience, severity peaked at day 22 (8.6 ± 1.3, N=9) before declining at later ages and was significantly greater than the value in UT animals of this age (5.4 ± 3.0, N=9). However, this difference did not persist over days 23-25. The facilitation of AGS severity from repeated sound stimulation was accompanied by a contrasting effect on latencies to running or convulsions, which were significantly longer in seizure-experienced rats at day 22 than in UT littermates. These results suggest that repetitive seizure induction in young GEPR-3s exacerbates the development of seizure severity, producing a kindling-like effect in this model of brainstem seizures, but prolongs the latency for seizure initiation.

377.16

DIFFERENCES IN AUDIOGENIC SEIZURE PRONENESS BETWEEN LINES OF CF-1 MICE FROM DIFFERENT SUPPLIERS. J.W. Collins and W.B Iturrian*. Pharmacology, Univ. of Georgia, Athens, GA 30602-2356.

Environmental noise sensitizes CF1 mice for audiogenic seizures upon subsequent exposure to the audiogenic stimulus (Lab. Animal Sci., 18:559, 1968). While rederiving an inbred CF1 line from Charles River CF1 parents, we surprisingly obtained 20 day old mice that uniformly exhibit audiogenic seizure proneness (asp) upon the initial auditory exposure (IAS). After failing to find environmental noise contamination, we obtained CF1 mice from another supplier (Harlan Sprague Dawley, Inc.) for comparison. Offspring between 16 and 20 days old were IAS-ed with a 102dB bell in an enclosed, circular, glass chamber and tested for sensitization-dependent seizure activity upon reexposure to the same bell 2 or 3 days later. Mice derived from Crl:CF1 BR show 92% asp with 50% tonic-clonic, while those derived from Hsd:NSA (CF1) exhibit 10% asp. The F1 hybrid cross of these lines are 17% asp. Nearly all mice (98%) from all three lines showed sensitization-dependent audiogenic seizures upon the second exposure. These results confirm a genetic anomaly for differences in asp in CF1 lines from different suppliers. Moreover, the study may be of genetic relevance for the asp loci hypothesis since DBA/2 x Crl:CF1 hybrids carry an uniquely high risk for asp and calcerous pericarditis (Pharmacol. 21:144, 1979). Furthermore, 20 day old CF1 mice seizure with a unilateral IAS in the presence of reserpine or naloxone (Fed. Proc. 38:257, 1979). We conclude that the genetics of the audiogenic seizure (and asp) is much more complex and perhaps more relevant to human epilepsy than generally acknowledged. Future genetic studies should examine the linkage of neurotransmitters, calcium binding proteins, and asymmetry in seizure susceptibility.

377.17

ROLE OF HYPERTONICITY IN BLOOD-BRAIN BARRIER (BBB) OPENING AS PART OF THE MECHANISM OF ACTION OF MONOSODIUM GLUTAMATE (MSG) IN AN EXPERIMENTAL MODEL OF GENERALIZED SEIZURES IN THE RAT. A. Ferla-Velasco*, G.G. Ortiz and M.D. Hulzar-Lara. Lab. of Exp. Neuropath. Centro Invest. Biomed. Occte. Instituto Mexicano del Seguro Social (IMSS), Guadalajara, Jal. and Div. Neurobiol., D.F. Biomed. Res. Ctr. IMSS, México, D. F. MEXICO

MSG has been used to induce convulsions in rodents when administered intraperitoneally (i.p.). Catecholamines and acetylcholine play and important role in the physiopathology of convulsions in this model.Due to high concentrations of MSG employed by the various authors,the aim of this work was to evaluate the effects of MSG solution (sol) on the integrity of the BBB in correlation with events of the preconvulsive period when MSG is i.p. injected to adult rats.Three groups of adult Wistar rats were i.p. injected with 0.9% NaCl (PSS),MSG (5 mg/g),and NaCl (equimolar to MSG sol),respectively.Plasma osmolarity was measured at 0,15,30 and 45 min after injections in all groups;and Evans blue was injected at those times through the right carotid artery to evaluate the dye passage to brain,and craneotomy was performed under general anesthesia. Normal values of osmolarity were seen when PSS was given; while those values progressively increased in the other groups,reaching at 45 min,463±14 and 385±8 mEq/L when MSG or NaCl equimolar to MSG were injected, respectively. When PSS was given, only the areas devoided of BBB were stained at all times studied.Right cerebral hemisphere was progressively stained since 15 min after MSG injection,while dye was evident at lesser extent in the group injected with NaCl(equimolar to MSG) at 30 and 45 min after the injection. It is concluded that hyperosmolarity of MSG sol opens the BBB allowing its free access to brain parenchyma, exerting a direct excitatory effect and influencing variations in other neurotransmitter systems, resulting in generalized cerebral hyperexcitability and convulsions.

377.18

CARDIAC HYPERTROPHY SECONDARY TO STATUS EPILEPTICUS IN THE RAT. B.K. Rubinstein, N.Y. Walton, and D.M. Treiman*. Neurology and Research Services, Wadsworth DVAMC and Department of Neurology, UCLA School of Medicine, Los Angeles, CA. 90024.

Status Epilepticus was induced in rats (N = 29) by sequential injections of lithium and pilocarpine. EEG was monitored continuously in the unrestrained rats via epidural screw electrodes. Seizure activity was aborted by administration of 4 mg/kg MK-801 followed by 10 mg/kg diazepam. The duration of status epilepticus varied from a few minutes to three hours. Animals were allowed to recover following status epilepticus for 8-12 days - intensive supportive care was required to enable complete recovery. Intracardiac perfusion was performed using 50 ml of saline followed by 50 ml of formalin. Hearts were excised, and all connective tissue was dissected away as well as both atria and aorta. Hearts were blotted and weighed. The hearts were then dessicated overnight and re-weighed.

Rats which had experienced status epilepticus of any duration had significantly larger hearts than did controls (wet weight t-test = 3.234, $p<.01$). The dried heart weights were also significantly different ($t = 2.524$, $p<.05$). The larger difference in wet versus dry weights suggests that the changes were the result of cellular hypertrophy. Control rats given all of the drugs, but not allowed to seize, did not differ from naive controls.

Cardiac hytpertrophy has not been reported, following either human or experimental status epilepticus and may represent a serious problem which deserves further study.

377.19
INCREASED SYNAPTIC NUMBERS IN THE MEDIAL PALLIUM OF FROGS WITH CISPLATIN-INDUCED EPILEPSY. K.S. Blisard*, D.A. Harrington, K.L. Taylor. Univ. of Cincinnati Med. Ctr., Cincinnati, OH, 45267-0529.

Frogs treated with a single systemic dose (10 mg/kg) of the anticancer agent cis-diamminedichloroplatinum II (cisplatin) develop recurrent generalized seizures 3-5 weeks after the injection. Minimal histologic changes are seen in the brain by light microscopy. We quantitated the number of synapses per 100 μ^2 in the telencephalic medial pallium (analogous to the mammalian hippocampus) in treated vs. control frogs. In frogs with seizures, there was a statistically significant increase in both the total number of synapses (9.7 $\pm$ 0.9 for experimental vs. 7.6 $\pm$ 1.4 for control) as well as in asymmetric synapses (8.2 $\pm$ 0.8 vs. 6.4 $\pm$ 1.4, respectively). There was little change in the number of symmetric synapses. These data may suggest that an increased level of excitatory neurotransmission contributes to the epileptic state in this model.

EPILEPSY: BASIC MECHANISMS III

378.1
EFFECT OF MEDIAL SEPTAL AREA INACTIVATION ON C-FOS-LIKE PROTEINS EXPRESSION INDUCED BY SOMAN. M. Denoyer, L. Quintin* and G. Blanchet. Dept. Neurotoxicology, C.R.S.S.A., B.P. 87, 38702 La Tronche, France.

Status epilepticus induced by the anticholinesterase soman can elicit c-fos production in the rat brain. We have recently reported that intraseptal application of atropine reduces the typical cholinergic signs of soman. We therefore hypothesized that the blockade of convulsions would be followed by a suppression of Fos production. Thus, this study examined the effect of intraseptal injection of atropine on the distribution of c-fos-like proteins immunoreactivity related to soman treatment. Imalgene-anesthetized rats were implanted with a guide canula above the medial septal area. After recovery, atropine were injected into the septum (20 μg/0.2 μl). Twenty min later, 0.9 LD_{50} soman (83 μg/kg, s.c.) was administred. Two hours later, rats underwent brain perfusion-fixation. The blockade of Fos induction revealed by immunohistochemistry was significant in most of the limbic structures but also in non-limbic areas. Thus, only a very poor labelling in the piriform and entorhinal cortices was still detected. Moreover, the laminar pattern of staining observed in non-treated frontoparietal and cingulate cortices had disappeared. The present data strongly suggest that hyperactivity of the cholinoceptive cells of the medial septal area is responsible for Fos production related to soman.

378.2
EFFECT OF TRANSECTIONS ON SEIZURES INITIATED FROM THE DEEP PREPIRIFORM CORTEX OF RATS. R. A. Browning*[1], R. Maggio and K. Gale. Dept. Pharmacology, Georgetown Univ. Sch. of Med., Wash. D.C. 20002 and [1]Dept. Physiology, Southern Illinois Univ. Sch. of Med., Carbondale, IL 62901.

Previous studies have shown that brainstem seizures can still occur after transections which separate forebrain from brainstem. In the present study we sought to determine whether forebrain-evoked electrographic seizures require brainstem connections for initiation and generalization. Male Sprague-Dawley rats (295-320 g), implanted with epidural electrodes underwent one of the following transections at the pre-, mid-, or postcollicular level: (1) brainstem (core), (2) complete brain, (3) incomplete transections (sparing pathways ventral to the pretectal nuclei) or (4) hemisection. All transections were performed under ether anesthesia and seizures were initiated 3 hours later by focal infusion of bicuculline (BIC, 118 pmoles/120 nl) into the area tempestas (AT) via a previously implanted guide cannula. Bilateral forebrain electrographic seizures were found to occur in the complete absence of connections between forebrain and brainstem, showing that the brainstem is not required for forebrain-evoked seizures. Additionally, it was found that focally-evoked forebrain seizures were suppressed by "complete" transections which also interrupted connections between AT and the caudal telencephalon (entorhinal cortex, subiculum and ventrolateral hippocampus). On the other hand, sensitivity to focally-evoked forebrain seizures was restored in rats with "complete" transections if carbachol was administered together with BIC, suggesting that removal of excitation from the caudal telencephalon is responsible for the effect of the "complete" transection. The finding that "complete" brain transections caudal to the telencephalon do not suppress focally-evoked forebrain seizures further emphasizes the importance of the connections between the caudal telencephalon and AT in determining the sensitivity of AT to bicuculline. The present findings support previous studies showing that seizure substrates in the forebrain and brainstem are separable and independent.

378.3
CORTICAL INFUSIONS OF THE CHOLINERGIC ANTAGONISTS ATROPINE AND PIRENZEPINE INDUCE EPILEPTIFORM SPIKING IN THE RAT. J. Payne, D.K. Bilkey, & C.L. Darlington*, Department of Psychology and the Neuroscience Centre, University of Otago, Dunedin, New Zealand.

Previous research has suggested a largely facilitatory action for the cholinergic system in epileptiform activity, with atropine and other antimuscarinics retarding or blocking these epileptiform effects. However, in this study, we have found that intracranial infusions (0.5 to 1 ul) of atropine sulphate (1 to 160 mM) into the perirhinal cortex produced high amplitude epileptiform spiking (approx 1-2 Hz) in 6 out of 7 freely moving adult Sprague Dawley rats. Spiking typically occurred within 4 minutes of the onset of the infusion and lasted for up to 4 hours, depending on dose. Similar epileptiform activity was also recorded following infusions into the temporal, frontal and pyriform cortices in 6 out of 9 rats. Concurrent recordings from regions distal to the area of infusion indicated that this activity remained largely isolated to the infused regions, although occasionally, propagation into other regions would occur and behavioural seizures identical in appearance to Stg 1-5 amygdala kindled seizures were observed.

To determine which muscarinic receptor subtype was mediating this effect, the specific antagonists pirenzepine (M1), gallamine triethiodide (M2), and p-fluoro-Hexahydro-sila-difenidol HCl (M3) were infused. Preliminary results indicate that only pirenzepine was effective in eliciting epileptiform activity. These data suggest a functionally inhibitory (possibly mediated by activation of a GABA interneuron) cortical cholinergic pathway. This pathway appears to utilise the M1 receptor subtype. Blockade of this receptor could result in disinhibition of excitatory pathways and the generation of epileptiform activity.

378.4
ANTICONVULSANT EFFECT OF SYSTEMIC FLUOXETINE ON FOCALLY-EVOKED LIMBIC MOTOR SEIZURES IN RATS. **S. Prendiville and K. Gale* Department of Pharmacology, Georgetown University Medical Center, Washington, DC 20007**

Fluoxetine, a serotonin (5HT) uptake inhibitor, was evaluated for anticonvulsant effects in a rat model of limbic motor seizures. Seizures were induced by focal microinjection of the $GABA_A$ receptor antagonist, bicuculline methiodide (118 pmol) unilaterally into 'area tempestas' (AT), an epileptogenic site in the deep prepiriform cortex of the rat. Fluoxetine (or saline in controls) was administered intraperitoneally 1 hr before seizure induction. Significant dose-dependent seizure protection was obtained with fluoxetine (5,10 and 20 mg/kg), with 50% protection following the 5 mg/kg dose. Suppression of electrographic seizure activity was concomitant with suppression of motor seizures. These findings support and extend recent observations of Dailey et al. (JPET 260:533, 1992) who found that fluoxetine exerted anticonvulsant actions against audiogenic seizures in genetically seizure-prone rats. Thus, pharmacologic enhancement of serotonergic transmission may reduce seizure susceptibility in a variety of experimental models.

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378.5

ENHANCEMENT OF SEROTONERGIC TRANSMISSION IN SUBSTANTIA NIGRA PRODUCES ANTICONVULSANT EFFECT.
A.PASINI*, A. TORTORELLA, K. GALE. Dept. of Pharmacol., Georgetown Univ. Med. Centr., Washington, D.C. 20007

The influence of serotonin transmission in substantia nigra (SN) was studied using a rat model of focally-evoked complex partial seizures with secondary generalization. A serotonin uptake inhibitor, fluoxetine, was injected into SN. When bilaterally microinjected into SN, fluoxetine (1.75, 3.5 and 7.0 nmol.) protected against convulsive seizures evoked by the focal injection of the GABA-A receptor antagonist, bicuculline methiodide (118 pmol) into a discrete epileptogenic site in the deep prepiriform cortex, area tempestas (AT), of the rat. Injection of fluoxetine unilaterally in SN or bilaterally into a site dorsal to SN was not anticonvulsant. Focal application of a serotonin receptor agonist, TFMPP, bilaterally in SN produced anticonvulsant effects comparable to fluoxetine. The anticonvulsant action of fluoxetine (3.5 nmol.) in SN was not reversed by the application of the GABA-A receptor antagonist, bicuculline methiodide (800 pmol) in SN. These data suggest that serotonergic transmission in SN exerts a seizure suppressing action which is independent of nigral GABA-A transmission. This raises the possibility that in SN, serotonin and GABA may act in an additive and/or mutually compensatory fashion to limit the development and propagation of seizure activity generated in limbic circuits.

378.6

CONVERSION OF EEG FROM DISCRETE TO CONTINUOUS SEIZURE ACTIVITY CAUSED BY ELEVATION OF BRAIN GABA DURING EXPERIMENTAL STATUS EPILEPTICUS.
N.Y. Walton* and D.M. Treiman. Neurology and Research Services, Wadsworth DVAMC and Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

We have reported that the EEG during untreated status epilepticus progresses from a pattern of discrete seizures to one of continuous ictal activity (Epilepsy Res 1990, 5:49-60) and that brain GABA levels increase progressively during status in the rat (Exp Neurol 1990, 108:61-70).

Rats were prepared with recording electrodes and cortical cobalt lesions . When the lesion became epileptogenic status was induced by injection of 5.5 mmol/kg homocysteine thiolactone. Tiagabine was injected IP after the second generalized tonic-clonic seizure (GTCS). EEG was monitored for 30 minutes following tiagabine injection, then the animals were anesthetized and blood and brain samples obtained.

The anticonvulsant effect of tiagabine was evident almost immediately - rats receiving 10 mg/kg or more had no further GTCS. However, within 15-20 minutes, the EEG began to display a continuous ictal pattern, consisting of rhythmic 3 Hz activity as well as spiking. This model rarely converts to a continuously ictal pattern in less than one hour following saline treatment, and the rapid conversion observed here was striking. We hypothesize that GABA receptor desensitization may be responsible for this, and may also cause the conversion in untreated status epilepticus.

378.7

EVIDENCE FOR NORADRENERGIC REGULATION OF FOREBRAIN SEIZURES : STUDIES OF DSP-4 IN SPRAGUE-DAWLEY RATS PRETREATED WITH DESIPRAMINE.
P.C. Jobe,* R.L. Burger, A.F. Bettendorf, J.W. Dailey, C. Wang, R.A. Browning and P.K. Mishra. Univ. of Illinois College of Medicine at Peoria and SIU School of Medicine, Carbondale, IL.

The role of norepinephrine in regulating brainstem seizures has been well documented. These seizures are characterized by running/bouncing clonus, and tonic extensor convulsions. Evidence for noradrenergic regulation of brainstem seizures comes partially from studies with genetic models of epilepsy which are characterized by innate noradrenergic deficits, and from selective lesioning of noradrenergic neurons and/or pathways. We have previously reported that the noradrenergic neurotoxin DSP-4 (N-(2-chloroethyl)-N-2-bromobenzylamine) reduces the threshold for forebrain and brainstem seizures. The present study was conducted to evaluate whether this effect of DSP-4 is due to its influence on noradrenergic system or through some other mechanism. Sixty female Sprague-Dawley rats (49-55 day old) received DSP-4 (50 mg/kg ip) after a pretreatment of desipramine (a norepinephrine reuptake inhibitor) or saline. A control group of 30 animals received two administrations of the vehicle. Three weeks later, these animals were tested for facial and forelimb clonus (FFC) threshold (convulsive current$_{50}$) via corneal electroshock stimulation.

The FFC CC$_{50}$ thresholds in the animals treated with saline plus DSP-4 were significantly reduced below control (vehicle-vehicle) values. Also the desipramine pretreated group failed to exhibit any reduction in FFC thresholds as indicated by the Litchfield and Wilcoxon test. These observations provide additional support for the noradrenergic hypothesis of forebrain seizure regulation. Supported in part by NIH grant # NS22672.

378.8

IMAGING OF SEIZURE SPREAD ACROSS CORTEX OF THE PERFUSED GUINEA-PIG BRAIN.
P. Federico*, S.G. Borg, and B.A. MacVicar. Neuroscience Research Group, The University of Calgary, Calgary, Alberta T2N 4N1 CANADA.

Neuronal activity in the guinea-pig isolated whole brain preparation is associated with detectable intrinsic optical signals. We used video imaging of reflectance changes combined with extracellular recordings to examine seizure spread across cortex of this preparation. Entorhinal cortex (EC) stimulation (5 Hz, 10 sec) decreased tissue reflectance in the EC in an area 2 - 3 mm around the stimulation site. This reflectance change recovered within 1 min after stimulation. After several stimulation trials in some preparations, seizure activity was evoked along with a persistent, large amplitude optical signal that was initially restricted to the EC for 10 sec. This optical signal then spread over 20 sec to the medial amygdala via the medial EC/subiculum. In some experiments, seizure activity was evoked in the EC or posterior piriform cortex by lateral olfactory tract stimulation (5 Hz, 10 sec). When seizure propagation occurred, it was directed toward the medial amygdala with the same time course as observed previously. Perfusion of the $GABA_A$ antagonist bicucculline (20 or 100 μM) resulted in spontaneous seizure activity in the EC that spread to the medial amygdala following the same path and time course as seizures evoked by electrical stimulation alone. Occasionally, spontaneous seizures were initiated in the posterior perirhinal cortex which later propagated anteriorly along the perirhinal cortex and also toward the entorhinal, amygdalar, and piriform cortices. Perfusion of the $GABA_B$ antagonist 2-hydroxysaclofen (100 μM) increased the area of a stimulus-induced seizure focus, while having no consistent effect on seizure propagation. Therefore, seizure spread across cortex can be examined using intrinsic imaging techniques. This spread is characteristically directed toward the medial amygdala and might be shaped by $GABA_A$ as opposed to $GABA_B$ receptor activation.

Supported by the Medical Research Council of Canada (MRC). Paolo Federico is an MRC and Alberta Heritage Foundation for Medical Research student.

378.9

Medial Thalamus:A possible pacemaker site for the generation of spike and wave discharges induced by gamma-hydroxybutyric acid in rats. P.K. Banerjee* and O.C.SneadIII, Dept. and Div. pf Neurology, Chilrens Hosp. 0f LA, Sch. of Med., USC,Los Angeles, CA 90027.

Gamma-hydroxybutyric acid(GHB) is a naturally occuring compound which induces experimental absence seizures in rats(Snead,Epilepsia,29:361, 1988).It is generally believed that the low frequency bilaterally synchronous spike wave discharges(SWD)that characterize absence seizures arise due to abnormal thalamocortical oscillations. We have earlier reported that specific thalamic(VPL/VPM,medial and reticularthalamus) and cortical(outer layer of frontal ctx) areas which participate in the generation of SWD induced by GHB constitute thalamocortical circuitry for the GHB model(Banerjee et.al. Soc. Neurosci. Abs. 17 1606,1991). The purpose of the present study was to investigate whether any of the thalamic acts as pacemaker site for the generation of SWD. For this porpose we lesione-d the thalamic sites (bilaterally) and recorded the SWD from the unlesioned thalamic and cortical sites. Lesions in medial thalamus suppressed the generation of SWD from both subcortical and cortical leads. In reticular nucleus lesioned animals, SWD was not abolished but the seizure duration was reduced. In the VPL/VPM lesioned group, neither SWD was abolished nor its duration was affected. These results suggest that MT may act as a pacemaker site in the GHB model of absence seizures.

378.10

DIFFERENTIAL PARTICIPATION OF INFERIOR COLLICULUS SUBNUCLEI IN THE NMDA-DEPENDENT AUDIOGENIC SEIZURES ELICITATION.
N. Garcia-Cairasco* and V.C. Terra. Department of Physiology, Ribeirão Preto School of Medicine, University of São Paulo, 14049, Ribeirão Preto-SP-Brazil.

Neurochemical alterations in the inferior colliculus (IC) include both GABA and NMDA, in audiogenic seizure (AS) susceptible rats. Because central (CIC) and cortical (CXIC) IC subnuclei seem to play a different role in AS, the present experiments were developed to compare AS sensitization by NMDA of AS resistant (R) rats and blockade of AS by NMDA antagonists in AS susceptible (S) rats, in CIC and CXIC. AS were evaluated by means of a quantitative behavioral cluster analysis (Garcia-Cairasco et al., Behav.Brain Res.48:49-56,1992. NMDA (2.0, 2.5 and 3.0µg/0.2µl) or phosphate buffer(PB;0.2µl) were microinjected unilaterally into CIC (n=30) and CXIC (n=35) 15 minutes before sound stimulation (110 dB) in R rats. AP7 (3.0 µg/0.2µl) or PB (0.2µl/side) were microinjected unilaterally 1 minute before NMDA in R rats(n=16), and bilaterally, 15 minutes before sound stimulation in S rats(n=15). NMDA induced spontaneous AS-like behaviors at 2.5 and 3.0 µg/0.2µl($p<0.01$). Also 2.5 µg/0.2µl NMDA was best potentiated by the sound, mimicking AS when it was applied into CXIC nucleus. Moreover, NMDA induced contralateral spontaneous tonic AS in CIC and bilateral spontaneous AS in the CXIC. In both subnuclei, the generalization was observed during the sound stimulation. AP7 blocked completely AS in S animals when applied into the CIC($p<0.01$) and partially when applied into the CXIC($p<0.01$). These results suggest that CIC and CXIC may have a NMDA-dependent epileptogenicity and that they play a different role in the sensorimotor transduction involved in AS.

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378.11

IN VIVO MAPPING OF KAINIC ACID INDUCED SEIZURES WITH VOLTAGE SENSITIVE DYE.

D.S. SACKS* and R.M. DASHEIFF. Dept. of Veteran Affairs, VAMC, University Dr., Pgh, Pa, 15240 and Univ. of Pittsburgh Epilepsy Center.

The functional relationship between electrical activity and brain function is a central concern in the study of epilepsy. By mapping the electrical activity of the neural pathways involved in the propagation of seizures, new structure-function relationships can be uncovered. The voltage sensitive dye (VSD) diO-C(2)-5 was used as a marker of this electrical activity during kainic acid (KA) induced seizures in awake animals. This technique when coupled with computerized image analysis, (Seizure, in press) allows a histological survey of the brain's neural activity during the dye injection period. The rats received either i.p. kainic acid (12 mg/kg) + saline vehicle, or saline alone prior to dye injection. EEG activity was recorded via hippocampal electrodes and the dye injection timed to coincide with seizure activity. The neural activity of the septum, amygdala, anterior thalamus, hippocampus, mammilary bodies, substantia nigra, and entorhinal, frontal and posterior cortices will be presented.

Previous work had shown that KA induces specific patterns of depolarization in the several neural structures previously surveyed. During the present study, we extend this survey to include other neural structures. It is our contention that the spatial pattern of polarization induced by the KA extend throughout the brain. The acute seizures as well as the chronic pathological changes induced by this agent have been shown to be similar to those seen in human temporal lobe epilepsy. This work lays the foundation to imaging human brain activity with VSD's labelled for PET or MRI.

378.12

MODEL OF PARTIAL EPILEPSY IN RAT PIRIFORM CORTEX REVEALS STAGES IN THE TRANSITION FROM FOCAL EPILEPTIFORM ACTIVITY TO ELECTROGRAPHIC SEIZURES. K.L.Ketchum* and L.B.Haberly. Dept. of Anatomy, Univ. of Wisc., Madison, WI 53706.

An acute anaesthetized preparation has been developed in which several characteristics of partial (focal) epilepsy can be consistently reproduced: spontaneous interictal-like activity, slow spreading recruitment of adjacent cortex into the generation of epileptiform activity, and self-sustained ictal-like activity that extends into the recruited area. Afferent shock stimuli were used to pace the frequency of epileptiform events resulting from disinhibition by picrotoxin injection of a focus in ant. piriform cortex (PC) of urethane anesthetized rats. Stimulation at 1/2 Hz evoked interictal-like events similar to those occurring spontaneously at 1/3 Hz. Current source-density (CSD) analysis and multiunit recording indicated that the regenerative process underlying these events was confined to the disinhibited focus. Increasing the rate to 1 Hz resulted in a large stereotyped increase in the amplitude of the epileptiform field potential in successive trials which propagated across post. PC at approximately 0.001 m/s. Following this transition the epileptiform population spike was no longer restricted to the focus; it propagated without decrement across PC at the conduction velocity of the caudally directed association fibers (0.3 m/s). During the transition the field potential within the focus was unchanged, suggesting that a change in excitability occurred outside the focus. At 2 Hz a second, long latency population spike was initiated caudally and propagated back into the focus -- a "secondary" initiation site for epileptiform activity developed at a posterior site. Following the delivery of 10 to 20 stimuli at 4 Hz, self-sustaining ictal-like activity developed throughout PC. The full sequence of changes could be evoked repeatedly during the same picrotoxin application, indicating that they were not a consequence of diffusion of picrotoxin from the injection site. Analysis of the different stages is being carried out with intracellular recording and CSD analysis. Supported by NINDS grant NS19865 to LBH and NRSA grant NS08328 to KLK.

378.13

DEVELOPMENTAL SENSITIVITY OF MODERATE AND SEVERE SEIZURE GENETICALLY EPILEPSY-PRONE RATS (GEPRs) TO FLUROTHYL SEIZURES. C.E. Reigel*. Department of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

The GEPR currently exists as two independently derived colonies of rats bred to exhibit specific audiogenic seizures (AGS). GEPR-3s exhibit clonic seizures whereas GEPR-9s exhibit tonic seizures in response to sound. GEPRs also exhibit a broad seizure sensitivity to a wide variety of electrical and chemical seizure provoking modalities. A series of developmental studies suggest AGS susceptibility depends on two independent traits, the development of an AGS focus and a more general neuropathological condition functioning as a seizure propagation mechanism. This seizure propagation mechanism is hypothesized to be responsible for the extreme sensitivity of the GEPR to other seizure-provoking manipulations. Franck et al. (Epilepsia 30:1-6, 1989) demonstrated GEPR-9s to be more sensitive to flurothyl seizures prior to the age of AGS susceptibility. The purpose of the present study was to extend the findings of Franck et al. to the GEPR-3, characterize the developmental sensitivity of the seizure propagation mechanism independent of the AGS focus and to determine if the seizure propagation mechanism developmentally precedes AGS. Clonic and tonic flurothyl thresholds were determined in GEPR-9s, GEPR-3s and controls at 13, 17, 21, 25, 30 and 45 days of age, ages preceding AGS susceptibility through maturation to adult AGS traits. Unexpectedly, flurothyl induced tonic seizures in both GEPR-3s and GEPR-9s at all ages, including ages in which GEPR-9s do not exhibit tonic AGS. Flurothyl thresholds were lower in both strains of GEPR than control and lower in GEPR-9s than GEPR-3s at all ages. The seizure propagation mechanism developmentally precedes AGS susceptibility in both strains.

378.14

NEUROPATHOLOGICAL ALTERATIONS IN GENETICALLY EPILEPSY-PRONE RATS (GEPRs) FOLLOWING STATUS EPILEPTICUS. F.J. Denaro* and C.E. Reigel. Depts. of Med. Surg. Neurol and Pharmacol., Texas Tech Univ. Health Sci. Ctr. Lubbock, TX 79430.

One strain of GEPR, the GEPR-9, has been bred to exhibit full tonic extensor convulsions in response to sound. A small percentage of these animals exhibit status epilepticus following an acoustically evoked seizure or a spontaneous seizure. Episodes of status epilepticus occur in both adult and juvenile GEPR-9s. In untreated adults, there is an approximate 50 percent mortality. One week after acoustically-induced status epilepticus without pharmacological intervention, adult GEPR-9s were overdosed with pentobarbital and perfused with formalin. Brains were removed and examined by a battery of histochemical stains (H&E, PAS, Nissl, Luxol Fast Blue, Bielschowsky and GFAP). Diffuse neuronal cell loss was identified in the cortex and striatum. However, in contrast to human findings, no Purkinje cell loss was noted in the cerebellum. Early hypoxic changes were found in the hippocampus. One subject also showed evidence of microhemorrhages and bleeding into the ventricle. In this brain, encrusted neurons were identified. Bielschowsky stain revealed swollen axons in the cortex, but no neurofibrillary changes or inclusions. GFAP stain revealed patchy gliosis; glial cells were often swollen and showed evidence of microvacuolation. Similar findings have been reported in animal models of status epilepticus that employ somewhat heroic exogenous manipulations (bicuculline, cobalt or kainate) to induce sustained seizure activity. Significantly, morphological changes observed following status epilepticus in the GEPR brain, a nervous system pathophysiologically epileptic, closely correspond to human neuropathology associated with status epilepticus.

378.15

CHANGES IN PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR LEVELS IN ADULT GENETICALLY EPILEPSY-PRONE RATS (GEPRs). P. J. Syapin*, R. Medley and C. E. Reigel. Dept. of Pharmacology, Texas Tech Univ. Health Sci. Center, Lubbock, TX 79430.

The GEPR was derived from Sprague-Dawley (SD) rats by selective breeding for audiogenic seizure (AGS) susceptibility. One strain, the GEPR-9, exhibits full tonic extensor convulsions in response to sound. Recently, it was reported that astrocyte cultures prepared from neonatal GEPR-9 cortex had a reduced number of peripheral-type benzodiazepine receptor (PBR) sites compared to cultures prepared from control cortex (Ducis, et al., Brain Res. 531:318, 1990). We have measured PBR sites in the adult GEPR-9 to determine if the results from astrocyte cultures extend to *in vivo* conditions. Four days after an AGS, brains, kidneys and adrenals were removed from age-matched male GEPR-9s and SD control rats, quick frozen in 0.32 M sucrose, and stored at -70°C until assayed. To measure PBR sites the samples were thawed, dissected (brain only), weighed, and homogenized in 50 mM Tris-HCl buffer (pH 7.5). A washed membrane fraction was prepared and used to quantify [H^3]PK11195 (0.3 - 12 nM) binding to the PBR. No statistically significant differences in either the affinity (K_D) or binding capacity (B_{max}) was found for the adrenals or cerebellum. A significant 20%-40% decrease in B_{max} was found in GEPR-9 kidney, hippocampus and cerebral cortex, while a large *increase* in B_{max} was observed in the olfactory bulbs. These results confirm and extend the report of Ducis et al., however the significance of these findings to the development and maintenance of an epileptic state is presently obscured. The observed CNS changes could be either a cause or an effect of seizure susceptibility, or neither. The decrease in kidney sites may indicate an increase in stress or anxiety in the GEPR-9 line. (Supported in part by grant AA07351 to PJS)

378.16

THE ROLE OF THE PERIRHINAL CORTEX IN LIMBIC MOTOR SEIZURES. A. Tortorella*, L. Hu, and K. Gale Department of Pharmacology, Georgetown University Medical Center, Washington, DC 20007.

The area tempestas (AT), an epileptogenic site in the prepiriform cortex, has been demonstrated to be involved in the generation of bilateral limbic motor seizures. Previous studies have shown neural projections from AT to the perirhinal cortex (PRC), an area 6-7 mm caudal to the level of AT, and this area shows marked activation of c-fos expression and glucose utilization in response to AT triggered seizures. In the present study, we have further explored the functional relationship between AT and PRC. Seizures evoked by microinjections of bicuculline-methiodide (236 pmol) into AT were suppressed by microinjections of muscimol (220 pmol) into PRC, but were not suppressed by muscimol injections 2 mm dorsal to PRC. Microinjections of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo-(F) quinoxaline (NBQX, 1 nmol), a selective antagonist of AMPA receptors, into PRC also attenuated AT-evoked seizures. Bilateral microinjections of NBQX (2 nmol) into PRC also protected against seizures evoked by systemic bicuculline (2 mg/kg, sc). These observations indicate a role of GABAergic and glutamatergic transmission in PRC for the mediation of seizures evoked from AT as well as for systemically evoked seizures.

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378.17

RECIPROCAL CONNECTIONS BETWEEN THE AREA TEMPESTAS AND THE PERIRHINAL CORTEX IN THE RAT. N. Sahibzada*[1,2], W. Chen[1], and K. Gale[2]. [1]Dept. of Psychology, Univ. District of Columbia and [2]Dept. of Pharmacology, Georgetown Univ. Medical Center, Washington DC 20007.

The area tempestas (AT) is a functionally distinct epileptogenic site in the deep prepiriform cortex. Previously, we reported that it has connections with a number of structures in the limbic forebrain (Neurosci. Absts. 201.2, 1992). The perirhinal cortex (PRC) is one such structure. The purpose of the present study was to characterize the cells of origin of the AT that project to the PRC, and the distribution of terminals in the AT that arise from the PRC. Using anterograde and retrograde flourescent tracers, bulk as well as discrete injections were made in the PRC immediately dorsal to the rhinal fissure.

Retrograde tracing demonstrated flourescent *cells* in the ipsilateral AT; following unilateral injection of Fluoro-Gold in PRC. Furthermore, dense label was encountered in the rostral endopiriform nucleus (EN), dorsomedial to the AT. In addition, sparse labeling was observed in external and lateral anterior olfactory nucleus and the lateral part of the intermediate olfactory tract. Anterograde tracing revealed flourescent *terminals* in AT following application of Fluoro-Ruby into PRC.

These data suggest that a reciprocal connection exists between the PRC and the ipsilateral AT, and to a lesser extent between the PRC and contralateral AT. Moreover, the existence of strong reciprocal connections between PRC and rostral EN raises the possibility that neural information from AT and EN may converge in the PRC.

Supported by HHS grant # NS28130

SCHIZOPHRENIA: NEUROCHEMISTRY

379.1

GAMMA-GLU-GLN AND EXCITATORY AMINO ACIDS (EAA) IN CEREBRO-SPINAL FLUID (CSF) OF UNTREATED SCHIZOPHRENIC PATIENTS. K.Q. Do*[1], C. Lauer[2], M. Cuenod[1] and F. Holsboer[2]. [1]Brain Res. Inst., Univ. of Zürich, Zürich, Switzerland; [2]Max-Planck-Inst. for Psychiatry, Munich, FRG.

Pathophysiological theories of schizophrenia have emphasized dysfunctions in dopaminergic (DA) systems, although a role of EAA systems has also been proposed. Lumbar CSF of 19 patients with schizophrenic disorders (according to DSM-III-R; all patients were off drugs for at least 1 year) and of 16 age- and sex-matched controls were analyzed by optimized precolumn o-phtalaldehyde derivatization HPLC. Performing an analysis of covariance with age and sex as covariates, taurine and tyrosine were found to be significantly decreased in the patients. The remaining amino acids evaluated (including aspartate, glutamate and sulfur containing EAA) as well as the catecholamines metabolites did not differ between patients and controls. The level of a compound (P15.5) which we identified as γ-glutamyl-glutamine (as it coeluted with the dipeptide at 3 different pH) was significantly decreased by 13-18% in the patients. While the results do not support a direct involvement of DA or EAA systems in those patients, they point out to a possible role of γ-Glu dipeptides in the pathogenesis of schizophrenic disorders.

379.2

CORRELATION OF ALTERED NAAG AND NAALADase LEVELS WITH LEVELS OF GLUTAMATE AND OTHER AMINO ACIDS IN SCHIZOPHRENIC BRAIN. L. A. Passani*, G. Tsai, J. Kleinman and J. T. Coyle. Dept. of Psychiatry, Harvard School of Medicine / MGH, Boston, MA.

We have previously reported significantly increased N-Acetyl-Aspartyl-Glutamate (NAAG) levels and decreased N-Acetylated Alpha-Linked Acidic Dipeptidase (NAALADase) activity in hippocampus, parahippocampus and orbital frontal cortex of schizophrenic brain. It has been demonstrated that NAAG, a putative neurotransmitter or modulator in glutamatergic pathways, may interact with both nonNMDA and NMDA glutamate (GLU) receptors. From these observations we hypothesized that NAAG may influence GLU-mediated neurotransmission and thereby alter Glu levels. In our present study, levels of GLU, aspartate (ASP), glycine (GLY), taurine (TAU) and GABA were determined by HPLC in eight different regions of schizophrenic, neuroleptic control and normal control brains using the specimens previously analyzed for NAAG and NAALADase (Tsai, et al., 1991). Preliminary results indicate significant decreases in GLU levels in hippocampus, orbital frontal cortex and cerebellum as compared to normal and neuroleptic controls. We also found decreases in the levels of ASP, GLY, TAU and GABA in several regions of schizophrenic brain. The fact that we observed close regional correlations between GLU and NAAG/NAALADase alterated areas suggest a possible causal link between levels of NAAG/NAALADase and GLU in disease-affected regions of schizophrenic brain.

379.3

MESOPONTINE CHOLINERGIC CELLS AND MESOLIMBIC DOPAMINE FUNCTIONS, INCLUDING SCHIZOPHRENIA. J.S. Yeomans*, Dept. of Psychology, Univ. Toronto, Toronto, Canada M5S 1A1.

Mesolimbic dopamine systems are believed important for positive symptoms, and cholinergic systems for negative symptoms of schizophrenia, due to the clinical effects of systemic drugs (Tandon & Greden, 1989). Recently, Ch5 and Ch6 cholinergic cells were found in twice normal numbers in a few schizophrenic brains (Karson et al., 1991). Also, increased AChE levels were found in several thalamic nuclei (McGeer & McGeer, 1977). Thus, some schizophrenics may have increased Ch5 cholinergic function. Ch5 cells appear to connect monosynaptically to A9 and A10 dopamine cells (e.g., Bolam et al., 1991; Lacey et al., 1991). We tested whether these connections could activate dopamine-related functions in rats by injecting muscarinic drugs near the Ch5 cells, and near the dopamine cells. Activation of cholinergic cells (e.g., by injecting scopolamine, 100 ug, near medial Ch5 cells) increased mesolimbic dopamine-related functions (i.e., activity, stereotypy, reward sensitivity) by several times, with only slight evidence of nigrostriatal activation (i.e., turning). These increases were blocked by atropine (60 ug) injected near A10 cells. Ch5 cholinergic overactivation may be a causal factor in positive symptoms (via mesolimbic dopamine activation), disorganized thinking (via thalamic activation, Steriade, 1990) and REM sensitivity (via pontine reticular formation connections) in some schizophrenics. (Supported by NSERCC grant to J.Y.)

379.4

NEUROPSYCHOLOGICAL IMPAIRMENT IN MONOZYGOTIC TWINS DISCORDANT AND CONCORDANT FOR SCHIZOPHRENIA. T. E. Goldberg*, E. F. Torrey, J. M. Gold, D. R. Weinberger. Clinical Brain Disorders Branch, National Institute of Mental Health, Washington, D.C. 20032

In this study, we examined neuropsychological performance in monozygotic (MZ) twins discordant (N=20) and concordant for schizophrenia (N=8) and normal MZ twins (N=7). We first contrasted unaffected and affected twins from the discordant pairs in order to assess disease specific impairment. The affected group performed significantly worse on tests of executive function, secondary memory, attention, psychomotor speed, and IQ. The profile of performance was suggestive of fronto-temporal dysfunction. Matched pair hit rate analyses suggested that cognitive impairment was not restricted to a group of outliers but was present throughout the distribution. Next, we contrasted affected twins from the discordant group and twins from the concordant group. No differences were observed. This result does not support the idea that putatively "genetically" determined concordant cases have more severe deficits than putatively "sporadic" cases in the discordant group. In the final comparison, designed to assess genetic risk for cognitive impairment in schizophrenia, we contrasted unaffected and normal twins. We observed subtle differences favoring the normal twins on tests of executive and memory function.

379.5

BRAIN PATHOMORPHOLOGY IN FIRST EPISODE SCHIZOPHRENIA. J.A.Lieberman*, G.DeGreef, M.Ashtari, B.Bogerts, R.Bilder. Dept. of Psychiatry, Long Island Jewish Medical Ctr. Albert Einstein College of Medicine, Glen Oaks, NY 11004.

The aims of this study were: 1) to characterize brain morphology in young first episode schizophrenics (SCZ) prior to drug treatment or the effects of chronic illness; 2) to determine the clinical correlates of brain pathomorphology; 3) to determine if brain pathomorphology was progressive in the course of SCZ.

Brain MRIs were acquired in 70 first episode treatment naive SCZ and matched normal subjects at hospital admission and 18 months follow-up on a Siemens Magnetom (1.0t) with a gradient echo pulse sequence (FLASH) providing 63 contiguous 3.1mm slices in the automated mensuration system. Results indicate that SCZ have significant volume differences in the hippocampus, temporal lobe and caudate nucleus (decreased), and the lateral and third ventricles (increased) which were greatest in the left hemisphere and in males. Temporolimbic volumes were correlated with positive psychotic symptoms while lateral ventricular enlargement was associated with primary negative symptoms and poor treatment response. Repeat MRIs are being analyzed.

Current findings multifocal pathology specifically associated with clinical features and are consistent with a neurodevelopmental etiology of SCZ.

379.6

SCHIZOPHRENIA OR MESOPONTINE NEURONOSIS? Authors: C.Karson*, R.Mrak, M.Husain, W.Griffin
Depts of Psychiatry, Pathology & Pediatrics, UAMS, Little Rock, AR 72205

A preliminary finding of increased NADPH-diaphorase (+) PPN neurons in schizophrenia led to Western immunoblot studies of protein indices of cholinergic activity (ChAT) and neurodevelopment (B-tubulin 2.1) vs degeneration (GFAP) in this disorder. Rostral pons was obtained from 5 deceased schizophrenics, mean age $\pm$ SD=61$\pm$8 years, and the same number of age and sex matched controls. **RESULTS:** In schizophrenia, pontine [B-tubulin] was increased at both 24 and 66 hrs of exposure (mean $\pm$ SD=0.06 $\pm$ 0.03 units of absorption vs 0.00 $\pm$ 0.00 units, $p<0.05$ and 0.40 $\pm$ 0.16 units vs 0.10 $\pm$ 0.03 units, $p<0.01$, respectively). ChAT and GFAP concentrations were not different between the 2 groups. **DISCUSSION:** The increased concentration of B-tubulin in schizophrenia is consistent with our earlier report of increased neuronal numbers in this brain region and problem(s) in early development. (-) results with ChAT may suggest that many of the NADPH-diaphorase (+) neurons found in schizophrenic PPN are not ChAT (+).

379.7

Abnormal Serine-Glycine Metabolism in the Mesial Temporal Lobes of Schizophrenics.
R. Waziri*, S. Baruah & A.D. Sherman. Dept. of Psychiatry, Univ. of Iowa Coll. Med., Iowa City, IA 52242.

High serine and glycine concentrations and decreased serine hydroxymethyl transferase (SHMT) activity in the plasma are biological markers for schizophrenia & psychosis (Waziri, et al., 1984). The purpose of this study is to find out whether these variables are also biological markers in the autopsied brains of schizophrenics. The present results are from samples of frozen autopsied brains obtained from the Wadsworth VA Hospital in Los Angeles and the MacLean Hospital in Belmont, MA. Conventional biochemical and HPLC techniques were used to assay SHMT and serine and glycine levels. LineWeaver-Burke plots were constructed to determine the apparent K_m of the enzyme. Serine and glycine concentrations in Brodmann area (BA) 34 and adjacent areas of brain samples from schizophrenics was significantly higher than those found in brains from controls ($p < 0.0005$ and $p < 0.001$, respectively). Serine and glycine concentrations in BA 22 did not differentiate between the brains of schizophrenics and controls. The apparent K_m of SHMT (for serine as substrate) in BA 34 and adjacent areas was significantly higher than in controls ($p < 0.002$). Abnormalities in the levels of the above variables could not be attributed exclusively to either the left or right hemisphere of the brain. These results provide evidence of abnormal serine and glycine metabolism in the mesial but not lateral temporal lobes of schizophrenics.

Conclusion: Because glycine and serine can enhance glutamatergic neurotransmission and neurotoxicity at the NMDA site, these results suggest that abnormally high levels of these amino acids may be a factor in the frequently observed neuropathology of mesial temporal lobes in schizophrenia, particularly in the vulnerable areas of limbic structures.

379.8

INCREASED NUMBERS OF GLUTAMATE-IMMUNOREACTIVE VERTICAL AXONS IN SUPERFICIAL LAMINAE OF CINGULATE CORTEX OF SCHIZOPHRENIC BRAIN. F.M. Benes*, I. Sorensen, S.L. Vincent, E.D. Bird, M. Sathi. Department of Psychiatry and the Program in Neuroscience, Harvard Medical School; Mailman Research Center, McLean Hospital, Belmont, MA 02178.

A recent post-mortem investigation has suggested that schizophrenia may involve a defect in associative information processing in limbic cortex because vertical processes visualized with antibodies against the neurofilament 200K subunit were increased in layers II and IIIa of anterior cingulate cortex (ACC) of schizophrenics (SZs). To explore this possibility further, an immunoperoxidase localization of glutamate has been found to visualize vertical fibers in superficial layers of ACC and prefrontal cortex (PFC) of normal (N = 15) and SZs (N = 17). Vertical fibers were distinguished according to small or large calibres and were differentially counted with a blind computer-assisted technique. In SZ ACC, the density of small calibre (77.8%; $p = 0.0025$), and to a lesser extent, large calibre (30%; $p = 0.02$) fibers was increased when compared to controls. There were no differences in the density of either small or large calibre vertical fibers in PFC. The potential effects of age, post-mortem interval, fixation and neuroleptic exposure do not account for the differences seen in ACC of SZs. Taking together their small calibre, vertical orientation, preferential localization in superficial layers and marked glutamate immunoreactivity, it seems plausible that the fibers showing an increased density in SZs may be glutamatergic afferents, possibly ones that are associative in nature. Overall, the data are consistent with earlier findings based on NFP200-immunoreactive axons and support the hypothesis that alterations of associative processing in ACC and may be one component to the pathophysiology of schizophrenia. Supported by MH00423 and the Scottish Rite Foundation.

379.9

REDUCTION IN [^{3}H]FLUNITRAZEPAM BINDING IN CINGULATE CORTEX AND HIPPOCAMPUS OF POSTMORTEM SCHIZOPHRENIC BRAINS. R.F. Squires*, E. Saederup, A. Laitha, and M. Palkovits, N.S. Kline Institute, Orangeburg, NY 10962

Cerebral atrophies and neuronal loss are seen in many cases of schizophrenia. Benes et al. have reported loss of small (inter)neurons from the anterior cingulate cortex (Arch. Gen. Psychiat., 48:996, 1991a) and loss of pyramidal cells from the CA1 region of the hippocampus (Schizophrenia Bull., 17:597, 1991b). We report here a 50% reduction of [^{3}H]flunitrazepam (FLU) (0.2 nM) binding in anterior cingulate cortex (P=0.005), 30% reduction in hippocampus (P=0.025), 38% in cerebellar cortex (P=0.025), and 27% in globus pallidus (P<0.02). In 12 other brain regions there were no significant differences between schizophrenic (n, 2 to 4) and non-schizophrenic (n, 3 to 9) brains with respect to [^{3}H]FLU binding using 10 uM clonazepam to define non-specific binding (which subtracts binding to "peripheral" sites). Cingulate cortex and hippocampus are part of the loop of Papez, together with mammillary bodies and anterior thalamic nucleus (Benes et al., 1991b). We speculated earlier that instances of psychoses may be associated with selective loss of glutamatergic neurons (Squires and Saederup, Neurochem. Res. 16:1099, 1991), and it is known that hippocampal pyramidal cells are glutamatergic. It is known that most of the small interneurons of the cortex are GABAergic, but some are glutamatergic (e.g., granular cells, which may consist partly of very small pyramidal cells). The neurotransmitters used by the small interneurons that disappear from the cingulate cortex of schizophrenic brains are not yet known (Benes et al., 1991a). We speculate that many of them may be glutamatergic and would normally exhibit high densities of [^{3}H]FLU binding sites.

380.1

EXCITOTOXIC LESIONS IN RAT STRIATUM PROTECT AGAINST SUBSEQUENT METHAMPHETAMINE-INDUCED DOPAMINE TERMINAL DAMAGE. S.J. O'Dell*, F.B. Weihmuller, R.J. McPherson and J.F. Marshall. Dept. of Psychobiology, University of California, Irvine, CA 92717.

Repeated administration of methamphetamine (m-AMPH)(4x 4 mg/kg, sc) produces a large increase in striatal dopamine (DA) overflow and subsequent damage to the DA terminals (measured as an average decrease in striatal DA content of 45% one week after m-AMPH). We have shown that unilateral striatal injections of quinolinic acid (QA) (15 ug/0.5ul) two to three weeks before a neurotoxic regimen of m-AMPH can protect the lesioned striatum from m-AMPH-induced DA terminal injury. In these animals, m-AMPH produced a decrease in striatal DA of 41% in the intact striatum one week later while the DA content of the QA-injected striatum was no different from that of control animals not exposed to m-AMPH. The QA lesions themselves had no significant effect on the integrity of striatal DA terminals as indicated by no differences in either DA content or [3H]mazindol binding between QA- and vehicle-injected striata. In previous studies we have found that the amount of DA overflow produced by neurotoxic m-AMPH regimens was positively correlated with the degree of subsequent DA depletions. Treatments which protected against m-AMPH neurotoxicity were found also to reduce m-AMPH-induced DA overflow. We are using in vivo microdialysis to determine if QA lesions also protect against m-AMPH-induced neurotoxicity by reducing the stimulant-induced DA overflow.

380.2

PRETREATMENT WITH L-DOPA MARKEDLY INCREASES METHAMPHETAMINE-INDUCED EXTRACELLULAR STRIATAL DOPAMINE OVERFLOW AND POTENTIATES TERMINAL DAMAGE. F.B. Weihmuller*, S.J. O'Dell, and J.F. Marshall. Dept. of Psychobiology, University of California, Irvine, CA 92717.

We have recently shown that repeated methamphetamine (m-AMPH) treatments produce a marked overflow in extracellular striatal dopamine (DA) and the extent of this overflow is correlated with the depletion of striatal DA measured one week later (O'Dell et al., 1991). We have tested this relationship by further increasing extracellular striatal DA overflow and observing the consequences on striatal DA content. Rats were pretreated with 70 mg/kg L-DOPA + 17.5 mg/kg carbidopa one hr before a single m-AMPH treatment (4 mg/kg) and DA terminal damage was measured one week later. Microdialysis measurement of striatal DA overflow showed that treatment with L-DOPA + carbidopa alone produced a small (2-fold), but consistent increase in striatal DA overflow. However, when this L-DOPA + carbidopa treatment was followed by m-AMPH, striatal DA overflow increased to as much as 60-fold over basal levels and remained elevated at 20-fold basal for the subsequent 9 hrs. Striatal tissue content measured 1 week later was reduced by 50 - 90% when compared to that of animals given a single, non-neurotoxic injection of m-AMPH. These findings further support the hypothesis that the amount of DA overflow produced by m-AMPH may be an important determinant of the extent of DA terminal injury produced.

380.3

REGIONAL DIFFERENCES IN THE INDUCTION OF C-FOS mRNA IN GERBIL BRAIN BY METHAMPHETAMINE. B.K. Tolliver, M.S. Kindy, and J.M. Carney*. Depts. of Pharmacology and Biochemistry, Univ. of Kentucky Col. of Med., Lexington KY 40536.

Methamphetamine (MA) has been shown to induce neuronal damage at doses which alter gene expression in the brain. The effects of MA on c-fos proto-oncogene expression in distinct regions of gerbil brain were studied. Levels of c-fos mRNA in cerebral cortex, cerebellum, brainstem, hippocampus, and striatum 60 minutes after i.p. injection of 1.0, 3.2, 10, or 20 mg/kg MA were assayed by Northern blot analysis. Levels of c-fos mRNA were also assayed at 10, 30, and 60 minutes following injection of 20 mg/kg MA. Methamphetamine induced c-fos in all brain regions studied but regional differences were found in the dose-dependency and time course of induction. In cortex, a dose-dependent induction of c-fos was maximal 60 min after 20 mg/kg of MA (107-fold induction over saline controls), intermediate at 30 minutes (41-fold induction) and least at 10 minutes postinjection (6-fold induction). Induction of c-fos in striatum was also dose-dependent but was maximal at 30 minutes (23-fold over control) and declined by 60 minutes (7-fold induction). Elevation of c-fos mRNA in cerebellum, brainstem, and hippocampus also peaked at 30 minutes postinjection. The present study therefore demonstrates that methamphetamine rapidly and transiently alters expression of the early inducible gene c-fos in gerbil brain in a dose-dependent fashion. While the precise role of c-fos elevation in response to methamphetamine remains unclear, such changes in gene expression may be involved in the long term neurotoxic effects of MA.

380.4

PROTECTION OF AMPHETAMINE-DERIVATIVE-INDUCED NEUROTOXICITY TO SEROTONIN NEURONS BY NITRIC OXIDE (NO) SYNTHESIS INHIBITION OR N-METHYL-D-ASPARTATE (NMDA) RECEPTOR ANTAGONISM. S. Benmansour & D.J. Brunswick*. Dept. of Psychiatry, Univ. Pa. Sch. of Med. & VA Med. Ctr., Phila., PA 19104.

Dextromethorphan, an NMDA receptor antagonist, prevents the neurotoxic effects of amphetamine-derivatives on serotonin (5-HT) neurons. Since activation of NMDA receptors leads to increases in the synthesis of NO, we investigated whether NO is involved in the neurotoxic effects of amphetamine derivatives. Groups of rats were pretreated with either L-N^G-nitroarginine (NO_2-arg) (50mg/kg b.i.d. for 2 days), an irreversible inhibitor of NO synthase, or MK-801 (2.5mg/kg), an NMDA receptor antagonist. p-Chloroamphetamine (pCA) (4mg/kg) was administered 40 hours after the last injection of NO_2-arg or 15min after MK-801. Other groups received either pCA or saline alone. Rats were killed 7 days after pCA or saline administration and neurotoxicity was assessed by quantitative autoradiography using [^{3}H]cyanoimipramine ([^{3}H]CN-IMI), a radioligand which binds to 5-HT uptake sites. pCA caused significant reductions in binding of [^{3}H]CN-IMI (40-65%) in all serotonergic terminal field areas examined. By contrast, rats treated with both MK-801 and pCA had no significant reductions in [^{3}H]CN-IMI binding. Similarly, in rats given both NO_2-arg and pCA, most areas showed no significant reductions in binding. Attenuation of the neurotoxic effects of fenfluramine (20mg/kg) was also seen following pretreatment with NO_2-arg. In separate experiments it was determined that the NO_2-arg treatment regimen produced 80% inhibition of NO synthase activity at the time of the administration of amphetamine-derivatives. These results indicate that NO may mediate neurotoxic effects of amphetamine-derivatives on 5-HT neurons. (Supported by Research Funds from the Dept. of Vet. Affairs and USPHS grant DA 05317).

380.5

EFFECTS OF NMDA ANTAGONISTS ON METHAMPHETAMINE-INDUCED SEROTONIN DEPLETION IN VARIOUS BRAIN REGIONS. T.Ohmori*, T.Koyama, S.Matsubara and I.Yamashita. Dept. of Psychiatry and Neurology, Hokkaido Univ. Sapporo 060 Japan

N-methyl-D-aspartate(NMDA) antagonists have been shown to prevent the toxic action of methamphetamine(MA) on the dopaminergic and serotonergic systems in the striatum. To investigate the involvement of NMDA receptors in the MA-induced damage of serotonin (5-HT) nerve terminals in various brain regions, we examined the effects of a non-competitive (MK-801) and a competitive (SDZ EAA494) antagonist on MA-induced 5-HT depletion in the rat frontal cortex (FC), nucleus accumbens (NA), dorsal striatum (ST), anterior hypothalamus (AH), amygdala (AM), and hippocampus (HI).

Four injections of MA (7.5 mg/kg, sc, at 2hr intervals) produced significant reduction in the levels of 5-HT in all areas examined (30-60 % of control values), although the levels of dopamine(DA) was significantly reduced only in ST. The monoamines were measured by HPLC-ECD one week after the MA treatment. Pretreatment with MK-801 (1mg/kg, ip, 30min before and 3.5hr after the first MA injection) or SDZ EAA494 (20 mg/kg, ip, 30min before, 1.5hr after, and 3.5hr after the first MA injection) almost completely reversed the MA-induced 5-HT depletion in all areas. The pretreatments also reversed the reduction of striatal DA levels. These results suggest that exitatory amino acids participate in the MA-induced damage to 5-HT nerve terminals in FC,NA,ST,AH,AM, and HI, as well as DA nerve terminals in ST.

380.6

INHIBITOR OF NITRIC OXIDE SYNTHESIS REDUCE THE NEUROTOXIC EFFECTS OF METHAMPHETAMINE IN MICE. JA Clikeman, S Wei, SA Turkanis*, and KT Finnegan. Depts of Psychiatry and Pharmacology, Univ. of Utah Sch. Med., Salt Lake City, UT 84148

Glutamate is reported to stimulate the synthesis of nitric oxide (NO) in rat brain slices via an NMDA receptor-dependent mechanism. Glutamate-induced neurotoxicity may involve increases in NO, as inhibitors of NO synthesis have recently been shown to prevent the damaging effects of glutamate in cortical cell culture. Methamphetamine (METH) damages CNS dopaminergic neurons in animals, and recent studies indicate that the neurotoxic effects of this agent, like glutamate, can be prevented by NMDA receptor antagonists. Based on these observations, we evaluated the ability of the nitric oxide synthase inhibitor, N^g-nitro-L-arginine methyl ester (L-NAME), to block the toxic effects of METH on nigrostriatal DA neurons in mice. Groups of mice (N=10) were treated with saline or 4 doses of METH (10 mg/kg/injection, each injection separated by an interval of 2 h), either alone or in combination with 2 doses of L-NAME (given 3 h apart), and then killed 1 week later for the assay of striatal DA by HPLC-EC. Mice given saline and then treated with METH showed a 65% decrease in the level of striatal DA. Substitution of increasing doses of L-NAME (37.5, 75, or 150 mg/kg/injection) for saline, however, provided a significant dose-related protection against the long-term, DA-depleting effects of the neurotoxin, although complete protection was not observed. In other experiments, pretreatment with the NO-generating compound, isosorbide dinitrate, restored the toxic effects of METH in L-NAME-treated mice. The findings suggest that NO may play an important role in the neurotoxic mechanism of action of METH.

380.7

DOPAMINE RECEPTOR (DAr) ANTAGONISTS PROBABLY DO NOT ACT AS DA REUPTAKE INHIBITORS IN THEIR PROTECTION AGAINST AMPHETAMINE INDUCED NEUROTOXICITY. GE Drucker, N Levine, R Song, JH Gordon & JZ Fields. Res Svce 151, VA Hosp, Hines IL 60141.

Continuous exposure (≥ 8 hr) of rodents or primates to amphetamine elicits decreases (-50% to -80%) in striatal DA and in markers for striatal DA terminals. In vivo administration of D2 DAr antagonists prevents this neurotoxicity. The simplest explanation is that the D2 DAr antagonists act at the post-synaptic D2 DAr, although in theory they could protect by inhibiting the DA reuptake pump. Steranka (Naunyn-Schmeid Arch Pharmacol 325 (1984) 198-204) found that i) sulpiride was an effective preventive agent and that ii) (+)-butaclamol (the more potent DAr antagonist) completely prevented neurotoxicity while (-)-butaclamol (the less active stereoisomer), was ineffective at an equal dose. In our studies, in contrast, sulpiride, even at 0.1 mM, failed to inhibit in vitro synaptosomal accumulation of [3H]DA. Second, the two enantiomers of butaclamol had essentially equal potencies in their ability to inhibit [3H]DA reuptake in vitro (IC-50 = 6 uM). Moreover, all of the D2 DAr antagonists tested had low potencies (uM range) for inhibiting [3H]DA uptake relative to their DAr blocking concentrations (nM range). We conclude that DAr antagonists probably do not protect via pre-synaptic DA reuptake inhibition. Since post-synaptic D2 DAr are thus the probable target, the mechanism of amphetamine neurotoxicity must be more complex than a simple direct toxicity to DA terminals. (Supported by VA Med Res & Ro1-NS26449)

380.8

LARGE DOSE REGIMEN OF METHAMPHETAMINE PRODUCES LONG LASTING DEFICITS IN THE ACQUISTION AND PERFORMANCE OF A REACTION TIME TASK. M.J. Baggott, J.B. Richards, K.E. Sabol, L.S. Seiden*. University of Chicago, Department of Pharm/Phys Sci., Chicago, IL 60637.

Male Sprague Dawley rats were trained to press a lever for a water reinforcer (.05 ml). After initial bar press training the rats were injected with 50 mg/kg of methamphetamine (MA) (3 times, 1 injection/8hr), or saline. After a 2 week recovery period, MA treated rats (N=11) and control rats (N=8) were tested for acquistion and performance of a reaction time (RT) task. The RT task required the rats to hold down a lever for a variable duration until a stimulus light was turned on. Releasing the lever after stimulus light onset was reinforced if the rats reacted faster than a criterion RT value. Reaction time was defined as time elapsed between stimulus onset and lever release. The criterion RT value was adjusted by computer for each rat so that approximately 3 of every 4 lever releases was reinforced. Each training session consisted of 100 trials or 1hr, whichever occurred first. The rats were trained on the task 5 days/week for 9 weeks. During the first week of training the mean RTs were not siginificantly different between the 2 groups (CONT=0.37±.03s; MA=0.40±.04s) (mean+S.E.M.). During subsequent training, the RTs for the control group became significantly faster while the RTs for the MA group did not change. By week 9 of training the mean RTs for the two groups were: CONT=0.22±.02s; MA=0.37±.03s. The rats were sacrificed 11 weeks after MA treatment. Dopamine tissue concentrations were significantly decreased in the caudate (66%) and nucleus accumbens/olfactory tubercle (67%) (% = treated/control). Serotonin tissue concentrations were significantly decreased in the nucleus accumbens/olfactory tubercle (36%), caudate (31%), somatosensory cortex (4%), amygdala (35%), and hippocampus (10%). MA treatment caused long lasting deficits in both neurotransmitter levels and behavioral output. (Supported by: NIDA DA-00085 and RSA-10562 L. Seiden)

380.9

CAUDATE/PUTAMEN (CPU) DOPAMINE (DA) AND GLUTAMATE (GLU) RELEASE AND CHANGES IN TOTAL DA AND 5HT CONTENT PRODUCED BY METHAMPHETAMINE (METH) ARE AFFECTED BY AGE. B. Gough*, R.R. Holson, W. Slikker Jr., G.W. Lipe, G.D. Newport and J.F. Bowyer. NCTR/FDA, Jefferson, AR 72079-9502.

Six and 12 mo. old female Sprague-Dawley rats were given 4 doses (i.p. at 2 hr intervals in a 23° C environment) of METH, and extra-cellular CPU DA, 5HT and GLU levels were determined at 20 min intervals using microdialysis and HPLC-EC up to 5 hr after the last METH dose. These rats were then sacrificed either 3 or 14 days after METH to determine total CPU DA and 5HT content. The 6 and 12 mo. old controls did not differ from each other in either total CPU DA and 5HT content or the extracellular CPU amino acids levels. In contrast, basal extracellular CPU DA and 5HT levels were only 50% as great in 12 mo. compared to 6 mo. old rats, and the increase in CPU DA, 5HT and GLU levels evoked by METH was also less than 50% as great in the 12 month old rats. The CPU GLU and Taurine levels did not differ between the controls in the 2 age groups but GLU levels were elevated in METH treated rats towards the end of dosing (particularly in the 12 mo. old rats). No late DA surge after the 4th dose of METH occurred in 6 or 12 mo. old female rats. Despite this blunted response of CPU DA release by METH in 12 mo. old rats, they had significantly greater reductions in total CPU DA and 5HT content 3 and 14 days post METH than 6 mo. old rats. In summary, METH toxicity may not be predicted solely by extracellular DA levels attained during METH exposure: Age and other factors also greatly influence METH toxicity.

381

SYMPOSIUM. MECHANISMS OF HIV-RELATED INJURY IN THE CENTRAL NERVOUS SYSTEM. Stuart A. Lipton, Harvard Med. Sch. (Chairperson); Clayton A. Wiley, UCSD; Douglas E. Brenneman, NIH-NICHD; Lynn Pulliam, UCSF; Melvyn Heyes, NIMH.

Both neuronal and glial injury are involved in the neurologic manifestations of AIDS even though neurons are not infected by HIV-1. **Dr. Wiley** will present an overview of the neuropathological findings of myelin pallor and neuronal loss with resulting clinical dementia. **Dr. Brenneman** will describe injury to rodent neurons in culture and in animal models induced by the envelope protein (gp120) of HIV-1. Neuronal damage may be indirect, mediated in part by gp120 effects on astrocytes. The effects of gp120 resemble those associated with antagonists of vasoactive intestinal peptide (VIP). **Dr. Pulliam** will describe the injury to astrocytes and cytological changes in neurons caused by gp120 in cultured human brain cell aggregates. She will also describe the neurotoxic and glial-toxic effects of as yet unidentified substances released from HIV-infected human macrophages. **Dr. Heyes** will describe the increase in quinolinic acid (an NMDA-like neurotoxin) measured in the cerebrospinal fluid of AIDS patients with dementia. **Dr. Lipton** will show that gp120 increases intracellular neuronal calcium levels, with subsequent neuronal injury in both rodent culture systems and animal models. The action of gp120 may be mediated in part by the release of NMDA-like neurotoxins from macrophages. Calcium channel antagonists and NMDA antagonists can protect from this gp120-induced neuronal injury. The findings of the various speakers will be linked by summarizing effects of HIV-related toxins, including gp120, on macrophages, astrocytes, and neurons.

382

SYMPOSIUM. COGNITIVE AND NEUROBIOLOGICAL CONSEQUENCES OF NORMAL AGING: FROM RATS TO PRIMATES. C. A. Barnes, Univ. of Arizona (Chairperson); M. Gallagher, University of North Carolina; L. de Toledo-Morrell, Rush-Presbyterian - St. Lukes Medical Center; P. R. Rapp, The Salk Institute; M. S. Albert, Massachusetts General Hospital.

This symposium reviews recent advances in understanding the neural basis of cognitive decline in normal aging. One experimental strategy that has proven particularly powerful in this regard involves the combined analysis of behavioral and neurobiological measures in the same subjects. This provides a basis for identifying those brain markers of aging that are most tightly coupled to senescent functional decline. Symposium topics were chosen to provide a focused survey of recent findings from investigations in rodents, monkeys, and humans, that have analyzed both behavioral and neurobiological measures in aged subjects. Rodent models for the study of cognitive decline in aging have focused on spatial learning tasks that are sensitive to the integrity of the hippocampal formation. Neurobiological markers that coincide with impairment are found in neurochemical, morphological, and electrophysiological studies of hippocampal circuitry. Such age-related changes are often region specific, and occur along with sparing of function and evidence for compensatory mechanisms. The cognitive consequences of aging will also be described for the nonhuman primate and related to neuroanatomical changes in the aged monkey brain. Again, individual differences in memory function are a prominent feature of aging that appear to be valuable predictors of the types of neural alterations observed. Finally, studies in healthy adults across the age range (30-80) will be discussed in terms of neuropsychological function, quantified electrophysiology, and measurements of CT and MRI scans. Recent longitudinal data provide additional information on the time course of these changes in humans.

385.1

ACTIONS OF NITRIC OXIDE IN THE MICROCIRCUITS OF THE GUINEA-PIG SMALL INTESTINAL MYENTERIC PLEXUS. K. Tamura, M. Schemann and J.D. Wood*. Tokai Univ., Isehara, Japan; Max-Planck Institut, Bad Nauheim, Germany; Dept. Physiol., The Ohio State Univ., Columbus, Ohio 43210.

Intracellular microelectrode recording was used to investigate the possible involvement of nitric oxide in the electrical and synaptic behavior of myenteric neurons in the small intestine. The nitric oxide synthase inhibitor, L-NAME (100 μM) was applied in the bathing medium for 20 min to identify actions on stimulus evoked slow EPSPs and fast nicotinic EPSPs. No effects of L-NAME on fundamental electrical behavior or excitatory synaptic events were found. Sodium nitroprusside was applied in the bathing solution as a source of nitric oxide. Sodium nitroprusside (10 μM to 1 mM) dose-dependently and reversibly suppressed or abolished slow excitatory postsynaptic potentials (slow EPSPs) in AH/Type 2 neurons. Slow EPSP-like responses to microejected substance P or serotonin were unaffected by sodium nitroprusside during suppression of the slow EPSPs. Pretreatment with either methylene blue (1 to 5 μM) or oxyhemoglobin (0.5 to 10 μM) reduced or prevented the inhibitory action of sodium nitroprusside on the slow EPSPs. Sodium nitroprusside did not significantly affect fast nicotinic EPSPs in the neurons. The principal action of nitric oxide in the microcircuits of the myenteric plexus appeared to be suppression of release of neurotransmitters for slow synaptic excitation. (Supported by International Scientific Research Grant Program 02044134 from M.E.S.C. of Japan)

385.2

INTERACTIVE METABOLIC STIMULATION BY ENDOTHELIN-1 AND NITRIC OXIDE IN THE RAT BRAIN. *P.M. Gross, D.F. Weaver, D.S. Wainman, F.J. Espinosa, R.J. Beninger* and S. Nag*, Departments of Surgery, Medicine, Chemistry, Physiology, Psychology & Pathology, Queen's University & Kingston General Hospital, Kingston, Canada K7L 3N6

Endothelin (ET) neuropeptides and biosynthetic mechanisms producing neuronal nitric oxide (NO) may coexist to modify neurotransmission and cellular activity. Using N_2O-halothane anesthetized rats, we applied the quantitative autoradiographic [^{14}C]deoxyglucose technique to assess the influence of ET and NO on focal rates of cerebral glucose metabolism (GM). The design employed lateral ventricular injections in separate rat groups to determine GM responses of individual periventricular structures to: 1) saline (3 μl), 2) ET-1 (9 pmol), 3) the NO donor, sodium nitroprusside (NP, 3 μmol), 4) the NO synthase inhibitor, N^G-monomethyl-L-arginine (NMMA, 4 μmol), 5) NP+ET, and 6) NMMA+ET. The main effects of each treatment were: ET - intense periventricular hypermetabolism (GM increases as high as +276% in the lateral septal n.) (*Neuropeptides* 21:211, 1992); NP - marked bilateral stimulation of white matter GM, e.g., corpus callosum (+239%), fornix (+94%), anterior commissure (+161%), and thalamic stria medullaris (+154%). In the ipsilateral choroid plexus, there was a 55% reduction in GM and histological evidence for cell damage; NMMA - no significant regional changes in GM, indicating that NO may not contribute to the basal metabolic state of the brain; NP+ET - topographically heterogeneous patterns of hypermetabolism (80% to 183% increases in GM) in the ipsilateral caudate and lateral septal n.; and NMMA+ET - inhibition of the hypermetabolic response to ET in the lateral septal n., results suggesting that ET may induce release of a metabolically-active NO in this structure. The findings indicate that intraventricular ET and NO have selective stimulatory effects on the GM of gray and white matter structures bordering the lateral ventricle.

Heart and Stroke Foundation of Ontario and Medical Research Council of Canada

385.3

NITRIC OXIDE AS A POSSIBLE SYNCHRONIZING FACTOR FOR PULSATILE LHRH RELEASE FROM IMMORTALIZED LHRH NEURONS PERIFUSED *IN VITRO*. M. Moretto, F. J. López, P. Petrusz* and A. Negro-Vilar. Reprod. Neuroendocr. Section, LMIN. National Institute of Environmental Health Sciences, NIH. Res. Tri. Pk., NC 27709.

The GT1-7 neuronal cell line secretes LHRH into the incubation medium in a pulsatile fashion, and is responsive to several stimuli. Nitric Oxide (NO) is best known for its relaxing properties as an endothelium-derived relaxing factor. Recent studies have provided compelling evidence indicating that NO may act also as a neurotransmitter depicting unique characteristics. As a gas, NO displays a extremely fast diffusion, which enables it to reach neighboring cells within a limited range. Those features may be important for the synchronization process in some specific neurosecretory systems like LHRH neurons. We tested this hypothesis in a perifusion system. GT1-7 cells were grown for four days on cytodex beads and then transferred to perifusion chambers. They were perifused for 4 h with Krebs-Ringer-Bicarbonate-Glucose buffer either alone or with 3 μM oxyhemoglobin, which binds NO. At the end of this period, cells were depolarized by 56 mM K$^+$-containing medium for 5 min. Fractions were collected at 5 min intervals and assayed for LHRH. The results were analyzed for pulsatility using the algorithm Detect. The continuous infusion of oxyhemoglobin (Hb) induced a marked reduction in pulse frequency without altering quantitative aspects of pulsatile LHRH secretion. In addition, an increased responsiveness to K$^+$ was observed in Hb-treated cells. Since Hb binds NO, thereby inhibiting its effects, it is tempting to suggest that NO participates in the genesis of a synchronous release of LHRH from these cells.

385.4

COLOCALIZATION OF GAD AND PARVALBUMIN IN THE MEDIAL SEPTUM OF THE RAT. **G. M. Peterson* and G. W. Lanford. Dept Anatomy & Cell Biology, East Carolina Univ Sch Med, Greenville, NC 27858**

The calcium-binding protein parvalbumin (Parv) is contained within most GABAergic neurons in the cerebral cortex, hippocampus and striatum. Within the medial septum (MS), a substantial number of neurons are immunoreactive for GABA (or GAD). Cells immunoreactive for Parv are similar in number and position to GABAergic cells within the MS, suggesting colocalization of both GABA and Parv. We have employed a double immunofluorescence staining technique to investigate the extent and distribution of colocalization. Sections through the MS were incubated successively in antisera to GAD and Parv. Using a GAD antibody (Chemicon) to the 67 kD form of GAD which does not require pretreatment with colchicine for the visualization of somata, GAD+ somata were labeled with a biotinylated secondary antibody and tagged with Cy3 conjugated to streptavidin (Jackson Labs). To visualize both GAD and Parv in the same section, Parv+ somata were labeled with an AMCA conjugated secondary antibody. Sections were examined throughout the rostral-caudal extent of the MS and the number of GAD only, Parv only and GAD + Parv neurons were counted. It was determined that the percentage of neurons containing both GAD and Parv ranged from 83% near the rostral pole to 43% at the caudal pole. Viewing the MS as a whole, 68% of the GAD+ cells contain Parv. Only a few cells were observed to contain Parv without GAD. These data indicate that, as has been shown for other regions in the forebrain, GAD and Parv are colocalized in the MS, but the extent of colocalization varies within the nucleus. Since most of the septal neurons which project to the hippocampus originate from the caudal half of the MS (Peterson, 1989) it is possible that those GAD cells which do not contain Parv are projection neurons whereas those in which GAD and Parv are colocalized are interneurons.

Supported by the Alzheimer's Association (IIRG-88-059).

385.5

RELATIONSHIP OF GABAERGIC AND CHOLINERGIC BASAL FOREBRAIN NEURONS AND THEIR PROJECTIONS TO THE MEDIODORSAL THALAMIC NUCLEUS IN THE CAT. I.Gritti*, M. Mariotti and M. Mancia. Institute of Human Physiology II, University of Milan, Milan, Italy I-20133.

Multiple lines of evidence indicate that cholinergic and GABAergic neurons located in the basal forebrain and preoptic region may influence cortical activities directly through cortical projections as well as indirectly through the subcortical connections involving the thalamus. In an effort to understand the potential role of the GABAergic neurons of the basal forebrain, we have investigated in the cat first their relationship to cholinergic neurons, and secondly their projections to the mediodorsal thalamic nucleus. In order to establish the relationship of neurons which synthesize GABA with those which synthesize acetylcholine (ACh) within the basal forebrain regions, a sequential double labelling immunohistochemical procedure was employed to stain glutamic acid decarboxylase (GAD) and choline acetyl transferase (ChAT), respectively. Through the entire basal forebrain, there was no evidence for colocalization of GAD and ChAT in the same cells. However a large population of distinct GAD+ neurons was found intermingled with ChAT+ cells through the cytoarchitectonically defined basal forebrain regions. GAD-immunoreactive neurons appeared to be morphologically similar to ChAT+ neurons although they were concentrated in slightly different positions of each subregion. Following injections of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) into the mediodorsal thalamic nucleus and combined processing for WGA-HRP and immunohistochemistry, a contingent of basal forebrain projecting neurons was found to contain ChAT, whereas a small contingent was found to contain GAD. These results demonstrate that a distinct population of GABA-synthesizing cells is intermingled with ACh-synthesizing cells across the basal forebrain. They reveal the existence of GABAergic projections from the basal forebrain to the mediodorsal nucleus, which may play an important role in the control of thalamocortical functions during sleep.

385.6

NOREPINEPHRINE RELEASE ELICITED *IN VIVO* BY LOCAL NMDA RECEPTOR STIMULATION. V. Robine, R. Valentino, G. Aston-Jones and J. Lehmann*. Division of Neurobiology, Department Mental Health Sciences, Hahnemann University, Philadelphia PA 19102.

Norepinephrine (NE) recovered in microdialysate obtained from frontal cortex of halothane-anesthetized rats was increased when N-methyl-D-aspartate (NMDA) was contained in the microdialysis medium. NE levels increased by a factor of 3.6 compared to prior baseline levels when 1 mM NMDA was applied for 30 minutes. This increase was largely reversible. Subsequent application of aconitine (0.3 mM, 30 minutes), which acts on voltage-sensitive sodium channels, evoked a second release of NE. The NMDA antagonist MK-801 (300 nM) blocked the NMDA-elicited increase but not the aconitine-elicited increase. However, this concentration of MK-801 also decreased the spontaneous release of NE, raising the possibility that there may be endogenous excitatory amino acid neurotransmission mediated by presynaptic NMDA receptors.

Supported by PHS grants MH49898, MH00840, and NS24698.

385.7

EFFECTS OF SEROTONERGIC (5-HT_2) BLOCKADE ON ENDOGENOUS DOPAMINE RELEASE MEASURED IN VIVO WITH POSITRON EMISSION TOMOGRAPHY (PET). S.L. Dewey,* G.S. Smith, J. Logan, J.D. Brodie, P.T. King, N. Pappas, R.R. MacGregor, T. Martin, D. Alexoff, C. Shea, J.S. Fowler and A.P. Wolf. Depts of Chem., Med., BNL, Upton, NY 11973 and Dept of Psych, NYU Med Center, NY, NY 10016.

In our continuing effort to investigate neurotransmitter interactions in vivo with PET we have examined the effects of serotonergic (5-HT_2) blockade on the striatal binding of ^{11}C-raclopride, a highly selective dopamine D_2 receptor ligand. Following a baseline scan with labelled raclopride in adult female baboons (Papio anubis, n=3) we pretreated these animals with altanserin (a highly selective 5-HT_2 receptor antagonist, Leysen and Gommeren, 1986; iv, 1.0 mg/kg, 45 mins preinjection) and performed a second scan with ^{11}C-raclopride. Striatal binding decreased by an average of 36% while there were no changes measured in the cerebellum or in the rate of systemic ^{11}C-raclopride metabolism. These changes greatly exceeded the test/retest variability (5%) observed with this radiotracer in the same animals under identical experimental conditions. This decrease in ^{11}C-raclopride binding is consistent with an increase in endogenous dopamine release following 5-HT_2 receptor blockade. These data support previous experimental findings that suggest that serotonin inhibits striatal dopamine (Spampinato, et al., 1985). Supported by DOE/OHER, NIH, NS-15638, NS-15380.

385.8

N-METHYL-D-ASPARTIC ACID DIFFERENTIALLY REGULATES DOPAMINE, GABA AND GLUTAMATE RELEASE IN THE DORSOLATERAL STRIATUM OF THE HALOTHANE ANAESTHETIZED RAT. AN IN VIVO MICRODIALYSIS STUDY. M. Morari, W. T. O'Connor, U. Ungerstedt and K. Fuxe*. Depts. of Pharmacology, Histology and Neurobiology, Karolinska Inst., S104-01, Stockholm, Sweden.

The effects of local perfusion with the glutamate (GLU) receptor agonist N-Methyl-D-Aspartic acid (NMDA) and the non competitive NMDA receptor antagonist MK-801 on extracellular dopamine DA, GABA and GLU levels in the dorsolateral striatum were monitored using in vivo microdialysis in the anaesthetized rat. Local perfusion (10 min) with both the 10-3M and 10-4M dose of NMDA increased striatal DA and GABA release while only the (10-3M) dose of NMDA was associated with a small and delayed increase in extracellular GLU levels. The NMDA induced effects were dose-dependently counteracted by simultaneous perfusion with MK-801 (10-6M and 10-5M). Both basal and NMDA (10-3M) induced increases in DA release were reduced in the presence of TTX (10um) and low Ca2+ (0.1mM) while both basal and NMDA stimulated GABA release were unaffected by these treatments. Both the basal and NMDA stimulated GLU release were enhanced following TTX, while perfusion with low Ca2+ reduced basal GLU levels and enhanced and prolonged the NMDA induced stimulation. These data provide evidence for a differential effect of NMDA receptor stimulation on these three striatal neurotransmitter systems, possibly reflecting direct and indirect actions mediated via striatal NMDA receptors.

385.9

HALOPERIDOL INCREASES NEUROMEDIN N AS WELL AS NEUROTENSIN CONCENTRATIONS IN SPECIFIC BRAIN REGIONS R. Clement, D. Griff, B. Banks, C. B. Nemeroff, P. Kitabgi and G. Bissette*.Departments of Psychiatry, Duke University Medical Center, Durham, NC 27710, Emory University Medical School, Atlanta, Georgia 30322, and IPMC, CNR, Valbonne, France

Mammalian gene sequences coding for neuromedin N (NMN) and neurotensin (NT) are contained on the same exon and are separated by a single pair of dibasic residues. It is likely that differential processing can occur, however, as the regional brain distributions of NMN and NT are often distinct. Because several classes of antipsychotic drugs have been shown to selectively increase NT in the rat nucleus accumbens and caudate nucleus, we examined the response of NMN neuronal systems to three weeks of daily haloperidol treatment. The effect of lithium alone or in concert with haloperidol and the effects of a D_2 receptor agonist, quinelorane, on regional NMN and NT concentrations were also examined in nine brain regions. Haloperidol significantly increased NMN and NT concentrations in the nucleus accumbens, caudate nucleus and pre-optic nucleus of the hypothalamus and this increase was significantly correlated ($p<.0001$) between the two peptides. Lithium alone did not alter NMN or NT concentrations in any brain region examined and lithium did not further increase haloperidol's effect when co-administered. Quinelorane did not decrease the regional concentrations of either peptide but did increase both NMN and NT in the nucleus accumbens. Neuroleptic drugs may induce some of their clinical antipsychotic effects through increases in NMN as well as neurotensin.

LIMBIC SYSTEM IV

386.1

PHYSIOLOGICAL PROPERTIES OF ANATOMICALLY IDENTIFIED AXO-AXONIC CELLS IN THE RAT HIPPOCAMPUS IN VITRO

E.H. Buhl*, Z.-S. Han, Z. Lörinczi and P. Somogyi. Medical Research Council, Anatomical Neuropharmacology Unit, Mansfield Rd., Oxford OX1 3TH, U.K.

In hippocampal slices of the rat non-pyramidal cells were physiologically characterized with biocytin filled electrodes and subsequently identified with correlated light and electron microscopy. Four neurons in area CA1, two in CA3 and two in the dentate area had the distinct appearance of chandelier cells with their axon forming symmetrical synaptic contacts exclusively with the axon initial segment of pyramidal cells, granule cells and mossy cells.

Axo-axonic cells (AACs) had a mean resting membrane potential of -68 mV, an average input resistance of 42 MΩ and a short time constant (6.3 msec). Short duration action potentials (AP; 0.39 msec at halfamplitude) were followed by a deep short-latency afterhyperpolarization (8.5 mV mean amplitude) as well as a depolarizing afterpotential which was most prominent in CA1. In response to depolarizing current AACs could fire at up to 388 Hz and showed a varying degree of spike frequency adaptation. AACs in CA1 had a unique firing pattern, displaying numerous "doublets", i.e. one AP followed by another at a very short latency, apparently triggered by the depolarizing afterpotential. In the presence of 1 µm TTX and 5 mM TEA depolarizing pulses triggered APs of small amplitude, slow rise and long duration, suggesting that they were mediated by calcium. Electrical activation of the Schaffer collaterals, perforant path fibers and the alveus evoked short-latency EPSPs with a mean rise time of 3.7 msec, while suprathreshold stimuli elicited 1-3 APs. Bath application of the non-NMDA glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 µM) reduced the EPSP amplitude by up to 96%. IPSPs were usually comprised of a short latency IPSP-A (max. amplitude at 28.9 msec latency) with a mean reversal potential of -67 mV, while the IPSP-B peaked at 125 msec latency. In conclusion, AACs (a) have a distinct firing pattern (b) receive synaptic input which is largely mediated by non-NMDA receptors (c) and are involved in feedforward inhibition.

386.2

CALRETININ IN THE PRIMATE HIPPOCAMPUS IS PRESENT IN INTRINSIC INHIBITORY AND AFFERENT EXCITATORY SYSTEMS.

C. Leranth*(1) and R. Nitsch (2). (1) Dept. of OB/GYN and Section of Neurobiol., Yale Univ. Sch. of Med., New Haven, CT 06510 and (2) Centr. of Morph. Univ. Clinic, Frankfurt, D-6000 Frankfurt/M. 70, FRG.

The calcium-binding protein calretinin (CR) is present in rodent hippocampal non-pyramidal cells including a presumably excitatory cell type specifically associated with the mossy fiber system. Our light and electron microscopic studies on the hippocampus of African green monkeys demonstrated that CR is exclusively present in non-pyramidal cells concentrated in the hilus subjacent to the granule cells and in both sides of the fissure. They showed smooth, aspiny dendrites extending to all hippocampal layers. Colocalization studies revealed that almost all of the hippocampal CR-containing neurons contain GABA. CR boutons were concentrated in the hilus and on both sides of the hippocampal fissure and established symmetric synaptic contacts. Conversely, CR-positive axons which formed a prominent band at the border of the dentate molecular and granule cell layer and in the CA2, established asymmetric contacts, and were found to be immunoreactive for substance P (SP). These axons disappeared 10 days after a fimbria-fornix transection. An HRP tracing study revealed their cells of origin in the tuberomammillary nucleus (TBM). These large projective neurons contained both CR and SP. These observations suggest that in contrast to the rat, in the primate hippocampus, there are no intrinsic excitatory CR neurons associated with the mossy fiber system, but rather an intrinsic inhibitory and an extrinsic, hipothalamic, excitatory pathway. Supported by NS26068; MH44866; HD2380; (C.L.) and DFG: Ni344/1-1 R.N.).

386.3

THE DEVELOPMENT OF THE MOSSY FIBER PLEXUS IS DELAYED IN THE HILUS OF THE DENTATE GYRUS AS COMPARED TO THAT IN CA3. C.E. Ribak* and M.S. Navetta, Dept. of Anatomy & Neurobiology, Univ. of Calif., Irvine, CA 92717.

Recent studies have shown that mossy fibers, the axons of granule cells, sprout into the inner molecular layer after seizures and hilar cell death in adult rats, but they do not sprout in 15 day old rats injected with kainic acid. The present study was made to examine the number of mossy fibers in the dentate gyrus at different postnatal ages to determine whether a lack of hilar cell loss and subsequent sprouting in 15 day old rats is due to a reduced mossy fiber input to hilar neurons. It is interesting to note that mossy fibers form mature synapses in CA3 before 14 days of age. Neo-Timm and electron microscopic preparations from rats at 7, 10, 12, 15, 22 and 30 days of age were examined. The adult pattern of Timm-labeled mossy fiber innervation was found for hilar basket cells at 22 and 30 days, but deep hilar cells were less densely innervated at 22 days than in the adult. At 10 and 15 days, basket cells in the rostral dentate gyrus showed more intragranular mossy fibers than those in the septal pole, and they appeared to be the first hilar cell type to become densely innervated by mossy fibers. Electron microscopy of the 7 day hilus showed only a few synapses formed by developing mossy fibers. At 12 and 15 days, the density of synapses formed by mossy fibers with dendrites in the hilus was less than half that found in adults. The relatively sparse innervation of hilar neurons in the 15 day old rat may contribute to their resistance to kainate-induced cell death because these cells have a reduced number of synapses and/or their associated glutamate receptors. The delayed growth of synapses in the hilus between 10-22 postnatal days may be due to this region being a thoroughfare for migrating granule cells.

386.4

INHIBITORY LATERAL SEPTUM→HYPOTHALAMUS PATHWAY; A PHA-L TRACING/GABA-IMMUNOGOLD EM STUDY. R.L. Jakab[1]* and C. Leranth[1,2]. Dept. of Ob/Gyn[1] & Sect. Neurobiol[2]., Yale University Sch. Med., New Haven, CT 06510

The lateral septum (LS) massively projects to the hypothalamus, and contains a large group of GABAergic somatospiny neurons. This study aimed to test whether the LS→hypothalamus pathway is GABAergic.

The anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) was applied to the location of the somatospiny neurons. Sections from the two major termination fields of PHA-L labeled LS efferents, the anterior and lateral hypothalamic nuclei, were processed for electron microscopy. GABA content of PHAL-labeled axons was determined by processing ultrathin sections for postembedding GABA-immunogold staining. All PHA-L-labeled axon terminals examined were immunoreactive for GABA, and formed symmetric synapses on GABA-positive or immunonegative profiles.

This GABAergic LS → hypothalamus projection may be responsible for the septal inhibition of neuroendocrine and autonomic functions. Our previous studies demonstrated that the somatospiny neurons (the parent cells of this inhibitory projection): 1) are strongly controlled by numerous peptidergic pericellular baskets of mostly hypothalamic origin (thus, forming an "autonomic disinhibitory circuit"); and 2) the same cells receive massive excitatory afferents from higher limbic areas, such as hippocampus and amygdala, which can activate the inhibitory LS → hypothalamus projection. This model may explain why septal stimulation suppresses, whereas septal lesion triggers autonomic responses. (Supported by NIH grants NS 26068 and HD 23830, C.L.).

386.5

ENTORHINAL CORTEX (EC) GENERATES 40 Hz HIPPOCAMPAL OSCILLATIONS.

S. Charpak*, D. Paré and R. R. Llinás, Department of Physiology and Biophysics, New York University Medical Center, 550 First Avenue, New York, N.Y. 10016.

Recurring episodes of 40 (± 10 Hz) field potential oscillations can be recorded from the hippocampal formation in anaesthetized guinea pigs (Ketamine-Xylazine). Laminar analyses of these oscillations and of EC-evoked field potentials in the hippocampus revealed that this synchronous oscillatory activity is generated by a rhythmic synaptic input to the stratum lacunosum-moleculare and the stratum moleculare of the fascia dentata. Because these layers constitute the targets of EC afferents, we performed field potential and single unit recordings in the EC. These experiments revealed the presence of 40 Hz EC oscillations accompanied by rhythmic neuronal discharges at the same frequency. Furthermore, injections of tetrodotoxin within EC boundaries abolished ipsilateral hippocampal and EC 40 Hz oscillations but preserved hippocampal synaptic responses to contralateral EC stimulation. In contrast, hippocampal removal sparing only its temporal pole did not suppress the 40 Hz EC oscillation. Moreover, knife cuts interrupting the connections between CA3 and CA1 did not abolish the 40 Hz oscillations recorded in the stratum lacunosum-moleculare. Taken together, these results suggest that the 40 Hz oscillations recorded in both the fascia dentata and the CA1 regions originate in the EC and are conveyed by a monosynaptic input. The existence of 40 Hz in the entorhinal-hippocampal circuit, which is linked with the association cortices, raises the issue of temporal binding of perception with the memory-affective axis.

386.6

EFFECTS OF VENTRAL HIPPOCAMPAL COMMISSUROTOMY ON RHYTHMIC SLOW - WAVE ACTIVITY (THETA) IN THE URETHANE ANAESTHETISED RAT. A.J. Heynen* and D.K. Bilkey, Department of Psychology and the Neuroscience Research Centre, University of Otago, Dunedin, New Zealand.

The present study examined the contribution of the hippocampal commissural system in the production of rhythmic slow-wave activity (RSA) in urethane anaesthetised Sprague Dawley rats (n=10). Bilateral recording electrodes were placed in the hippocampus of each hemisphere in order to perform depth profiles of RSA during high frequency stimulation of the nucleus pontis oralis. Stimulating electrodes were also placed bilaterally in the CA3 region in order to activate the commissural/associational projection onto CA1 pyramidal cells. Lesions of the ventral hippocampal commissure (VHC) were performed by sectioning with a scouten knife.

Prior to transection of the VHC, single pulse stimulation of the CA3 region reliably produced field responses in both the ipsilateral and contralateral CA1 regions. Depth profiles of RSA demonstrated a superficial amplitude maximum in stratum oriens and a second deep maximum at the level of the hipppocampal fissure. Following transection of the VHC, field responses were no longer elicited in contralateral recordings, whereas ipsilateral field responses remained unaltered. Depth profiles conducted through the hippocampus of both hemispheres revealed a dramatic reduction of amplitude, or in some cases, a complete loss of RSA recorded in the stratum oriens region. A reduction in amplitude through the stratum radiatum was also observed, however the RSA recorded at the fissure remained unaltered. The present results suggest that a major portion of the RSA field recorded from the CA1 region during urethane anaesthesia depends on activity originating in the contralateral hippocampus. (Supported by New Zealand HRC Grant 91/98).

386.7

INVOLVEMENT OF A GABAERGIC COMPONENT IN THE INHIBITORY EFFECT INDUCED BY VENTRAL TEGMENTAL AREA STIMULATION ON THE PREFRONTAL CORTEX.

R. Godbout[1], S. Pirot[2], J. Mantz[2], J. Glowinski[2] and A.M. Thierry[2].
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The medial prefrontal cortex (PFC) receives dopaminergic (DA) and non-DA projections from the ventral tegmental area (VTA). The present study sought an electrophysiological evidence for the existence of a GABAergic component in PFC inhibition induced by VTA stimulation or DA iontophoretic application. Experiments were performed under ketamine anesthesia in control rats and in animals treated with αMPT in order to deplete DA stores. The iontophoretic applications of DA and GABA induced an inhibition of spontaneous firing in 70% of 84 cells and 100% of 133 cells, respectively. The DA receptor antagonist sulpiride blocked 89% of DA inhibitions but none of GABA inhibitions; the GABA receptor antagonist bicuculline blocked 100% of GABA inhibitions and 57% of DA inhibitions. Electrical stimulation (1Hz) of the VTA inhibited 86% of 196 cells in control rats (D=112ms); iontophoretic application of sulpiride blocked 54% of VTA inhibitions (n=41); bicuculline blocked 51% of VTA inhibitions (n=68). In αMPT rats, only 66 of 169 PFC cells (39%) were inhibited by VTA stimulation (D=94ms); sulpiride was no longer effective in reversing VTA inhibitions while bicuculline reversed 95% of VTA inhibitions. The present results suggest that: 1) the DA-induced inhibition of PFC cells involves cortical GABAergic interneurones; 2) the non-DA mesocortical system also exerts an inhibitory influence on PFC cells and appears to be GABAergic.

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386.8

RHYTHMIC SLOW ACTIVITY IN EEG OF REPTILIAN AMYGDALA. J.C. Prechtl* and T.H. Bullock. Dept. of Neurosciences & Neurobiology Unit, Scripps Institution of Oceanography, U.C.S.D., La Jolla, CA. 92093-0201

Homologues of limbic structures have been established in non-mammals from ontogeny, connectivity and cytoarchitecture but little is known of their comparative physiology. The mammalian amygdala is distinguished by slow EEG rhythms that have recently been related to affective behavior. We used pond turtles (*Pseudemys scripta*) implanted with 6-8 electrodes to examine for an analogous EEG profile in the reptile. Electrode targets were: the central amygdala, the thalamus, the dorsal ventricular ridge (DVR), and the dorsal cortex (DC). The turtles were awake and restrained. Unstimulated EEGs were recorded with a band pass of 0.1-50 Hz. The multi-channel record was examined by eye and culled of movement artifacts after which power spectral analyses were applied.

The power spectrum from electrodes in or near the central amygdala yielded higher densities than those from the DC and DVR placements throughout the 1-15 Hz range and particularly between 1-4 Hz. On average, power density values in the 1-4 Hz range were nearly four times as large as those of DC or DVR, including comparably deep DVR loci. Results of placements in or near the thalamus or ventral part of the medial cortex were varied but always showed less overall power than amygdalar sites. Amygdalar EEGs included two conspicuous rhythms: one of broad (160 ± 8 ms) negative "sharp" waves of 90 ± 5 μV peak-to-peak (p-p) that occurred at a frequency of 4-5 per sec, and another that consisted of smaller but similarly shaped contiguous waves at a frequency of 7-8 per sec. Power spectra of epochs containing exclusively slow or fast amygdalar rhythms show peaks between 1-7 Hz or 7-12 Hz respectively.

DC loci show 17-25 Hz spindles lasting > 900 ms, associated with slow negative shifts. Spindles occur as often as once every 4 sec with 50-100 μV (p-p) amplitudes. Spindles cause 12-25 Hz power spectrum peaks exceeding those peaks in the amygdalar spectrum. The spectra from most DVR loci resemble those obtained from DC depths where spindles were not observed.

In view of the wide differences in brain morphology and metabolism between mammals and ectotherms, analogous activities are impressive. Autonomic and behavioral measures may uncover functional parallels.

386.9

OPTICAL ACTIVITY OF DORSAL HIPPOCAMPUS IN FREELY BEHAVING CATS ASSOCIATED WITH EXTRACELLULAR VOLUME MANIPULATION. G.R. Poe*, D.A. Nitz, F.M.Bairamova, D.M.Rector and R.M.Harper. Department of Anatomy and Cell Biology and the Interdepartmental Neuroscience Program, UCLA School of Medicine, Los Angeles, CA, 90024.

Application of hyperosmotic solutions to hippocampal slices *in vitro* increases extracellular volume and decreases cell size, whereas hypoosmotic solutions effect the opposite result. MacVicar and Hochman hypothesized that stimulation-induced changes in 700 nm reflectance in the dorsal hippocampal slice result from cell swelling accompanying increased neural activity. We examined the possibility that reflectance changes found in the dorsal hippocampus of freely behaving animals stem from variations in extracellular space and cell volume which accompany neural activity.

Adult cats were anesthetized and stereotaxically fitted with an optical monitoring device and microdialysis cannulae in the dorsal hippocampus. After recovery, 20 mM sucrose and 100% double distilled H_20 were successively applied to the CA1-CA2 region in the freely behaving cat via passive diffusion through a microdialysis membrane probe. Images were digitized at 3 s intervals with respect to the ECG R-wave. Hypoosmotic (H_2O) trials resulted in decreased 700 nm light reflectance relative to baseline Ringer's solution conditions, and hyperosmotic (sucrose) trials resulted in increased reflectance (ANOVA, $\alpha = 0.05$). Thus, conditions which increase the extracellular volume fraction and decrease cell size are associated with increased reflectance, whereas circumstances which decrease the extracellular volume fraction and increase cell size correlate with decreased reflectance.

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386.10

ASSESSMENT OF DORSAL HIPPOCAMPAL OPTICAL PROPERTIES CONCURRENT WITH ELECTROPHYSIOLOGICAL MEASURES AT HIGH TEMPORAL AND SPATIAL RESOLUTION. D. M. Rector*, G. R. Poe, and R. M. Harper. Department of Anatomy & Cell Biology and the Interdepartmental Neuroscience Program, UCLA School of Medicine, Los Angeles, CA., 90024.

Optical measurement of neural tissue activity requires high temporal resolution to ensure adequate correlation with concurrent electrophysiological events occurring at high frequencies. The introduction of inexpensive dedicated image processors allow microcomputers to digitize and store standard video images continuously at a rate of 60 frames/sec. To assess the correlation between slow wave electrical activity and optical density of neural tissue, a 1.6 mm diameter coherent fiber optic imaging probe, with attached macrowires for recording slow wave electrical events, was placed over the dorsal hippocampus in freely behaving cats. A miniature CCD video camera attached to the distal end of the probe generated video images from reflected 700 nm light. Images were digitized continuously at a rate of 60 Hz and saved to a mass storage device. A multiplexer was designed to interlace video signals with up to 32 electrophysiological channels which were simultaneously digitized and stored with the images. Subsequent analysis involved retrieval of images and cross correlation of image characteristics with electrical events. The physiological record was scored for sleep state, and discrete epochs were identified with characteristic patterns of hippocampal rhythmical slow wave activity of REM sleep and low frequency waves of quiet sleep. Reflected light amplitude correlated temporally with individual theta waves (4-7 Hz) during REM sleep and with slow waves (0-3 Hz) during quiet sleep.

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386.11

EFFECTS OF INTRA-ACCUMBENS MICROINJECTIONS OF AN NMDA ANTAGONIST IN AN OPEN FIELD TASK C.S. Maldonado-Irizarry *and A.E. Kelley Dept. Psychology, Northeastern University, Boston, MA 02115.

Anatomical experiments have demonstrated the existence of a pathway originating in the hippocampal formation (subiculum) and projecting to the nucleus accumbens (NAcc). There is some evidence that this pathway, which is primarily coded by glutamate, plays an important role in mediating locomotion and exploratory behavior (Mogenson & Nielsen, 1984). To further investigate this hypothesis, rats were tested in an open field task following microinjections of amino-5-phosphonovaleric acid (AP-5), a competitive NMDA antagonist (0, 0.05, 0.5, 1.0μg/0.5μl) into the NAcc. In this procedure, rats were previously habituated to the apparatus and locomotion, rearing and grooming were measured. AP-5 significantly decreased peripheral locomotion. No other behaviors were affected. A control experiment indicated a certain degree of site specificity; AP-5 injections into the anterior dorsal striatum did not significantly affect locomotion. Interestingly, this effect may be rate- or environment-dependent; similar NAcc AP-5 infusions increased motor activity in photocell cages. In a further test of exploration of novel objects in the open field, NAcc infusions of AP-5 (0, 0.1, 1.0 μg/0.5 μl) had no effect on exploration of objects (specific exploration). Taken together, these findings suggest that the hippocampal-accumbens projection may mediate diversive exploration (responses that bring the animal into contact with distal stimuli) but does not play a role in specific exploration (investigatory responses to specific stimuli).

OTHER FACTORS AND TROPHIC AGENTS: BDNF, NT3

387.1

NEUROTROPHINS AND MOTONEURON SURVIVAL DURING AVIAN DEVELOPMENT. R. W. Oppenheim*, D. Prevette, Y. A. Barde, R. Kolbeck. Dept. of Neurobiology and Anatomy, Wake Forest University, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1010, and Dept. of Neurobiochemistry, Max Plank Institute for Psychiatry, Munich, Germany.

Developing spinal motoneurons (MNs) express neurotrophin receptors and retrogradely transport at least some members of the NGF family of neurotrophins. However, previous in vitro studies with NGF, NT-3 and BDNF, as well as in vivo studies with NGF have consistently failed to observe any effect of these agents on spinal MN survival during development. We have used an in vivo bioassay (naturally occurring cell death) to further explore the role of NGF, NT-3, BDNF and NT-4/5 in MN development and survival. In accord with previous (including our own) findings, NGF was without effect on MN survival. Preliminary results indicate that NT-3 is also ineffective in preventing naturally occurring MN death in vivo. By contrast, BDNF not only promoted the in vivo survival of sensory neurons in the dorsal root and nodose ganglia (as expected), but also rescued significant numbers of spinal MNs from cell death. Experiments with NT-4/5 are still in progress and the role of all of these neurotrophins in regulating the cell death of other populations of central and peripheral neurons is being examined. The present data suggest that at least one member of the NGF family of neurotrophins (BDNF) may play a role in the normal in vivo development and survival of neuronal populations (e.g. MNs) other than those in peripheral sensory ganglia.

387.2

MULTIPLE PROMOTERS DIRECT TISSUE-SPECIFIC AND SEIZURE-INDUCED EXPRESSION OF THE RAT BDNF GENE. T. Timmusk, K. Palm, M. Metsis*, M. Saarma and H. Persson. Lab of Mol. Gen., Inst. of Chem. Phys. and Biophys., Akadeemia 23, EE0026 Tallinn, Estonia, Dept. of Med. Chem. (II), Lab. of Mol. Neurobiol., Karolinska Inst., Box 60400, S-10401 Stockholm, Sweden and Inst. of Biotec., Univ. of Helsinki, Karvaamokuja 3, SF-00380, Helsinki, Finland.

Here we show that the rat BDNF gene spans more than 40 kilobases of genomic DNA and consists of three short 5′-untranslated exons and one 3′ protein coding exon. Three similar size mRNAs of approximately 1.6 kb, containing three different 5′-untranslated exons spliced to the same 3′-exon, are transcribed from the BDNF gene. The use of an alternative polyadenylation site in the 3′-exon generates three additional approximately 4.2 kb long mRNAs with the same 5′-ends as the shorter transcripts. The 5′-flanking regions of exons I and III contain TATA box-like elements and the 5′-flanking region of exon II is similar to TATA-less promoters. Several transcription initiation sites were mapped in these regions and transfection of promoter-reporter gene constructs confirmed that these sequences act as promoters. BDNF mRNAs containing exons I and II were expressed only, or preferentially, in the brain, while exon III containing mRNA is the predominant form in lung and heart. Exon II containing mRNA was markedly increased in the brain after seizures evoked by injection of kainic acid while only modest increases were seen for the other two forms of the BDNF mRNA. Combined, the data argue that alternative usage of three promoters within the BDNF gene and differential splicing control tissue specific and seizure-induced expression of BDNF mRNA.

387.3

EXPRESSION OF RECOMBINANT HUMAN NEUROTROPHIC FACTORS: STUDIES OF MUTATED FORMS AND THEIR BIOLOGICAL ACTIVITIES. K. Kullander, A. Kylberg, L. Lärkfors and T. Ebendal*. Department of Developmental Biology, Uppsala University, Biomedical Center, S-751 23 Uppsala, Sweden.

PCR was used to isolate DNA fragments encoding nerve growth factor (NGF), neurotrophin-3 (NT-3), brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF). cDNA from cultured human embryo primary brain cells were used to derive full-length DNA fragments that were subsequently sequenced and subcloned into the vector pXM for transient expression in COS cells. The neurotrophic activities of the various culture media were tested in bioassays using chicken embryo ganglia and found to be similar to those observed in our earlier work with mouse, rat and chicken neurotrophins. In particular, NT-3 strongly stimulated neurite outgrowth from the Remak ganglion but only a weak response was observed in paravertebral sympathetic ganglia. We are now undertaking a study of chimeric proteins where parts of the human NT-3 sequence have been replaced by the corresponding amino acids of NGF in order to determine receptor specificities. These mutations involve differences in chemical character of the replaced amino acids. They may also, from the three-dimensional structure of NGF, involve regions of binding to the *trk*-family of high-affinity receptors but not to the low-affinity NGF receptor, according to previous data. The human CNTF was not secreted from the COS cells but found in the cell homogenate after transfection. To overcome this lack of secretion, we have designed a CNTF which includes the 20-amino-acid signal peptide from NGF. Upon transfection, this construct resulted in the secretion of biologically active CNTF. These recombinant trophic factors were also tested for their c-fos induction in cultured E16 rat cerebellar neurons. (Granted by the Swedish Science and Medical Research Councils, and the Swedish EPA).

387.4

POTENTIATION OF THE EFFICACY OF DEVELOPING SYNAPSES BY NEUROTROPHINS. A.M. Lohof*, Z. Xie, N.Y. Ip+ and M-m. Poo. Dept. of Biol. Sci., Columbia Univ., N.Y., N.Y. and +Regeneron Pharmaceuticals, Inc., Tarrytown, N.Y.

The neurotrophins are a family of factors with well-known effects on neuronal survival and neurite growth. In the present study, we examined their actions on synaptic transmission. Spontaneous and evoked synaptic currents were recorded from developing neuromuscular synapses in *Xenopus* cultures before and immediately after extracellular application of 50 ng/ml of brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) or nerve growth factor (NGF). BDNF and NT-3 induced marked potentiation of spontaneous synaptic activity, while NGF was without effect. The frequency of spontaneous synaptic currents (SSCs) showed an average 6.4 - fold (n=8) and 5.5 - fold (n=5) increase in response to BDNF and NT-3, respectively. The effect of NT-3 had an earlier onset than that of BDNF. The range of SSC amplitudes remained unchanged, indicating that the increase in SSC frequency reflects potentiation of spontaneous ACh secretion from the presynaptic nerve terminal rather than changes in the postsynaptic ACh sensitivity. BDNF and NT-3 also potentiated the impulse-evoked synaptic response. During the first hour of exposure to BDNF and NT-3, the mean amplitude of the evoked synaptic currents increased 30 % (n=6) and 32 % (n=5), respectively. These results indicate that neurotrophins can produce acute physiological effects on synaptic functions and may play a role in promoting synapse maturation.

387.5

CHANGES IN THE EXPRESSION OF THE NEUROTROPHINS BDNF AND NT3 MAY MEDIATE THE DEPENDENCE OF HIPPOCAMPAL NEURONS ON GLUCOCORTICOID LEVELS Timothy L. Denton, Millicent N. Dugich-Djordjevic, Nancy R. Nichols, Cynthia J. Davenport*+, Caleb E. Finch and Franz Hefti, Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089 and +Central Research Division, Pfizer Inc., Groton, CT 06340

Chronic stress and the elevated levels of glucocorticoids associated with it can cause degeneration in the hippocampal pyramidal neurons of both primates (Sapolsky, 1989) and rodents (Landfield, 1991). This damage resembles the degeneration that is characteristic of the aged human brain. Contrariwise the granule neurons of the dentate in young rats require adrenal steroids for survival (Sloviter, 1990). The highest levels BDNF are expressed in hippocampal pyramidal cells, while NT3 is the principal neurotrophin of the dentate. We investigated the effects of adrenal steroids on BDNF and NT3 mRNA levels. We found that male F344 rats showed a statistically significant decline (36%) in hippocampal BDNF mRNA by Northern blot analysis when treated with corticosterone (CORT) at 10 mg/day for three days compared with intact animals. The decline was confirmed as being in both dentate and pyramidal layers by in situ hybridization analysis. Interestingly, in the same experiment, NT3 mRNA levels show a statistically significant decrease (25%) in response to adrenalectomy. Our results suggest that BDNF and NT3, by being regulated in different directions, may mediate the degenerative effect of both excessive adrenal cortical hormones, as well as their lack, on hippocampal neurons.

387.6

CUTANEOUS AND MUSCLE AFFERENTS IN VITRO: DIFFERENT REQUIREMENTS FOR SURVIVAL FACTORS . P.LoPresti* and S.A.Scott, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook , NY 11794.

Characterization of the diversity of neurons is essential for understanding the mechanism by which neuronal diversity arises. One aspect of the diversity of dorsal root ganglion (DRG) neurons is their response to different neurotrophic factors. NGF, BDNF and NT-3 each supports the *in vitro* survival of subsets of DRG neurons, but the identity of the responding subsets is unknown. In this study we ask which neurotrophic factors support cutaneous and muscle afferents *in vitro*. These populations of DRG neurons have been labeled by injecting fast DiI into either a cutaneous (cutaneous femoralis medialis) or a muscle (femorotibialis/iliotibialis) nerve in E9 chick embryos. We then established cultures of lumbosacral DRG 2 and 3, in which approximately 2% of neurons were labeled. Cultures were supplemented with either NGF, BDNF or NT-3. Survival of labeled and unlabeled neurons was determined at 24 and at 48 hours. Preliminary data suggest that the major survival factor for cutaneous afferents is NGF and to a lesser extent BDNF. In contrast, the major survival factor for muscle afferents appears to be BDNF, with minor but consistent support from NT-3 and NGF.

To determine whether different factors support the same or separate neurons within cutaneous or muscle afferents, we will test whether the survival effects of different trophic factors are additive. (Supported by NIH NS16067)

387.7

BOTH NMDA-SENSITIVE AND NON-NMDA GLUTAMATE RECEPTOR AGONISTS INCREASE THE BDNF mRNA EXPRESSION IN CEREBELLAR GRANULE NEURONS IN CULTURE. J.M. Rimland*, M. Favaron, R.M. Manev, M.C. Comelli and H. Manev, FIDIA Research Laboratories, 35031 Abano Terme, Italy.

We have used primary cultures of rat cerebellar granule neurons to characterize the influence of the excitatory glutamatergic neuronal transmission on the expression of mRNAs for the brain-derived neurotrophic factor (BDNF). 8-12-day old cultures responded to an increased level of KCl in the medium (depolarization) or the removal of Mg^{2+} from the medium by enhancing the basal expession of the BDNF mRNAs (4 and 1.6 kb bands). The increase was blocked by MK-801 (the noncompetitive antagonist of N-methyl-D-aspartate (NMDA)-sensitive glutamate receptors. NMDA itself potentiated the expression of the BDNF mRNAs dose dependently. The effect of NMDA was blocked by MK-801, but not by CNQX, the antagonist of the non-NMDA glutamate receptors. In situ hybridization revealed that this increase occurred within individual neurons without enhancing the number of cells expressing the mRNA. The levels of the BDNF mRNAs can also be elevated by the non-NMDA agonists kainate, AMPA and quisqualate. Here we showed for the first time that the primary culture of cerebellar granule neurons could be a suitable model to study the mechanisms involved in the regulation of BDNF expression. The model can also be used to evaluate the putative pharmacological approaches.

387.8

Neurotrophin-3 Binds to Brain Sections with High Affinity and with a Topography Distinctly Different from NGF. C. A. Altar*, M. Criden, J. A. Siuciak, R. M. Lindsay, P. S. DiStefano, Regeneron Pharmaceuticals, Tarrytown, NY 10591.

The binding of biologically active, [^{125}I]-labeled neurotrophin-3 (NT-3) was studied with dry film and emulsion autoradiography. The equilibrium binding of 300 pM [^{125}I]NT-3 to rat brain sections was reversible and inhibited by unlabeled NT-3 (IC_{50} of 420 pM). [125I]NT-3 bound saturably, with high affinity (K_d of 269 pM), and with a capacity (B_{max}) of 26 fmol/mg prot that exceeded that of NGF by 3-fold. The association rate constant for [125I]NT-3 was 0.0074 x 10^9 M^{-1} min^{-1} and the dissociation rate constant was 19.4 x 10^{-4} min^{-1}, yielding a K_d value (k_{-1}/k_1) of 227 $\pm$ 28 pM. As with NGF , [125I]NT-3 bound to a second site with lower affinity (K_d of 2.8 nM) but with much higher capacity (B_{max} of 170 fmol/mg prot). [125I]NT-3 binding was not blocked by NGF, CNTF, K252a, or serum proteins, but was competed for by BDNF in a distinctly biphasic manner (IC_{50} values of 230 pM and 37 nM). [125I]NT-3 binding topography was clearly different from [125I]NGF. Highest binding was in layers 1 and 2 of rat (and human) neocortex, strata oriens and radiatum of hippocampus, molecular dentate gyrus, nu. of the lateral olfactory tract, entorhinal cortex, olfactory bulb, anteromedial thalamus, and olfactory tubercle. Moderate binding densities were present in retina, layers 4-6 of neocortex, amygdala, substantia nigra, DRG, spinal cord, and in rat and human caudate-putamen. Binding was absent from many brain regions and peripheral organs assessed with whole body autoradiography. [125I]NT-3 binds to CNS structures distinctly unlike [125I]NGF and thus is likely to affect brain and spinal cord regions not affected by NGF.

387.9

GLIAL CELLS IN THE MATURE RODENT CNS EXPRESS TRK RECEPTOR PROTEINS. L.F. Kromer*, R.H. Fryer, and D.R. Kaplan. Dept. of Anat. & Cell Biol., Georgetown University Medical Center, Washington, DC 20007 and ABL-Basic Res. Program, the National Cancer Institute, Frederick, MD 21702

Recently, the *trk* family of tyrosine kinase receptors have been identified as high affinity receptors for the neurotrophins NGF, BDNF, and NT-3. These trophic factors are thought to be specific for neuronal elements within the CNS which are presumed to possess specific *trk* receptor subtypes. Thus, the objective of the present study was to determine whether receptors for these neurotrophins also are present on glial cells within the CNS. For these experiments a double immunofluorescence technique was used to colocalize GFAP and *trk* receptors on astrocytes. Using a pan-*trk* antibody that recognizes a highly conserved intracellular domain present in *trk*A, *trk*B and *trk*C, it was possible to colocalize *trk* receptors on CNS astrocytes in the postnatal and adult rat. On the day of birth (P0), most astrocytes lacked GFAP and *trk* staining except within the glial endfeet at the pial border. By P10, astrocytes within white matter tracts strongly stain for GFAP and *trk*. In the adult CNS all astrocytes that stain for GFAP also possess weak *trk* staining which is concentrated in glial endfeet along blood vessels and the ependymal and pial surfaces. Those astrocytes within myelinated pathways expressed the most intense *trk* immunoreactivity. These observations suggest that during the postnatal maturation of astrocytes there is an induction of *trk* protein that coincides with the expression of GFAP. Current experiments are investigating the functional significance of these receptors.

387.10

NEUROTROPHIN-3 RECEPTORS IN THE DEVELOPING RETINA OF THE CHICK. A. Rodríguez-Tébar*, E. de la Rosa and A. Arribas. Inst. Cajal de Neurobiología, Doctor Arce 37, E-28002 Madrid, Spain.

The aim of this study was to characterize the receptor(s) for neurotrophin 3 (NT-3R) in the chick retina at various developmental stages. High affinity binding of [^{125}I]-NT-3 was found during the entire period of investigation, between embryonic days (E) 4 and 15, with little change in the dissociation konstant (k_D = 1.3x10^{-11} M). Estimation of receptor numbers per cell revealed two distinct peaks of NT-3R expression, one around E6-7 (at the onset of neuron differentiation), and another one at E12-14 (when neuron differentiation is already complete in the chick retina). The NT-3Rs expressed by retinal cells at the age of E7 and E14 may not be identical. This is suggested by differences in the pattern of inhibition with other neurotrophins and by the results of crosslinking experiments. The latter disclosed a 130 kDa band with trk-like immunoreactivity, both at E7 and E14. However, application of [^{125}I]-NT-3 at increasing concentrations showed that this band was a high affinity NT-3R only at E14, but not E7 (k_D: 2x10^{-11} and >2x 10^{-10} M, respectively). Age-dependent differences were found also in physiological actions of NT-3 on retinal cells in short-term culture. At E7 NT-3 arrested proliferation and stimulated differentiation of neuronepithelial cells. In contrast, trophic support of amacrine and ganglion cells was exerted by NT-3 at E14 .

These results suggest that, by diversity of specific neurotrophin receptors, a particular neurotrophin may induce distinct effects on retinal cells at different stages of development.

387.11

INDUCTION OF TRUNCATED *trk*B NEUROTROPHIN RECEPTORS IN HIPPOCAMPAL GLIAL CELLS DURING INJURY-INDUCED AXONAL SPROUTING. Klaus D. Beck*, Thomas H. McNeill, Caleb E. Finch, Franz Hefti, and Jonathan R. Day. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

Neurotrophin receptors are formed by products of *trk*-related tyrosine protein kinases. At least for NGF, high affinity binding requires *trk* and the low affinity NGF receptor protein. *trk*B forms functional BDNF receptors, whereas NT-3 is capable of interacting with *trk*, *trk*B, and *trk*C. NT-5 was reported to bind to *trk*B and possibly also to *trk*. BDNF and *trk*B expression is prominent in the adult brain, particularly in cortex and hippocampus, and may be confined to neuronal cells. To test the hypothesis, suggested by the high abundance of BDNF and *trk*B mRNA in the hippocampus, that BDNF mechanisms play a crucial role in adult plasticity of this structure, we used defined lesions of hippocampal afferents which cause synaptic rearrangements in the hippocampal dentate gyrus. Combined lesions of perforant path and fimbria remove inputs from entorhinal cortex and septum and produce robust sprouting of axons of the commissural/assocation pathways into the molecular layer of the hippocampal dentate gyrus. We showed that this neuroplasticity correlates in time and place with the induction of *trk*B mRNA synthesis in non-neuronal cells in the dentate outer molecular layer. The induced mRNA species codes for a truncated *trk*B receptor lacking the cytoplasmic protein kinase domain. These findings suggest that *trk*B molecules expressed on the surface of glial cells, possibly serving as neurotrophin presenting molecules, play an important role for axonal growth in the central nervous system.

POTASSIUM CHANNEL PERMEATION AND GATING

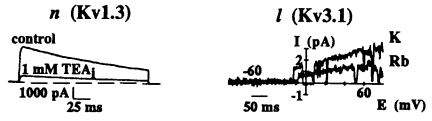
388.1

DOES A SINGLE RESIDUE IN THE PORE DETERMINE SENSITIVITY TO TEA_i, SINGLE CHANNEL CONDUCTANCE, AND ION SELECTIVITY OF T-CELL K CHANNELS? S. Grissmer, J. Aiyar*, R. Wymore, A. Nguyen, R. Spencer, and K. G. Chandy. Dept. Physiol. & Biophys., UC Irvine, CA 92717.

The genes encoding two voltage-gated K channels found in T cells, types *n* and *l*, have been identified as Kv1.3 (Grissmer *et al.*, 1990. *PNAS* 87:9411) and Kv3.1 (Chandy *et al.*, 1992. *Biophysical Journal* 61:A257), respectively. These two channel proteins differ by only one residue in the deep pore (P-region).

Kv1.3	W	W	A	V	V	T	M	T	T	V	G	Y	G	D	D	M
Kv3.1	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-

This residue has been recently suggested to determine sensitivity to TEA_i, single channel conductance, γ, and ion selectivity of K channels (Kirsch *et al.*, 1992. *Neuron* 8:499). To test this hypothesis we compared the properties of the native types *n* and *l* channels in lymphocytes and the cloned channels expressed in oocytes. Type *n* currents were reduced ~85% by 1.0 mM TEA_i (see figure), whereas type *l* currents were resistant to block by TEA_i (>10 mM). γ of type *n* and *l* channels was 11 pS and 27 pS, respectively (K_i = 156 mM, K_o = 4.5 mM). γ of type *n* channels did not change if K_i was replaced by Rb_i, however, γ of type *l* channels was reduced to 14 pS (see figure). These data suggest that L (Kv3.1) determines higher K vs. Rb conductance, whereas V (Kv1.3) confers TEA_i sensitivity. Mutagenesis studies exchanging these residues between Kv1.3 and Kv3.1 are in progress to delineate the properties associated with these residues.



388.2

HYDRATION POTENTIAL OF A SINGLE PORE RESIDUE AS DETERMINANT OF ION SELECTIVITY IN K^+ CHANNELS. J.A. Drewe*, M. Taglialatela, G.E. Kirsch#, M. De Biasi, H.A. Hartmann and A.M. Brown. Depts. of Molecular Physiology and Biophysics and # Anesthesiology, Baylor College of Medicine.

A single residue at position 374 in Kv2.1, regulates K^+:Rb^+ selectivity (Kirsch et al, Biophys. J. 1992). To understand the mechanism, site-directed mutagenesis was used to introduce substitutions at this position in Kv2.1 and in a chimera of Kv2.1 and Kv3.1 (Hartmann et al, Science, 1991). When cRNA transcribed from the different mutations was injected into *Xenopus* oocytes, 0.4-10ng, only L,V,T,I,S,C and F produced ion permeating channels. For this limited group, K^+ and Rb^+ conductances correlated inversely. Not expressing currents were the smallest residues (G,A), the aromatics (Y,W) as well as the charged, strongly hydrophilic residues (D,E,R,K,Q). Not examined were N,H,M and P. The results suggest that, at position 374, selectivity for K^+ and Rb^+ better correlates with hydrophilicity i.e. hydration potential, not volume or hydrophobicity of the introduced residue. Supported by NIH grants NS29473 to G.E. Kirsch and NS23877 and HL37044 to A.M. Brown.

388.3

BLOCK BY HYDROXYLAMINE REQUIRES AN INTERACTION BETWEEN A PUTATIVE PORE SITE AND THE N-TERMINAL OF THE SHAKER K+ CHANNEL. *Andrea Yool* and Thomas Schwarz,* Dept. of Molecular & Cellular Physiol., Stanford Univ. Med. Center, Stanford CA 94305

A single site mutation (T441S) in H5 of the Shaker K+ channel has been shown previously to alter ion permeation (Yool & Schwarz 91 *Nature* 349:700) and internal TEA binding (Yellen & al 91 *Science* 251:939). These results suggest that T441 contributes to the structure of the ionic pore. In support of this hypothesis, we have found that the wild and T441S channels differ in sensitivity to block by the substituted ammonium derivative, hydroxylamine ($HONH_3$+). Channels were expressed in *Xenopus* oocytes and analyzed by 2-electrode voltage and patch clamp. In the mutant T441S, but not in wild type, $HONH_3$+ appeared to stabilize the inactivated state mediated by the N-terminal. No block by $HONH_3$+ was observed in the non-inactivating mutant ΔN-T441S (N-terminal deletion 6-84). The dose-dependent block of outward current of T441S was half-maximal with 10 mM external $HONH_3$+ (90mM NaCl, 10mM $HONH_3Cl$, 4.3mM $MgCl_2$, 5.0mM Hepes), and correlated with a left-shift (by 10-15 mV) in the prepulse inactivation curve. Both the block of current and the shift in prepulse inactivation for T441S were prevented by the substitution of 2-10 mM external Na+ with permeant ions (K+, NH_4+ or Rb+). Our results support the hypothesis that T441S alters the affinity of a putative ion binding site for NH_4+ and ammonium derivatives. The interaction between the site T441S, the ion $HONH_3$+, and the N-terminal inactivation particle (a cytoplasmic domain of the channel, Zagotta & al 90 *Science* 250:568) suggests that the ion is trapped by the inactivation particle at the internal side of the pore. (Supported by NIH GM42376 and a postdoctoral fellowship from the Natl. Multiple Sclerosis Society)

388.4

MUTATIONAL ANALYSIS EXAMINING THE PERMEATION PATHWAY OF THE *SHAKER* POTASSIUM CHANNEL. G.A. Lopez*, Y.N. Jan & L.Y. Jan. Howard Hughes Medical Institute and Departments of Physiology and Biochemistry, University of California, San Francisco, San Francisco, CA 94143-0724.

To identify regions of the protein which may contribute to the internal mouth and pore lining structure of the *Shaker* potassium channel, we have made multiple single amino acid substitution mutations in the proposed sixth transmembrane segment (S6) and adjacent intracellular regions of the protein because sequence comparison reveals that some of the residues in the S6 domain are 100% conserved in all cloned potassium channels. Mutant channels were characterized at the single-channel level using inside-out patches with bi-ionic conditions to determine if the single-channel conductance and/or selectivity profile of the channel had been altered. We have found that several conservative substitution mutations in S6 affect the single channel conductance. For example, mutations at positions L468 and L472 decrease the single channel conductance. In addition to single amino acid substitutions, we have made chimeric channels using the S6 domain from other sub-families in order to identify residues which might contribute to the different single channel conductances observed with rat Shal1 (Kv4.2) and NGK2 (Kv3.1).

388.5

EFFECT OF MUTATIONS IN *SHAKER* S4-S5 CYTOPLASMIC LOOP AND S5 SEGMENT ON ION SELECTIVITY P.A. Slesinger*, E.Y. Isacoff, Y.N. Jan and L.Y. Jan. Depts. of Biochem. and Physiology, Univ. of Calif. Med. School. San Francisco, CA 94143.

Voltage-gated potassium channels transport ions across membranes at extremely fast rates, yet maintain very high specificity by incorporating "selectivity filters" along the ion-conduction pathway. Although recent mutagenesis experiments have shown that the H5 (p, SS1/SS2) region connecting S5 and S6 transmembrane domains contains elements important for single-channel conductance and ion selectivity, it is possible that other regions of the channel comprise part of the ion conducting pore. Because mutations in the cytoplasmic loop connecting S4-S5 segments alter the single-channel conductance, suggesting that this loop lies near the internal mouth of the channel, we examined the effect of mutations in this loop as well as the neighboring S5 segment on ion selectivity. Permeability ratios for Rb and NH_4 relative to internal K were determined by measuring the reversal potential in excised patches containing macroscopic currents and using a voltage ramp protocol. Most mutations produced no effect on either P_{Rb}/P_K (0.64) or P_{NH4}/P_K (0.1) ratios, except for L385A & S392C mutations in the loop and G397A in S5 which increased the permeability to Rb but not to NH_4. We are currently examining whether other amino acid substitutions at these residues alter Rb selectivity.

388.6

MODULATION OF SLOW INACTIVATION OF Kv3, A DELAYED RECTIFIER TYPE K+ CHANNEL. J. Kupper, S. Marom and I.B. Levitan*. Dept. of Biochemistry and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

Using the patch clamp configuration to record macroscopic currents through Kv3 channels expressed in *Xenopus* oocytes, we find that the behavior of the channels changes dramatically upon moving from the on-cell to the cell-free mode. In the on-cell mode, when the membrane is pulsed repeatedly to the same depolarized potential, the successive current traces show no change in size and kinetics. In the off-cell mode, the current decreases substantially from one pulse to another. Kv2, a highly homologous channel, does not show this change under the same conditions. One way to describe the macroscopic behavior of the Kv3 channel in the cell-free configuration is to assume that the channel can enter a long-lasting nonconducting state. TEA is known to reduce the rate of slow inactivation in *Shaker* H4 K+ channels when applied from the outside (Choi, K.L. et al. *PNAS* **88 (1991)**5092-5095). External TEA also interferes with the decrease in Kv3 current with repeated pulses, suggesting that the long-lasting nonconducting state in Kv3 is equivalent to the slow inactivated state in *Shaker*. Studies with chimeric channels indicate that the structural components involved in this phenomenon are upstream from the pore region.

388.7

MODULATION BY cAMP OF THE DELAYED RECTIFIER CHANNEL Kv3 EXPRESSED IN *Xenopus* OOCYTES. J. Acosta-Urquidi, C. Chavkin*, J. Redell, N. Davidson§ and H.A. Lester§. Dept. Pharmacol. Univ. Washington, Seattle, WA 98195 and §Div. Biol. 156-29, Caltech, Pasadena, CA 91125.

Using standard two-electrode voltage-clamp techniques, we studied the effects of the membrane permeable cAMP analogue 8-chlorophenylthio-cAMP (8CPTcAMP), on the cloned delayed rectifier Kv3 (Swanson et al. Neuron 4: 929, 1990). As previously described, Kv3 RNA expresses well in oocytes; its current is steeply voltage-dependent, activates between -35 to -20 mV, and is blocked by 5 mM 4-AP. We found that Kv3 is insensitive to Ba^{++}, reaches peak in 40-50 ms at +20 mV, decays exponentially to steady-state in 2-3 s, and exhibits twin-pulse inactivation requiring 20-25 s rest for 100% recovery. 8CPTcAMP (150-300 μM) produced a variable change in peak amplitudes: both increases (19±2.4%, N=13) and decreases (30.6±7%, N=9) were observed. In contrast, 8CPTcAMP consistently slowed the kinetics of activation and of inactivation (τ_{off} 434 vs. 540 ms, P<0.01, N=5). Steady-state inactivation curves were shifted positive ($h_{0.5}$: -22.8±3, vs -16.5±3.2 mV, P < 0.01, N=6). Tail analysis (E_{rev} -70 to -80 mV) revealed a slower decay rate after 8CPTcAMP (30.5±4, vs. 20± 9 ms, P<0.05, N=6). Supported by DA-04123. We thank R. Swanson for generously providing the Kv3 clone.

388.8

MUTATIONS OF A SINGLE PORE RESIDUE INTRODUCE FAST INACTIVATION INTO SLOWLY INACTIVATING K+ CHANNELS. M. De Biasi*, H.A. Hartmann, M. Taglialatela, J.A. Drewe, C.C. Shieh, A.M. Brown and G.E. Kirsch#. Departments of Molecular Physiology and Biophysics, and Anesthesiology#. Baylor College of Medicine, Houston, TX 77030.

Fast inactivation was introduced by point mutations at position 369 in Kv2.1 and a chimeric K+ channel (CHM), in which the pore region of Kv3.1 replaced that of Kv2.1. This form of inactivation called pore or P-type inactivation shares with Shaker C-type inactivation the property that extracellular TEA slows inactivation. Additional features of P-type inactivation are its modulation by extracellular K+, its sensitivity to side chain substitutions and its removal by a unique second mutation of pore residue L374 to Val (Kirsch, et al.; this meeting). Side chain substitutions in Kv2.1 and CHM, respectively, accelerated the rate of inactivation in the following order: Leu>>Ser≥Thr>Ile>Tyr>His and Ile≥Ser>Thr>Cys>Val. Although no correlation was found between side chain polarity and the rate of inactivation produced, all the polar residues markedly reduced ouward K+ conductance. These results support the notion that residues lining the pore modulate inactivation gating. Supported by NIH grants NS29473 (G.E. Kirsch) and NS23877 and HL37044 (A.M. Brown).

388.9

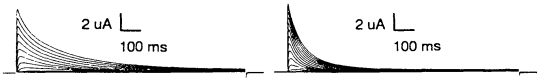
THE SODIUM CHANNEL III-IV LINKER FUNCTIONS AS AN AMINO TERMINAL INACTIVATION PARTICLE IN THE MK1 POTASSIUM CHANNEL. D.E. Patton[1]*, J.W. West[2], W.A. Catterall[2], and A.L. Goldin[1]. [1]Dept. of Microbiology & Molecular Genetics, U. California, Irvine, CA 92717 and [2]Dept. of Pharmacology, U. Washington, Seattle, WA 98195.

The short cytoplasmic linker that connects domains III and IV of the voltage-gated sodium channel is thought to be involved in the fast inactivation process. One hypothesis is that the linker acts as an inactivation particle in a manner similar to the amino terminal region of the *Shaker* B potassium channel. To test this hypothesis, we constructed a series of chimeric ion channels using as the parent channel MK1 (Kv1.1), which lacks the fast "N-type" inactivation process. To determine if MK1 possesses a functional receptor for the ShH4 amino terminal inactivation particle, we first constructed a chimeric channel in which the first 57 amino acids of the ShH4 potassium channel were attached to the amino terminus of MK1. Macroscopic currents through these channels expressed in *Xenopus* oocytes were characterized using two microelectrode voltage clamp techniques. The macroscopic inactivation kinetics of the ShH4/MK1 chimera were very similar to the wild type ShH4 potassium channel, suggesting that MK1 contains a functional receptor for the inactivation particle. To test the hypothesis that the sodium channel III-IV linker acts as an inactivation particle, we constructed a chimeric channel in which the sodium channel III-IV linker was attached to the amino terminus of MK1 (NaMK1). This chimeric channel also inactivated with kinetics similar to the wild type ShH4 channel, suggesting that the III-IV linker of the sodium channel and the amino terminal inactivation particle region of the ShH4 potassium channel may serve similar functions in the inactivation process. We are currently introducing mutations in the III-IV linker portion of the NaMK1 chimera which have dramatic effects on sodium channel inactivation to test if the same region of the III-IV linker is essential for fast inactivation in these two channels.

388.10

MODULATION OF KINETIC PROPERTIES OF A CLONED MAMMALIAN A-TYPE K^+ CHANNEL BY BRAIN POLY(A)$^+$ mRNA. L.D. Chabala*[1], N. Bakry[1] and M. Covarrubias[2], Depts. of Medicine[1] and Pathology & Cell Biology[2], Jefferson Medical College, Phila., PA 19107.

The mouse brain cDNA clone mShal encodes a transient A-type K^+ current that can be expressed in Xenopus oocytes and studied with a two-electrode voltage clamp. Expressed currents show rapid and slow phases of inactivation (A). When mShal cRNA is coinjected along with a low molecular weight (LMW) fraction of brain poly(A)$^+$ mRNA (2-4 kb), currents show mostly a rapid phase of inactivation (B). These findings were confirmed using "macro" patch recordings. We did not observe any current size-dependent kinetics for control or coinjected oocytes ($2<I_p<15$ μA). Results are consistent with expression of a modulatory factor, such as an enzyme, by LMW poly(A), which is not expressed endogenously by oocytes. The LMW poly(A) also results in sizable up-regulation of mShal current. Oocytes in A and B (same batch) were injected with ≈2 and ≈0.5 ng mShal cRNA. Oocyte in B was coinjected with ≈50 ng LMW poly(A). V_c = -90 to +50 mV.



A. Control mShal B. Coinjected mShal+poly(A)

388.11

PROTEIN KINASE C ACTIVATORS ENHANCE A MAMMALIAN A-TYPE K^+ CURRENT EXPRESSED IN *XENOPUS* OOCYTES.
Covarrubias, M.[1], Pak, M.[2] and Sorensen, R.[3]* Departments of Pathology and Cell Biology[1] and Medicine[3], Jefferson Medical College, Philadelphia, PA 19107 and NIH[2], Bethesda, MD 20892.

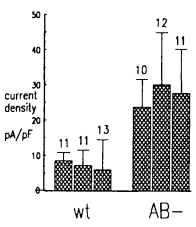
Using *Xenopus* oocytes as expression system, we have studied the effects of PKC activation on the properties of a mammalian A-type K^+ channel coded by *mShal*1. In voltage-clamp experiments we found that 12-*O*-tetradecanoylphorbol 13 acetate (TPA, 10-100 nM) or 1-oleoyl 2-acetylglycerol (OAG, 20 μM) enhance the peak *mShal*1 current and the effect reaches a maximum 5-10 min after the activators were perfused in the bath solution (%enhancement: 53±32, n=7 and 26±11, n=4, with TPA, 20 nM and OAG, 20 μM, respectively). PKC activation does not affect the kinetics or voltage sensitivity of *mShal*1 currents. The inactive 4α-phorbol ester does not affect the current and the effect of TPA was inhibited by a 5-10 min pretreatment of the oocytes with a selective PKC inhibitor (Calphostin C, 1 uM). Furthermore, we found that the current coded by *Shal2* (the *Drosophila* homolog) was also enhanced by PKC activation (92%, n=2). This is in contrast to *Shaker* currents which were only inhibited by TPA (20-100 nM). Currently, we are preparing deletion mutants and point mutations to identify the site most likely associated with the *Shal* current enhancement induced by PKC activation.

388.12

CHANGES IN K^+ CURRENT IN NORMAL AND PROTEIN KINASE A-DEFICIENT CELL LINES. M. M. Bosma*, M. L. Allen, and B. L. Tempel, GRECC, Seattle VA Med. Ctr. and Depts. of Medicine and Pharmacology, U.W. School of Medicine, Seattle, WA 98195.

MK1 K^+ channels were stably transfected into Chinese Hamster Ovary (CHO) cells and their electrophysiological properties studied. Expressed currents were voltage-dependent, activating within 25 ms and showing less than 15% inactivation during a 500 ms pulse to +40 mV. Appreciable current was first seen between -50 and -40 mV, and maximal conductance was reached at 0 mV in mammalian Ringer. Currents were reversibly blocked by external tetraethylammonium (Ki = 5 mM), 4-aminopyridine (Ki = 3 mM) and dendrotoxin (Ki = 2.5 nM). Cells transfected with a control plasmid (vector only) or an antisense plasmid expressed no K^+ current.

This channel was also stably transfected into a CHO cell line which is co-transfected with a plasmid containing a dominant mutant regulatory subunit of Protein Kinase A (PKA), causing a reduction of approximately 90% of normal PKA activity . Under these conditions, MK1 current density was up-regulated almost 2.5 fold. The histogram shows current density for 6 different cell lines, three with normal PKA activity (wt), and three with the mutant PKA (AB-). The number of cells is shown above each bar. The molecular mechanism of this regulation is under investigation. Supported by Mellon Foundation, HL44948 and NS27206.



VISUAL DEVELOPMENT: NEURONAL AND SYNAPTIC DEVELOPMENT

389.1

DENSITY GRADIENTS AND LATERALLY DISPLACED DENDRITES OF RETINAL GANGLION CELLS. R. Linden*, Instituto de Biofisica da UFRJ, Rio de Janeiro, Brazil.

Dendrites of retinal ganglion cells (RGC) in adult mammals tend to distribute mainly towards areas of low RGC density after lesions, and also in the retina of normal cats. In normal rats, however, the dendrites of adult RGC do not point away from central retina, which was attributed to a shallow centro-peripheral (C-P) gradient of RGC density. In HRP-labeled whole-mounts of adult rat retinae, we studied the relationship between lateral displacement of dendrites and gradients of RGC density created by lesions of the contralateral optic tract made at birth. After degeneration of RGC with crossed projections, the dendrites of ipsilateral RGC are laterally displaced towards the depleted nasal retina. However, the temporal to nasal (T-N) gradient created by the lesions was less steep than the normal C-P gradient. When RGC density gradients were examined at 5 days after birth (PND5), the induced T-N gradient in operated rats was also less steep than the C-P gradient of normal adults. In normal rat retinae at PND5, ganglion cells are homogeneously distributed. The results indicate that the time course, rather than the magnitude of C-P gradients of cell density determines the appearance of laterally-displaced dendrites in mammalian retinal ganglion cells. *(CNPq, FINEP)*

389.2

DENDRITIC FIELD DEVELOPMENT OF RETINAL GANGLION CELLS (RGC) IN THE CAT AFTER DAMAGE TO VISUAL CORTEX AT BIRTH. A.J. Weber*and R.E. Kalil, Waisman Center and Dept. of Comparative Biosciences, Center for Neuroscience and Dept. of Ophthalmology, Univ. of Wisconsin, Madison, WI 53706.

Competitive interactions among RGC dendrites during development are thought to influence their mature organization. Since damage to visual cortex in newborn cats leads to the selective retrograde degeneration of about 75% of beta RGC, we decided to investigate whether this massive degeneration of a single class of RGC in infant cats affects the subsequent dendritic development of spared RGC. Therefore, we labeled more than 110 individual RGC in living retinae with Lucifer Yellow. These cells were sampled from one nondegenerated and 12 degenerated hemiretinae in adult cats with unilateral damage to visual cortex at birth, and the spatial organization of their dendrites was analyzed with video-enhanced microscopy.

Most labeled RGC could be classified morphologically as alpha, beta or gamma cells according to standard criteria. Measurements of dendritic field size and orientation indicate that the arbors of alpha and gamma RGC are not unequivocally abnormal. While dendritic arbors of surviving beta RGC tend to be larger than those of control cells, current sample sizes are small, 8 and 11 cells respectively. In summary, these results suggest that a pronounced loss of beta RGC during infancy may not produce major alterations in the dendritic development of those ganglion cells that are spared.

389.3

CORRELATED SPONTANEOUS CALCIUM BURSTING IN THE DEVELOPING RETINA. R.O.L. Wong, A. Chernjavsky, S. J Smith and C.J. Shatz. Depts. of Neurobiol., and Molec. & Cell Physiol., Stanford University, CA 94305.

Ganglion cells of the neonatal ferret retina spontaneously fire sodium action potentials in rhythmic bursts, which are correlated between neighbors in the form of an excitatory wave (Meister et al, *Science*, 252:139,1991). To learn more about the spatiotemporal pattern of bursting activity in the retina, we used single wavelength (380 nm exc.) *in vitro* measurements of Fura-2 stained neonatal retinae (P5-P15) to monitor changes in intracellular calcium. Cells in the ganglion cell layer (GCL) underwent spontaneously generated increases in $[Ca^{++}]_i$ that lasted 5 to 8 secs; these increases occured periodically, every 25 to 60 secs. This temporal pattern is identical to that seen with recordings of action potentials. Another similarity to action potential recordings is that the "calcium bursting" of neighbouring cells in the GCL was correlated. Even though not all cells in the GCL were active during periods of spontaneous bursting, the remaining cells could increase $[Ca^{++}]_i$ with perfusion of 100 µM glutamate, indicating they are capable of responding to applied transmitter. Calcium bursting ceased in the presence of 10-25µM tetrodotoxin. By contrast, the pattern of calcium bursting was not altered in the presence of 0.1-1 mM of kynurenic acid, suggesting that glutamate is not involved in sustaining the spontaneous activity. Retinal cells in the inner nuclear layer were also periodically bursting and their activity was correlated with that of cells located in the GCL.Taken together, our observations suggest that the spontaneous changes in $[Ca^{++}]_i$ require Na^+ dependent action potentials and that the correlated activity between developing retinal neurons is likely to be mediated by a specific network of connections rather than by the non-specific release and diffusion of glutamate into the extracellular space. Supported by: NHMRC C.J. Martin Fell. to ROLW, NSF IBN 9212640 to CJS, R01NS28587 to SJS, P50MH480101 to SJS, CJS.

389.4

JUNCTIONAL COUPLING BETWEEN NEURONS IN THE DEVELOPING MAMMALIAN RETINA. A. A. Penn, R.O.L. Wong and C.J. Shatz*. Dept. of Neurobiolgy , Stanford Univ. School of Medicine, Stanford ,CA 94305 and Dept of Molecular and Cell Biology, UC Berkeley, CA 94720.

The ganglion cells of the developing ferret retina during the first 3 postnatal weeks spontaneously generate waves of electrical activity that travel across the retina in the absence of mature photoreceptors (Meister et al., Science, 252: 139, 1991). These waves of correlated activity may be generated by a neural circuit in the retina. Here we have explored the possibility that gap junctions could represent a morphological substrate for the correlations. Retinal ganglion cells in a living *in vitro* preparation from P10 to P30 ferrets were injected with neurobiotin and lucifer yellow, tracers known to pass through gap junctions. At all ages studied lucifer yellow entirely filled the soma and processes of the injected cell only. In contrast, from P12 onwards neurobiotin filled not only the injected cell but also frequently a constellation of other cells within the dendritic tree. When injected ganglion cells were classified by type, alpha cells are seen to be tracer coupled to amacrine cells (including displaced amacrines) and by P21 they were also coupled to other alpha ganglion cells, in a pattern resembling that seen in the adult (Vaney, Neurosci. Lett. 125: 127,1991). Beta cells were apparently not coupled at any time nor to any other cell. These observations indicate that junctions able to pass the lower molecular weight neurobiotin are present in the inner plexiform layer during the period when the firing of retinal ganglion cells is highly correlated. Such junctions could play a role in synchronizing the activity of neighboring ganglion cells during development. However, since the junctions are known to exist in adulthood but the waves of activity disappear by P30, other changes in retinal circuitry are likely to contribute to the disappearance of the waves. Supported by GM 07365 to A.A.P., NHMRC C.J. Martin Fellowship to R.O.L.W., and NSF IBN 9212640 and the March of Dimes to C.J.S.

389.5

GENESIS OF RETINAL GANGLION CELLS AND THE CHIASMATIC COURSE OF THEIR AXONS IN THE FERRET B.E. Reese[1]*, G.E. Baker[2], W.F. Thompson[1], and J.D. Peduzzi[3]. [1]Neuroscience Research Institute and Dept of Psychology, University of California, Santa Barbara, CA 93106; [2]Dept of Physiology, University of California, San Francisco, CA 94143; [3]Dept of Physiological Optics, University of Alabama, Birmingham, AL 35294

Retinal ganglion cell classes in non-primate mammals differ greatly in their decussation patterns. The axons of medium-sized cells partially decussate at the optic chiasm, so that those from the nasal retina project contralaterally while those from temporal retina project ipsilaterally, whereas the axons of most small-sized cells project contralaterally, irrespective of retinal origin. We have examined the hypothesis that these different decussation patterns are governed by a time-dependent mechanism defining the chiasmatic course of growing optic axons.

In one study, fetal ferrets were exposed to ^{3}H-thymidine at different gestational stages in order to label early and late cohorts of retinal ganglion cells at two months after birth. Early (E-26) and late (E-32) injections of ^{3}H-thymidine each label ganglion cells across the surface of both the nasal and temporal retina. Early injections label exclusively medium-sized cells while later injections label primarily small-sized cells. Since medium-sized cells in the temporal retina project ipsilaterally while the majority of small-sized cells project contralaterally, time of genesis may determine pathway choice at the optic chiasm.

The axons of recently generated ganglion cells take 3-4 days to reach the optic tract. In a second study therefore, fetuses were fixed with paraformaldehyde on E-30 or E-36, and then given implants of DiI into either one optic tract or into the temporal retina to label the two eyes or optic tracts, respectively. Implants into the temporal retina on E-30 anterogradely label fibers only in the ipsilateral optic tract. Consistent with this anterograde result, implants into one optic tract at the same age retrogradely label cells in the ipsilateral temporal retina but only the contralateral nasal retina. In contrast, implants into the temporal retina at E-36 anterogradely label both the ipsilateral and contralateral optic tracts, while implants into one optic tract retrogradely label both temporal retinae as well as the contralateral nasal retina.

These results indicate that the line of decussation is well-defined for only the early generated cells, and that it is produced by selective guidance of the early-arriving temporal retinal axons into the ipsilateral optic tract. This mechanism weakens as development proceeds, leading to the complete decussation of later-generated cells. (Supported by grants from the NIH: EY-08415 and EY-01318)

389.6

ACTIVITY-DEPENDENT ENHANCEMENT OF SYNAPTIC TRANSMISSION WITHIN THE DEVELOPING LGN. R. Mooney*, D.V. Madison and C.J. Shatz. Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

The adult pattern of segregated eye input to the mammalian lateral geniculate nucleus (LGN) arises as a consequence of an activity-dependent rearrangement of synapses from retinal ganglion cell (RGC) axons, which initially are intermixed with each other. To study the synaptic mechanisms capable of supporting such activity-dependent reorganization, we used an *in vitro* slice preparation of the neonatal ferret LGN, in which retinogeniculate synaptic transmission could be monitored physiologically. Whole cell voltage-clamp recordings were obtained from LGN neurons with perforated patch techniques. Electrical stimulation of the optic tract elicited both short latency inward currents (at V_m=-70 mV), which were blocked by a combination of CNQX (10 µM) and D-APV (50 µM), and longer latency currents, which were eliminated by picrotoxin (50-75 µM). To examine the activity-dependent properties of the short latency glutamatergic EPSC, recordings were performed in picrotoxin. Under these conditions, three to six bursts of high frequency stimulation of the optic tract often (17 of 37 cells) produced marked increases in the EPSC amplitude. This enhancement persisted up to two hours, and was observed in slices prepared from P6 to P30 animals. These observations indicate that synapses within the retinogeniculate pathway exhibit activity-dependent changes in strength. Thus, such changes may underlie the process of synaptic rearrangement that accompanies the segregation of RGC axons into eye-specific layers. Supported by H.H. Whitney Foundation to R.M., March of Dimes and NSF IBN 9212640 to C.J.S., and NIHMH 48108 to D.V.M. and C.J.S.

389.7

DEVELOPMENT OF ELECTROPHYSIOLOGICAL MEMBRANE PROPERTIES AND SYNAPTIC RESPONSES OF FERRET LGNd CELLS. A.S. Ramoa* and D.A. McCormick. Yale Univ. Med. Sch. New Haven, CT

Establishment of the mature pattern of retino-geniculate connectivity requires the development of electrophysiological activity in LGNd (dorsal lateral geniculate nucleus) neurons. In the present study, we used whole cell patch-clamp recordings *in vitro* to examine the time course of development of the intrinsic membrane properties and synaptic (optic tract) inputs of ferret LGNd cells.

Several changes in electrophysiological properties of LGNd cells were noted between postnatal day 1 (P1) and P25. Although P1-5 cells could generate long-duration (500 msec) and high frequency (up to 50 Hz) trains of action potentials to depolarization, individual action potentials became taller in amplitude, faster in rate of rise and fall, and shorter in duration with age. Early postnatal cells could also generate a high threshold Ca ++ current but not the low threshold Ca++ spike that characterizes cells P5 or older. In addition, P1-5 cells lacked the hyperpolarization-activated cation current Ih. Activation of retinal afferents resulted in longer duration EPSPs earlier in life. IPSPs only appeared several days after birth. Thus, the postnatal development of intrinsic membrane properties and synaptic responses in LGNd neurons is coordinated to enhance synaptic-driven action potential activity at an early age, even before endogenous rhythmic burst firing develops.

389.8

ELECTROPHYSIOLOGICAL PROPERTIES OF DEVELOPING NEURONS RECORDED IN SLICES OF FERRET LGN C.A. White*, M. Esguerra and M. Sur. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139

During the first postnatal month, retinogeniculate axons segregate into eye-specific laminae and On and Off sublaminae. Eye-specific laminae are formed by postnatal day (P) 14 (Linden et al., '81) and On and Off sublaminae a week or more later. Using 4M potassium acetate electrodes, we recorded intracellularly from 45 A-layer neurons in slices of LGN between P7 and P33, during the period of lamination and sublamination. Resting membrane potential (mean=62.4 mV), input resistance (58.5 MΩ) and time constant (7.0 ms) did not change significantly during the period. At all ages cells were capable of firing action potentials and had low threshold potentials when excited from hyperpolarized membrane potentials. On the average action potentials became shorter in duration (from 4.4 ms to 1.7 ms) and larger in amplitude (from 56.9 mV to 77.5 mV) with age. In cells recorded with 4M cesium acetate, the spikes broadened significantly and a typical 8-20 ms afterhyperpolarization was not seen. Young and older neurons shared similar spike train characteristics, producing more spikes with shorter latency (maximum frequency 123 Hz) as current intensity increased. Optic tract stimulation produced EPSPs, IPSPs and EPSP-IPSP pairs beginning at the youngest ages. Preliminary experiments indicate that the EPSPs are reduced by the NMDA and non-NMDA glutamate receptor antagonists d-APV and NBQX, respectively, and that the IPSPs are reduced by the $GABA_A$ receptor antagonist bicuculline methiodide. Supported by EY 06297 (C.A.W.) and EY 07023 (M.S.).

389.9

GLUTAMATE - INDUCED CALCIUM RESPONSES OF DEVELOPING SUBPLATE CELLS. K. Herrmann* and C.J. Shatz. Division of Neurobiology, Dept. of Molecular and Cell Biology, U.C. Berkeley, CA 94720.

In neocortical development, the subplate is a complex neuropil filled with subplate neurons and synapses, some of which are supplied by waiting thalamic axons. To further understand and characterize the possible interactions between the waiting axons and their postsynaptic partners, we used Fura-2 imaging techniques. Since glutamate is thought to be used by thalamic axons in adults, glutamate-induced changes in the calcium responses of subplate cells were monitored in acute slices of neocortex taken from neonatal ferrets. Many cells in the subplate were well stained with Fura-2, but in acute slices they showed very little spontaneous activity. However, application of 100 µM glutamate induced large increases in the intracellular Ca^{++} concentration $[Ca^{++}]_i$, which could be mimicked by 100 µM NMDA. During application of glutamate and NMDA, some cells responded with a transient rise in $[Ca^{++}]_i$, while other cells underwent oscillations. Responses were abolished in the presence of 2.5 µM TTX, or upon application of 100µM glutamate in the presence of 50 µM APV. These observations indicate that glutamate elicits heterogeneous responses, suggesting the presence of diverse cell types in this zone, as has been demonstrated for the cortical plate in rats (Yuste & Katz 1991, Neuron 6, 333). In addition, these changes in $[Ca^{++}]_i$ depend upon activation of voltage sensitive Na^+ channels via NMDA receptors. Since migrating neurons are not thought to express NMDA receptors (Lo Turco et al.1991, J. Neurosci 11, 792), and astrocytes are virtually absent until about P4 in ferrets, it is likely that the observed changes in $[Ca^{++}]_i$ originate primarily in subplate neurons. If so, then subplate neurons are capable of responding to release of neurotransmitters such as glutamate during these early developmental times. Supported by the Alzheimer's Association and NIH EY02858.

389.10

GROWTH BEHAVIOR OF THALAMIC AXONS ON CORTICAL MEMBRANES STUDIED WITH TIME-LAPSE VIDEO MICROSCOPY. M. Hübener*[1], M. Götz[1], S. Klostermann[2], and J. Bolz[1]. [1]Friedrich-Miescher Labor der Max-Planck Gesellschaft and [2]Max-Planck Institut für Entwicklungsbiologie, Spemannstr., 7400 Tübingen, Germany.

Thalamocortical afferents arrive in the cortex before their target cells in layer 4 are born. The fibers wait in the subplate zone and enter the grey matter only after layer 4 cells have migrated to their final position. It has been shown recently that specific growth promoting molecules up-regulated during cortical development allow thalamic fibers to invade the cortex (Götz et al., Soc. Neurosci. Abstr. **17**, 898, 1991). We have now used time-lapse video microscopy to study the growth of thalamic axons on cortical membranes from different developmental stages, and their behavior at borders between different substrates. There were clear differences in the growth behavior of thalamic axons on cortical membranes prepared from embryonic (E16) or postnatal (P7) rats. On P7 membranes fibers grew at an average rate of 30 µm/h. Collapse of growth-cones was observed frequently after contact with other axons, but only rarely in the absence of fiber-fiber interactions. In contrast, axons grew slower on E16 membranes (16 µm/h) and growth-cone collapse often occurred without contact with another axon, suggesting that substrate properties are responsible for this behavior. In experiments where thalamic axons were given a choice between alternating lanes of E16 and P7 cortical membranes, a higher number of fibers grew on P7 membranes. Fibers extending on P7 membranes and encountering a border with E16 membranes often reacted with a growth-cone collapse and retraction. In many cases axons performed sharp turns after contact with E16 membranes and continued their growth on P7 membranes. Only a few fibers crossed the borders between the two substrates without any visible change in growth behavior. These observations show that the trajectories of thalamic axons can be oriented by growth-permissive molecules expressed on cortical membranes. Supported in part by DFG.

389.11

NEURONAL DOMAINS IN DEVELOPING NEOCORTEX: MECHANISMS OF COACTIVATION. R. Yuste*, D. A. Nelson, A. Peinado and L. C. Katz. Dept. of Neurobiology, Duke University, Durham, NC 27710.

Correlations in the spontaneous activity of neighboring neurons may play a critical role in the emergence of cortical connectivity. By imaging slices of neonatal rat visual and somatosensory cortex with the Ca^{2+} sensitive dye fura-2, we observe that discrete regions of neurons spontaneously undergo synchronous $[Ca^{2+}]_i$ changes, defining neuronal "domains." In tangential slices domains have similar sizes, 50 - 120 µm in diameter, discrete borders, and tile the cortex with little overlap with neighboring domains. In coronal slices they have radial shapes, resembling columns. These spatial properties suggests that domains represent modular units in developing cortex that could contribute to the formation of the adult functional architecture.

To study the mechanisms responsible for the coactivation of neurons within a domain, we performed high-speed optical recordings (33 msec time resolution). The activation of all neurons in a domain took less than 1 sec; the resulting increases in $[Ca^{2+}]_i$ persisted for 10 - 25 sec. The rate of spread of the coactivation ranged from 49 to 382 µm/sec (mean = 128 ± 71 µm/sec, n = 27). These speeds are faster than estimates of free ionic diffusion, but are compatible with electrical activation or regenerative second messenger waves (Meyer, *Cell* 64:675, 1991). Thus, passive diffusion of calcium cannot account for the coactivation. In addition, domains occurred in TTX, suggesting that their coactivation is not due to sodium spikes and conventional synaptic transmission, but may be mediated instead by gap-junctions. In agreement with this, perfusion of 12 slices with the gap-junction blocker 1-octanol (1 mM) reversibly blocked 69 ± 19 % of the domains. Also, consistent with gap-junctional coupling, intracellular injections of the tracer biotin ethylenediamine into single neurons resulted in the labeling of numerous neighboring neurons (see next abstract).

Based on these data, we hypothesize that the domains are due to the spontaneous activation of one or a few "trigger" neurons which in turn activate a restricted number of secondary neurons through gap-junctions.

389.12

EXTENSIVE NEURONAL DYE-COUPLING IN NEOCORTEX DURING THE TIME WHEN CIRCUITS ARE FIRST FORMED. A. Peinado*, R. Yuste and L. C. Katz. Dept. of Neurobiology, Duke University, Durham, NC 27710.

The formation of local circuits in the developing cerebral cortex involves many kinds of neuronal interactions mediated by conventional synaptic transmission. Another way for neurons to transmit signals is through gap-junctions. In cerebral cortex, these have been difficult to observe with conventional gap-junction tracers such as Lucifer yellow (LY), although there are reports of coupling among small groups of up to 9 neurons using LY in rat before day 10 (Connors et al, J. Neurosci. 3:773 '83) and among groups of ventricular zone neuroblasts (LoTurco & Kriegstein, Science 252:563 '91). Using a smaller intracellular tracer, biotin ethylenediamine (BE) (Vaney, Neurosci. Lett., 125:187, '91), in rat visual and somatosensory cortex, we find that coupling is widespread among neurons during the first 2 postnatal weeks.

We injected single neurons with BE or LY in cortical slices. BE was visualized using the ABC method (Vector) and viewed with Nomarski optics. LY was visualized similarly, using a biotin-conjugated anti-LY antibody to amplify its signal.

We find that after injection of biotin ethylenediamine into single neurons, the tracer either remains confined to that cell or spreads to many nearby neurons (range= 21-35 cells; mean= 27 cells; ages P7-9) where it labels their soma and proximal dendrites. In contrast, LY usually labels a single neuron and occasionally 2 or 3.

Coupling is already extensive by P5, the earliest time tested, and can be seen until P14, but not later. Injections on P5 often generate a columnar arrangement of labeled cells, 50-70 µm wide, spanning several cortical layers. At later ages most coupled neurons are found either inside the dendritic field of the injected neuron, where dendro-somatic contacts can be seen, or close enough to it to be within range of dendro-dendritic contact. Coupling is only rarely associated with axons.

These results, together with those in the previous abstract, suggest that exchange of small molecules and ions through dendritic gap junctions may be a means for neighboring cortical neurons to achieve temporal coordination of their responses during the period of circuit formation .

CELL MIGRATION AND MOTILITY I

390.1

Biochemical Differentiation of Migratory Gonadotropin-Releasing Hormone (GnRH) Cells in the Mouse. I. Livne*, M. J. Gibson, and A. J. Silverman. Dept. of Medicine, Mt. Sinai Sch. of Med., New York, N. Y. 10029 & Dept. of Anat. & Cell Biol., Columbia Univ., New York, N. Y. 10032.

GnRH cells are first detected in the olfactory placode on E11.5 in the mouse and migrate across the nasal septum on E12.5-E15.5, taking residence within the CNS on E16.5. We have studied several aspects of the biochemical and morphological differentiation of GnRH neurons and the cellular associations that they make during their migratory process. On E12.5, when most of the cells are still in the nasal septum, only 15% of the population can process the pro-GnRH precursor to the amidated decapeptide. Two days later (E14.5), when the majority of GnRH cells have advanced into the forebrain, 79% contain mature GnRH. In keeping with these observations, EM analysis indicated that E12.5 GnRH neurons are only lightly stained and the reaction product is confined to the outer nuclear envelope and the rough endoplasmic reticulum (RER). By E14.5 cells in the nasal septum have more reaction product in the RER and some of the Golgi cisternae are also positive. Immunoreactive neurosecretory granules also appear at this stage. While GnRH neurons migrate in the nasal septum they remain within the confines of the olfactory and vomeronasal (VMO) axonal fascicles. Immunoreactive cells maintain close apposition with each other and with the axons and their associated glia which ensheathe these fascicles. Once in the forebrain, GnRH neurons no longer maintain close association with each other, nor do they follow any defined anatomical structure. These findings indicate that although GnRH cells express their unique neuropeptide early in their ontogeny, their differentiation continues and is coordinated with their migration. The migration of these neurons across the nasal septum relies on axonal fascicles of the olfactory and VMO nerves. In the forebrain, however, GnRH cells must utilize alternative guiding mechanisms to complete their migration. Supported by HD 10665 and NS 20335.

390.2

CELLS DESTINED FOR THE OLFACTORY BULB ORIGINATE IN THE TELENCEPHALIC SUBVENTRICULAR ZONE AND REACH THEIR TARGET BY MIGRATING ALONG A RESTRICTED LONGITUDINAL PATHWAY. M.B. Luskin*, E. Breding, K.E. Miller, M. Maultsby. Dept. of Anatomy and Cell Biology & Program in Neuroscience, Emory Univ. Sch. of Med., Atlanta,GA 30322.

In the neonatal rodent forebrain, glial cells are derived from the subventricular zone (SVZ) which surrounds the lateral ventricles. We are further examining the spatial and temporal patterns of migration of cells originating in different regions of the SVZ.

To determine the course of migration of the neurons and glia arising in the anterior part of the SVZ (SVZa), a retroviral lineage tracer containing the reporter gene *E. coli* β-galactosidase (*lacZ*) was injected into the SVZa of rat pups on postnatal day 0 (P0) or P1. Animals were perfused either 1 day or 1 - 5 weeks later and the brains were sectioned and stained with X-Gal to reveal the position of $lacZ^+$ cells.

The number and distribution of $lacZ^+$ cells were dependent upon injection site and survival time. One day after injection, small numbers of $lacZ^+$ cells appeared near the injection site (1.0-1.9 mm lateral to midline, 1.5-2.0 mm anterior to Bregma, 1.5-2.0 mm below the pial surface), whereas after one week, there were numerous $lacZ^+$ cells throughout the subependymal zone (SEZ), which formed a continuous stream of cells extending longitudinally from the injection site to the center of the olfactory bulb (OB). Two weeks after injection, the majority (97%) of $lacZ^+$ cells had migrated from the SEZ of the OB radially into overlying cellular layers. By 4 weeks, <30 % of the post-migratory $lacZ^+$ cells were located in the glomerular layer, while the majority (>60 %) populated the granule cell layer. Retroviral injections more posteriorly (0.3-1.0 mm anterior to Bregma) in the SVZ seldom labeled cells in the OB.

Our experiments show that progenitor cells situated in the SVZa generate cells destined for the OB and that their progeny reach the OB by proceeding initially, in a longitudinal direction, along a confined pathway and then proceed radially outward into the OB. Supported by Pew Charitable Trusts, NIH and March of Dimes.